Increased Thromboxane Biosynthesis in Type IIa Hypercholesterolemia

Giovanni Davi, MD; Maurizio Averna, MD; Isabella Catalano, MD; Carlo Barbagallo, MD; Antonella Ganci, MD; Alberto Notarbartolo, MD; Giovanni Ciabattoni, MD; and Carlo Patrono, MD

**Background.** Increased platelet thromboxane (TX)A₂ production has been described in type IIa hypercholesterolemia. To verify the relevance of these capacity-related measurements to the actual rate of TXA₂ biosynthesis in vivo, we studied the urinary excretion of its major enzymatic metabolites in 46 patients with type IIa hypercholesterolemia and 20 age-matched controls.

**Methods and Results.** Urinary 11-dehydro-TXB₂ and 2,3-dinor-TXB₂ were measured by previously validated radioimmunoassays. The excretion rate of 11-dehydro-TXB₂ was significantly (p<0.001) higher in patients (68.7±35.1 ng/hr, mean±SD) than in controls (22.4±9.4 ng/hr), with metabolite excretion >2 SD of the normal mean in 74% of the patients. Urinary 11-dehydro-TXB₂ was significantly (p<0.01) correlated with the threshold aggregating concentration of collagen (r=-0.641) and arachidonate (r=-0.734) and with agonist-induced platelet TXB₂ production in vitro (r=0.647 and 0.748, respectively). Moreover, a statistically significant correlation (r=0.673, p<0.001, n=66) was found between 11-dehydro-TXB₂ excretion and total plasma cholesterol. The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor simvastatin (20 mg/day for 6 months) significantly reduced cholesterol levels by 22–28% and urinary 11-dehydro-TXB₂ excretion by 32–42% in 10 patients. However, the reduction in the latter did not correlate with the reduction in the former and may have resulted from a nonspecific effect of simvastatin. Moreover, selective inhibition of platelet cyclooxygenase activity by low-dose aspirin (50 mg/day for 7 days) was associated with cumulative inhibition of 11-dehydro-TXB₂ excretion by approximately 70% in six patients.

**Conclusions.** TXA₂ biosynthesis is enhanced in the majority of patients with type IIa hypercholesterolemia; this is, at least in part, a consequence of abnormal cholesterol levels, as suggested by the correlation between the two. Low-dose aspirin can largely suppress increased metabolite excretion, thus suggesting that it reflects TXA₂-dependent platelet activation in vivo. (*Circulation* 1992;85:1792–1798)

**KEY WORDS** • thromboxane • metabolites • simvastatin • aspirin

A high incidence of atherosclerosis and thrombotic complications has been associated with type IIa hypercholesterolemia. Because platelet hyper-reactivity has been considered responsible or at least contributing to acute thromboembolic complications such as myocardial infarction and ischemic stroke, several studies have assessed platelet function in type IIa hyperlipoproteinemia. Increased sensitivity of platelets to the aggregatory effects of various agonists and higher production of arachidonic acid metabolites have been described in hypercholesterolemia in association with increased activity of platelet phospholipases.

Washed platelets from type IIa hypercholesterolemic subjects incubated with exogenous arachidonate produce higher amounts of thromboxane (TX)B₂, which correlates linearly with cholesterol levels. This abnormal platelet function has been related to the cholesterol content of platelets, possibly reflecting an exchange between plasma lipoproteins and platelets. In fact, platelets artificially enriched with cholesterol show enhanced sensitivity to aggregating agents and higher conversion of labeled arachidonate to TXB₂.

Consistent with this hypothesis is the finding that the platelet content of cholesterol and phospholipids is significantly higher in patients with type IIa hypercholesterolemia than in controls and that this altered lipid composition is associated with enhanced production of TXA₂ by platelets.

In the present study, we sought to determine whether the formation of this potent platelet agonist is altered in vivo through measurements of a major enzymatic metabolite of TXB₂ in the urine of patients with type IIa hypercholesterolemia and matched controls. Our results provide biochemical evidence for the occurrence of TXA₂-dependent platelet activation in vivo and for its relation to the plasma cholesterol level. Moreover, we demonstrate that enhanced TXA₂ biosynthesis can be...
partially reduced by simvastatin, a selective inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and largely suppressed by low doses of aspirin, a selective inhibitor of the enzyme prostaglandin (PG)G/H synthase.

### Methods

#### Subjects

Forty-six patients with type IIa hypercholesterolemia (19 women and 27 men; age, 47±14 years; range, 20–66 years) and 20 healthy volunteers (10 women and 10 men; age, 42±13 years; range, 26–65 years) were studied on several occasions between March 1988 and May 1990. Informed consent was obtained from each participating subject. Type IIa hypercholesterolemia was defined in accordance with World Health Organization criteria, based on the determination of plasma total cholesterol and triglyceride levels as well as lipoprotein fractionation. Patients with diabetes mellitus, impaired hepatic function, secondary hypercholesterolemia, history of alcoholism (drug abuse), concomitant treatment with anticoagulants, antiplatelet drugs, corticosteroids, and theophylline were excluded. Moreover, we excluded patients with a history and/or clinical examination positive for evidence of macrovascular complications as judged on the basis of clinical symptoms, ECG monitoring during exercise, Holter monitoring, carotid Doppler testing, and Doppler echographic study of the lower limbs. Patients with renal disease (creatinine clearance <80 ml/min, serum creatinine >2 mg/dl, urine albumin excretion >300 mg/day) were also excluded. None of the patients had taken any drugs known to affect lipid metabolism or platelet function for at least 4 weeks before the start of the study. Patients were on a lipid-lowering diet (30% lipids, 52% carbohydrates, 18% proteins) with a polyunsaturated/saturated ratio of about 1.3 for at least 8 weeks before the study.

Nine patients were current smokers, and 37 never smoked or were ex-smokers who stopped smoking at least 2 years before the study. All healthy subjects were nonsmokers.

#### Design of the Studies

In the first study, a cross-sectional comparison of thromboxane metabolite excretion and platelet function was performed between the patients and controls. Both patients and controls were studied on an outpatient basis and fasted overnight before the study. Blood samples were obtained for measurement of platelet aggregation and TXB2 synthesis in vitro. Urine was collected during the 12 hours before blood sampling; the samples were frozen immediately and kept at -20°C until extraction. Paired measurements of platelet synthesis of TXB2, and aggregation in vitro and thromboxane metabolite excretion in vivo were obtained in 20 patients and 20 controls. The 20 patients (11 women and nine men) were nonsmokers.

To investigate prospectively the relation between blood cholesterol levels and thromboxane biosynthesis in vivo, we assessed the effects of a persistent reduction in cholesterol synthesis induced by an HMG-CoA reductase inhibitor in 10 of the above patients (seven men, three women; 26–55 years of age). Two of these patients were current smokers. The 10 patients were selected on the basis of a) indication to lipid-lowering drug therapy; b) no contraindication to simvastatin; c) willingness to participate in the study. The sample size was determined on the basis of the theoretical magnitude of thromboxane inhibition that could be reasonably expected from the correlation. Urine and blood samples were obtained before and after 1, 3, and 6 months of simvastatin (Merck Sharp & Dohme) treatment at a dose of 20 mg/day. The diet was controlled and remained unchanged throughout the study.

A third study was designed to examine the relative contribution of platelets to the enhanced excretion of 11-dehydro-TXB2. For this purpose, six patients (four men, two women; 23–58 years of age; four current smokers) were given aspirin (Bayer) at a dose of 50 mg/day for 7 days, and 12-hour urine collections were obtained immediately before and on the third and seventh days of treatment. The six patients were selected on the basis of a) no contraindication to aspirin treatment and b) willingness to participate in the study. The sample size was determined on the basis of the expected reduction in thromboxane metabolite excretion, assuming a predominant platelet source of enhanced TXA2 biosynthesis. In the same patients, the reproducibility of 11-dehydro-TXB2 excretion was assessed by obtaining three additional urine samples during the week preceding aspirin administration. The studies were approved by the internal medicine review board of our institution.

### Lipid and Platelet Function Measurements

Patients attended our lipid clinic in the morning after an overnight fast. All blood samples for lipid, lipoprotein, and apolipoprotein analyses were drawn into sodium and potassium EDTA (1 mg/ml). Cholesterol and triglycerides were determined enzymatically (Boehringer-Mannheim kit). High density lipoprotein (HDL) cholesterol was determined by the phosphotungstic acid (PTA)/MgCl2 precipitation method (Boehringer-Mannheim). Low density lipoprotein (LDL) cholesterol was calculated by Friedewald’s formula (LDL-C = TC – TG/5 – HDL-C). Apolipoprotein B was quantified by Mancini’s radial immunodiffusion method using plates with polyclonal antibodies (Daichi, Tokyo).

For platelet studies, blood was collected into 3.8% sodium citrate (1 ml for 9 ml of blood). Platelet-rich plasma and platelet-poor plasma were prepared as previously described. Platelet aggregation was measured in an ELVI 840 (Logos, Milan, Italy) aggregometer according to the method of Born after adjusting the number of platelets for each individual sample. For each agonist, the threshold aggregating concentration was defined as the lowest concentration of the agent that caused at least a 50% increase in light transmittance within 3 minutes. Platelet thromboxane synthesis was measured as previously described.

#### Urinary 11-dehydro-TXB2 and 2,3-dinor-TXB2 Assays

Measurement of urinary 11-dehydro-TXB2 and 2,3-dinor-TXB2 was performed by previously validated radioimmunoassay (RIA) techniques. Immunoreactive 11-dehydro-TXB2 was extracted from 20-ml
TABLE 1. Plasma Lipid and Apolipoprotein Levels in Type IIa Hypercholesterolemic Patients and Age-Matched Controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients (n=46)</th>
<th>Smokers (n=9)</th>
<th>Nonsmokers (n=37)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma cholesterol (mg/dl)</td>
<td>297±44</td>
<td>323±75*</td>
<td>291±29</td>
<td>182±23</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dl)</td>
<td>143±51</td>
<td>143±71</td>
<td>143±45</td>
<td>91±34</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>44±12</td>
<td>37±7*</td>
<td>46±13</td>
<td>45±10</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>224±44</td>
<td>257±70†</td>
<td>217±30</td>
<td>119±21</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dl)</td>
<td>178±36</td>
<td>212±64‡</td>
<td>171±21</td>
<td>100±20</td>
</tr>
<tr>
<td>Total cholesterol/HDL ratio</td>
<td>7.3±2.3</td>
<td>9.1±3.0‡</td>
<td>6.8±1.8</td>
<td>4.2±1.0</td>
</tr>
<tr>
<td>LDL cholesterol/HDL ratio</td>
<td>5.6±2.1</td>
<td>7.3±2.7‡</td>
<td>5.1±1.7</td>
<td>2.8±0.9</td>
</tr>
</tbody>
</table>

Values are mean±SD.
HDL, high density lipoprotein; LDL, low density lipoprotein.
*p<0.05 smokers vs. nonsmokers.
†p<0.01 smokers vs. nonsmokers.
‡p<0.005 smokers vs. nonsmokers.
*p<0.001 for all comparisons of controls vs. patients (except for HDL cholesterol, NS).

Statistical Analysis
Data were analyzed by nonparametric methods to avoid assumptions on the distribution of the measured variables. An ANOVA was performed by the Kruskall-Wallis method. Subsequent pairwise comparisons were made by the Mann-Whitney U test. Associations of eicosanoid measurements with other biochemical and functional measurements were assessed by stepwise regression analysis and multiple linear regression. All values are reported as mean±1 SD. Statistical significance was defined as p<0.05.

Results
Thromboxane Biosynthesis and Platelet Function

Table 1 shows the mean lipid and apolipoprotein levels of patients and controls. Untreated type IIa hypercholesterolemic patients exhibited significantly (p<0.001) higher lipid levels than healthy subjects. Among hypercholesterolemic patients, there were statistically significant differences between smokers and nonsmokers in all lipid and lipoprotein measurements except for comparable triglyceride levels (Table 1).

Platelets from patients with type IIa hypercholesterolemia required significantly (p<0.01) less collagen and arachidonate to aggregate and synthesized greater amounts of TXB₂ in response to both agonists than did platelets from healthy subjects (Table 2).

The urinary excretion rate of 11-dehydro-TXB₂ averaged 22.4±9.4 ng/hr in the control group and 68.7±35.1 ng/hr in hypercholesterolemic patients (p<0.001). No statistically significant difference was found between men and women in either group. Among the patients, smokers had a significantly (p<0.01) higher excretion rate than nonsmokers (94.7±39.4, n=9 versus 62.4±30.8, n=37). In 34 (74%) of the 46 patients, metabolite excretion was more than 2 SD above the normal mean (Figure 1). To comparatively explore the metabolic disposition of endogenously released TXB₂...
through dehydrogenation of the hemiacetal alcohol group at C-11 vis-a-vis β-oxidation,22 we measured the urinary excretion of 2,3-dinor-TXB₂ in eight patients with type IIa hypercholesterolemia, encompassing excretory rates of 11-dehydro-TXB₂ between 8.8 and 83.5 ng/hr. A highly significant correlation was found between these paired measurements (r=0.929, p<0.001, n=8), with the ratio of 11-dehydro-TXB₂ to 2,3-dinor-TXB₂ averaging 1.4±0.4 (range, 0.6-2.1).

In both patients and controls, urinary 11-dehydro-TXB₂ was significantly (p<0.01) correlated with the threshold aggregating concentrations of collagen (r=-0.641) and arachidonate (r=-0.734) and with agonist-induced platelet TXB₂ production in vitro (r=0.647 and 0.748 for collagen and arachidonate, respectively). As shown in Figure 2, a statistically significant correlation (r=0.673, n=66, p<0.001) was found between 11-dehydro-TXB₂ excretion rates and total plasma cholesterol levels.

**Influence of Cholesterol Reduction**

Inhibition of cholesterol biosynthesis by the HMG-CoA reductase inhibitor simvastatin (20 mg/day for 6 months) was associated with statistically significant average reductions in both blood cholesterol levels by 22–28% and urinary 11-dehydro-TXB₂ excretion by 33–42% in 10 patients with type IIa hypercholesterolemia. Lipid changes are detailed in Table 3. The observed mean reduction in thromboxane metabolite excretion was consistent with the expected change that would be predicted by the correlation shown in Figure 2 if enhanced thromboxane biosynthesis were at least in part a consequence of elevated blood cholesterol levels. However, the response to cholesterol lowering by simvastatin was not homogeneous. Thus, in individual patients, thromboxane biosynthesis was reduced by as low as 10% to as high as 75% at any time point during simvastatin therapy, with no statistically significant correlation with the reduction in cholesterol levels (Table 4).

### Table 2. Indexes of Platelet Function in Patients With Type IIa Hypercholesterolemia and Controls

<table>
<thead>
<tr>
<th>Index</th>
<th>Controls* (n=20)</th>
<th>Patients† (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen threshold (μg/ml)</td>
<td>0.92±0.24</td>
<td>0.40±0.22</td>
</tr>
<tr>
<td>Arachidonate threshold (mmol/l)</td>
<td>0.89±0.21</td>
<td>0.47±0.27</td>
</tr>
<tr>
<td>Collagen-induced TXB₂ synthesis (ng/10⁸ platelets)</td>
<td>17.1±2.7</td>
<td>26.4±7.3</td>
</tr>
<tr>
<td>Arachidonate-induced TXB₂ synthesis (ng/10⁸ platelets)</td>
<td>31.4±7.6</td>
<td>59.2±15.2</td>
</tr>
</tbody>
</table>

Values are mean±SD. TXB₂, thromboxane B₂.

*Ten women, 10 men; all nonsmokers; total plasma cholesterol, 182±23 mg/dl; urinary 11-dehydro-TXB₂, 22.4±9.4 ng/hr.

†Eleven women, nine men; all nonsmokers; total plasma cholesterol, 313±51 mg/dl; urinary 11-dehydro-TXB₂, 74.7±40.7 ng/hr.

### Table 3. Plasma Lipid and Apolipoprotein Levels in Patients With Type IIa Hypercholesterolemia Before and After 6-Month Treatment With Simvastatin 20 mg/day

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before (mg/dl)</th>
<th>After (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma cholesterol</td>
<td>351±46</td>
<td>252±39*</td>
</tr>
<tr>
<td>Plasma triglycerides</td>
<td>166±53</td>
<td>132±24</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>40±14</td>
<td>42±10</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>278±45</td>
<td>183±39*</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>197±36</td>
<td>137±21*</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; LDL, low density lipoprotein. Values are mean±SD, n=10.

*p<0.001.
Effect of Low-Dose Aspirin

To characterize the enhanced excretion of 11-dehydro-TXB₂ in patients with type IIa hypercholesterolemia as being of platelet or nonplatelet origin, we evaluated the short-term effects of a platelet-selective regimen of aspirin therapy (50 mg/day for 7 days) on the degree of suppression of metabolite excretion in six patients. The lipid profile of these patients was: total plasma cholesterol, 304±21 mg/dl; plasma triglycerides, 125±25 mg/dl; HDL cholesterol, 48±7 mg/dl; LDL cholesterol, 231±23 mg/dl; apolipoprotein B, 176±8 mg/dl. Before aspirin administration, the rate of 11-dehydro-TXB₂ excretion averaged 78.5±43.8 ng/hr, with an intrasubject coefficient of variation of 10±3% as measured on four consecutive urine samples. After 3 days of aspirin administration, metabolite excretion was significantly (p<0.01) reduced to 40.0±21.4 ng/hr and at the end of 1 week was further reduced to 24.9±12.5 ng/hr (Figure 3). This finding is consistent with the cumulative nature of acetylation of platelet PGG/H synthase¹⁷ and inhibition of thromboxane biosynthesis¹⁸ by low-dose aspirin in healthy subjects.

Discussion

Several lines of evidence suggest that platelet hyperreactivity might be an important factor that contributes to the enhanced risk of thrombotic complications associated with a selective increase in plasma LDL cholesterol (reviewed in Reference 19). Thus, earlier studies in patients with type IIa hypercholesterolemia consistently demonstrated enhanced platelet aggregation in response to a variety of agonists and increased conversion of arachidonate through the platelet PGG/H synthase pathway to form proaggregatory eicosanoids such as PGH₂ and TXA₂.⁴⁻⁸

The results of the present study provide in vivo evidence of platelet activation in this type of patients in the absence of other cardiovascular risk factors known to influence TXA₂ biosynthesis.²⁰ The in vitro measurements of platelet aggregation and TXB₂ synthesis confirm and extend previous findings in patients with the same condition.¹⁹ However, both measurements represent capacity-related indexes and by no means reflect the actual occurrence of TXA₂-dependent platelet activation in vivo.²¹ Thus, the present study addressed the issue of the actual rate of TXA₂ biosynthesis in vivo through measurements of a major urinary metabolite, i.e., 11-dehydro-TXB₂.¹⁵ Approximately three quarters of our patients excreted two to seven times the level of metabolite measured in controls. In six patients in whom four different urine collections were obtained on consecutive days before aspirin administration, a relatively small intrasubject coefficient of variation (7–13%) was calculated, indicating a highly reproducible metabolic alteration. Because enhanced 11-dehydro-TXB₂ excretion might reflect either increased formation of TXA₂ or a shift in its metabolic disposition, we also performed paired measurements of 11-dehydro-TXB₂ and 2,3-dinor-TXB₂ in eight patients. The two metabolites reflect independent pathways of enzymatic degra-

![Figure 3](http://circ.ahajournals.org/figures/figure3.png)

**Figure 3.** Bar graph shows effects of low-dose aspirin (50 mg/day for 7 days) on urinary 11-dehydro-thromboxane (TX)B₂ excretion in six patients with type IIa hypercholesterolemia. Mean±SD values are represented for measurements performed before and during aspirin administration. Metabolite excretion was significantly (p<0.01) lower on the third and seventh days of aspirin administration compared with pretreatment measurements.
resisting a major source of enhanced TXA\textsubscript{2} production in type IIa hypercholesterolemia. In this context, it is interesting to note that the reduction in the risk of myocardial infarction associated with the long-term use of aspirin in a large population of healthy male physicians was apparent at all levels of cholesterol, including those $\geq 260$ mg/100 ml.\textsuperscript{33} Although subgroup analysis suggests that the benefit was greatest at low levels of cholesterol, this may have reflected the small number of events in the $<159$ mg/100 ml group. Even in a trial as large as the Physicians Health Study, reliable identification of subgroups of subjects among whom treatment is particularly advantageous (or among whom it is ineffective) is unlikely to be possible.\textsuperscript{34}

**Summary**

Our studies demonstrate that TXA\textsubscript{2}-dependent platelet activation does occur in vivo in the majority of patients with type IIa hypercholesterolemia without macrovascular complications. Whether inhibition of cholesterol biosynthesis may reduce the frequency or intensity of platelet activation remains to be firmly established. Moreover, low-dose aspirin may largely suppress enhanced TXA\textsubscript{2} formation in this type of patients. We would like to suggest that TXA\textsubscript{2}-dependent platelet activation may represent a transduction mechanism linking this lipid disorder to the enhanced risk of vascular occlusive complications. This hypothesis can be tested by a controlled clinical trial of low-dose aspirin in hypercholesterolemic patients having major vascular events (i.e., myocardial infarction, stroke, and vascular death) as primary end points.

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**References**


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G Davì, M Averna, I Catalano, C Barbàgallo, A Ganci, A Notarbartolo, G Ciabattoni and C Patrono

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