Modification of the core lipids of low density lipoproteins produces selective alterations in the expression of apoB-100 epitopes

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Abstract The conformation of the apolipoprotein B-100 associated with low density lipoproteins (LDL) is not fixed. Rather, the conformations of several regions are subject to alteration by a variety of metabolic and therapeutic perturbations that change either the lipid compositions and/or sizes of LDL particles. However, because these perturbations simultaneously alter several structural-compositional features of the particles it has been difficult to relate structural-compositional features of LDL to apoB-100 conformations. Furthermore, in in vivo studies several days pass between samplings, thus different sets of particles are studied before and after experimental perturbation. In the present experiments more discrete perturbations of LDL were obtained in vitro by incubating LDL with very low density lipoproteins (VLDL) in the presence of partially purified human plasma lipid transfer proteins. The conformations of apoB on the LDL particles then were examined *a*) by probing epitope expression and *b*) by examining interactions between LDL and LDL-receptors in cultured human fibroblasts. During incubations with VLDL and lipid transfer proteins, the diameters of LDL particles decreased; the percentage composition of LDL triglycerides increased three- to fivefold; concomitantly, cholesteryl esters decreased. Lipid transfer protein was required for the transfer to occur and the magnitude of the increase in LDL-triglycerides depended upon the duration of incubation, the ratio of VLDL/LDL, and unknown properties specific to the various LDL preparations. The fact that the triglyceride contents of all LDL preparations were not identically affected suggests that initial packaging of the core region may affect capacity for lipid exchange. With increasing core triglyceride contents, all LDL preparations manifested progressively decreased immunoreactivities, noted in competitive enzyme linked immunoassays using a panel of monoclonal anti-apoB antibodies. The regions encompassing amino acids 690 to 797 (MAb CC3.4) and 4082 to 4348 (MAb B6C3) appeared to be selectively affected. These regions are at a distance from the putative LDL-receptor recognition region on either side of amino acid 3249. Epitope expression between amino acids 3214 and 3506 (MAb B1B3) and 3506 and 3635 (MAb B1B6) were not affected. Affinities of interactions between LDL and LDL-receptors of cultured fibroblasts also were progressively decreased with increasing LDL-triglyceride enrichment, even though epitopes relatively close to the LDL-receptor recognition domain were apparently not affected. The data suggest that altering the core lipids of LDL leads to changes in the sizes of LDL particles and in the conformation of several regions of apoB-100. The data also indicate that conformation of epitopes involves a smaller region than the conformation of the LDL-receptor recognition region and, finally, that regions at a distance from the putative LDL-receptor recognition domain of apoB-100 may modulate the interactions of apoB-100 with the LDL-receptor. —**Kinoshita, M., E. S. Krul, and G. Schonfeld.** Modification of the core lipids of low density lipoproteins produces selective alterations in the expression of apoB-100 epitopes. *J. Lipid Res.* 1990. **31:** 701–708.

Supplementary key words triglycerides • lipid transfer protein • LDL-receptor

The conformation of apoproteins on lipoproteins can be altered by perturbing their compositions and/or structures either in vivo or in vitro (1). We have been studying the effects of lipid-lowering drugs and experimental diets on apoB-containing lipoprotein particles in order to understand the relationship between the compositions and/or structures of the lipoproteins on the one hand, and the conformations of their apoproteins on the other (2, 3). Altered conformations can be detected *a*) by physical methods which, unfortunately, do not identify the specific regions undergoing the alterations; *b*) by susceptibility to tryptic digestion; *c*) by functional tests, such as assays of LDL–LDL-receptor interactions (4–6); or *d*) by immunochemical methods (1). The latter three techniques, which can distinguish regional alterations in conformation, have

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apoB, apolipoprotein B; LTP, lipid transfer protein; TG, triglyceride; PL, phospholipids; PMSF, phenylmethylsulfonylfluoride; SBTI, soybean trypsin inhibitor; PPACK, D-phenylalanyl-L-propyl-L-arginine chloromethylketone; FPLC, fast protein liquid chromatography; PC, phosphatidylcholine; LPDS, lipoprotein-deficient serum; PAGE, polyacrylamide gel electrophoresis; GGE, nondenaturing gradient gel electrophoresis; MAb monoclonal antibody; BSA, bovine serum albumin; PBS, phosphate-buffered saline; CE, cholesteryl ester; ED_{50}, effective dose of antigen required to achieve 50% inhibition of binding of tracer radiolabeled antigen to antibody; IC_{50}, concentration required to achieve 50% inhibition.

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been used in our studies. Diet or drug-induced alterations of LDL composition and/or structure alter the expressions of some apoB epitopes while other epitopes are not affected. Similarly, some perturbations of LDL selectively change the interactions of LDL particles with LDL-receptors (3, 4). However, in these experiments performed in vivo it has not been possible to ascertain which structural-compositional features are related to changes in the expression of any given apoB epitope. This is because it is difficult to produce specific, quantitative perturbations of LDL in vivo. However, incubation of LDL in vitro with lipids may produce graded changes in the chemical compositions of LDL particles (4). In the set of experiments described herein, the triglyceride (TG) contents of LDL were systematically increased by incubating LDL with VLDL, in the presence of lipid transfer protein (7). The TG-modified LDL particles were then characterized with respect to their compositions, diameters, immunoreactivities (with monoclonal antibodies), and interactions with fibroblasts.

MATERIALS AND METHODS

Materials

L-3-Phosphatidyl [N-methyl-^{3}H]choline (1,2-dipalmitoyl) ([^{3}H]PL) was purchased from Amersham. Phenylmethylsulfonyl fluoride (PMSF), dithionitrobenzoic acid, soybean trypsin inhibitor (SBTI), and benzamidine were purchased from Sigma (St. Louis, MO). D-Phenylalanyl-L-propyl-L-arginine chloromethylketone (PPACK) was purchased from Calbiochem (La Jolla, CA). Microtiter plates were purchased from Nunc (Denmark) and the Fast Protein Liquid Chromatography (FPLC) apparatus, Superose 6 column, and phenyl-Sepharose 4B were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden).

Lipoprotein preparation

Blood from fasted donors was drawn into tubes containing EDTA (1.0 mg/ml). Plasma was separated by centrifugation and 20 mM PPACK and 0.1 mg/ml Gentamicin were added to the plasma. Very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) were obtained from plasma by sequential ultracentrifugation in a Beckman 50.2 Ti rotor, as density lower than 1.006 g/ml (VLDL), density between 1.02 and 1.05 g/ml (LDL), and 1.063 and 1.19 g/ml (HDL), respectively (8). VLDL, LDL, and HDL were dialyzed against 50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA.

Purification of lipid transfer proteins (LTP)

The procedure was as reported by Tall, Abreu, and Shuman (9). The d > 1.19 g/ml fraction was isolated from human plasma by ultracentrifugation at 45,000 rpm for 44 h in a Beckman 50.2 Ti rotor. The infranatant fraction was applied to a phenyl-Sepharose 4B column (2.0 × 30 cm) preequilibrated with 10 mM Tris (pH 8.0), 150 mM NaCl at a flow rate of 40 ml/h. The column was washed with 10 mM Tris (pH 8.0), 150 mM NaCl. The bound proteins were eluted with distilled water with 0.02% NaN_3 and 1 mg/ml EDTA (pH was adjusted to 8.0 with NH_4OH). The eluted fractions were adjusted to 50 mM sodium acetate by addition of 1/19 volume of 1 M sodium acetate (pH 4.5). The precipitates were removed by low speed centrifugation and the supernatant was applied to a carboxymethylcellulose column (2.0 × 30 cm CM52) (Whatman, England) equilibrated with 50 mM sodium acetate (pH 4.5) at flow rate 30 ml/h. The bound proteins were eluted with 120 mM NaCl in 50 mM sodium acetate (pH 4.5). The eluted proteins were dialyzed against 10 mM Tris (pH 7.4), 150 mM NaCl, concentrated, and analyzed by SDS-gel electrophoresis.

Characterization of LTP

Proteins concentrated to 300–1000 μg/ml protein and analyzed by 3–15% SDS-PAGE showed four protein bands. The major protein (~75% of Coomassie blue-stained area) in this fraction had a molecular weight of about 62,000 which corresponds to the molecular weight reported for LTPI (9, 10).

Preparation of [^{3}H]phosphatidylcholine (PC)-HDL

HDL was dialyzed against 150 mM NaCl, 10 mM Tris (pH 7.4), 1 mM EDTA, and 0.05% NaN_3. One μCi of [^{3}H]PC (1,2-dipalmitoyl-*sn*-phospho-[N-methyl-^{3}H]choline: 76 Ci/mmol) was dried under nitrogen atmosphere and dissolved in a volume of 95% ethanol that was less than 0.1% of the HDL volume. The [^{3}H]PC ethanolic solution was slowly added beneath the surface of the HDL with gentle stirring. The mixture was then incubated 4 h at room temperature followed by dialysis against 10 mM Tris (pH 7.4), 150 mM NaCl, and 1 mM EDTA.

Measurement of LTP activity

[^{3}H]PC-HDL and LDL were mixed in the test tubes (approximately 1:5, HDL-PC mass:LDL-PC mass). Next, the test LTP sample or control buffer (50 mM Tris, 150 mM NaCl, pH 7.4) were added to each tube. After all tubes were brought to a volume of 600 μl with control buffer, they were incubated at 37°C for 20 h. The reaction was stopped by immersion of the test tubes in ice. Chilled pooled plasma (400 μl) was added to each tube followed by the addition of 125 μl heparin–$MnCl_2$ (10). The test tubes were incubated for 30 min at 4°C. The apoB-containing lipoproteins aggregated with heparin–$MnCl_2$ and were sedimented by centrifugation at 3,000 rpm for 30 min at 4°C. Aliquots (750 μl) of each supernatant

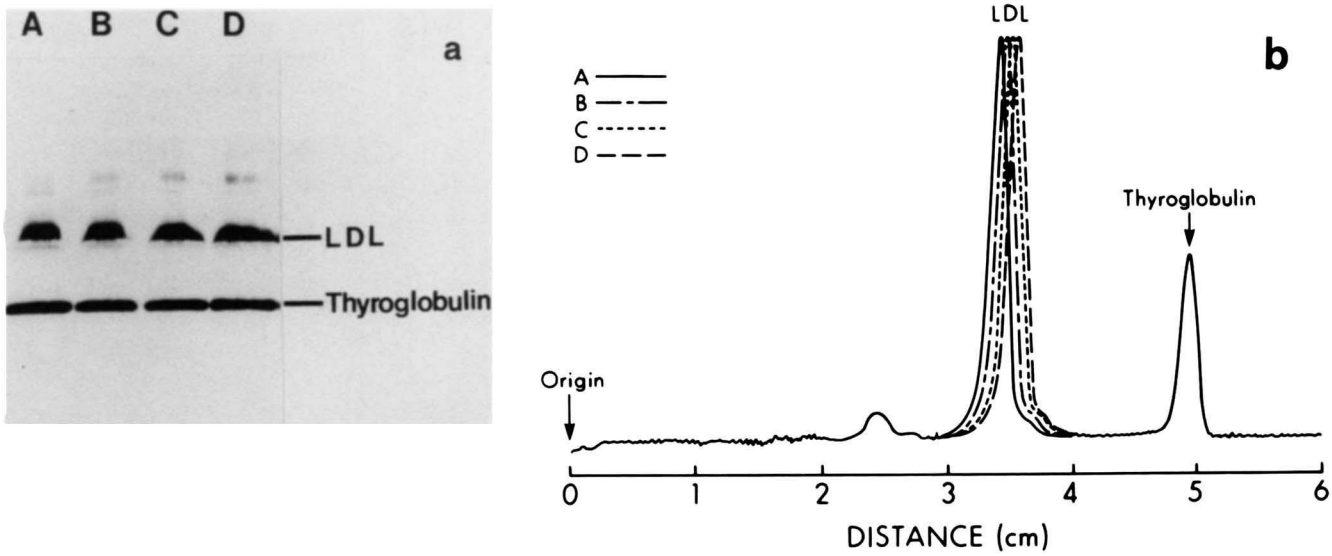

Fig. 1. a: Two to sixteen GGE analysis of LDL particles with different TG-contents. Four LDLs with different TG-contents (% of total particle weight) (e.g., A, 7%; B, 13%; C, 17%; D, 20%) were applied to nondenaturing gradient polyacrylamide gels for particle size analysis. Migrations of LDL and thyroglobulin (internal standard) are indicated by arrows. b: Results of laser densitometric scanning of the gel in (a). The gel was scanned by an ULTROSCAN (LKB, Sweden). Origins and thyroglobulin peaks are superimposed on each other to compare the differing migration distances of the LDLs.

were counted in a scintillation counter. LTP activity was expressed as percentage of [^{3}H]PC transferred from HDL to LDL/volume per incubation time. The range of transfer activity was 0.19–0.28 %/μl · h^{-1}. Since the partial purification does not separate LTPI (cholesteryl ester transfer protein) from LTPII (phospholipid transfer protein), the activity detected in our assay may have reflected the activities of both LTPs.

Transfer of TG from VLDL to LDL

VLDL and LDL were incubated with 5–10 μg partially purified LTP at 37°C for 20 h in 0.15 M NaCl, 50 mM Tris, pH 7.4, containing 2 mM dithionitrobenzoic acid, 1 mM PMSF, 1 mM benzamidine, 2.8 TIU/ml aprotinin, 0.05% soybean trypsin inhibitors, and 0.002% PPACK. Protein ratios of VLDL to LDL were varied between 0.1 and 2.0. After the incubation, VLDL, LDL, and LTP were separated from each other immediately by ultracentrifugation as described by Chait et al. (7). Control LDL was prepared by the incubation of LDL with VLDL but without LTP, or LDL with LTP but without VLDL under the same conditions.

Cell binding of lipoproteins by fibroblasts in culture

Human fibroblasts (GM0203), normal for LDL-receptor binding activity, were obtained from the NIH Cell Repository. LDL binding assays using human fibroblast cultures were performed according to the methods of Goldstein and Brown (5) and Ostlund et al. (6). Fibroblasts were grown in culture dishes and experiments were begun after incubation in lipoprotein-deficient serum (LPDS) for 48 h. In the experiment, LDL was added to the cell cultures in media containing 10% LPDS, simultaneously with a constant amount of reference ^{125}I-labeled LDL. The ability of these preparations to inhibit the binding of reference ^{125}I-labeled LDL to cells was compared at 4°C for 4 h. Lipoprotein binding was determined after fibroblasts were washed, dissolved in 0.1 M sodium hydroxide, and aliquots were assayed for cell-associated radioactivity as described previously (11). Additional aliquots were taken to determine total cell protein. Nonspecific binding was determined as the amount of bound or cell-associated radioactivity after incubation of ^{125}I-labeled LDL in the presence of 50-fold excess of nonlabeled homologous lipoprotein.

Polyacrylamide gel electrophoresis (PAGE)

Purified LTP and apolipoproteins were analyzed by SDS-PAGE by the method of Laemmli (12). In some cases, LDL preparations were analyzed for particle size distribution by nondenaturing gradient polyacrylamide gel electrophoresis (GGE) on 2–16% polyacrylamide gels.

Enzyme-linked competitive immunoassay using anti-apoB monoclonal antibodies (MAbs)

Competitive inhibition of LDL binding to anti-apoB MAb was performed in 96-well microtiter plates. Control LDL (150 μl containing 1 μg protein/ml) were loaded into each well and incubated overnight at room temperature. Wells were then washed with phosphate-buffered saline (PBS) and blocked with 3% BSA-PBS for more than 1 h.

Serial dilutions of the LDLs in 1% BSA-PBS were added to wells followed by a constant amount of MAbs and incubated overnight at room temperature. After incubation, each well was washed and horseradish peroxidase-labeled goat anti-mouse IgG (150 μl of 1:1000 dilution, Fisher Biotek) was added to each well and incubated for 4 h at room temperature. After this, 150 μl of *o*-phenylenediamine dihydrochloride (0.67 mg/ml in 0.1 M sodium citrate [pH 5.0]) was added for color development and the reaction was stopped by the addition of 75 μl of 4 N H_2SO_4. Absorbances in each well were read on a MR600 Microplate Reader (Dynatech) at 490 nm using a reference wavelength of 630 nm. ED_{50} values were calculated by ALLFIT program (13). The open circles and closed triangles each indicate a different preparation of LDL. Linear regression curves and correlation coefficients (r) were calculated using the Sigma Plot computer program (Jandel, Corte Madera, CA). Levels of significance are indicated in parentheses.

Other methods

Protein was measured by the method of Lowry et al. (14), using the National Bureau of Standards BSA as a standard. Unesterified and esterified cholesterol, phospholipids, and triglycerides were measured using enzymatic kits (Wako Fine Chemicals, Dallas, TX).

RESULTS

VLDL-LDL incubations and characterization of TG-rich LDL

Initially, incubations of LDL with VLDL, in the presence of LTP were conducted at 37°C at VLDL/LDL protein ratios of 2/1, for 20 h. LDL became enriched in triglycerides and lost cholesterol, while VLDL acquired cholesterol lost triglycerides (**Table 1**). The mean cholesteryl ester (CE)/TG ratio in LDL fell from 4.07 to 2.22, while in VLDL it rose from 0.12 to 0.17. There were no significant differences in the ratios of cholesteryl esters/total cholesterol or in the amounts of phospholipids. The lipid composition of two control LDLs incubated without LTP or without VLDL were not significantly different from those of the freshly isolated lipoproteins. When VLDL/LDL protein ratios were varied stepwise between 0.0–2.0/1.0 while the time of incubations with LTP was held constant at 20 h, LDL-triglyceride contents rose stepwise from 4.3 to 13.4% (% mass) (**Table 2A**). In two other experiments using different LDL donors but carried out under the same conditions, LDL-triglyceride contents rose from 7 to 20% and from 1.9 to 10.2%. For one LDL preparation, varying the incubation times between 1 and 6 h while VLDL/LDL ratios were held at 2/1, raised LDL-triglyceride contents from 1.5 to 4.3% (Table 2B). In each incubation reciprocal changes were seen in the contents of LDL-cholesterol, but there were no significant or consistent changes in the other components of LDL. No degradation of apoB occurred during these incubations as judged by SDS-PAGE analysis, either in control or LTP-treated LDL (not shown).

To assess the effects of TG acquisition and cholesterol loss on LDL size, the LDL samples were applied to 2–16% GGE gels for electrophoresis. LDL with relatively higher TG-contents had smaller diameters than the LDL with lower TG-contents (**Fig. 1a and b**).

In competitive binding assays using several monoclonal antibodies directed against apoB-100, TG-enriched LDL bound with less affinity (larger ED_{50} values) than control LDL to two of the MAbs (B6C3 and CC3.4), but TG-enriched LDL bound with the same affinity as control LDL to six other MAbs (**Fig. 2a and b; Table 3**). Slopes of curves were not significantly or consistently different between control and TG-enriched LDLs. This pattern of immunoreactivity was consistent in three LDL preparations tested.

There were significant direct correlations between ED_{50} values obtained with MAbs B6C3 and CC3.4 and the TG-contents of several LDL preparations. For MAb B6C3, $y = 0.474x + 4.02$, $r = 0.760$ ($P < 0.02$), and for MAb CC3.4, $y = 0.101x + 0.718$, $r = 0.630$ ($P < 0.03$),

TABLE 1. Effect of coincubation of VLDL, LDL, and LTP on lipoprotein composition

Lipoprotein Composition	VLDL				LDL			
	No LTP		With LTP		No LTP		With LTP	
	% mass							
Protein	7.7	7.7	7.6	7.6	26.7	27.1	28.7	29.1
Triglyceride (TG)	63.2	63.1	61.5	59.6	7.2	7.5	12.3	11.7
Cholesteryl ester (CE)	7.5	7.7	9.0	11.3	30.7	29.1	25.9	27.4
Free cholesterol	5.6	5.7	5.8	5.4	9.8	9.8	9.2	9.5
Phospholipid	16.0	16.0	16.1	16.2	25.5	26.5	23.8	22.3
CE/TG (mass ratio)	0.12	0.12	0.15	0.19	4.2	3.9	2.1	2.3

Results represent two different preparations of VLDL and LDL. Values represent the means of duplicate determinations. VLDL and LDL (protein ratio 2:1) were incubated with or without LTP at 37°C for 20 h.

TABLE 2. Effects of VLDL/LDL protein ratio and time of incubation of LDL composition

Lipoprotein	A: VLDL/LDL Protein Ratio[a]				B: Incubation Time[b]			
Composition	0:1	0.1:1	0.5:1	2:1	0 h	1 h	3 h	6 h
				% mass				
Protein	28.2	28.4	28.8	28.9	30.1	28.2	29.0	28.7
Triglyceride (TG)	4.3	10.0	13.3	13.4	1.5	2.8	3.0	4.4
Cholesteryl ester (CE)	29.4	24.9	22.0	23.8	30.6	31.0	29.3	28.4
Free cholesterol	12.5	11.2	10.4	9.2	10.2	10.9	10.8	11.1
Phospholipids	25.6	25.5	25.4	24.6	27.5	27.0	27.9	27.4
CE/TG (mass ratio)	6.89	2.49	1.65	1.77	18.93	10.76	9.64	6.57

[a]Incubations were carried out for 20 h at 37°C at the indicted VLDL/LDL protein ratios. Values represent the means of duplicate determinations.
[b]The VLDL/LDL protein ratio was 2:1 and incubations were carried out for indicated times at 37°C. Values represent the means of duplicate determinations.

where y = ED_{50} (μg/ml) and x = LDL-TG (% of LDL mass). There was no significant correlation between ED_{50} values and the percent mass of phospholipids.

TG-rich LDLs also were bound with lower affinity to the LDL-receptors of fibroblasts compared to LDLs with lower TG-contents (**Fig. 3**). There appeared to be significant correlation between 50% inhibitory concentrations (IC_{50}) and the TG-contents of LDLs (Fig. 3, inset), although the slopes of the curves were not identical for the two LDL preparations tested.

DISCUSSION

Our aim was to assess the effects on epitope expression of perturbing the core regions of LDL by enriching them with triglycerides and depleting them of cholesteryl esters. Partially purified LTP was used to effect the lipid transfers that occurred only in the presence of LTP. The amount of exchange occurring depended on the proportion of VLDL to LDL and the time of incubation. Also, each of the LDL preparations isolated from different donors acquired different amounts of triglycerides, even under similar conditions of incubation with the same LTP and VLDL preparations (Tables 1 and 2), suggesting that the initial packing of lipid in the LDL particles probably affected the rate and the total amount of triglycerides that could be assimilated. LDL became smaller in size as they acquired more TG and lost cholesteryl esters (Fig. 1). This is consistent with the small sizes of TG-rich LDL observed in patients with hypertriglyceridemia (2).

Two modalities were used to probe the conformation of apoB-100 in TG-enriched LDL: *a*) immunochemistry, and *b*) LDL-cell interactions. Each modality was able to detect some alterations in apoB-100 conformation.

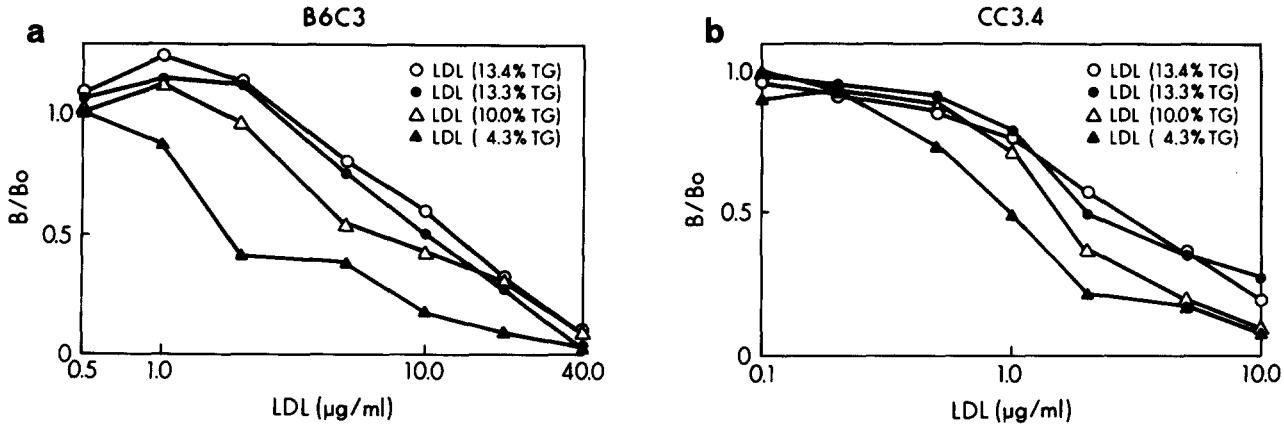

Fig. 2. Competitive inhibition of LDL binding to anti-apoB MAb B6C3 (a) or anti-apoB MAb CC3.4 (b). Control LDL (150 μl containing 1 μg protein/ml) were incubated in 96-well plates overnight at room temperature. After blocking the nonspecific protein binding sites on the wells with 3% BSA-PBS, serial dilutions of LDLs with different TG-contents in 1% BSA-PBS were added to wells followed by a constant, predetermined amount of MAb and incubated overnight at room temperature. After incubation, each well was washed and horseradish peroxidase-labeled goat anti-mouse IgG was added to each well and incubated for 4 h at room temperature. After this, 150 μl of *o*-phenylenediamine dihydrochloride was added for color development and the reaction was stopped by the addition of 75 μl of 4 N H_2SO_4. B/Bo represents the ratio of MAb binding in the presence of the added LDLs divided by the binding observed in the absence of any added LDL. Each point was determined in duplicate.

TABLE 3. Slopes and ED_{50} (midpoint isotherm) values of competition curves

LDL#1		13.4% TG-LDL	10.0% TG-LDL	4.3% TG-LDL[a]
C1.4	ED_{50}	0.40 ± 0.05	0.47 ± 0.06	0.44 ± 0.05
	Slope	1.05 ± 0.16	1.22 ± 0.19	1.47 ± 0.23
C4D1	ED_{50}	2.83 ± 0.28	2.45 ± 0.21	3.00 ± 0.21
	Slope	1.12 ± 0.14	1.09 ± 0.11	1.57 ± 0.16
CC3.4	ED_{50}	2.87 ± 0.30	1.68 ± 0.14	0.97 ± 0.10
	Slope	1.05 ± 0.13	1.49 ± 0.17	1.22 ± 0.15
D7.2	ED_{50}	2.34 ± 0.37	2.36 ± 0.36	2.64 ± 0.42
	Slope	0.79 ± 0.10	0.86 ± 0.11	1.01 ± 0.13
29EE-2	ED_{50}	0.54 ± 0.06	0.60 ± 0.06	0.57 ± 0.08
	Slope	1.04 ± 0.13	1.11 ± 0.13	1.02 ± 0.15
B1B6	ED_{50}	0.24 ± 0.03	0.22 ± 0.02	0.23 ± 0.02
	Slope	0.92 ± 0.09	0.96 ± 0.09	1.13 ± 0.11
B1B3	ED_{50}	0.35 ± 0.04	0.32 ± 0.04	0.33 ± 0.03
	Slope	1.04 ± 0.10	0.90 ± 0.10	1.08 ± 0.11
B6C3	ED_{50}	12.44 ± 1.56	8.10 ± 1.21	5.66 ± 0.89
	Slope	1.67 ± 0.36	1.27 ± 0.25	1.26 ± 0.25

LDL#2		20% TG-LDL	17% TG-LDL	13% TG-LDL	7% TG-LDL[a]
CC3.4	ED_{50}	2.81 ± 0.33	1.62 ± 0.19	1.06 ± 0.11	0.84 ± 0.09
	Slope	0.92 ± 0.12	0.83 ± 0.09	1.12 ± 0.13	0.97 ± 0.17
B6C3	ED_{50}	13.86 ± 1.49	8.55 ± 1.22	13.61 ± 2.02	6.24 ± 0.71
	Slope	1.48 ± 0.26	1.09 ± 0.20	0.93 ± 0.18	1.43 ± 0.23

LDL#3		10% TG-LDL	8% TG-LDL	2% TG-LDL[a]
CC3.4	ED_{50}	1.88 ± 0.23	2.61 ± 0.27	0.70 ± 0.10
	Slope	1.08 ± 0.14	1.25 ± 0.15	0.96 ± 0.12

Results of three different LDL preparations are shown. ED_{50} values and slopes were calculated by the ALLFIT program (13). ED_{50} values are given as μg/ml. ED_{50} and slope values represent means ± SD for points run in triplicate. (see Fig. 3).

[a]Indicates the TG-contents for controls (i.e., LDL not treated with LTP or VLDL).

In fibroblast binding assays, the ability of TG-enriched LDL to compete for binding of ^{125}I-labeled control LDL decreased in proportion to the degree of TG-enrichment (Fig. 3). These results are in agreement with those of Chait et al. (7) who noted a progressive decrease in degradation of ^{125}I-labeled modified LDL with increasing TG-content.

Although our results indicate a modulation of the conformation of the LDL-receptor recognition site on apoB-100, no significant changes in the immunoreactivities of these TG-enriched LDLs with MAbs B1B6 and B1B3 were noted (Table 3), despite the assignment of the epitopes of these MAbs near to the putative LDL-receptor recognition site of apoB-100 (15, 16). However, distant epitopes (MAbs B6C3, amino acids 4082 to 4348, and CC3.4, amino acids 690 to 797) were modulated. It is interesting to note that lipolysis of phospholipids, a perturbation of the surface region of LDL, induced conformational changes in a region around the epitope recognized by CC3.4 (amino acid residues 401–582, recognized by MAb C4D1) in some LDL preparations (4).

Based on the specificities of the MAbs and the findings of Chait et al. (7) and Aviram et al. (17) (see below), differences in binding of MAbs B1B3 and B1B6 to TG-modified LDLs may have been anticipated, but similar dissociations between binding of LDL to receptors and changes in MAb reactivity have been observed before in

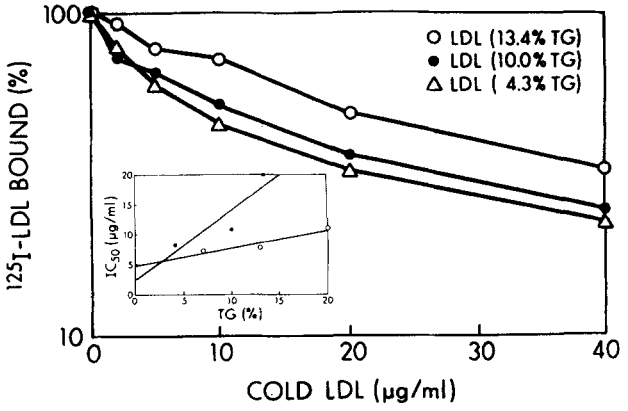

Fig. 3. Inhibition of binding at 4°C of ^{125}I-labeled LDL to LDL receptors of fibroblasts by LDLs with different TG-contents. The cell binding experiment was carried out as described in Methods. A constant amount of ^{125}I-labeled LDL (4.0 μg/ml) (TG-content = 4.3%) was added to all culture wells with or without increasing concentrations of three preparations of LDLs (LDL of 13.4%, 10.0%, and 4.3% TG-content, respectively). ^{125}I-Labeled LDL binding in the absence of competitor was set to 100%. Coefficients of variation for replicates were < 10%. (Inset) Correlation between IC_{50} (concentration of LDL protein required to achieve 50% inhibition of ^{125}I-labeled binding to fibroblasts) and TG-content of LDL. IC_{50} was calculated from regression curves fitted to competitive inhibition data. Closed and opened circles indicate different LDL preparations tested in two different cell experiments using two different preparations of ^{125}I-labeled LDL. A regression curve for each experiment is shown. Closed circles represent the same LDL samples used as competitors in Fig. 3. Open circles represent another preparation of LDL particles used as competitors whose TG-contents were 7, 13, 17, and 20%, respectively.

this laboratory. For example, after feeding a high carbohydrate, nearly fat-free diet for 7 days to normolipidemic individuals, their LDLs became smaller in diameter and relatively enriched in triglycerides (3). These LDLs were bound and degraded more avidly by cultured fibroblasts. While ED_{50} values in RIAs containing MAb B1B3 were decreased, no changes in ED_{50} were noted with MAb B1B6(3). Also, after phospholipase A_2 treatment of LDL, the phospholipid depletion of LDL particles caused significant changes in fibroblast LDL-receptor recognition, yet there were only minimal to no changes in MAb B1B6 and B1B3 binding (4), while ED_{50s} with MAb B6C3 increased greatly. One interpretation of the present and previous results is that the binding of a given MAb may depend on the specific conformation of a relatively small region of apoB-100, which remains unchanged in the experiments, whereas optimal LDL-receptor recognition may involve not only the recognition site itself but also areas of the apoB-100 molecule distant from the recognition domain whose conformations did change. The importance of distant regions for optimal LDL-receptor recognition is also supported by our findings in a patient with hypobetalipoproteinemia whose LDL contained a truncated form of apoB-100 characterized by a deletion of 11% of the carboxylterminal region, including the MAb B6C3 epitope (18, 19). This apoB-89-LDL was bound and degraded by normal fibroblasts with enhanced affinity, while the binding of MAb B1B6 was unaltered. Thus, it is possible to have changes in binding of apoB-100 to the LDL-receptor without concomitant changes at epitopes near the LDL-receptor recognition region.

Results compatible to those reported herein were published by Aviram et al. (17) while this work was in progress. Those workers used ^{13}C-NMR spectroscopy and one anti-apoB MAb used in the current study that is able to inhibit LDL binding to fibroblasts. They concluded from the NMR data that the conformation of apoB was indeed altered by TG-enrichment of LDL-cores. However, the NMR data were unable to provide data on the specific regions undergoing the conformational changes. Their cell binding studies indicated that the region around the LDL-receptor recognition domain of apoB-100 was altered by TG-enrichment of LDL. However, as noted above, they also noted slight changes in ED_{50} using our MAb B1B6, while we did not. The reasons for the discrepancy are not obvious to us. Nevertheless, our data confirm and extend their observations to more MAbs that probed other regions of the molecule. It is clear that several specific regions of apoB-100 are altered as a result of increasing the TG-content of LDL in vitro and this is correlated with altered functional behavior of the LDL particle.

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