Thrombin Binding and Response in Platelets From Patients With Dyslipoproteinemias: Increased Stimulus–Response Coupling in Type II Hyperlipoproteinemia

By Joan T. Harmon, Narendra N. Tandon, Jeffrey M. Hoeg, and G.A. Jamieson

Platelets were obtained from patients with various hyperlipidemias (familial hypercholesterolemia, Tangier disease) to ascertain relationships among plasma lipids, platelet lipids, thrombin binding and thromb-in-induced platelet aggregation, and to compare these data with those previously obtained on stimulus–response coupling in platelets following in vitro modification of membrane microviscosity. Washed platelets were studied for their ability to bind 125I-thrombin in the range of 10^-10 to 10^-6 mol/L (10 mU/mL to 100 U/mL) and to aggregate with thrombin at concentrations <10^-4 mol/L (100 mU/mL). The values for binding and aggregation in eight patients from six kindreds with familial hypercholesterolemia, taken as a group, fell in the low normal range. If divided into two groups, patients with overt cardiovascular disease bound normal amounts of thrombin but were more responsive to it, whereas patients without overt cardiovascular disease bound lower amounts of thrombin but gave an aggregation response in the normal range. These results suggest that platelet hyperresponsiveness in familial hypercholesterolemia arises from an alteration in the coupling mechanism between thrombin binding and response such that platelets from patients with familial hypercholesterolemia are able to respond with lower receptor occupancy than is the case with normal platelets. Thrombin binding and aggregation were within normal ranges for platelets from abetalipoproteinemia patients (N = 4) and type V hyperlipoproteinemia (N = 2), although in the latter case the response appeared to be less at very low thrombin concentrations (<30 mU/mL). Thrombin binding was elevated in Tangier disease (N = 3) but with lower responsiveness at lower thrombin concentrations. Thrombin binding was also elevated in LCAT deficiency (N = 2), and one patient showed increased and another showed decreased aggregation responses. In general, increased plasma cholesterol levels resulted in increased stimulus–response coupling (type II), whereas increased triglyceride levels resulted in decreased coupling (type V, Tangier). and there was no apparent alteration in the coupling mechanism with overall reduction in plasma lipid levels as in abetalipoproteinemia.

It is generally accepted that platelets play an important role in atherosclerosis and atherogenesis. The high incidence of atherosclerosis and its thrombotic complications in certain types of hyperlipoproteinemias has led to several studies of platelet activation in the dyslipoproteinemias. Relatively few of these studies have used thrombin as the aggregating agent, and none has addressed the question of thrombin binding and stimulus–response coupling in platelets from patients with dyslipoproteinemias.

In normal platelets, activation by thrombin appears to involve binding of the agonist to surface receptors and a subsequent effector step leading to platelet activation and aggregation. Three different types of binding sites have been identified: a small number of sites (~50) of high affinity for thrombin [kilodalton (kd) ~ 0.3 nmol/L], a larger number of sites (~2,000) of moderate affinity (kd ~ 10 nmol/L), and a large number of sites (~600,000) of very low affinity (kd ~ 3 umol/L). When platelet membrane microviscosity is increased by incubation in vitro with cholesterol-rich liposomes there is a corresponding increase in platelet aggregability and an increase in the number of thrombin receptors. The increase in number is accompanied by a decrease in affinity but no change in stimulus–response coupling; conversely, a decrease in membrane microviscosity is accompanied by decreased aggregability and a decreased number of binding sites with increased affinity.

Elevations in the cholesterol–phospholipid ratio and, by extension, in the membrane microviscosity have been reported in type II hyperlipoproteinemias. Because of the implications of these changes with respect to thrombin-induced activation and stimulus–response coupling, the present study was undertaken to ascertain the relationships among plasma lipids, platelet lipids, thrombin binding to platelets, and thrombin-induced platelet aggregation in humans as an extension of our previous studies on in vitro modification of membrane microviscosity. We have examined the in vivo effects of hyperlipidemia on platelet–thrombin interactions using platelets isolated from patients with increased concentrations of VLDL (type V hyperlipoproteinemia, N = 2), LDL (homozygous familial hypercholesterolemia, type II hyperlipoproteinemia, N = 8), and free cholesterol (lecithin–cholesterol acyltransferase [LCAT] deficiency, N = 2). In addition, platelets were studied from patients with the congenital hypolipidemic conditions: absence of VLDL and LDL (abetalipoproteinemia, N = 4) and the absence of HDL (Tangier disease, N = 3). Because these inborn errors of metabolism are rare, with <100 patients being known in any category, the above numbers constitute a reasonably representative sampling.

MATERIALS AND METHODS

Normal Volunteers

Control blood samples were obtained from normal volunteers who were either participating in metabolic turnover studies at the National Institutes of Health or were laboratory personnel.

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mean age was 23.1 years (range 19 to 33 years). Of the 15 normal volunteers, 8 were men and 7 were women. At least one control exam was carried out on each day that a patient sample was examined.

Patients

Patients with types II and V hyperlipoproteinemia demonstrated the typical clinical and biochemical criteria for these respective diagnoses.14 The diagnoses of the following specific genetic diseases were based on standard criteria: homozygous familial hypercholesterolemia (HFH),15 Tangier disease,16 abetalipoproteinemia,17 and LCAT deficiency.14 The fasting plasma lipid and lipoprotein concentrations of the study patients are given in Table 1.

The LCAT-deficient patients were not on any medications (including aspirin). The patients with Tangier disease, abetalipoproteinemia, and type V hyperlipoproteinemia had not taken any lipid-lowering medications or aspirin for at least 6 weeks prior to blood sampling. The patients with HFH refrained from taking aspirin for at least 2 weeks prior to blood sampling and were taking the medications listed in Table 2 at the time of these studies.

Most of the patients with HFH were examined on at least two occasions. Patient Jo.P. was examined three times; patients V.G., Ja.P., D.P., and T.H. were each examined twice, although aggregation studies were not carried out on the two latter patients; and patients V.H., T.P., and L.R. were each examined once for both binding and aggregation.

Lipoprotein Quantitation

Blood was obtained from all study subjects in the supine position after a 12- to 14-hour overnight fast. After blood was collected in 0.01% EDTA, plasma was separated by centrifugation at 800 g for 30 minutes at 4 °C. Plasma total cholesterol and triglyceride concentrations were determined by an enzymic, colorimetric assay in a spectrophotometer (Beckman, Palo Alto, Calif), and the VLDL was separated from the plasma cholesterol concentration.19 Plasma dextrose was obtained from individual patients and normal volunteers. Platelet-rich plasma (PRP) was obtained by centrifugation at 1,600 g for 10 minutes. The platelets were resuspended and washed in citrate wash buffer (11 mmol/L of dextrose; 128 mmol/L of NaCl; 4.26 mmol/L of Na2HPO4; 4.77 mmol/L of trisodium citrate; and 2.35 mmol/L of citric acid, pH 6.5) supplemented with 0.35% bovine serum albumin as previously described.15 After three washes, the platelets were either resuspended in Tris-binding buffer (136 mmol/L of NaCl; 25 mmol/L of Tris; and 0.6% polyethylene glycol 6,000, pH 7.4) for thrombin-binding studies or in modified Tyrode's buffer (136 mmol/L of NaCl; 5.5 mmol/L glucose; 2.7 mmol/L of KCl; 0.07 mmol/L of Na2HPO4; and 0.01 mmol/L of NaHCO3, pH 7.4) for studies on thrombin-induced aggregation.

Thrombin-induced Aggregation

Aggregation of washed platelets in response to increasing concentrations of thrombin (10 to 100 mU/mL) was measured in a Payton aggregometer (Payton Associates, Buffalo) at 37 °C as previously described.15 Dose-response curves were constructed for each individual, and the thrombin concentration required for half-maximal aggregation was determined. Data were normally distributed and, where more than two patients were available, significance was calculated by Student's t test.

Thrombin Binding Assay

Platelets (4 × 10⁹/mL) were incubated with ¹²⁵I-thrombin (10⁻¹⁰ mol/L), without or with increasing concentrations of unlabeled thrombin (10⁻⁸ to 10⁻⁶ mol/L), in Tris binding buffer for 10 minutes at 22 °C as previously described.15 Under these conditions, the thrombin binding reached steady state, and thrombin degrada-

<table>
<thead>
<tr>
<th>Table 1. Plasma Lipids of Patient Classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Type II</td>
</tr>
<tr>
<td>Type V</td>
</tr>
<tr>
<td>Tangier disease</td>
</tr>
<tr>
<td>Abetalipoproteinemia</td>
</tr>
<tr>
<td>LCAT deficiency</td>
</tr>
</tbody>
</table>

Values are given as the means ± the range (N = 2) or SD (N > 2). LCAT, lecithin-cholesterol acyltransferase.

Table 2. Clinical Features of Homozygous Familial Hypercholesterolemic Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>LDL Receptor Activity (%)</th>
<th>Treatment</th>
<th>Cardiovascular Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.P.</td>
<td>7</td>
<td>M</td>
<td>4</td>
<td>Portacaval shunt</td>
<td>Femoral bruits and cardiac catheterization</td>
</tr>
<tr>
<td>V.G.</td>
<td>6</td>
<td>F</td>
<td>1</td>
<td>Portacaval shunt</td>
<td>None evident by exercise stress test</td>
</tr>
<tr>
<td>Ja.P.</td>
<td>22</td>
<td>F</td>
<td>29</td>
<td>Diet only</td>
<td>Carotid femoral bruits and cardiac catheterization</td>
</tr>
<tr>
<td>Jo.P.</td>
<td>20</td>
<td>F</td>
<td>24</td>
<td>Cholestyramine</td>
<td>None evident by cardiac catheterization</td>
</tr>
<tr>
<td>T.P.</td>
<td>26</td>
<td>M</td>
<td>26</td>
<td>Cholestyramine</td>
<td>Femoral bruits and cardiac catheterization</td>
</tr>
<tr>
<td>V.H.</td>
<td>22</td>
<td>F</td>
<td>6</td>
<td>Diet only</td>
<td>Femoral bruits and cardiac catheterization</td>
</tr>
<tr>
<td>L.R.</td>
<td>26</td>
<td>F</td>
<td>14</td>
<td>Diet only</td>
<td>None evident by stress test</td>
</tr>
<tr>
<td>T.H.</td>
<td>13</td>
<td>F</td>
<td>11</td>
<td>Portacaval shunt</td>
<td>None evident by cardiac catheterization</td>
</tr>
</tbody>
</table>

Platelet Preparation

Fresh blood (50 mL) anticoagulated with citrate/phosphate/dextrose was obtained from individual patients and normal volunteers. Platelet-rich plasma (PRP) was obtained by centrifugation at 800 g for ten minutes at 22 °C. The pH of the PRP was adjusted to 6.5 with citric acid and the platelets were sedimented by centrifugation at 1,600 g for ten minutes. The platelets were resuspended and washed in citrate wash buffer (11 mmol/L of dextrose; 128 mmol/L of NaCl; 4.26 mmol/L of Na2HPO4; 4.77 mmol/L of trisodium citrate; and 2.35 mmol/L of citric acid, pH 6.5) supplemented with 0.35% bovine serum albumin as previously described.15
RESULTS

A 0.30
I-
0.25
II
I
0.20
0.15
III
II
I
0.10
0.05
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Table 3. Platelet Lipid Composition

<table>
<thead>
<tr>
<th>Subjects</th>
<th>C*</th>
<th>PL*</th>
<th>C:PL†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (12)</td>
<td>1.129 ± 217</td>
<td>3.505 ± 670</td>
<td>0.64 ± 0.066</td>
</tr>
<tr>
<td>Type II (11)</td>
<td>1.093 ± 235</td>
<td>3.096 ± 614</td>
<td>0.71 ± 0.084‡</td>
</tr>
<tr>
<td>Type V (2)</td>
<td>1.140 ± 60</td>
<td>4.000 ± 300</td>
<td>0.58 ± 0.021</td>
</tr>
<tr>
<td>Tangier disease (1)</td>
<td>980</td>
<td>3,250</td>
<td>0.57</td>
</tr>
<tr>
<td>Abetalipoproteinemia (4)</td>
<td>1.287 ± 277</td>
<td>3,960 ± 315</td>
<td>0.65 ± 0.11</td>
</tr>
<tr>
<td>LCAT deficiency (2)</td>
<td>1.150 ± 30</td>
<td>3,875 ± 125</td>
<td>0.60 ± 0.034</td>
</tr>
</tbody>
</table>

C, cholesterol; P, phospholipid; C:PL, cholesterol-phospholipid ratio.

Numbers in parentheses are the numbers of different determinations; sufficient platelets were available for lipid assay with only one of the three Tangier patients. Four of the type II patients (T.H., Ja.P., Jo.P., and V.G.) were assayed on two occasions.

*Values are given in micrograms per 10^10 platelets ± range (N = 2) or SD (N > 2).
†Values are given as the molar ratios ± range (N = 2) or SD (N > 2).
‡Significantly different from normal (P < .05).

Lipid and Lipoprotein Levels

The values for plasma lipids in the patients correspond with values previously obtained in defining these disease classes (Table 1): specifically, the type II patients had elevations in total and LDL cholesterol concentrations; type V had elevations in concentrations of VLDL cholesterol and triglycerides; the three patients with Tangier disease had low concentrations of total plasma and HDL cholesterol but elevated triglyceride concentrations; and the four patients with abetalipoproteinemia had low concentrations of total cholesterol (virtually all of which was in HDL) and low triglyceride concentrations. The LCAT-deficient patients had elevated concentrations of triglycerides but low concentrations of HDL cholesterol. Because platelet reactivity has been correlated with membrane microviscosity,12,23 which is
proportional to the cholesterol–phospholipid ratio (C:PL). Analytical data for platelet cholesterol, phospholipid and the C:PL ratio are given in Table 3. There were no significant differences from normal controls in the platelet cholesterol levels and platelet phospholipid levels in any of the patient groups. However, there was a significant difference (P < .05) between the platelet C–PL ratio of normal volunteers and the platelet C–PL ratio of type II hyperlipoproteinemia patients but not with those of the other patient groups.

Thrombin Binding and Aggregation

Normal Volunteers

On each day that a patient sample was studied, at least one sample from a normal volunteer was also studied. On several occasions, the same normal volunteer was used to determine between-assay variations. Results indicated no significant difference in the thrombin-binding isotherms obtained on two separate occasions and that the thrombin-induced dose–response curves (performed on the same days as the binding assay) were reproducible within the limits of this type of assay (Fig 1). No differences were observed dependent on the sex of the normal volunteer with regard to thrombin binding or thrombin-induced aggregation (data not shown).

Patients

Type II. Comparison of the thrombin-binding isotherms of platelets from eight type II (HFH) patients with those of normal controls showed that the data were within the normal range (±2 SD) albeit at the lower end. Similarly, the concentration dependence of aggregation showed an apparently random spread in relation to the aggregation data obtained with normal controls. However, closer examination showed differences in these parameters if the data were analyzed on the basis of the presence or absence of cardiovascular disease. The clinical features of these patients are given in Table 2. Four of the patients did not have overt cardiovascular disease, and their platelets bound lower amounts of thrombin than did those of normal controls (Fig 2A); however, the observed aggregation was within the normal range (Fig 2B), and the thrombin concentration required for half-maximal aggregation (24 ± 5 mU/mL in eight determinations on the four patients) was identical with that of normal controls (23 ± 7 mU/mL; N = 14). On the other hand, four of the patients had cardiovascular disease, as indicated by femoral bruises and confirmed by cardiac catheterization. The thrombin-binding isotherm for these patients fell within the range of normal controls (Fig 2C), but platelets from these patients were hyperaggregable, as shown by the leftward shift of the concentration-dependence data (Fig 2D) and the fact that the thrombin concentration for half-maximal aggregation was decreased to 16 ± 3 (P = 0.089; N = 4). That is, patients without cardiovascular disease bound lower amounts of thrombin than did normal control subjects but gave an aggregation response in the normal range whereas patients with cardiovascular disease bound normal amounts of thrombin but showed an increased aggregation response.

Computer analysis of the thrombin-binding isotherms resulted in the calculated binding parameters shown in Table 4. The amount of thrombin bound at a particular site is a function of the number of sites (R) and the affinity (K) of that site for thrombin. Thus, the product of these parameters (KR) is a better reflection of the amount of ligand bound than is either parameter (K or R) alone. It is the theoretical maximum binding to a particular site at infinite dilution of the labeled ligand and can be more precisely determined than can either the affinity or the receptor number. There is a statistically significant decrease in the KR parameters for each of the three binding sites on platelets from HFH patients without cardiovascular disease in comparison to these parameters for platelets from normal volunteers (Table 5). Thus, there appears to be a decrease in binding to all three binding sites on platelets from HFH patients without cardiovascular disease, whereas normal levels are observed following the onset of cardiovascular disease.

Type V. Platelets from the two patients with type V hyperlipoproteinemia showed a thrombin-binding isotherm similar to that of normal platelets (Fig 2E). However, there was a significant increase in the KR4 parameter, suggesting an increase in binding to the high-affinity binding site. These platelets appeared to be less responsive to low thrombin concentrations (<30 mU/mL) than those of normal volunteers but responded normally to higher thrombin concentrations (Fig 2F), and the thrombin concentration for half-maximal aggregation was calculated to be 30 ± 1 mU/mL.

LCAT deficiency. Only two patients with LCAT deficiency were available for study. The thrombin-binding isotherm for these two patients (Fig 2K) fell at the +2 SD line for normal controls in the range of the low-affinity receptor (thrombin concentration > 10−7 mol/L) but was ~50% above the range of normal controls at lower thrombin concentrations, indicating greater binding of thrombin to the high-affinity and moderate-affinity sites. There was a significant increase in KR parameters for each of the three binding sites. The aggregation patterns for these two patients (Fig 2L) were difficult to reconcile with the thrombin binding data: one patient showed an approximately threefold greater sensitivity to thrombin than did normal volunteers, as indicated by aggregation response points, which all fell to the left of the normal response curve. Conversely, the other patient showed a reduced response to thrombin, with all of the aggregation points falling to the right of the normal response curve. All data taken together indicated that the thrombin concentration for half-maximal aggregation was 24 ± 12 mU/mL.

In addition to the above studies, thrombin binding and aggregation have also been examined in washed platelets from patients suffering from the hypolipoproteinemias, Tangier disease, and abetalipoproteinemia.

Tangier disease. Platelets from the three patients with Tangier disease showed a thrombin-binding isotherm that was ~2 SD above the mean of 15 normal controls over the thrombin concentrations corresponding to high-affinity and moderate-affinity receptors, but the difference appeared to be less marked at high thrombin concentrations corresponding to the low-affinity receptor (Fig 2G). The increase in binding is reflected by significant increases in the KR4 and KR2 parameters. The aggregation data suggested that Tan-
Fig 2. Thrombin binding and thrombin-induced aggregation of platelets obtained from patients with dyslipoproteinemias. Left column of figures (A,C,E,G,I, and K): thrombin-binding isotherms; shaded area denotes mean ± 2 SD of results obtained using platelets from 15 normal volunteers. Right column of figures (B,D,F,H,J, and L): thrombin-induced aggregation response data; shaded area denotes mean ± 1 SD of results obtained using platelets from 15 normal volunteers. Data points were obtained with platelets from HFH patients without cardiovascular disease (A and B), homozygous familial hypercholesterolemia (HFH) patients with cardiovascular disease (C and D), patients with type V hyperlipoproteinemia (E and F), patients with Tangier disease (G and H), patients with abetalipoproteinemia (I and J) and patients with lecithin-cholesterol acyltransferase (LCAT) deficiency (K and L).
gier disease platelets were somewhat less responsive to thrombin than were those of normal control subjects, and that this difference was most marked at lower thrombin concentrations (Fig 2H). This difference was also indicated in the thrombin concentration required for half-maximal aggregation (34 ± 10 in three determinations; \( P = .041 \)).

Abetalipoproteinemia. The thrombin-binding isotherm for the four patients with abetalipoproteinemia fell almost exactly on the ± 2 SD line of normals (Fig 2I). There were no significant differences from normal volunteers in the KR parameters. Aggregation data were scattered, with no detectable differences from data for normal subjects (Fig 2J), and a half-maximal aggregation value of 20 ± 6 mU/mL (\( N = 4 \)) was calculated.

Correlations. The positive correlation between total plasma cholesterol concentration and the cholesterol content of low-density lipoprotein (LDL) has been reported in many studies. We have also observed this correlation in the present study. Other correlations which were found to be statistically significant (\( P < .01 \)) in the present work include: (a) the positive association between the platelet C:PL ratio and the total plasma concentration of cholesterol or the cholesterol concentration in the LDL, and (b) the indirect correlation between the platelet C:PL ratio and the total amount of thrombin bound to the platelet (\( K'R_1 + K'R_2 + K'R_3 \)). There is also an indirect correlation (\( P = .064 \)) between the percentage of LDL receptor activity on fibroblasts and the concentration of thrombin necessary to elicit a 50% aggregation response with platelets from HFH patients. The physiologic relevance of these correlations has not been determined.

### DISCUSSION

Several studies have reported on the aggregability of platelets in dyslipoproteinemias. However, agonist binding to platelets and stimulus–response coupling have not been studied, and in only a few cases has platelet responsiveness to thrombin been examined. A further variable in aggregation studies has been the use of platelets in plasma or gel-filtered platelets, which has prevented differentiation of intrinsic aspects of platelet response from secondary effects due to plasma components, especially lipoproteins that are known to interact with platelets and affect platelet function.\(^2\) To clarify several of these points, we studied the binding of thrombin and thrombin-induced aggregation using washed platelets from a variety of patients with hyperlipoproteinemias and hypolipoproteinemias.

The cholesterol and phospholipid analysis of platelets from patients with LCAT deficiency and type V hyperlipoproteinemia do not appear to have been previously reported. For normal control subjects and HFH patients, our values are in good agreement with those previously reported,\(^3\) as is the case for the single patient with Tangier disease whose platelet lipids were analyzed.\(^4\) The values previously reported for cholesterol content in patients with abetalipoproteinemia (2,390 ± 106 \( \mu g/10^9 \) platelets; \( N = 2 \)) are almost twice those found in the present work (1,287 ± 277; \( N = 4 \)) and yielded an unusually high C:PL ratio of 1.47; this value may be questioned since it is much higher than C:PL ratios found in any of the other dyslipoproteinemias which range from −0.6 in type V to −0.7 in HFH patients and is even higher than values reported for platelets artificially enriched in

### Table 4. Thrombin-Binding Parameters

<table>
<thead>
<tr>
<th>Subjects</th>
<th>( K_1 (\text{nmol/L}) )</th>
<th>( K_2 (\text{nmol/L}) )</th>
<th>( K_3 (\text{nmol/L}) )</th>
<th>( R_1^* )</th>
<th>( R_2^* )</th>
<th>( R_3^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.84 ± 0.3</td>
<td>51 ± 20</td>
<td>1.2 ± 0.3</td>
<td>89 ± 30</td>
<td>4,900 ± 2,000</td>
<td>120,000 ± 12,000</td>
</tr>
<tr>
<td>Type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No disease</td>
<td>0.73 ± 0.6</td>
<td>40 ± 30</td>
<td>1.6 ± 0.7</td>
<td>53 ± 50</td>
<td>3,100 ± 2,000</td>
<td>120,000 ± 30,000</td>
</tr>
<tr>
<td>Disease</td>
<td>0.45 ± 0.2</td>
<td>51 ± 30</td>
<td>1.4 ± 0.6</td>
<td>57 ± 30</td>
<td>5,000 ± 3,000</td>
<td>120,000 ± 30,000</td>
</tr>
<tr>
<td>Type V</td>
<td>1.1 ± 0.5</td>
<td>81 ± 70</td>
<td>0.90 ± 0.5</td>
<td>150 ± 60</td>
<td>6,800 ± 8,000</td>
<td>84,000 ± 10,000</td>
</tr>
<tr>
<td>Tangier</td>
<td>0.73 ± 0.5</td>
<td>67 ± 40</td>
<td>3.0 ± 3</td>
<td>110 ± 80</td>
<td>10,000 ± 8,000</td>
<td>280,000 ± 220,000</td>
</tr>
<tr>
<td>Abetalipoproteinemia</td>
<td>0.39 ± 0.4</td>
<td>33 ± 10</td>
<td>0.91 ± 0.2</td>
<td>31 ± 30</td>
<td>3,900 ± 2,000</td>
<td>120,000 ± 10,000</td>
</tr>
<tr>
<td>LCAT deficiency</td>
<td>0.72 ± 0.6</td>
<td>23 ± 16</td>
<td>1.1 ± 0.3</td>
<td>140 ± 100</td>
<td>3,900 ± 2,000</td>
<td>130,000 ± 20,000</td>
</tr>
</tbody>
</table>

### Table 5.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>( K_1R_1 )</th>
<th>( K_2R_2 )</th>
<th>( K_3R_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.071 ± 0.0005</td>
<td>0.063 ± 0.0006</td>
<td>0.065 ± 0.001</td>
</tr>
<tr>
<td>Type II</td>
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</tr>
<tr>
<td>No disease</td>
<td>0.048* ± 0.002</td>
<td>0.051* ± 0.001</td>
<td>0.051* ± 0.002</td>
</tr>
<tr>
<td>Disease</td>
<td>0.084 ± 0.01</td>
<td>0.065 ± 0.01</td>
<td>0.057 ± 0.01</td>
</tr>
<tr>
<td>Type V</td>
<td>0.086* ± 0.001</td>
<td>0.056 ± 0.006</td>
<td>0.062 ± 0.01</td>
</tr>
<tr>
<td>Tangier</td>
<td>0.10* ± 0.003</td>
<td>0.10* ± 0.004</td>
<td>0.062 ± 0.007</td>
</tr>
<tr>
<td>Abetalipoproteinemia</td>
<td>0.053 ± 0.02</td>
<td>0.080 ± 0.008</td>
<td>0.089 ± 0.01</td>
</tr>
<tr>
<td>LCAT deficiency</td>
<td>0.13* ± 0.006</td>
<td>0.11* ± 0.005</td>
<td>0.082* ± 0.002</td>
</tr>
</tbody>
</table>

LCAT, lecithin-cholesterol acyltransferase.

Affinities for each binding site are given as the dissociation constant. Values are given as the mean ± SE and are the product of the association constant \((\text{mol/L})^{-1}\)/\((\text{mol/L})\) and the number of binding sites (M).

*Significantly different from normals \( P < .0005 \).
cholesterol by incubation with cholesterol-rich liposomes (~0.9). Platelets from HFH patients have previously been reported to show increased rate and amplitude of aggregation with moderate concentrations of thrombin (500 mU/mL), whether as platelet-rich plasma or as plasma-free gel-filtered platelets, which has been taken to indicate an intrinsic platelet abnormality in hyperlipoproteinemia.

In the present work, we were not able to show any abnormalities in thrombin binding or aggregation of washed platelets from HFH patients as a whole. However, useful analysis was achieved by dividing the patients into two groups, those with and those without overt cardiovascular disease. Platelets from HFH patients with cardiovascular disease bound normal amounts of thrombin but were more responsive to the bound thrombin than were normal platelets. On the other hand, platelets from HFH patients without cardiovascular disease bound lower amounts of thrombin than did normal platelets but still gave aggregation responses in the normal range. These results suggest that in both platelet groups there is an alteration in the coupling mechanism which links the thrombin-binding sites to the response system which enables thrombin, with these platelets, to elicit a response with lower receptor occupancy than is possible in normal platelets. The separation of the patients into two groups shows that significant clinical changes correlate with measurable biochemical changes which are obscured if the data are taken as a whole.

All HFH patients eventually develop cardiovascular disease. Thus, the phenotypic characteristic of the platelet-thrombin interaction in these patients is probably that seen in the group prior to the onset of cardiovascular disease, namely, a decreased binding of thrombin but an increased responsiveness to the thrombin bound so that normal aggregation results. The onset of cardiovascular disease could result from a shift in this phenotypic characteristic to the second situation, in which normal amounts of thrombin are being bound and the increased responsiveness of HFH patients is manifesting itself as increased aggregability. However, there appear to be other, possibly more plausible, explanations: damage to the internal surface of blood vessels could result in a mild degree of platelet activation, and this activation could itself result in a synergistic response during further exposure to thrombin. Alternatively, platelets from hypercholesterolemic patients with cardiovascular disease have been reported to have a shorter survival time than that of platelets from hypercholesterolemic patients without cardiovascular disease. Thus, the shift in the binding isotherm and in the dose–response curve seen with platelets from HFH patients with cardiovascular disease could be the result of an increased population of younger platelets, which have been reported to respond to lower concentrations of agonists, rather than the result of inherent differences in platelets in the two patient groups. A corollary of this latter interpretation is that the number of thrombin receptors on platelets would be expected to be inversely proportional to platelet age.

These results using platelets from patients with HFH differ from those obtained in studies on thrombin binding and responsiveness following in vitro manipulation of the platelet C:PL ratio. In the latter case, the hyperresponsiveness arises from increased expression of thrombin receptors without any change in stimulus–response coupling whereas, in the present situation, that hyperresponsiveness in both groups of HFH patients is due to an increase in stimulus–response coupling.

Patients with type V hyperlipoproteinemia, Tangier disease, or LCAT deficiency have varying lipoprotein concentrations, but all have an increased plasma triglyceride concentration. The platelets from patients in these three disease groups all showed increased binding of thrombin to the high-affinity binding site (K,R1, Table 5) and demonstrated a reduced sensitivity to low concentrations of thrombin. These data suggest that elevated plasma triglyceride concentrations may cause the uncoupling of the high-affinity thrombin binding site from the response system. However, reduced plasma cholesterol levels and triglyceride levels in platelets from abetalipoproteinemia patients do not appear to alter either interaction with thrombin or thrombin responsiveness, suggesting that stimulus–response coupling is similar to that in platelets from normal volunteers. The recent studies of Aviram et al9 offer further evidence that triglyceride levels (associated with chylomicrons) are inhibitory to platelet function. Their studies report a normal response of gel-filtered platelets from type V hyperlipoproteinemic patients to 200 mU/mL of thrombin, and the results are very similar to those reported here. However, they did not examine the responsiveness of these platelets to lower thrombin concentrations (<30 mU/mL), which in our study appeared to demonstrate a reduced platelet responsiveness. Further studies are necessary to define clearly the effects of plasma triglyceride levels on platelet-thrombin interactions.

In conclusion, we used platelets from patients with various, rare, inborn errors of lipid metabolism to extend our earlier studies which showed that in vitro manipulation of C:PL ratios resulted in increased aggregation due to increased expression of thrombin receptors with no alteration in stimulus–response coupling. Three major conclusions may be drawn from the present work: (a) In vivo elevations of C:PL ratios in both groups of HFH patients resulted in increased aggregation response due to increases in stimulus–response coupling; (b) this increased stimulus–response coupling occurred in patients both with and without overt cardiovascular disease; and (c) normal C:PL ratios in other dyslipoproteinemic patients are not necessarily accompanied by normal thrombin binding or normal aggregation response. Finally, these studies show that in vitro manipulation of C:PL ratios, while perhaps providing useful information on membrane function, does not provide an adequate model for physiopathologic situations.

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Thrombin binding and response in platelets from patients with dyslipoproteinemias: increased stimulus-response coupling in type II hyperlipoproteinemia

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