

Native and modified low-density-lipoprotein interaction with human platelets in normal and homozygous familial-hypercholesterolaemic subjects

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The binding of low-density lipoproteins (LDL) as well as LDL modified by cyclohexanedione (CHD-LDL) to gel-filtered platelets (GFP) and its effect on platelet function were studied in normal and in homozygous familial hypercholesterolaemic (HFH) subjects. Only normal-derived LDL could significantly compete with normal ^{125}I -labelled LDL for binding to normal platelets. When GFP from normal subjects were incubated with normal LDL at concentrations of 25–200 μg of protein/ml, platelet aggregation in the presence of thrombin (0.5 i.u./ml) was increased by 65–186%. CHD-LDL, at similar concentrations, caused the opposite effect and decreased platelet aggregation by 26–47%. Both LDL and CHD-LDL (100 $\mu\text{g}/\text{ml}$) from HFH patients, when incubated with normal GFP, caused a significant reduction in platelet aggregation (33 and 50% respectively). When HFH-derived platelets were used, both patient LDL and CHD-LDL (but not the normal lipoprotein) could markedly compete with the patient ^{125}I -labelled LDL for binding to the platelets. LDL and CHD-LDL (100 $\mu\text{g}/\text{ml}$) from normal subjects decreased aggregation of HFH-platelets by 52 and 85% respectively, while corresponding concentrations of LDL derived from HFH subjects (HFH-LDL) and CHD-LDL derived from HFH subjects (CHD-HFH-LDL) increased platelet aggregation by 165 and 65% respectively. The present results support the following conclusions: (1) platelet activation by LDL in normal subjects is through the arginine-rich apoprotein-binding site; (2) more than one binding site for LDL exists on platelets; (3) under certain circumstances, LDL binding can cause a reduction in platelet activity; (4) specificity for LDL binding to the platelets resides in different regions of the lipoprotein in HFH and in normal subjects. We have thus suggested a model for LDL-platelet interaction in normal and in HFH subjects.

Hypercholesterolaemia and blood platelet function are interrelated and play a major role in atherosclerosis (Mustard & Packham, 1975; Ross & Glomset, 1976). The effect of plasma low-density lipoproteins (LDL) on platelet function has been provided by studies *in vitro* and investigations of patients with familial hypercholesterolaemia (FH). Patients with this disorder, characterized by

Abbreviations used: FH, familial hypercholesterolaemia; HFH, homozygous familial hypercholesterolaemia; CHD, cyclohexanedione; GFP, gel-filtered platelets; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

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excessive amounts of LDL due to a partial (heterozygous) or complete (homozygous) absence of LDL receptors in various cell cultures (Goldstein *et al.*, 1983), have been shown to have platelet hyperactivity, both *in vitro* (Carvalho *et al.*, 1974; Colman, 1978; Aviram & Brook, 1982) and *in vivo* (Joist *et al.*, 1974; Zahavi *et al.*, 1982). Recently it has been shown that platelet hyperactivity in these patients is mediated through the presence of some factors present in FH patients' plasma (Aviram & Brook, 1982) and that at least one of these factors might be LDL (Viener *et al.*, 1984). We and others (Aviram *et al.*, 1981; Koller *et al.*, 1982) have demonstrated that normal platelets possess specific receptors for lipoproteins and that, as a consequence of platelet-lipoprotein interaction, there is a change in platelet cholesterol content as

well as platelet function (Aviram & Brook, 1981a, 1983a,b,c, 1984). The binding to normal platelets seems to be dependent on the presence of a number of functionally significant, positively charged arginine or lysine residues on the lipoprotein surface (Aviram *et al.*, 1981). When normal LDL is subjected to a specific chemical modification with cyclohexane-1,2-dione, receptor-lipoprotein interactions are abolished (Mahley *et al.*, 1977; Aviram *et al.*, 1981). In contrast with HFH fibroblasts, which apparently exhibit no specific LDL binding (Goldstein *et al.*, 1983), HFH platelets exhibit specific accumulation of LDL (Aviram & Brook, 1981b). Differences in the lipoprotein composition between normal and FH subjects have been described (Brook *et al.*, 1981; Viener *et al.*, 1984), and the platelet composition has been shown to be abnormal in these patients (Shastri *et al.*, 1980; Nordoy & Rodest, 1971).

Although results of experiments *in vitro* support the conclusion that some platelet functions are affected by the lipid content of their environment (Shattil *et al.*, 1975), it has not been proven that this effect is through the receptors mentioned above. The goal of the present study was to examine two interrelated and important lines of investigation, the first dealing with significance of the LDL binding sites and the latter dealing with the differences between normal- and HFH-derived platelet LDL binding sites.

Materials and methods

Subjects

Healthy normal volunteers included five female and 17 male subjects, whose ages ranged from 23 to 55 years (mean 29 years); they were on no medications and had normal plasma lipid concentrations [plasma cholesterol, 4.8 ± 0.7 mmol/l (mean \pm s.d.); plasma triacylglycerols, 1.13 ± 0.23 mmol/l].

The patients, two males and one female, aged 20, 15 and 25 years respectively, were homozygous for the familial form of type II hyperlipoproteinemia, determined by plasma lipid concentrations, lipoprotein electrophoresis and quantification (Hatch & Lees, 1968) as well as family history. These patients had plasma cholesterol levels of 13.62, 14.39 and 15.65 mmol/l and triglyceride levels of 0.90, 1.04 and 1.11 mmol/l. All had marked hypercholesterolaemia; tendon, planar and tuberous xanthomata and, in at least two patients, premature atherosclerosis. They were treated by plasma exchange on an outpatient basis at 3-4-weekly intervals. None of the subjects had taken any medication for at least 2 weeks preceding the experiments. Informed consent for the study was obtained from each subject.

On each analysis when comparing patients with controls, age-matched pairs were studied. Venous blood was collected, after a 14h fast, through silicone-treated needles into plastic syringes. The blood was added to plastic tubes containing disodium EDTA (1 mmol/l final concn.) for lipoprotein separation. For platelet preparation, 9 vol. of blood were added to 1 vol. of 3.8% (w/v) sodium citrate.

Preparation of lipoproteins

Plasma lipoproteins were separated by discontinuous-density-gradient ultracentrifugation (Aviram, 1983). The density of 4 ml samples of plasma was raised to 1.25 kg/l with KBr. A 4 ml portion of a density solution of 1.04 kg/l was then carefully laid over the plasma sample, followed by 4 ml of NaCl ($\rho = 1.006$ kg/l). Ultracentrifugation was performed in an SW-41 rotor in a Beckman L2-65B preparative ultracentrifuge for 48 h at 4°C and 35000 rev./min. LDL was then carefully removed and dialysed extensively against NaCl (150 mmol/l), pH 8.6. The purity of the LDL fraction was analysed by cellulose acetate electrophoresis, Ouchterlony double immunodiffusion and immunoelectrophoresis for all the experiments. The lipoproteins were pure and free of other plasma proteins. Lipoprotein protein concentration was determined by the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard. The cholesterol and triglycerol content of each lipoprotein was determined by enzymic methods on a centrifugal fast analyser (Gemsac, Fairfield, NJ, U.S.A.).

Part of the LDL prepared as above was chemically modified by cyclohexanedione (Mahley *et al.*, 1977). A 1 ml portion of LDL (5 mg/ml) was added to 2 ml of cyclohexane-1,2-dione (0.15 mmol/l) in sodium borate (0.2 mmol/l), pH 8.1, and incubated for 2 h at 35°C. The CHD-modified LDL (CHD-LDL) was dialysed against NaCl solution ($\rho = 1.006$ kg/l) with EDTA (1 mmol/l), pH 8.6, and filtered through a 0.22 μ m-pore-size Millipore filter before use.

CHD-modified albumin was prepared in a manner similar to that used for CHD-LDL, albumin (5 mg/ml) being used instead of LDL.

LDL accumulation by platelets

Gel-filtered platelets (100000/ μ l) were incubated with 125 I-labelled LDL (25 μ g of protein/ml; 175-215 c.p.m./mg of protein) for 30 min at 37°C with increasing concentrations of LDL or CHD-LDL. Platelet samples were washed with 5 ml of HEPES buffer [HEPES (5 mmol/l), NaCl (137 mmol/l), MgCl₂ (1.2 mmol/l), NaHCO₃ (12 mmol/l), NaH₂PO₄·H₂O (0.4 mmol/l), KCl (2.7 mmol/l) and glucose (0.6 mmol/l), pH 7.4] four

times, and the resuspended pellet (0.5 ml) was overlaid on 1.0 ml of foetal-calf serum and spun down for 5 min at 8000g in an Eppendorf 3200 minicentrifuge. The supernatant was removed, and the pellet was then counted for a radioactivity in a scintillation counter.

Platelet preparation

Platelet-rich plasma was prepared from citrated whole blood by low-speed centrifugation for 10 min at 23°C and 200g. GFP were isolated by chromatography (Aviram *et al.*, 1981) of 5 ml of platelet-rich plasma on a Bio-Gel A 50-M (50–100 mesh; Bio-Rad Laboratories) column (27 mm × 90 mm) pre-equilibrated with Hepes buffer, pH 7.4. Fractions (1 ml) were collected during elution with the buffer at a flow rate of 1 ml/min, and 60% of the platelets were recovered in the GFP pool that was collected as a visible, turbid peak. The platelets were morphologically intact, contained less than 1% nucleated cells and erythrocytes and were free of lipoproteins and plasma proteins [as determined by double immunodiffusion against anti-lipoproteins, anti-albumin and anti-(whole serum)].

Incubation procedure

GFP (100000/μl) from normal or HFH subjects were incubated for 30 min at 37°C with lipoproteins (LDL or CHD-LDL) from normal or HFH subjects, or with CHD-albumin, at concentrations ranging from 25 to 200 μg of protein/ml. The incubation media contained Hepes buffer. At the end of the incubation, thrombin (0.5 i.u./ml)-induced platelet aggregation and [¹⁴C]serotonin release were determined

Platelet aggregation

Platelet aggregation was studied in a dual aggregometer (Chronolog Corp. Havertown, PA, U.S.A.) (Born, 1969). Bovine thrombin (Parke-Davis Co., Detroit, MI, U.S.A.) was used as the aggregating agent.

Before the addition of the aggregating agent, CaCl₂ (2 mmol/l) and fibrinogen (1 mg/ml) were added to the analysed incubation suspension, as well as to the blank sample. The percentage transmittance of GFP was recorded as zero and that of its appropriate blank as 100. Results of platelet aggregation were expressed either as the percentage of aggregation amplitude during the first 10 s of aggregation, termed 'platelet-aggregation velocity', or as the time interval between the addition of thrombin to the analysed suspension and the change in the optical transmittance of the suspension ('latent time'). All the results are expressed as the ratio between the velocity or latent time when the lipoprotein was incubated with GFP and that of GFP alone.

Platelet [¹⁴C]serotonin release

Platelet [¹⁴C]serotonin release was determined by a modification of the method of Jerushalmy & Zucker (1966). GFP (100000/μl) were incubated with 0.2 μCi of [¹⁴C]serotonin/ml (New England Nuclear; NEC-225) for 30 min at 37°C and then centrifuged at 1700g for 10 min at room temperature. The platelet pellet was then resuspended in an equivalent volume of Hepes buffer and incubated with the lipoprotein. At the end of the incubation, 450 μl samples were prepared, and either saline or thrombin (0.5 i.u./ml) was added (50 μl) and incubated for 10 min at 37°C with constant stirring. A 400 μl portion of EDTA (100 mmol/l) was then added in order to prevent re-uptake of [¹⁴C]-serotonin.

A sample was taken for total-radioactivity counting, and the remaining platelet suspension was centrifuged for 10 min at 4°C and 8000g. Portions of the supernatant of both samples were counted for radioactivity in a liquid-scintillation counter, with toluene/Triton X-100 as a scintillation fluid. Platelet [¹⁴C]serotonin release was calculated as a percentage of the radioactivity released by thrombin.

Statistics

Statistical analysis of the data was performed in all cases by the Wilcoxon rank test. All the results, unless otherwise noted, are expressed as the means ± s.e.m., and each experiment was repeated three times.

Results

The cell surface of normal GFP was shown to have 1500 binding sites for normal ¹²⁵I-LDL (Aviram & Brook, 1983a; Aviram *et al.*, 1981; Koller *et al.*, 1982). The degree of binding of normal ¹²⁵I-LDL to normal platelets was 30–40% lower (Aviram & Brook, 1981b).

When normal GFP were incubated with normal ¹²⁵I-labelled LDL, normal-derived LDL (but not CHD-LDL) at 1 mg of protein/ml reduced platelet accumulation of the iodinated LDL by 80%. Both LDL and CHD-LDL, when derived from patients with HFH, could only minimally compete with ¹²⁵I-labelled LDL for accumulation by the normal platelets (Fig. 1). When similar experiments were done using platelets derived from HFH patients, the patient LDL as well as its CHD-LDL reduced the patient ¹²⁵I-labelled LDL binding by 80%. However, normal LDL or CHD-LDL reduced the platelet accumulation of the patient ¹²⁵I-labelled LDL by only 5%.

Fig. 2 illustrates the effect of native LDL as compared with CHD-LDL (both derived from

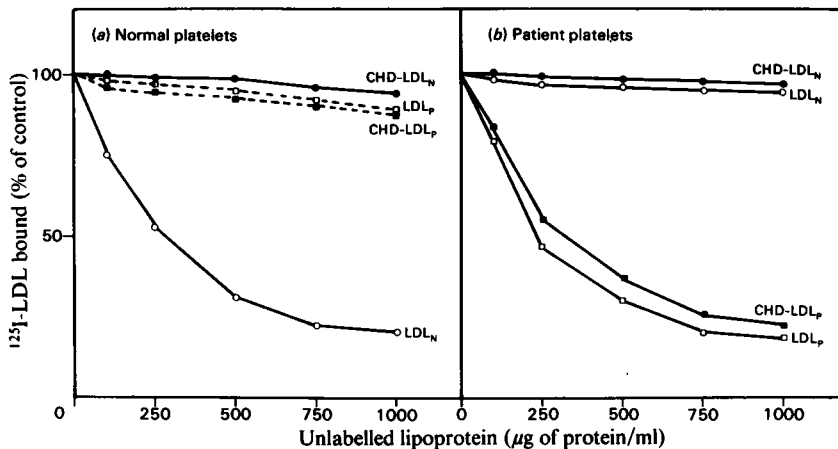


Fig. 1. Ability of LDL and CHD-LDL to compete with ^{125}I -labelled LDL for accumulation by platelets in normal and in HFH subjects

(a) Normal GFP were incubated with ^{125}I -labelled normal LDL ($25\ \mu\text{g}/\text{ml}$) in the presence of increasing concentrations of unlabelled normal LDL (LDL_N) and CHD-LDL (CHD-LDL_N) as well as with unlabelled HFH-derived LDL and CHD-LDL (LDL_P and CHD-LDL_P). The accumulation of ^{125}I -labelled LDL was determined after 30 min at 37°C . (b) HFH-derived GFP ($100000/\mu\text{l}$) were incubated with ^{125}I -labelled HFH-derived LDL in the presence of increasing concentrations of LDL_N , CHD-LDL_N , LDL_P and CHD-LDL_P , as described above. The results represent the means for three independent experiments.

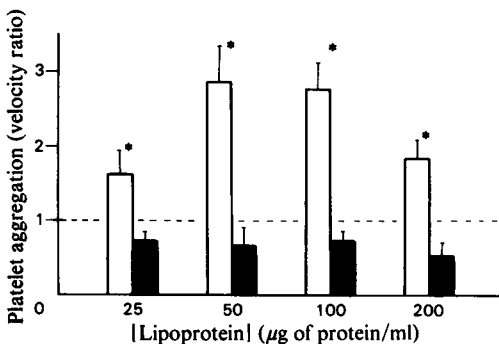


Fig. 2. Effect of LDL and of CHD-LDL derived from plasma of normal subjects on aggregation of normal platelets. Platelet aggregation induced by thrombin ($0.5\ \text{i.u./ml}$) was studied after incubation of normal GFP ($100000/\mu\text{l}$) for 30 min at 37°C with increasing concentrations of LDL (\square) or CHD-LDL (\blacksquare) derived from normal subjects. Platelet aggregation is expressed as the ratio of the aggregation velocity in the presence of the lipoprotein to that obtained with GFP alone. The bars represent means \pm S.E.M. for three independent experiments; * $P < 0.01$ (LDL versus CHD-LDL).

normolipaemic subjects) on platelet aggregation in normal subjects. LDL when incubated with normal platelets caused a significant increase in thrombin-induced platelet-aggregation velocity. Platelet aggregation increased by 65, 186, 178 and

91% when GFP was incubated with lipoprotein concentrations of 25, 50, 100 and $200\ \mu\text{g}$ of protein/ml respectively. The effect of LDL on platelet aggregation was greatest at $50\ \mu\text{g}$ of protein/ml, and as the concentration rose, this effect diminished.

When platelets were incubated with CHD-LDL, a fall in platelet aggregation by 26, 33, 25 and 47% at lipoprotein concentrations of 25, 50, 100 and $200\ \mu\text{g}$ of protein/ml respectively was found.

Platelet aggregation expressed by latent time gave a similar pattern. LDL caused a decline in latent time in comparison with GFP alone by 10 ± 0.9 and $4 \pm 0.8\%$ (mean \pm S.D.) at concentrations of 50 and $200\ \mu\text{g}$ of protein/ml respectively. CHD-LDL had an opposite effect on latent time, causing an increase (in comparison to GFP alone) of 44 ± 1.4 and $18 \pm 3.8\%$ (mean \pm S.D.) at concentrations of 50 and $200\ \mu\text{g}$ of protein/ml respectively.

CHD-albumin had almost no effect on platelet aggregation. The ratio between platelet-aggregation velocity with and without HD-albumin was 100 ± 0 , 90 ± 11 and $94 \pm 0.6\%$ (mean \pm S.D.) at CHD-albumin concentrations of 25, 70 and $170\ \mu\text{g}$ of protein/ml respectively.

Similar results were obtained when we examined platelet [^{14}C]serotonin release in response to incubation with LDL and CHD-LDL derived from normolipaemic subjects. LDL at concentrations of 50 and $100\ \mu\text{g}$ of protein/ml caused an

increase of 77 ± 6 and $57 \pm 0.6\%$ (mean \pm s.d.) respectively in comparison with GFP alone. CHD-LDL at concentrations of 50 and $100 \mu\text{g}$ of protein/ml caused a decrease of 59 ± 3 and $52 \pm 2\%$ (mean \pm s.d.) respectively.

When LDL derived from HFH patients was incubated with GFP from normal subjects, it caused a marked decline in platelet-aggregation velocity (Fig. 3a), not only in comparison with the effect of LDL derived from normal subjects, but also compared with the control containing GFP alone. At protein concentrations of 25, 50, 100 and $200 \mu\text{g}/\text{ml}$ of LDL derived from HFH patients, the decrease in platelet aggregation was 33, 30, 33 and 24% respectively. In the range of concentrations

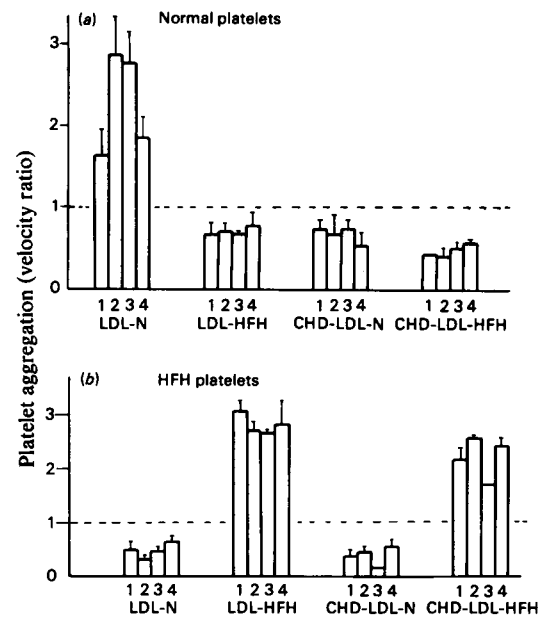


Fig. 3. Effect of LDL and of CHD-LDL derived from either normal (a) or HFH (b) platelets

Platelet aggregation induced by thrombin (0.5 i.u./ml) was studied after incubation of normal GFP (a) or HFH-GFP (b) for 30 min at 37°C with increasing concentrations of LDL or CHD-LDL (1, 2, 3 and 4 represent concentrations of 25, 50, 100 and $200 \mu\text{g}$ of protein/ml respectively) derived from normal or HFH subjects. Platelet aggregation is expressed as the ratio of the aggregation velocity in the presence of the lipoprotein to that obtained with GFP alone. The bars represent means \pm s.e.m. of three independent experiments. The significance between normal LDL (LDL-N) and the other lipoproteins in each concentration was $P < 0.01$ for normal GFP (a), but when using HFH-GFP (b) the normal LDL and CHD-LDL (but not the patient CHD-LDL) caused significantly lower ($P < 0.01$) aggregation in comparison with the patient LDL.

we examined, no trend in platelet activity could be seen; this, too, is in contrast with what was seen when normal LDL was used.

CHD-LDL derived from HFH patients caused effects similar to those seen with CHD-LDL derived from normal subjects (Fig. 3a). Platelet aggregation decreased, in comparison with the control containing GFP alone, by 57, 60, 50 and 44% at CHD-LDL-HFH concentrations of 25, 50, 100 and $200 \mu\text{g}$ of protein/ml respectively. This effect was greater than that caused by CHD-LDL obtained from normal subjects.

Platelets obtained from HFH patients were affected by LDL and CHD-LDL in a different manner (Fig. 3b). LDL from these patients caused a significant increase in platelet-aggregation velocity, whereas LDL from normal subjects caused a significant decrease. Lipoprotein concentrations of 25, 50, 100 and $200 \mu\text{g}$ of protein/ml caused a decrease of 50, 66, 52 and 35% respectively, when normal LDL was used, but caused an increase of 205, 170, 165 and 180%, respectively when LDL derived from HFH patients was added to HFH-GFP.

After incubating platelets derived from HFH patients with CHD-LDL obtained from normal individuals, a diminution in the aggregation response was observed compared with the control containing no lipoprotein in the medium (Fig. 3b). An increment in the aggregation response was observed when CHD-LDL from HFH patients was incubated with these platelets. Lipoprotein concentrations of 25, 50, 100 and $200 \mu\text{g}$ of protein/ml caused an increment of 115, 155, 65 and 140% respectively when CHD-LDL derived from HFH patients was added, but when the lipoprotein was CHD-LDL obtained from normal subjects, the results showed a decline in platelet aggregation by 63, 55, 85 and 42% respectively.

Discussion

The LDL high-affinity binding sites on platelets and on other cell surfaces in normal subjects are specific for arginine-rich residues of the apoprotein moiety (Mahley *et al.*, 1977; Aviram *et al.*, 1981). It was assumed that, if the LDL-platelet interaction is through these high-affinity binding sites, then by using cyclohexanedione-modified LDL, unable to bind through the arginine residues, the lipoprotein would lose its effect on the platelets. Surprisingly, the results in this study show not only that CHD-LDL had an effect on platelets, but that, in contrast with LDL, it caused a decline in platelet activity. This effect of CHD-LDL (Fig. 2) and the previous observation (Mahley *et al.*, 1977), that CHD itself can cause a decrease in receptor binding, made it pertinent to prove that the results

shown for CHD-LDL were not actually the effect of CHD alone. By using cyclohexanedione-modified albumin, prepared in a manner and at concentrations similar to those used with CHD-LDL, it was shown that CHD had a minor desensitizing effect (up to 10%), if at all, on platelet activity at the concentrations used in our study. Competitive inhibition of labelled LDL (derived from normal subjects) binding to normal platelets (Fig. 1) showed that LDL high-affinity binding sites were specific for the arginine residues of LDL derived from normal subjects. In HFH patients, Fig. 1 shows that the binding sites were specific for LDL derived from these patients, but probably not to the arginine residues.

The addition of lipoproteins to GFP did not give rise to aggregation, but affected the aggregation response to thrombin. Shattil *et al.* (1975) showed that the enrichment of platelet plasma membranes with cholesterol increased platelet sensitivity. Transfer of phospholipids between platelets and lipoproteins has been demonstrated (Bereziet *et al.*, 1978) and shown to be a process short enough to be completed in the time lipoproteins were incubated with platelets in the present study. The cholesterol content of the platelet plasma membrane is directly correlated with the plasma cholesterol level (Shattil *et al.*, 1975), and it has recently been shown that the LDL cholesterol/protein ratio, which has a significant effect on platelet activity (Viener *et al.*, 1984), differs in HFH and in normal subjects (Jadhav & Thompson, 1979). A possible role of the LDL binding sites may be the transfer of lipids between LDL and the platelet plasma membrane, causing a change in the physicochemical properties of the membrane, with consequent sensitization of the platelets to aggregating agents (Shattil *et al.*, 1975).

No strict correlation has been proved between LDL binding and its effect on platelet activity. The complete displacement of bound labelled LDL is at a dose much higher (Fig. 1) than that needed for maximum effect on platelet aggregation (Figs. 2 and 3). Furthermore, as mentioned above, the effect of LDL might be through the transfer of lipid and not related to the apoprotein bound to the cell surface, making it even less likely that the dose-response relation is identical with the extent of LDL binding. Nevertheless, our study suggests the existence of two unrelated binding sites differing in their affinity for LDL. This is due to our observation (Fig. 2) that, in normal subjects, LDL at relatively low concentrations caused an increase in platelet activity in a pattern supporting the existence of a saturable binding site. At higher concentrations (above 100 µg of protein/ml), there was a decrease in LDL's activating effect on platelet aggregation and [¹⁴C]serotonin release, suggest-

ing that, at higher concentrations, saturation of the high-affinity binding sites had occurred and that the decrease in platelet activity was due to the effect of the other binding sites that cause platelet inhibition. In HFH patients (Fig. 3a), CHD modification had only a minor effect on platelet-LDL interaction, and the effect of the lipoprotein did not decrease with increasing concentrations of the lipoprotein. We therefore conclude that at least two binding sites exist on human platelets, that the high-affinity binding sites differ between normal and HFH subjects, and that the high-affinity binding sites on the cell surface of the latter probably reach a maximal effect at higher concentrations of LDL than in normal subjects.

Low-affinity binding sites have been shown to exist on fibroblast cells and are significant only at relatively high LDL concentrations (Steinberg, 1978). On platelets, these binding sites, we propose, act similarly to, or are identical with, those of HDL and possibly function as an acceptor of cholesterol from the plasma membrane (Weinstein *et al.*, 1976). In a recent report, Aviram & Brook (1983a) showed in normal platelets that labelled LDL uptake by platelets is only minimally affected by unlabelled HDL, whereas the accumulation of a similar concentration of labelled HDL was significantly inhibited by unlabelled LDL. This observation can be explained by competitive inhibition between HDL and LDL on the low-affinity binding sites. Therefore, unlabelled HDL would have only a minor effect on labelled LDL accumulation, due to LDL's ability to bind to high-affinity sites. On the other, hand, unlabelled LDL would have a marked effect on labelled HDL binding (to low-affinity binding sites).

The results of cross experiments using lipoproteins, from HFH patients or from normal subjects, to platelets derived from normal (Fig. 3a) or HFH (Fig. 3b) subjects support the existence of two different high-affinity binding sites and a different LDL apoprotein composition or configuration in normal and HFH subjects. Furthermore, the results support the existence of only one type of low-affinity binding site, showing no specificity of normal LDL in comparison with HFH-LDL, and having an inhibitory effect on platelet activity. A model based on the data obtained in the present study is illustrated in Fig. 4. It seems reasonable to assume that the effect of the high-affinity binding site is dominant and that only at relatively high concentrations does the low-affinity binding site have any significant effect; therefore, when a lipoprotein can bind to both sites, the net effect will be an increase in platelet activity (Fig. 4), at least at relatively low lipoprotein concentrations. CHD-LDL derived from normal subjects probably interacts only through the low-affinity binding sites of

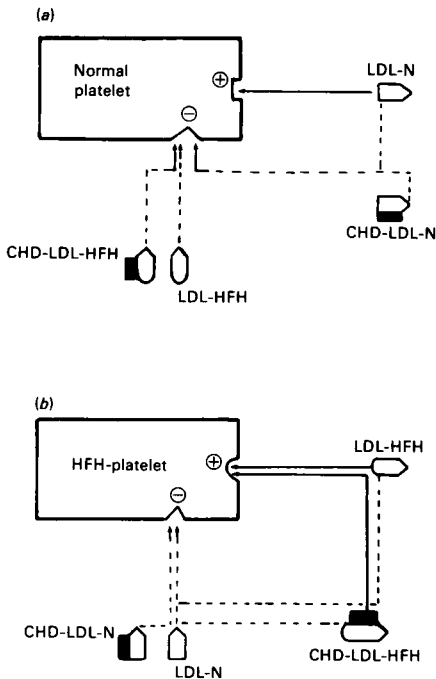


Fig. 4. Hypothetical model for platelet binding sites of LDL in normal and in HFH subjects

(a) Interaction of normal platelets with normal LDL causes platelet activation, whereas interaction of the low-affinity binding site with lipoprotein causes inhibition. LDL from HFH patients (LDL-HFH) cannot interact with the specific 'activating' binding site and therefore causes inhibition by interacting with the low-affinity binding site. CHD-LDL from both normal and HFH subjects (CHD-LDL-N and CHD-LDL-HFH) interacts only with the low-affinity binding site, therefore also causing inhibition of aggregation. \uparrow , main binding sites for normal LDL; \sim , low-affinity binding sites for LDL; (+), (—), activation (-), (----), inhibition. (b) HFH platelets. The elliptical socket (\sim) represents the specific 'activating' binding site for LDL of HFH platelets. This binding site differs from that of normal platelets, and therefore CHD has no effect on LDL binding in HFH subjects. The low-affinity binding site is identical in HFH and normal subjects. LDL derived from normal subjects cannot interact with the specific binding site; therefore both LDL and CHD-LDL from normal subjects, which interact only through the low-affinity binding sites, inhibit platelet aggregation.

normal- or HFH-derived platelets, causing a decrease in their activity. On the other hand, CHD-LDL derived from HFH patients could interact through the high-affinity binding sites of HFH-derived platelets, causing an increase in their activity in the presence of thrombin. In a

recent report (Viener *et al.*, 1984), it was shown that incubating normal platelets with FH-derived LDL increased platelet activity to a greater extent than when normal-derived LDL was used. It is possible that, in these patients (not like in HFH patients), their abnormal LDL composition results in a preferred activation of the high-affinity binding sites over that caused by normal lipoprotein. The different effect of LDL derived from normal or HFH subjects on platelet function is probably related to differences in LDL composition. Such a difference has been observed, but has not been shown to be in the apoprotein moiety, believed to be the key to cell-recognition sites (Knight & Soutar, 1982). It is likely that, although cell recognition is through the apoprotein moiety, it is the lipid composition that influences platelet activation. Although it has been reported that fibroblasts derived from normal subjects do not differentiate in LDL degradation between those derived from normal or HFH patients (Goldstein *et al.*, 1983), Knight & Soutar (1982) have reported that CHD modification inhibits normal LDL binding to fibroblasts to a greater extent than HFH-derived LDL binding. This is in agreement with our observations that only in normal subjects do the arginine residues play an important part in LDL binding. CHD modification of HFH-derived LDL, but not of normal LDL, increased its degradation by macrophages (Knight & Soutar, 1982), and this too suggests that LDL differs in HFH patients, in comparison with LDL derived from normal subjects, with regard to cell recognition.

Our study thus demonstrates the significance of the LDL binding sites, since LDL binding is related to platelet activation by the lipoprotein.

Platelets from HFH subjects do possess LDL binding sites, but these sites are different qualitatively from those found in normal subjects. The altered patient platelet LDL binding sites might be responsible for platelet hyperactivity in these patients.

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