Binding affinity and particle size of LDL in subjects with moderate hypercholesterolemia: relationship with in vivo LDL metabolism

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Abstract The aim of this study was to examine relationships between low density lipoprotein (LDL) metabolism, in vitro binding, and particle size. Twenty-four study subjects, 17 men and 7 women, had elevated plasma total cholesterol (TC), ranging from 174 to 252 mg/dl, and LDL cholesterol (LDLC) ranging from 113 to 195 mg/dl after 12 weeks on a Step I diet. The fractional clearance rate (FCR) for LDL ranged from 0.233 to 0.619 pools/day (0.366 ± 0.021) and was significantly correlated with plasma triglycerides (TG) (P < 0.05). Although there was no relation between FCR and binding in the study group as a whole, those subjects with an FCR within the normal range (>0.45, n = 20), showed a significant negative correlation between FCR and the KD for LDL binding, (r = -0.52). A subset of four subjects with an elevated FCR (>0.45) had higher production rate (PR) (P < 0.005) and a significant positive correlation between the KD for LDL binding and FCR (P < 0.05). LDL size varied from 240.0 to 265.8 Å and was significantly inversely correlated with plasma TG (P < 0.001) but there was no relation between LDL size and metabolism or binding affinity. Thus, there appears to be a correlation between binding affinity and clearance for subjects who had normal LDL production and clearance rates. On the other hand, mechanisms other than binding affinity appear to influence clearance in subjects with elevated rates of production and clearance. — Yamane, K., S. Kataoka, N-A. Le, M. Paidi, W. J. Howard, J. S. Hannah, and B. V. Howard. Binding affinity and particle size of LDL in subjects with moderate hypercholesterolemia: relationship with in vivo LDL metabolism. J. Lipid Res. 1996. 37: 1646-1654.

Supplementary key words fractional clearance rate • LDL cholesterol • LDL heterogeneity • LDL receptor

It is well established that an elevated concentration of low density lipoprotein (LDL) cholesterol is a major risk factor for the development of atherosclerosis (1). Thus, considerable research has been conducted to determine how LDL is metabolized with the aim of devising strategies to reduce its concentration. Metabolic studies have shown that there is wide variation in LDL clearance among moderately hypercholesterolemic subjects (2, 3) and on an even larger scale among populations (4). This may be due to a number of factors such as coexisting elevated triglycerides (5) or genetic variation of apoB (6). LDL metabolic studies that have been used to assess the mechanism of action of interventions designed to increase clearance have shown that the effects of an intervention on LDL metabolism can be demonstrated either in vivo or in vitro (3, 7, 8). However, none of the previous studies have examined both LDL metabolism in vivo and binding in vitro in the same subjects.

Reduced LDL clearance may be due either to reduced activity of the LDL receptor or to low affinity of circulating LDL for the receptors (9). Although a reduction in the number of functional LDL receptors has been shown to reduce LDL clearance in some patients, these patients account for a small high risk group with familial hypercholesterolemia (10, 11). The explanation for reduced clearance is less clear for the majority of subjects with moderate hypercholesterolemia. Several pharmacological studies have demonstrated that it is possible to increase LDL clearance in vivo (3, 12) while others using the same or a similar agent show that in vitro binding can be enhanced (13). Thus, there may be a relation between increased LDL clearance and improved LDL receptor binding in subjects with moderate hyperlipidemia; however, this has not been examined thoroughly because the in vivo and in vitro studies have not generally been conducted in concert.

Abbreviations: BMI, body mass index; FCR, fractional clearance rate; FFM, fat-free mass; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; C, cholesterol; IDL, intermediate density lipoprotein; KD, dissociation constant; LDL, low density lipoprotein; LPDS, lipoprotein-deficient serum; PR, production rate; SDS, sodium dodecyl sulfate; TC, total cholesterol; TG, triglyceride; VLDL, very low density lipoprotein.

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LDL is known to be heterogeneous in size and density (14, 15), and this variation, within and between subjects, may contribute to differences in metabolism. The relationship between LDL size and metabolic clearance has been studied more fully than that of clearance and binding; however, the findings have not been consistent. A number of studies suggest that dense LDL is slow to clear (12,16). On the other hand, Vega and Grundy (5) reported that the denser LDL from subjects with primary hypertriglyceridemia was cleared rapidly.

The purpose of this study was to examine the relationships between LDL metabolism, binding affinity, and particle size in a study across a wide range of cholesterol concentrations in order to determine to what extent binding affinity and LDL size influence LDL metabolism in vivo.

METHODS

Study subjects

The 17 men and 7 women participating in this study, ranged in age from 27 to 55 years, and had borderline elevated plasma LDL cholesterol levels, at least 130 mg/dl at baseline, before diet stabilization. The participants were recruited for a study of a pharmacological intervention, but the present report presents the data for baseline studies only. Entrance criteria included a plasma triglyceride level <300 mg/dl and body fat <30% in men and <35% in women. All subjects gave written informed consent for the study, which was reviewed and approved by the Institutional Review Board of the Washington Hospital Center. None had evidence of cardiovascular, gastrointestinal, liver, renal, metabolic, or endocrine diseases and none were taking any medications known to affect lipid metabolism. None of the women were pregnant or lactating.

Experimental design

Subjects were counseled on how to modify their diets according to the American Heart Association Step I diet (17) and followed this for a period of 12 weeks after which entrance criteria were reevaluated. Subjects continued on the Step I diet for an additional 16 weeks, at the end of which fasting lipids were remeasured and a large volume of venous blood was drawn for LDL isolation to be used for a metabolic study, an LDL binding assay, and measurement of LDL size.

Lipid and lipoprotein analyses

Blood was collected in tubes containing K$_2$EDTA (1.5 mg/ml final concentration) after an overnight fast and plasma was separated immediately by centrifugation (1,700 g, 10 min, 4°C). Triglyceride and total cholesterol concentrations were measured enzymatically as described by Wahlefield (18) and Allain et al. (19) on a Hitachi 705 automated chemistry analyzer (Hitachi, Tokyo, Japan) using reagents from Boehringer Mannheim Diagnostics, (Indianapolis, IN). VLDL (d < 1.006 g/ml) was isolated by ultracentrifugation (59,000 rpm, 16 h, 15°C) in a Beckman 50.4 Ti rotor (Beckman, Fullerton, CA) and cholesterol was measured in the infranatant fraction (d >1.006 g/ml). High density lipoprotein (HDL) cholesterol was measured after precipitation of apoB-containing lipoproteins on the whole plasma with heparin and MnCl$_2$. LDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol in the infranatant fraction (d >1.006 g/ml). The Medlantic Research Institute Laboratory participates in the Centers for Disease Control–National Heart, Lung, and Blood Institute Lipid Standardization Program and has met the criteria of precision and accuracy for the measurement of total cholesterol, triglyceride and HDL cholesterol.

LDL isolation

After an overnight fast, approximately 100 ml of venous blood was collected from each subject in plasmapheresis bags under sterile conditions using non-pyrogenic reagents. Plasma was separated immediately by centrifugation (800 g, 30 min, 10°C) and adjusted to d 1.020 g/ml using a solution of NaBr in 150 mM NaCl, 1 mM EDTA, d 1.350 g/ml (NaBr–NaCl), and VLDL and intermediate density lipoprotein (IDL) were removed after ultracentrifugation (50,000 rpm, 20 h, 15°C) in a 60 Ti rotor. The infranatant was adjusted to d 1.060 g/ml and LDL was isolated by centrifugation (50,000 rpm, 20 h, 15°C). LDL was rewashed and concentrated by adjusting to d 1.070 g/ml and centrifuging (40,000 rpm, 20 h, 15°C) in a 40 Ti rotor.

Isolated LDL was dialyzed against 150 mM NaCl containing 0.1 mM EDTA, 1 mM NaH$_2$PO$_4$, pH 7.4 (buffer A) for several hours at 4°C and divided into aliquots. LDL for the metabolic study was freshly iodinated and used while the remainder was frozen at -70°C for binding assays, LDL size, cholesterol, and apoB measurement. Hara and Howard (20) previously showed that there was no difference in binding between fresh and frozen LDL.

Pooled LDL used in the binding assays for radiotracer and nonspecific binding was prepared from several bags of frozen plasma (American Red Cross) using the same procedure described above and frozen aliquots were used throughout the studies. LDL protein was measured by the Lowry method as modified by Markwell et al. (21).
Radiiodination of LDL

Autologous LDL for the metabolic study and pooled human LDL for tracer in the binding assays were similarly labeled with $^{125}$I by the iodine monochloride method (22) as modified by Bilheimer, Eisenberg, and Levy (23). Unbound iodine was removed by extensive dialysis against buffer A. The labeling efficiency, the specific activity, the proportion of unbound iodine, and the degree of lipid labeling averaged 31.1 ± 2.8%, 52 ± 5 cpm/ng of LDL protein, 0.95 ± 0.03%, and 6.3 ± 1.1% (mean ± SEM), respectively, for the metabolic studies. Iodination values for the binding studies were similar: 21.6 ± 4.3%, 233 ± 77 cpm/ng of LDL protein, 0.60 ± 0.14%, and 7.3 ± 2.0%, respectively.

LDL apoB metabolic study

Measurement of the kinetics of LDL apoB was performed by a previously described method (24) with minor modification. $^{125}$I-labeled LDL was diluted with sterile 5% human serum albumin (Albuminar, Armour Pharmaceutical, Kankakee, IL) to obtain a final activity of 10 μCi/ml and passed through a Millipore filter (Millex-GV, 0.22 μm). Sterility and lack of pyrogenicity were confirmed in aliquots of the final preparation. All subjects received 150 mg of potassium iodine daily. Twenty-five μCi of autologous $^{125}$I-labeled LDL was injected intravenously after an overnight fast. Venous blood samples (10 ml) were collected at -30, 15, 30, and 60 min; 3, 6, 8, 24, 30, and 48 h; and 3, 5, 7, 9, 11, 13, and 14 days after injection. The validity of this sampling schedule was established previously (25). Subjects followed the Step I diet during the course of the metabolic study, except that on the day of injection breakfast and lunch were replaced by low fat midmorning and midafternoon meals.

Plasma was measured for radioactivity and the $^{125}$I counts were plotted against time to obtain a decay curve for calculation of fractional clearance rate (FCR) for LDL apoB according to a two-pool compartmental model (10).

ApoB in LDL was measured by immunoturbidimetric assays (26) using monospecific antibody for human apoB (Incstar Corporation, Stillwater, MN). This measurement has a CV of 5.0%. LDL apoB concentration in plasma was then estimated by multiplying the concentration of LDL cholesterol in plasma by the LDL apoB to cholesterol ratio as determined in the LDL fraction. The average ratio of the LDL apoB to cholesterol was 0.71 ± 0.01 (SEM). Plasma volume was determined as a function of body weight using the following equation (27): plasma volume (L) = 0.045 (L/kg) × fat-free mass (kg) + 0.61 (L/kg) × fat mass (kg). LDL apoB pool size was calculated as the product of the plasma volume and the concentration of LDL apoB. The production rate for LDL apoB was determined by multiplying the FCR for LDL apoB by the pool size and was expressed per kilogram of fat-free mass, as calculated from % body fat (Model 101, RJL Systems, Detroit, MI) because of the wide range of % body fat in the study participants.

Determination of LDL binding, internalization, and degradation

To assess binding, LDL from each subject was tested in a competition assay using $^{125}$I-labeled pooled human LDL as tracer (28). The TR 715-19, a mutant Chinese hamster ovary cell line that expresses a transfected human LDL receptor, was grown in culture medium (Ham's F-12 nutrient mixture supplemented with 20 mM HEPES, pH 7.4, and 2 mM L-glutamine), 4% (v/v) newborn calf lipoprotein-deficient serum (LPDS), 1% (v/v) fetal calf serum, 20 μM mevinolin, and 200 μM mevalonate. For binding experiments, cells were seeded at a concentration of 4 to 6 x 10⁴ cells per dish into petri dishes (60 x 15 mm) containing culture medium supplemented with 10% fetal calf serum. On day 3, cells received culture medium supplemented with 5% newborn calf LPDS, 0.1 μg/ml 25-hydroxycholesterol, and 10 μg/ml cholesterol. On day 4, cells were transferred to assay medium (Eagle's minimum essential medium, 10 mM HEPES, pH 7.4, 24 mM bicarbonate, 1% nonessential amino acids, and 10% human LPDS). The assay medium contained 1 μg/ml $^{125}$I-labeled LDL protein and 0, 1.5, 3.0, 6.0, 9.0, 19.0, 39.0, or 59.0 μg/ml nonlabeled LDL protein from each subject in triplicate dishes. Dishes were incubated at 37°C in a CO₂ incubator for 2 h. The binding, internalization, and degradation were determined according to procedures described by Goldstein, Basu, and Brown (29), modified as described previously (20). To determine nonspecific binding, 500 μg/ml nonlabeled pooled LDL protein was added. The apparent dissociation constants (Kᵣ) were computed from Scatchard analysis (30) of the binding data using a linear regression method. When a single LDL was assayed repeatedly, the binding assay had a CV of 28%.

LDL size measurement

Gradient gel electrophoresis on 2.5-16% nondenaturing polyacrylamide gels (Isolab Inc. Akron, OH) was performed to determine LDL size by a minor modification of the method of McNamara et al. (31). Frozen LDL samples, previously shown to give the same results as fresh samples, were thawed and stained with 1.3% Sudan Black B in ethylene glycol overnight at 4°C prior to
electrophoresis. Gels were pre-run at 120 V for 30 min in advance and pre-stained samples were electrophoresed at 8-10°C using 90 mM Tris buffer containing 80 mM boric acid and 3 mM Na2EDTA at pH 8.3. The voltage was first adjusted to 50 V for 15 min, then to 100 V for 15 min, and finally to 150 V for 20 h. Gels were scanned at 638 nm on an LKB Ultrascan XL laser densitometer (Pharmacia LKB Biotechnology, Piscataway, NJ). Gels were calibrated using two LDL pools whose size was confirmed by analytical ultracentrifugation (courtesy of Dr. R. Krauss, Berkeley, CA). The size reported is the diameter for the major peak. The CV for three control pools (248, 259, 274 Å) were 1.8, 1.2, 1.1%, respectively.

Statistical analysis

Data are presented as mean ± SEM. Pearson's product-moment correlation analysis was used to examine relations between lipid, lipoprotein, binding, and metabolic variables. The significance of the differences between groups was compared by using the Mann-Whitney test for unpaired values in a post-hoc analysis of subjects with high rates of LDL clearance compared to the remainder.

RESULTS

Demographic characteristics, and plasma lipid and lipoprotein concentrations of study subjects are shown in Table 1. The group had moderate hypercholesterolemia (230 ± 5 mg/dl). Two subjects had LDL cholesterol levels less than 130 mg/dl, the lower limit for eligibility, after 12 weeks on the AHA Step I diet but were not excluded from these analyses because they increased the range for LDL cholesterol. Subjects ranged in age from 27 to 55 years and had a wide range of BMI, from 20.1 to 33.8. Triglyceride concentrations also varied greatly, from 48 to 211 mg/dl.

LDL metabolic, binding and LDL particle size data are summarized in Table 1. The mean FCR for LDL apoB was 0.366 ± 0.021 and varied from 0.223 to 0.619 pools/day. FCR was significantly correlated with plasma triglyceride (r = 0.44, P = 0.03) but not with LDL cholesterol (r = 0.03, P = 0.86). The production rate (PR) for LDL apoB, expressed per kg of fat-free mass, was positively correlated with plasma total cholesterol (r = 0.53, P = 0.01) (Fig. 1A) and LDL cholesterol (r = 0.40, P = 0.05) (Fig. 1B). Plasma triglyceride was also correlated with LDL PR (r = 0.44, P = 0.03).

For the study group as a whole, LDL binding affinity did not correlate with clearance (r = -0.20, P = 0.54) or production rate (r = -0.08, P = 0.71). On further analysis, four subjects with unusually high clearance rates according to the criteria of Kesäniemi, Vega, and Grundy (32) were identified and analyzed separately. The cutoff point used for the subgroup classification was an FCR of greater than 0.45 pools/day. The published range for LDL apoB FCR for normal subjects is 0.287-0.462 pools/day (32). Despite the similarity of the LDL cholesterol and apoB levels, subjects with high FCRs had significantly higher production rates for LDL apoB, 32.6 ± 2.0 versus 18.0 ± 0.7 mg/kg fat-free mass • day, (P = 0.002). Plasma total cholesterol and triglyceride were also higher in the high FCR group (P = 0.037 and 0.063, respectively). In subjects with FCRs in the normal range there was a significant negative correlation between Kd and FCR (Fig. 2; r = -0.52, P = 0.020), whereas in the four subjects with high FCR, Kd was positively associated with FCR (Fig. 2; r = 0.96, P = 0.045). Therefore, using 0.45 as the upper limit of normal, two different relationships are shown in Fig. 2.

Average LDL size ranged from 240.0 to 265.8 Å (253.9 ± 1.3) and was inversely correlated with plasma triglyceride (r = -0.65, P = 0.001) but not with LDL cholesterol (r = 0.25, P = 0.26). LDL size had a weak inverse relation to production rate (r = -0.33, P = 0.13) (Fig. 3A) but was not related to FCR (r = -0.21, P = 0.35) (Fig. 3B). No relation was found between LDL size and binding, r = 0.17, P = 0.82 (Fig. 3C).

DISCUSSION

Lipids, lipoproteins, and in vivo LDL metabolism

There was a wide range of cholesterol concentrations in subjects recruited to participate in this study. Triglycerides also varied across a wide range but were below the level considered to be hypertriglyceridemic (<250 mg/dl), and LDL FCR ranged from 0.22 to 0.62 pools/day. Thus, there was enough variation within the study group, as well as a large number of participants, to make comparisons among lipid concentrations, LDL metabolism, and binding.

In this group of subjects as a whole, LDL production rates were correlated with LDL cholesterol concentrations although clearance was not. The correlation between LDL cholesterol and apoB was high, r = 0.87, therefore, the correlations for apoB are very similar to those for LDL cholesterol. Other studies of hypercholesterolemic subjects have also shown significant relations between LDL cholesterol levels and production (33, 34), and concluded that hypercholesterolemia may be due largely to overproduction in individuals without significant defects in receptor function.

In the present study, both LDL apoB clearance and production rates were correlated with plasma

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TABLE 1. Individual values for plasma lipids and lipoproteins, metabolic study, and binding study

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The data for the LDL metabolic study are presented in four columns: FCR, fractional clearance rate, PR, production rate, PR adjusted for body weight, and PR adjusted for fat-free mass (FFM). The $K_d$ (dissociation constant) values in the last three columns are for binding (B), internalization (I), and degradation (D).
triglycerides. This is consistent with an earlier report of a group of subjects with a wide range of cholesterol concentrations confirming that LDL metabolism is greatly influenced by its precursor VLDL (35). Because the apoB/E receptor is a mediator of clearance for VLDL remnants as well as LDL, LDL production is dependent on the rate of VLDL production and clearance.

**Relation between in vivo metabolism and in vitro binding**

This study has presented the first opportunity to examine the relation between in vivo kinetics and LDL binding affinity in vitro. LDL is cleared primarily through receptor-mediated clearance; therefore, we examined the relation between binding affinity and FCR in this group of subjects. The results show a complex set of interactions between the in vivo and in vitro metabolism of LDL.

There was no correlation between LDL clearance and binding affinity for the study group as a whole. Based on published values establishing an upper limit cutoff for normal FCR at 0.45 pools/day, we separated the subjects into two groups based on their FCR. In the 20 subjects with an FCR in the normal range, a significant correlation was observed between LDL binding and clearance. These data suggest that LDL with less affinity for the LDL receptor are cleared more slowly in vivo and that in individuals with normal clearance rates binding affinity may be an important regulator of LDL clearance. Similar results were reported by Turner et al. (34) who showed that subjects with moderately elevated cholesterol had reduced clearance and decreased LDL degradation compared to subjects with normal cholesterol concentrations. Indirect support for this conclusion also comes from Vega and Grundy (35), who reported that in 5 of 15 patients with primary moderate hypercholesterolemia, autologous LDL, expected to have reduced binding affinity, was cleared more slowly than homologous LDL.

The four individuals with high rates of LDL clearance and production showed an opposite relation between binding and FCR compared to the subjects with normal clearance. In this group of subjects, rapid production was accompanied by rapid clearance which implies that some compensatory mechanism was at work to accelerate clearance. In these individuals, clearance may be largely dependent on the number of receptors rather than the affinity of LDL for the receptor. In addition, these four subjects appeared to have elevated triglycerides, suggesting that VLDL metabolism was

![Fig. 1. Relation between production rate for LDL apoB and triglyceride (1A), and LDL cholesterol (1B).](image)

![Fig. 2. Relation between Kd for binding and FCR for LDL apoB. Subjects were divided into two groups according to their FCR values. The upper limit for normal was an FCR of 0.45 pools/day. The points below an FCR of 0.45, in the lower half of the graph, represent subjects with an FCR in the normal range. The graph shows a negative correlation between FCR and the Kd for LDL binding for those subjects in the normal range while those with a high FCR showed a positive correlation.](image)
important in regulating LDL metabolism in this group. However, this relation was not significant probably due to the limited number of subjects.

**LDL size, metabolism, and binding**

Recent data suggesting that individuals with predominantly small, dense LDL are at increased risk for cardiovascular disease (36–38) prompted interest in assessing the role of LDL size and its effect on LDL metabolism. In the present study, no correlation between LDL particle size and metabolism was found despite the expected inverse correlation between LDL size and plasma triglyceride concentrations (37). LDL size was weakly related to the rate of LDL production, with individuals with higher triglycerides and smaller LDL having higher LDL production rates. The exact metabolic basis for this relation is still unknown.

Several studies have shown that an intervention that increases LDL clearance is associated with an increase in LDL size (12, 13). On the other hand, Vega et al. (2, 5) reported that dense LDL were cleared more rapidly in subjects with either primary hypertriglyceridemia or primary hypercholesterolemia. The subjects in the present study had only moderately elevated cholesterol levels however.

No relation between LDL size and in vitro binding affinity was observed in the present study. LDL size was inversely correlated with plasma triglyceride, but none of the patients were hypertriglyceridemic. Thus, LDL isolated from subjects with normal triglyceride concentrations may not vary enough in conformation or composition to alter binding affinity. However, subfractionation of LDL isolated from normolipidemic subjects by density gradient ultracentrifugation (20, 39, 40) has suggested that LDL size can affect binding affinity, with the majority of studies showing that LDL isolated from the middle range binds the most avidly to the receptor. Thus, the binding of LDL to its receptor may be affected by the size of the lipoprotein particle as well as by the distribution of its components.

In conclusion, the results of this study suggest that LDL binding affinity may influence LDL clearance. Although LDL size and binding affinity did not correlate
with LDL FCR in the whole study group, subgroup analysis suggested that binding affinity significantly affects clearance in patients in whom the number of particles is low. On the other hand, in subjects where LDL production is elevated, receptor number may have a greater influence on clearance: these subjects are likely to have a combined hyperlipidemia. This study reinforces the concept that there are various causes for hypercholesterolemia and that properties of LDL particles such as LDL size and binding may not always control LDL clearance due to metabolic variation among subjects (2).

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REFERENCES


Yamane et al. LDL size, binding, and metabolism in hypercholesterolemia


