# Resistance of Native and Circulating Modified Low-Density Lipoproteins in Human Blood to Association

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The resistance of native and circulating modified low-density lipoproteins from human blood to spontaneous and polyethylene glycol-induced association was studied by recording light transmission fluctuations. Circulating modified low-density lipoproteins were less resistant to association than native low-density lipoproteins. Polyethylene glycol-induced association of low-density lipoproteins was irreversible. Our results suggest that atherogenic activity of circulating modified low-density lipoproteins is associated with their increased predisposition to irreversible association.

**Key Words:** *low-density lipoproteins; modified low-density lipoproteins; association of lipoproteins; aggregation of lipoproteins; atherosclerosis*

Accumulation of intracellular lipids in the vascular wall is typical for early stages of atherosclerosis. Undoubtedly, circulating low-density lipoproteins (LDL) serve as a source of lipids [1]. Modified LDL of human blood can increase accumulation of lipids in cultured human smooth muscle cells and macrophages [5,6,10,15]. These LDL are characterized by changed chemical composition (low content of sialic acid, cholesterol esters, phospholipids, and antioxidants and high content of lysophospholipids), small size, high specific density and negative surface charge, presence of modified $NH_2$ groups in the protein, and predisposition to oxidation and aggregation. They were called circulating modified LDL (cmLDL) [14]. It should be emphasized that cmLDL and *in vitro* modified LDL are characterized by increased atherogenicity and are predisposed to association [7,8,10,12,13]. Accumulation of intracellular lipids became more pronounced with the increase in the mean size of LDL [12-14]. It was hypothesized that *in vivo* changes in physicochemical properties of cmLDL are followed by the decrease in their resistance to association. Aggregates of cmLDL are easily engulfed by cells of the vascular intima and stimulate lipoidosis [12].

Here we evaluated the role of LDL association in the early stage of atherosclerotic vascular damage. The resistance of native (nLDL) and cmLDL to association was studied by recording light transmission fluctuations (LTF). We also determined reversibility of this process.

## MATERIALS AND METHODS

Total LDL fraction was isolated by two-step ultracentrifugation in a NaBr density gradient [11]. nLDL and cmLDL were separated by lectin chromatography on a column packed with *Ricinus communis* agglutinin agarose (Boehringer Mannheim GmbH) [14].

Association of LDL was assayed by recording LTF of a laser beam at 860 nm [12]. To study aggregation capacity of LDL, these particles were freed

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from aggregates by filtering through a Nalgene filter (pore size 0.45 μ). LDL were incubated in isotonic phosphate buffer (Gibco) containing 0.2 g/liter KCl, 0.2 g/liter $KH_2PO_4$, 8 g/liter NaCl, and 1.15 g/liter $Na_2HPO_4$ (pH 7.2) at 37°C. The buffer included 1 mg/ml ethylenediaminetetraacetic acid (EDTA). Association was induced by adding polyethylene glycol (PEG) with a molecular weight of 6000 Da (Sigma) [2,4]. LTF were recorded at fixed time intervals.

To study reversibility of association, nLDL and cmLDL were filtered through a filter (pore size 0.45 μ) and incubated in isotonic phosphate buffer containing 1 mg/ml EDTA and 5% PEG at 37°C. After incubation LDL were dialyzed against buffer at 4°C for 12 or 24 h. The use of cellulose dialysis tubes (Sigma) allowed us to dialyze polymers with a molecular weight of below 12,400 Da. LTF in LDL samples were recorded before and after dialysis.

Differences between LTF of nLDL and cmLDL were estimated by the methods of variational analysis. The differences were significant at $p<0.05$.

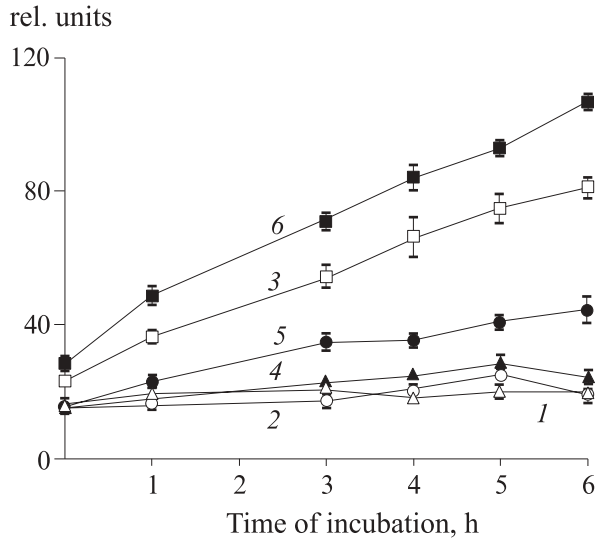


**Fig. 1.** Kinetic curves for light transmission fluctuations in the suspension of nLDL (*1-3*) and cmLDL (*4-6*) in the absence (*1*, *4*) and presence of 2% PEG (*2*, *5*) or 6% PEG (*3*, *6*). LDL concentration 0.2 mg protein/ml; incubation at 37°C. Ordinate: light transmission fluctuations, rel. units.

## RESULTS

Published data show that PEG modifies physicochemical properties of an aqueous phase on the surface of natural protein-lipid complexes (*e.g.*, LDL) and promotes their aggregation and fusion [2-4,9]. Association of LDL is determined by the state of particle surfaces. Changes in the particle surface produced by various factors affect the resistance of LDL to aggregation [2]. These data suggest that cmLDL differ from nLDL not only in physicochemical properties [14], but also in the resistance to PEG-induced association.

As differentiated from nLDL, cmLDL underwent slow aggregation during incubation at 37°C (Fig. 1, *1*, *4*). After 6-h incubation with 2% PEG, LTF increased by 2.5 times in the suspension of cmLDL, but remained unchanged in the suspension of nLDL (Fig. 1, *2*, *5*). The rate of LDL association increased with the increase in PEG concentration to 6%. LTF in the suspension of cmLDL and nLDL increased by 4.5 and 3.4 times, respectively, over 6-h incubation (Fig. 1, *3*, *6*).

Filtration of aggregates from LDL significantly decelerated association of nLDL and cmLDL (Fig. 2).

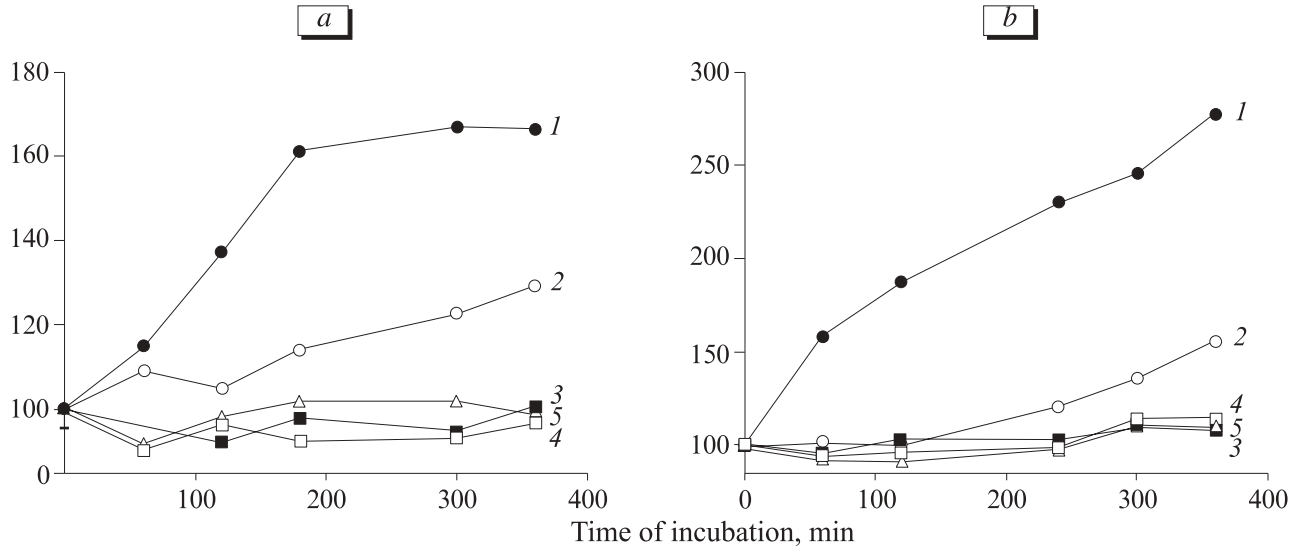


**Fig. 2.** Effect of prefiltration of the suspension of nLDL (*a*) and cmLDL (*b*) on light transmission fluctuations: control, no filtration (*1*); prefiltration through filters with a pore size of 1.0 (*2*), 0.45 (*3*), 0.22 (*4*), or 0.1 μ (*5*). LDL concentration 0.1 mg protein/ml; PEG concentration 6%; incubation at 37°C. Ordinate: light transmission fluctuations, % of baseline level (start of incubation).

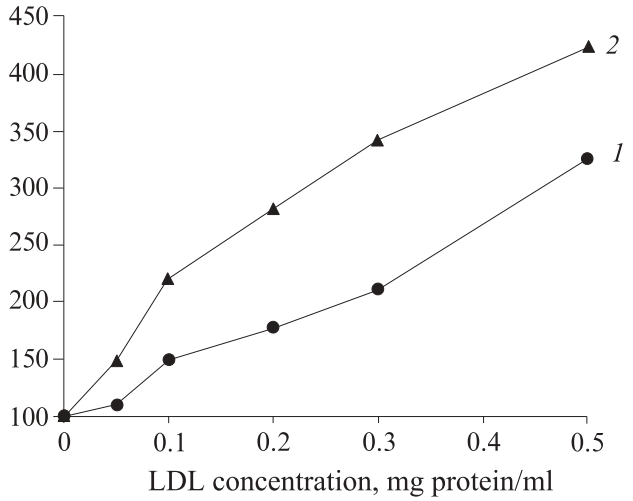


**Fig. 3.** Dependence of light transmission fluctuations in the suspension of nLDL (*1*) and cmLDL (*2*) on LDL concentration. Incubation at 37°C for 6 h. PEG concentration 5%. Ordinate: light transmission fluctuations, % of baseline level (start of incubation).

LTF in the suspension of nLDL and cmLDL rapidly increased in the presence of 6% PEG (Fig. 2, *1*). The rate of association significantly decreased, when the suspension of nLDL and cmLDL was prefiltered through filters with a pore size of 1 μ (Fig. 2, *2*). nLDL and cmLDL lost aggregation activity when pore size was decreased to 0.45 μ.

It can be hypothesized that microaggregates present in the suspension of LDL play a role of aggregation centers and stimulate this process. LDL were filtered through filters with a pore size of 0.45 μ immediately before the experiment to exclude possible effects of microaggregates on association.

LTF of nLDL and cmLDL, as well as the mean size of aggregates, increased with increasing in LDL concentration (Fig. 3). However, cmLDL were less resistant to PEG-induced association than nLDL.

These data show that cmLDL are highly predisposed to association (*e.g.*, PEG-induced association). To study reversibility of this process, nLDL and cmLDL were prefiltered through filters with a pore size of 0.45 μ, incubated in the presence of PEG, and dialyzed to remove PEG. If PEG-induced association of LDL is a reversible process, the removal of PEG should be followed by partial disaggregation of associated LDL. Incubation with PEG was accompanied by association of nLDL and, particularly, cmLDL. It was manifested in a significant increase in LTF after 3-h incubation (Table 1). The removal of PEG by dialysis did not decreased LTF and mean size of aggregates. By contrast, LTF tended to increase after 12-h dialysis. Dialysis for 24 h was followed by a significant increase in LTF. The data show that PEG-induced association of nLDL and cmLDL is an irreversible process.

Our results indicate that cmLDL are less resistant to association that nLDL. It should be emphasized that association is an irreversible process. These results are consistent with published data [11,12] that human blood cmLDL can be easily engulfed by cultured smooth muscle cells in human aortic intima and macrophages. Moreover, accumulation of LDL in cells increases with increasing in the size of particles. *In vivo* multiple modification and other methods for modification of LDL (*e.g.*, oxidation, treatment with malonic dialdehyde, and glycosylation) increase their predisposition to association [12,13]. It can be hypothesized that general structural changes in LDL are followed by association of particles and acquisition of atherogenic activity. These changes are manifested in lipid accumulation in cells of the vascular intima.

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**TABLE 1.** Effect of Dialysis of Associated nLDL and cmLDL Formed after Preincubation of nLDL and cmLDL with 5% PEG on LTF (*M*±*m*, *n*=5)

| Incubation with PEG, h | nLDL | | | cmLDL | | |
|---|---|---|---|---|---|---|
| | before dialysis | after dialysis | | before dialysis | after dialysis | |
| | | 12 h | 24 h | | 12 h | 24 h |
| 0 (control) | 27±2 | — | — | 44±3 | — | — |
| 0.5 | 31±4 | — | — | 49±8 | — | — |
| 3 | 44±9* | 61±12 | 96±20++ | 85±11*+ | 109±21 | 142±25++ |
| 6 | 48±11* | 68±14 | 80±12++ | 122±13*+ | 111±18 | 101±14 |

**Note.** $p<0.05$: *compared to the control; +compared to nLDL; ++compared to the parameter before dialysis.

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