Normal Levels of Fibrinopeptide A in Patients with Primary Hyperlipidemia

Hymie L. Nossel, Frank Rees Smith, Alan H. Seplowitz, Ralph B. Dell, Robert R. Sciacca, Clarence Merskey, and DeWitt S. Goodman

SUMMARY Fibrinopeptide A (FPA) levels were measured in a group of 130 controls and patients with various types of primary hyperlipidemia to investigate whether an increased steady state level of thrombin activity is present in hyperlipidemic patients. In a subset of 56 subjects, levels of clotting factors II, VII, and X were measured as well. FPA levels in hyperlipidemic patients were not significantly different from those of control subjects. Furthermore, on multiple regression analysis, no significant relationships were found between FPA levels and the concentrations of serum cholesterol or triglyceride, or log triglyceride levels. Statistically significant relationships were found between all three clotting factor levels and triglyceride concentration. The correlation coefficients for these relationships, however, were low, so that the correlations are of questionable pathophysiological significance. A weak relationship also was found between the plasma levels of cholesterol and of factor II. Thus, although small increases in various clotting factors may be found in patients with hyperlipidemia, plasma FPA levels are normal. These data indicate that hyperlipidemia is not associated with a steady state of increased thrombin activity in vivo in humans. Circ Res 45: 347-350, 1979

THE relationship between hyperlipidemia and atherosclerosis is well established. It is clear that the hemostatic system is involved in cardiovascular disease as a complication of atherosclerosis. Recently, evidence has been accumulating as well for a possible contributory role of the hemostatic system in the pathogenesis of atherosclerosis (Moore et al., 1976; Harker et al., 1976; Friedman et al., 1977). Most of this evidence has been obtained from studies on experimental animals and points to the possible involvement of platelets in the atherogenic process.

Much less experimental evidence exists for involvement of the coagulation system, as distinct from platelets, in the pathogenesis of atherosclerosis. Evidence for a possible effect of hyperlipidemia on the coagulation system has been reported by a number of investigators. Bajaj and colleagues (1976) found that very low density lipoproteins accelerated prothrombin activation in a purified system. Kim and colleagues (1976) reported short whole blood coagulation and prothrombin times as well as high coagulation factor levels and plasma triglycerides in rats with congenital hyperlipidemia as compared with hypolipidemic rats. Similar though less striking differences were found in squirrel monkeys with high and low cholesterol values. Constantino and colleagues (1977) reported an association between vitamin K-dependent coagulation factor levels and plasma lipid levels in humans, although previous studies had yielded contradictory results (Merskey and Marcus, 1963).

Recently developed hemostasis tests measure the products of in vivo reactions rather than in vitro reactivity. One study reported evidence suggesting the presence of increased intravascular coagulation in patients with hyperlipidemia (Carvalho et al., 1976). We now report the results of measurements in hyperlipidemic patients of the plasma levels of fibrinopeptide A (FPA), the initial specific cleavage product of thrombin proteolysis of fibrinogen. Plasma fibrinopeptide A levels have been found to be a sensitive index of venous thromboembolism (Yudelman et al., 1978).

Methods

One hundred and thirty volunteer subjects and patients consented to and participated in this study. All were studied as outpatients. Institutional and DHEW rules for protection of human subjects were observed. The characteristics of the control group and the patients with primary hyperlipidemia are shown in Table 1. The diagnoses of hyperlipidemia were based on an arbitrary working definition of levels of cholesterol in excess of 275 mg/dl and/or triglyceride in excess of 160 mg/dl after a 12-hour fast. Patients were classified as having hypercholesterolemia, hypertriglyceridemia, or both (mixed hyperlipidemia). Free-flowing venous blood was collected via 21-gauge needles by one of two investigators (F.R.S. and A.H.S.). Tourniquets were released promptly after venipuncture and the blood
collected into 10-ml Vacutainer tubes (Becton-Dickinson Company) without anticoagulant for lipid measurements, into 10-ml Vacutainer tubes containing 1 ml 0.15 M saline with 1000 U heparin and 1000 U TrasyloI for FPA, and into Vacutainer tubes containing one-tenth volume 3.8% trisodium citrate for factor II, VII, and X measurements. Cholesterol and triglyceride concentrations were measured in serum with a Technicon Auto Analyzer I (Technicon Instruments Corp.), using the method N-24a for cholesterol (Block et al., 1965) and a modification (Noble and Campbell 1970) of the Kessler and Lederer (1965) technique for triglycerides. Tubes containing blood for FPA and clotting factor measurements were inverted several times and then maintained at room temperature until centrifuged at 1500 g at 4°C for 20 minutes.

Plasma for FPA measurement was processed and assayed as previously described (Nossel et al., 1974). A specific radioimmunoassay technique was used that can detect plasma FPA concentrations of 0.2 pmol/ml. The coefficient of variation of the test used as determined by measuring 10 separately processed samples of a single sample of blood was 20%. Plasma for coagulation factor measurements was stored in polystyrene tubes at -60°C until assayed. Factors II, VII, and X were assayed as described elsewhere (Constantino et al., 1977). In the coagulation assays, control and hyperlipidemic plasma were compared with results from samples from a standardized pool of citrated plasma previously obtained from healthy donors and stored under similar conditions.

Subjects were divided into nonoverlapping patient groups according to the levels of serum cholesterol and triglyceride. Mean values and standard deviations were calculated for all variables within each patient group (see Table 1). A multiple regression analysis then was performed to search for differences in FPA or in clotting factor levels between these four groups. (Steel and Torrie, 1960). Regression analysis takes advantage of the continuous distribution of cholesterol and triglyceride levels in the study population, and hence is a more sensitive and powerful way to analyze for the effects of cholesterol, triglyceride, age, and sex on FPA or clotting factors levels than simply to test for differences between groups. Statistically significant group-to-group differences in FPA or in factors II, VII, and X levels, if present, would be detected as statistically significant slopes in multiple regression analysis. In the complete data analysis carried out, each of the coagulation parameters was the "y" or dependent variable, and serum levels of cholesterol, triglyceride or log triglyceride, age, and sex were the potential "x" or independent variables.

**Results**

There were 130 subjects for whom serum cholesterol, triglyceride, and FPA levels were measured (Table 1). No significant relationships between FPA levels and either serum cholesterol and triglyceride concentration or serum cholesterol and log triglyceride concentration were found by multiple regression analysis. Furthermore, age and sex were not found to be significant determinants of FPA level.

There were 56 subjects (a subset of the 130) for whom the plasma concentrations of clotting factors II, VII, and X were measured in addition to FPA, cholesterol, and triglyceride concentrations (Table 2). In this study group, a relationship was found between all three clotting factor levels and triglyceride concentration that was significant statistically ($P < 0.01$). The correlation coefficients for these relationships were: 0.47 for factor II, 0.40 for factor VII, and 0.37 for factor X. These findings indicate that, at best, triglyceride is related only weakly to clotting factor activity, as only 22% of the variation in clotting factor concentration was accounted for by triglyceride. (Note that the square of the correlation coefficient ($r^2$) indicates the extent to which the independent variable—in this case the triglyceride level—can account for the variation in the dependent variable—in this case the clotting factor levels.) The significance level increased only slightly using log TG (highest correlation coefficient 0.53). There was also a weak relationship between plasma concentration of factor II and plasma cholesterol ($P = 0.05$), but cholesterol did not affect significantly factor VII and X levels. Neither age nor sex significantly influenced any clotting factor level.

**Discussion**

The relationship between atherosclerosis, hyperlipidemia, and the hemostatic system has intrigued investigators since the rival theories of Virchow and Rokitansky (Duguid 1946). The linkage arises in the first place from the presence of lipid and fibrin

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**Table 1**  Fibrinopeptide A Levels in 130 Normal and Hyperlipidemic Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (years)</th>
<th>Cholesterol (mg/dl serum)</th>
<th>TG (mg/dl serum)</th>
<th>Log TG</th>
<th>FPA (pmol/ml plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32</td>
<td>45.4 ± 12.8</td>
<td>226 ± 33</td>
<td>124 ± 49</td>
<td>2.052 ± 0.206</td>
<td>0.65 ± 0.41</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>27</td>
<td>52.3 ± 11.7</td>
<td>335 ± 69</td>
<td>124 ± 43</td>
<td>2.061 ± 0.176</td>
<td>0.74 ± 0.49</td>
</tr>
<tr>
<td>Mixed hyperlipidemia</td>
<td>29</td>
<td>51.7 ± 11.9</td>
<td>314 ± 52</td>
<td>677 ± 723</td>
<td>2.665 ± 0.349</td>
<td>0.90 ± 0.80</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>42</td>
<td>47.1 ± 10.8</td>
<td>219 ± 33</td>
<td>296 ± 207</td>
<td>2.556 ± 0.178</td>
<td>0.70 ± 0.50</td>
</tr>
</tbody>
</table>

* Mean ± 1 SD values are presented. The abbreviations are: n = number of subjects, TG = triglyceride.
TABLE 2  Fibrinopeptide A and Factor II, VII, and X Levels in 56 Normal and Hyperlipidemic Subjects

<table>
<thead>
<tr>
<th>Cholesterol (mg/dl serum)</th>
<th>TG (mg/dl serum)</th>
<th>Factor†</th>
<th>FPA (pmol/ml plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>231 ± 38</td>
<td>129 ± 51</td>
<td>98.0 ± 14.7</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>376 ± 92</td>
<td>124 ± 43</td>
<td>108.0 ± 11.5</td>
</tr>
<tr>
<td>Mixed hyperlipidemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>300 ± 25</td>
<td>565 ± 606</td>
<td>115.1 ± 16.5</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>228 ± 27</td>
<td>455 ± 283</td>
<td>106.1 ± 14.9</td>
</tr>
</tbody>
</table>

* Mean ± 1 sd values are presented. The abbreviations are: n = number of subjects, TG = triglyceride.
† Factor II, VII, and X levels are presented as percent of normal value (the latter derived from a normal, standard pool; see Methods).

Fibrinopeptide A in Primary Hyperlipidemia

in atherosclerotic lesions. Animal experiments and epidemiological studies have established the relationship between hyperlipidemia and atherosclerosis, and pathological thrombosis as a complication of atherosclerosis seems clear. In the last few years, animal experimentation has stimulated interest in a potential role for platelets in atherogenesis. The results of these experiments include: the discovery of a platelet-derived growth factor for smooth muscle cells, following recognition of smooth muscle proliferation as an essential component of atherosclerosis (Ross et al., 1974, 1976; Ross and Vogel, 1978); the implication of platelets in the arterial complications of homocystinemia (Harker et al., 1974, 1976); the finding that thrombocytopenia prevents smooth muscle proliferation in injured rabbit aortae (Friedman et al., 1977); and the protection afforded pigs against developing raised atherosclerotic lesions by the presence of von Willebrand’s disease, in which platelet reaction with the arterial subendothelium is defective (Fuster et al., 1978). In contrast, there is little reproducible evidence of altered hemostasis in patients with either hyperlipidemia or atherosclerosis. In vitro fibrinolytic and coagulation factor activity have been studied. Conflicting data regarding fibrinolytic activity have been found in patients with coronary artery disease (Nestel, 1960; Merskey et al., 1960; Goldrick, 1961; Ogston, 1962) or hyperlipidemia (Merskey and Marcus, 1963; Rosing et al., 1973; Brakman et al., 1974.) Increased levels of the vitamin K-dependent coagulation factors (factors II, VII, IX and X) have been reported in congenitally hyperlipidemic rats and monkeys with induced hyperlipidemia (Kim et al., 1976) and in hyperlipidemic humans (Constantino et al., 1977). The tests used in these studies reflect in vitro behavior and newer tests that reflect in vivo proteolysis have also been carried out in hyperlipidemia. Thus, Carvalho and colleagues (1976) have reported evidence for increased intravascular coagulation in types II and IV hyperlipidemia.

The results of the studies presented here are consistent with the slight increases in coagulation factors in some categories of hyperlipidemia reported by Constantino et al. (1977). In particular, in the present studies hypertriglyceridemia was associated with increased levels of factor II, VII, and X. The increases, however, were not large. Moreover, the degree of association, although statistically significant, was relatively weak, with correlation coefficients varying from 0.37 to 0.47. These r values indicate that only from 14% to 22% of the variation in clotting factor concentration could be accounted for by variations in triglyceride levels. Thus, the pathophysiological significance of these relationships is unclear. The lack of a significant elevation of plasma FPA levels observed here in any group of individuals with hyperlipidemia is clearly inconsistent with the interpretation of Carvalho and colleagues (1976) that their data implicate intravascular coagulation in type II and IV hyperlipidemia. It may be mentioned that the mean FPA level in normal individuals reported in this study (0.65 pmol/ml) is similar to that reported in seven other studies made in six different laboratories (Nossel, 1976). It is by no means certain that FPA levels and abnormal fibrinogen behavior on agarose columns are equivalent measures of thrombin proteolysis of fibrinogen. It has been inferred that earlier-eluting fibrinogen derivatives represent fibrin complexed with fibrinogen (Fletcher et al., 1970), yet there is evidence that this may not be so (Chang and Bang, 1977). The degree of abnormal fibrinogen chromatographic behavior observed by Carvalho et al. (1976) was similar to that found in acute postoperative thrombosis (Fletcher et al., 1977). Mean FPA levels were elevated more than 6-fold in patients with thromboembolism when compared with a control group (Yudelman et al., 1978). FPA has a 3-minute half-life in plasma, whereas that of fibrin is many hours. In a steady state of production, the relative increases of FPA and fibrin concentration should, however, be similar. The question whether elevated plasma lipid levels could alter the elution behavior of fibrinogen on agarose columns might be raised, but the finding that clofibrate reversed the abnormal fibrinogen behavior without substantially altering lipid levels may argue against this explanation. Another possibility for discrepant findings is that the patient groups were not similar. The patients in the present study were asymptomatic and may have had less extensive atherosclerosis than those studied by Carvalho and colleagues. Whatever the reasons for the discrepant interpretations, our data show no evidence for steady
state increased thrombin proteolysis of fibrinogen in patients with hyperlipidemia. This finding calls into question the nature of the fibrin(ogen) deposits in atherosclerotic plaques. The deposits might well be fibrinogen rather than fibrin (Shainoff and Page, 1972).

Animal experiments implicate platelets rather than thrombin in atherogenesis. Nevertheless, there are many relationships between platelets and the coagulation system. Von Willebrand protein is required for platelet reaction with the subendothelium (Baumgartner, 1977), fibrinogen modifies platelet reaction with collagen (Lyman et al., 1971), and thrombin, as well as cleaving fibrinopeptides from fibrinogen, binds to platelets specifically and induces the platelet release reaction. Hence, it would be important in studies on patients with arteriosclerosis to determine whether in vivo platelet activation, as manifest by release of platelet-specific proteins, was associated with elevated FPA levels and hence probably due to thrombin, or with normal FPA levels and hence independent of thrombin.

Studies have been reported showing both a shortened platelet life-span in patients with arterial disease (Harker and Slichter, 1972), and platelet hyperresponsiveness in patients with hyperlipidemia, (Carvalho et al., 1974). These observations suggest that measurements of plasma levels of platelet-specific proteins may be of value in adding to our understanding of the pathophysiology of hyperlipidemia and atherosclerosis.

References


Normal levels of fibrinopeptide A in patients with primary hyperlipidemia.
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