Platelet Deposition at High Shear Rates Is Enhanced by High Plasma Cholesterol Levels
In Vivo Study in the Rabbit Model
Juan Jose Badimon, Lina Badimon, Vincent T. Turitto, and Valentin Fuster

We have studied the effects of high plasma cholesterol levels on platelet-vessel wall interactions under high shear rate conditions typical of the apex of stenotic arteries (2,600 sec⁻¹). Hypercholesterolemia was induced by feeding rabbits a 0.5% cholesterol-rich diet for 60 days. Platelet deposition was studied by use of an annular perfusion chamber and de-endothelialized abdominal rabbit aortas as substrates. After ingestion of the atherogenic diet, the experimental group of animals developed severe hypercholesterolemia, platelets became more fluid as determined by steady-state fluorescence anisotropy (p<0.05), and red blood cell deformability was decreased (p<0.001) when compared with normal controls. The fatty acid composition of platelet membranes showed an increase in the percentage of the long-chain saturated fatty acids (palmitic, C16:0, and stearic, C18:0) that may account for the lower polyunsaturated/saturated fatty acid ratio observed in the hyperlipemic animals. Total platelet deposition was significantly increased (p<0.05) in the hyperlipemic group as compared with the control group at 5 minutes' perfusion time, becoming less evident at 20 minutes' perfusion time. Our results suggest that the presence of hyperlipidemia may contribute to acute thrombosis by enhancing platelet-vessel wall interaction.


High levels of plasma lipids have been recognized as a major risk factor in the development and progression of atherosclerosis, a disease that is often the underlying cause of clinically relevant thrombosis.1–5

The process of thrombosis, especially in the vicinity of advanced atherosclerotic lesions, is complex, involving platelets, coagulation proteins, the condition of the vessel wall, and local blood flow conditions. For example, it is believed that rupture or ulceration of an atherosclerotic plaque may precipitate the growth of platelet and fibrinaceous elements in an already narrowed lumen.1,6 The localization of these thrombotic masses may well be modulated by local flow conditions.7 In this context, we8 have recently demonstrated that the high shear rate areas, typical of flow at the apex of stenotic regions, are the preferred sites for platelet thrombus formation on the injured vessel wall. Thus, both the degree of vascular damage and local flow conditions are important parameters for the study of clinically relevant thrombosis.

Dietary fat intake has also been shown to influence not only the development of atherosclerosis but also platelet function in experimental animal models and humans.4,5 Platelets from hyperlipemic patients have an increased cholesterol content in the membrane and show enhanced aggregability, release reaction, and thromboxane formation.9–12

However, the relation between plasma lipids and platelets with respect to the process of thrombosis has not been fully explored. While numerous studies have been conducted over the past 10 years demonstrating the mechanisms by which various plasma proteins influence platelet-vessel wall interactions, few studies have considered the role of lipoproteins in this process.

In this study, we have used a well-established annular perfusion system for studying the effects of increased plasma levels of cholesterol on platelet deposition and thrombus formation on normal rabbit subendothelium under controlled flow conditions. The results of our study indicate that severe hypercholesterolemia significantly increases platelet-vessel wall interactions, a finding that could be important in the thrombotic events associated with advanced atherosclerosis.

Methods

Animal Model
Adult New Zealand White rabbits (3.0±0.5 kg body weight; mean±SD) were selected by plasma
levels of triglycerides and total and high density lipoprotein (HDL) cholesterol values. The animals were housed in the Animal Research Center facilities of our institution. They were individually caged in stainless-steel wire-bottomed cages in a room controlled to 20±2°C, 50±10% humidity, and a 12-hour light/dark cycle. The animal care facility is accredited by the American Association for Accreditation of Animal Laboratory Care, and all procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

Hyperlipidemia was induced by feeding the animals a 0.5% cholesterol-rich diet. The hyperlipidemic diet was prepared by spraying normal rabbit chow (HF#5226, Ralston Purina Company, St. Louis, Mo.) with cholesterol dissolved in ethyl ether and allowing the solvent to evaporate in a fume hood. Water was provided ad libitum.13,14

**Experimental Design**

Age-matched rabbits were randomly divided into two groups: group 1 was fed the cholesterol-rich diet for 60 days (hyperlipidemic group, n=7), and group 2 (control group, n=5) was fed standard rabbit chow throughout the entire study.

On the day of the procedure, rabbits were anesthetized with ketamine plus Rompun (Haver, Mobay Corp., Shawnee, Kan.) 5 and 35 mg/kg, respectively i.m. The carotid artery was cannulated, and 10 ml blood was collected in acid–citrate–dextrose-saline for preparation of labeled platelets. Platelets were labeled with 111In-tropolone in plasma as previously described.15 The final pellet of radiolabeled platelets was resuspended in citrated platelet-poor plasma and added directly to the blood for perfusion. Blood for perfusion was collected into 90 mM sodium citrate (9 mM final concentration in blood) and was then recirculated by a roller pump (Masterflex model 7013, Cole-Palmer Instruments Co., Chicago, Ill.) through the perfusion chamber, which was prefilled with PBS, pH 7.4, for either 5 or 20 minutes at a flow rate of 40 ml/min (wall shear rate of 2,600 sec⁻¹). After exposure to blood, the perfused vessels were rinsed with PBS and placed in 2.5% glutaraldehyde (vol/vol) in 0.1 M cacodylate buffer, pH 7.4, at room temperature while still on the rod. After 5 minutes, the specimens were removed from the rod and transferred to fresh fixative at 4°C for 1 hour, after which they were rinsed with Tris buffer and placed in a gamma counter (Auto Gamma model 5560, United Technologies–Packard, Downers Grove, Ill.) for evaluation of radioactivity. After evaluation of radioactivity, the specimens were stored in 7% sucrose solution in PBS for up to 1 week before being embedded in Epon blocks. Storage, postfixation, and embedding of the exposed vessel segments, as well as cutting and staining of the sections, have been previously described.17

**Evaluation of Platelet Interaction With Vessel Wall**

Platelet–vessel wall interaction was determined by both radioisotopic and morphological techniques. The total number of platelets deposited on the exposed specimens was calculated from the platelet count and the 111In activity on the perfused area and in blood by means of our previously described methodology.18 Results are reported as platelets per vessel segment.

**Vessel segments were evaluated morphometrically by light microscopy.**19 Platelet interaction with the subendothelium was calculated systematically at 10-μm intervals for two axial positions located approximately 8 and 13 mm from the proximal end of the vessel segments. The results of the determinations were averaged. As previously defined,17 contact (C) platelets are platelets that are attached to but not spread on the subendothelium. Spread (S) platelets are platelets that have spread on the subendothelial surface. Thrombi (T) are defined as platelet thrombi 5 μm or more high attached to the surface by means of spread platelets. Platelet adhesion defined as C plus S (C+S) and T formation are expressed as a percentage of the total number of evaluations (=1,000) per vessel segment.

**Plasma Lipid Analysis**

Blood samples were collected in EDTA-containing tubes (1.5 mg/ml). All the animals were tested for their baseline and final levels of total and HDL cholesterol and triglycerides after 12–15 hours of fasting. Plasma was immediately obtained by low-speed centrifugation at 4°C. Cholesterol content was determined using a Beckman enzymatic kit (Dry Stat, Beckman Instruments, Inc., Carlsbad, Calif.). The triglyceride content of plasma was measured using an enzymatic kit (Seragen Diagnostics, Indianapolis, Ind.). HDL cholesterol was measured after precipitation of apolipoprotein (apo) B-containing lipoproteins by polyethylene glycol-6000.19,20 as we have previously described.13,14

**Fluidity Measurements**

Platelet membrane fluidity was determined by the method of fluorescence polarization.21 Steady-state fluorescence polarization measurements were per-
formed on an Amino-Bowman polarization spectrofluorometer (Houston, Tex.) arranged in a conventional T configuration. A circulating bath was connected to the thermostat-equipped compartment, and the temperature was monitored directly in the cuvette with a thermocouple microprobe connected to a digital thermometer. The compound 1,6-diphenyl-1,3,5-hexatriene (DPH) was used as the fluorescence probe. For probe incorporation, DPH was dissolved in tetrahydrofuran at a concentration of 1.5 mM. The platelets were suspended in 6 ml 154 mM NaCl, 100 mM sodium phosphate buffer, pH 7.4, at a concentration of 5 x 10^10 platelets/ml. At this platelet concentration, light scattering was minimal. The platelet suspension was divided into two equal aliquots. To one aliquot, 1 μl of the stock DPH solution was added ([DPH]=0.5 μM); the other aliquot served as the sample blank. Both aliquots were incubated at 37°C for 20 minutes. The excitation and emission wavelengths were set at 357 and 430 nm, respectively. Steady-state fluorescence polarization was expressed in terms of anisotropy (r) computed according to the following equation:

\[
r = \frac{I_0 - I_1}{I_0 + 2I_1}
\]

where \(I_0\) = fluorescence intensity with emission and excitation polarizers parallel and \(I_1\) = fluorescence intensity with the polarizers perpendicular. The data were corrected for unequal transmission of differently polarized light and intrinsic fluorescence. The latter correction was made by subtracting the fluorescence of the sample blank from the fluorescence of the sample containing DPH. The grating correction factor of Chen and Bowman was used to correct for the depolarization effect of grating monochromators.

**Analysis of Fatty Acid Composition of Platelets**

Blood samples were collected in acid-citrate-dextrose. Platelet-rich plasma was obtained by centrifugation of the anticoagulated whole blood at 170g for 10 minutes. A platelet pellet was obtained by centrifuging the platelet-rich plasma at 1,800g for 10 minutes. The platelet pellets were washed twice with 5 ml chloroform. This platelet concentrate was subjected to repeated freeze-thawing (three times) to rupture the cell membranes. Five milliliters methanol was added to the lysed platelets and incubated for 10 minutes at room temperature in a capped glass vial. An additional 5 ml chloroform was added to the suspension, briefly vortexed, and filtered through a 0.2-μm nylon-66 filter. The vial and filter were washed with 5 ml chloroform/methanol (2:1; vol/vol), containing 0.2% BHT. After addition of saturated NaCl solution (5 ml), the mixture was vortexed for 15 seconds and incubated at 20°C for 30 minutes. The mixture was finally centrifuged at 1,800g and 4°C for 30 minutes. The upper aqueous phase was discarded, and the lower organic phase was filtered again with a nylon-66 filter. The filter and centrifuge tube were washed twice with 5 ml chloroform. This platelet lipid extract was dried under vacuum (Buchi Rotovapor-R, Westbury, N.Y.), reconstituted in 4 ml chloroform, separated into two equal portions, and dried with a gentle N2 stream before being stored at -70°C.

Fatty acid derivatization was performed according to Miwa et al. In brief, the platelet extract was reconstituted with 2-3 ml chloroform and transferred to a 7-ml capped glass vial. An internal standard (100 μl heptadecanoic acid) was then added. The chloroform was removed under an N2 stream, 100 μl 2.5 M KOH in water/ethanol (2:8) was added, and the mixture was briefly vortexed and incubated for 10 minutes in a 90°C water bath. The samples were allowed to equilibrate in a room-temperature water bath. Thereafter, 200 μl 2-nitrophenylhydrazine was added and briefly vortexed, and 400 μl 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide pyridine solution was added; the mixture was briefly vortexed and incubated at 60°C for 20 minutes. The samples were incubated an additional 15 minutes at 60°C after addition of 100/μl KOH. The samples were brought to room temperature and injected into the HPLC system.

**Red Blood Cell Deformability**

An ektacytometer (Technicon Instruments, Tarrytown, N.Y.) was used to measure cell deformability of blood from normal and cholesterol-fed rabbits. Blood collected into 1% EDTA solution was diluted by a factor of 200 with 2.5% polyvinylpyrrolidone (PVP, Sigma Chemical Co., St. Louis, Mo.) in PBS, pH 7.4, 290 mosm. The cytometer is essentially a concentric cylindrical viscometer whose outer cylinder was rotated over a range of rotational speeds, so that the blood diluted in PVP, which was placed in the narrow gap between the viscometer walls, was
exposed to a continuously varying shear stress. A laser beam deflected through the cell suspension formed a characteristic diffraction pattern. The elongation index, EI, a quantitative measurement of cell deformability, was determined from the light intensity obtained from the major (A) and minor (B) indexes of the elliptical diffraction pattern produced by the deformed cells such that

\[ EI = \frac{A - B}{A + B} \]

**Hematological Analysis and Animal Surveillance**

Platelet number, size, and distribution were determined with a Coulter P260 platelet analyzer (Coulter Electronics, Hialeah, Fla.) as we have previously reported.\(^29\) Rabbit platelets and red blood cells are smaller than those of humans; therefore, new thresholds had to be defined in the counter to analyze rabbit blood. After a preliminary study, lower and upper thresholds of 1.73 \(\mu m^3\) and 15.7 \(\mu m^3\), respectively, were determined to include the smallest platelets and to avoid counting the micro-red blood cells as large platelets. Hematocrit was also measured by the microhematocrit technique (Readacrit centrifuge, Clay Adams, Parsippany, N.J.).

Animals were closely followed during the experimental period. Their daily food intake was recorded and their weight taken once a week to detect any sudden weight loss.

**Statistical Analysis**

Results are expressed as mean±SEM unless otherwise stated. The Student–Fisher \(t\) test or the Mann–Whitney \(U\) test for nonparametric data was used to compare differences between groups. The tests were two-sided, and the level of significance was chosen as \(p<0.05\).

**Results**

**Plasma Lipids**

Plasma lipid levels are presented in Table 1. The group receiving the cholesterol-rich diet responded with a marked increase (\(p<0.001\)) in plasma total cholesterol concentration compared with that of the control group. There were no significant differences in triglyceride levels between the two groups.

We have also studied the cholesterol distribution among the different classes of plasma lipoproteins (Table 2). The group receiving the cholesterol-rich diet showed high concentrations of cholesterol in the apo B-containing lipoproteins (very low density lipoproteins, \(d<1.006 \text{ g/ml}\); LDL, \(d=1.006-1.063 \text{ g/ml}\); and HDL, \(d=1.063-1.21 \text{ g/ml}\)) and a decrease in the HDL fraction when compared with that of the group receiving regular rabbit chow (Table 2).

**Platelet Membrane Fluidity**

Platelet membrane fluidity was measured by steady-state fluorescence anisotropy (\(r_5\)) in DPH-labeled platelets from the two groups of animals. It was found that anisotropy was significantly lower (\(p<0.05\)) in the cholesterol-fed group (0.199±0.01) than in the control animals receiving standard rabbit chow (0.217±0.01). These results suggest that the platelet membranes of the hypercholesterolemic rabbits have become more fluid due to the dietary treatment, presumably because of cholesterol incorporation.

**Platelet–Vessel Wall Interactions**

Platelet–vessel wall interactions on de-endothelialized rabbit aortas exposed to blood at a wall shear rate of 2,600 sec\(^{-1}\) was studied at perfusion times of 5 and 20 minutes. The deposition of platelets was determined by two different evaluation methods and is presented in Figure 1 and Table 3.

Radioisotopic \(^{111}\)In-labeled platelets. The cholesterol-fed group showed a significant increase in total platelet deposition on subendothelium at a perfusion time of 5 minutes as compared with the group fed

![FIGURE 1. Bar graph of platelet–vessel wall interaction as determined by the radioisotopic \(^{111}\)In-labeled platelets method. Results are expressed as platelet deposition \(\times 10^6\) vessel as a function of perfusion time (min). ■, Control group; □, hyperlipemic group.](http://atvb.ahajournals.org/)

**TABLE 1. Plasma Lipid Levels**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total cholesterol</th>
<th>HDL cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>66±4</td>
<td>25±4</td>
<td>60±8</td>
</tr>
<tr>
<td>Hyperlipidemic</td>
<td>1,510±140</td>
<td>61±6</td>
<td>64±9</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein.

Values are expressed as mg/dl (mean±SEM).

**TABLE 2. Cholesterol Distribution Among Different Plasma Lipoproteins**

<table>
<thead>
<tr>
<th>Group</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18±3</td>
<td>45±6</td>
<td>37±4</td>
</tr>
<tr>
<td>Hyperlipidemic</td>
<td>68±21</td>
<td>27±8</td>
<td>4±1</td>
</tr>
</tbody>
</table>

VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

Values are expressed as percent of total plasma cholesterol, mean±SEM, VLDL, \(d<1.006 \text{ g/ml}\); LDL, \(d=1.006-1.063 \text{ g/ml}\); and HDL, \(d=1.063-1.21 \text{ g/ml}\).
TABLE 3. Platelet-Vessel Wall Interaction (Morphometric Analysis)

<table>
<thead>
<tr>
<th>Time/group</th>
<th>Hematocrit</th>
<th>Platelet adhesion</th>
<th>&gt;5-μm thrombus</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-minute perfusion time</td>
<td>Control</td>
<td>33±1.7</td>
<td>22±7.1</td>
</tr>
<tr>
<td></td>
<td>Hyperlipemic</td>
<td>25±0.9</td>
<td>45.1±14*</td>
</tr>
<tr>
<td>20-minute perfusion time</td>
<td>Control</td>
<td>33±1.7</td>
<td>68±14</td>
</tr>
<tr>
<td></td>
<td>Hyperlipemic</td>
<td>25±0.9</td>
<td>35±12</td>
</tr>
</tbody>
</table>

Values are expressed as percent (mean±SEM). *p<0.05.

standard rabbit chow. The effect of the atherogenic diet on platelet deposition became less evident at perfusion times of 20 minutes (Figure 1).

Morphometric analysis. Platelet adhesion (C+S) was increased in the cholesterol-fed group versus the standard diet–fed group at the 5-minute perfusion time, as was T formation. At 20 minutes' perfusion time, the differences were not significantly different (Table 3).

Fatty Acid Composition of Platelet Membrane Phospholipids

The fatty acid composition of platelets in various treatment groups of animals is presented in Table 4. The increase in the polyunsaturated/saturated fatty acid (P/S) ratio observed in cholesterol-fed animals is mostly due to the increase in the long-chain saturated fatty acids, specifically palmitic (C16:0) and stearic (C18:0) acids. Despite the marked increase in arachidonic acid (C20:4), the results show a decrease in the P/S ratio in the cholesterol-fed animals, indicating an increase in the percentage of saturated fatty acid as a consequence of the ingestion of the hyperlipemic diet.

Red Blood Cell Deformability

A typical EI for red blood cells over a range of rotational speeds in the ektacytometer is shown in Figure 2. The red blood cells are gradually deformed by the increasing shear forces, such that EI increases up to a maximum value, EI_max, after which no further deformation is observed. Animals fed the atherogenic diet exhibited significantly (p<0.001) reduced values of EI_max (0.46±0.01) compared with normal controls (0.30±0.02). These results suggest a decrease in red blood cell deformability as a consequence of the hyperlipidemia.

Discussion

In the present investigation, we have studied the effects of a cholesterol-rich diet on platelet–vessel wall interactions under controlled flow conditions mimicking wall shear rates typical of values normally found in the microvasculature or in stenotic arteries (2,600 sec^{-1}). Such rheological conditions have proved sensitive for observing defects in C+S and T formation.7,16,20,31

At these high shear rate conditions, we have shown that increased levels of plasma lipids, such as those

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Group</th>
<th>Control</th>
<th>Hyperlipemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>8±0.1</td>
<td>2.6±0.5</td>
<td></td>
</tr>
<tr>
<td>14:1</td>
<td>12.2±0.8</td>
<td>6.1±4</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>17.4±1.4</td>
<td>24±5</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>14.5±1.5</td>
<td>13±1.7</td>
<td></td>
</tr>
<tr>
<td>18:2</td>
<td>17±1.9</td>
<td>21.6±1.6</td>
<td></td>
</tr>
<tr>
<td>18:3</td>
<td>ND</td>
<td>1.3±1</td>
<td></td>
</tr>
<tr>
<td>20:3</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>20:4</td>
<td>7.3±2.6</td>
<td>14.5±1.3</td>
<td></td>
</tr>
<tr>
<td>20:5</td>
<td>9.8±3.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>20:6</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>P/S ratio</td>
<td>1.6</td>
<td>1.28</td>
<td></td>
</tr>
</tbody>
</table>

P/S, polyunsaturated/saturated; ND, not determined.

Mean values±SEM are expressed as percent of total fatty acid composition.
obtained with a high-cholesterol diet in experimental animals, can induce marked alterations in the ability of platelets to deposit on normal aortic subendothelium. The enhanced deposition of platelets on vessel walls in these animals, as indicated in Figure 1, is apparent at perfusion times of both 5 and 20 minutes in the cholesterol-fed animals and appears, from Table 3, to be related predominantly to the altered levels of platelet T on the vessel wall rather than on increased levels of C+S.

The specific mechanism underlying the enhanced platelet-vessel wall interaction in hypercholesterolemic animals is not clear at present. As indicated above, these shear conditions were chosen because they have proved to be sensitive for measuring differences in platelet reactivity in various bleeding disorders. Thus, the differences presently observed are believed to be related to alterations in platelet function due to the hyperlipemic diet. At present, it is not clear what the basis for such changes might be. We have found that cholesterol feeding did modify platelet membrane fluidity, as defined by the reciprocal of the steady-state fluorescence anisotropy, r. According to the “lipid fluidity” concept, lower values of r in DPH-labeled platelets indicate an increase in platelet membrane fluidity in hyperlipemic animals compared with the normolipemic group. Since platelet adhesion is a membrane-mediated process, it is conceivable that modifications in the platelet membrane may be altered by such changes; however, few studies in this area have been conducted to determine the effects of such changes on thrombosis.

A direct influence of membrane fluidity on platelet aggregation has been observed by some authors, whereas an inverse relation has been shown between inhibited T formation and a dietary manipulation that resulted in increased membrane fluidity in rats. Our observation with respect to platelet fluidity is in agreement with those of several other groups, which have reported that dietary fats induce changes in platelet membrane fluidity. However, our report of increased platelet membrane fluidity in animals maintained on a low-cholesterol diet for 60 days is in contrast to the decreases in membrane fluidity induced by in vitro cholesterol loading of platelets. No explanation for the difference is apparent; however, the finding suggests that some caution should be exercised when interpreting changes in platelet fluidity by in vitro cholesterol incorporation techniques.

We have also studied the fatty acid composition of platelets. The cholesterol-fed group showed an increase in the percentage of the long-chain saturated fatty acids, palmitic (16:0) and stearic (18:0) acids, which have been shown to stimulate platelet aggregation in rats and to increase arachidonic acid content (20:4). These increases in the long-chain saturated fatty acids may account for the lower P/S ratio observed in the cholesterol-fed animals, but its effect on platelet deposition remains unknown. The net decrease in unsaturated fatty acid was 4.6%, while the increase in saturated fatty acid was 6.1%. Recently, Dalal et al have also reported an increase in arachidonic acid in platelets from cholesterol-fed rabbits as compared with control animals. They also reported that hyperlipemic platelets were hyperreactive when challenged with low concentrations of collagen.

There are several complicating factors influencing our observation of an enhanced platelet-vessel wall interaction due to hyperlipidemia. First, the animals on the atherogenic diet had a significant reduction in their hematocrit as indicated in Table 3. Under these rheological conditions of high shear rate, red blood cell concentration has been shown to strongly influence platelet-vessel wall interaction. At these wall shear conditions and levels of hematocrit (20–40%), red blood cells may contribute, both physically and chemically, to the overall deposition mechanism. In addition, recent studies have shown that the local concentration of platelets in the vicinity of the vessel wall may be significantly affected by changes in red blood cell concentration. However, with respect to the precise mechanism, the decrease in red blood cells would head to a reduced platelet-vessel wall interaction. Thus, the finding of an increased platelet deposition, even at reduced hematocrit numbers, suggests that changes in platelet-vessel wall interactions induced by the hyperlipemic diet would be even more marked if studied at similar hematocrit values. Another additional factor that could contribute to the increased platelet deposition is the decreased deformability of red blood cells in animals maintained on the cholesterol-rich diet. Aarts and colleagues have also shown that a decrease in red blood cell deformability substantially enhances platelet adhesion to the subendothelium. These authors induced less deformable red blood cells by in vitro incubation with cholesterol.

Therefore, it is likely that the enhanced platelet deposition observed presently may be related, at least in part, to an impaired deformability of red blood cell membranes, perhaps due to the uptake of cholesterol by red blood cells. An additional consideration is that red blood cell shape, which has been reported to be altered by cholesterol feeding, may also be affecting the local transport of platelets to the vessel wall. Further studies will be needed to determine the extent to which red blood cell deformability (versus a possible contribution of red blood cell shape) contributes to the enhanced deposition.

In summary, the association of high plasma lipid levels as attained through a high-cholesterol diet may contribute to acute thrombosis through a mechanism by which platelet-vessel wall interactions are enhanced. Such an effect may be modulated by changes in platelet reactivity or in various red blood cell parameters such as deformability and/or shape. Additional studies will need to be performed to delineate the unknown mechanisms that may be modified in the enhanced platelet deposition. Since these
mechanisms may include not only platelet function considerations but also red blood cell properties, a more thorough investigation concerning the contribution of each system would be useful for the eventual design of interventional strategies appropriate for patients whose advanced atherosclerosis is accompanied by high cholesterol levels and who, therefore, have a potentially enhanced thrombotic risk. While the present studies cannot be directly extrapolated to humans due to obvious species differences (very high lipid levels), the present observation suggests that further studies would be appropriate in humans.

References

26. Miyake H, Yamamoto H: Improved method of determination of biologically important C10:0–C22:6 fatty acids as their 2-ni-
37. Tandon N, Maramou JT, Rodbard D, Jamieson GA: Thrombo-


**KEY WORDS** • platelet deposition • lipids • hyperlipidemia • thrombosis • shear rate • platelets • lipoproteins
Platelet deposition at high shear rates is enhanced by high plasma cholesterol levels. In vivo study in the rabbit model.

J J Badimon, L Badimon, V T Turitto and V Fuster

doi: 10.1161/01.ATV.11.2.395

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/11/2/395

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at: http://atvb.ahajournals.org//subscriptions/