Lipolytic Activity of Post-Heparin Plasma in Hyperlipemia and Hypolipemia

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The demonstration of a heparin-stimulated lipemia-clearing (lipolytic) factor in the plasma of animals and man suggests that a deficiency of this factor may lead to the production of hyperglyceridemia in atherosclerotic patients. A number of investigators have compared the lipolytic activity of plasma from atherosclerotic patients and from normal individuals after giving them small doses of heparin intravenously. Some reported lower activities in the atherosclerotic patients; others did not find a significant difference. Much of this difference may be explained by: (1) lack of a standardized method for assaying post-heparin plasma lipolytic activity (PPLA), (2) failure to recognize the influences of dietary fat and calories upon the level of the plasma enzyme activity, and (3) failure to differentiate the clinical types of hyperlipidemia.

The purposes of this study are (1) to define more accurately the relationship between PPLA and the production of hyperglyceridemia, and (2) to determine the conditions that may alter the activity levels of plasma lipolytic enzyme in man.

Methods

GROUPS STUDIED

(a) Normal Subjects

This group consisted of 30 young, healthy normolipemic subjects with plasma triglyceride concentrations lower than 180 mg/100 ml, and included 20 males and 10 females, ranging from 19 to 28 years of age. These subjects were technicians, doctors, and medical students. All had been eating a normal American diet for many weeks prior to the study.

(b) Hyperglyceridemic Patients

Twenty-four hyperglyceridemic patients, including 18 males and 6 females, ranging from 4 to 62 years of age, were divided into two groups according to their clinical manifestations and to their responses to the fat and carbohydrate contents in the diet.

(1) Twenty-two Patients Having "Mixed" Hyperglyceridemia. In these, both fat and carbohydrate calories contributed to the hyperglyceridemia. They showed variable responses to low fat diet initially but their serum triglyceride levels remained elevated on a high carbohydrate intake. It has been shown in these patients, with an expanded triglyceride pool of endogenously synthesized triglyceride, that the fractional removal rate of the absorbed food fats is decreased, with resulting accumulation in the blood stream. This "mixed" hyperglyceridemia group included the "carbohydrate-induced" hyperglyceridemia as described by Ahrens and his associates. None of the patients had diabetes mellitus. All except four were known to have either coronary or peripheral arterial disease, or both. Ten of them had tuberous xanthomata. Their turbid plasma did not separate.

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after centrifugation, into a creamy layer at the top.

(2) Fat-induced Group Consisting of Two Patients, a Four-year-old Boy and a Sixteen-year-old Girl. Both patients developed acute abdominal pain, enlargement of liver and spleen, and eruptive xanthomata at the time of hospitalization. A thick layer of fat "creamed out" on top of the turbid plasma upon centrifugation. On a low fat diet, the hyperglyceridemia improved dramatically. Plasma lipids and PPLA of the available members of the families of these two patients were also studied. No history of early onset of atherosclerosis was elicited in these two families.

(c) Patients with Low Beta-lipoprotein Concentrations and No Beta-lipoproteins

E.G., a 42-year-old male with low beta-lipoprotein concentration, acanthocytosis, steatorrhea, and neuromuscular dystrophy, and his similarly afflicted brother, two sisters, and mother, were studied. A 15-year-old girl, with no beta-lipoproteins and the full accompaniment of symptoms of this syndrome was similarly tested. She was originally studied and reported by DiGeorge and Mabry.18

(d) Patients with Hypolipemia Due to Impaired Digestion or Absorption of Fat

Five children with cystic fibrosis of the pancreas, two patients with idiopathic steatorrhea, and one patient with chronic pancreatitis and pancreatic insufficiency constituted this group with impaired intestinal fat digestion or absorption, and malnutrition.

TECHNIQUES

Six patients with "mixed" hyperglyceridemia were selected for strict dietary control and study. During the initial period of two to four weeks of observation, all patients were on a self-chosen diet, estimated by the dietitian to contain more than 100 g of fat and 2500 calories per day. The patients were then admitted to the hospital and given rice and fruit diet for two to three weeks. They were encouraged to consume enough food to maintain their original body weights. In the third period, the daily dietary fat was decreased to 40 to 50 g and the total caloric intake was reduced to 1800 to 2000 calories per day.

The first blood sample was taken from each subject for cholesterol and triglyceride determinations after 12 to 14 hours of fasting and immediately prior to the injection of 0.1 mg/kg body wt of heparin intravenously. A second blood sample was taken 9 to 10 minutes after the injection. Three to four drops of 1 mg/ml heparin solution were added to each 10 ml of blood as anticoagulant. The specimen was cooled immedi-
Two determinations of PPLA and of cholesterol and triglyceride concentrations were made on each patient in the initial period of study. The studies were again made on these patients one, two, and three weeks after the start of the rice diet; and were repeated at three to six-week intervals after they were placed on a 40 to 50-g fat, low caloric diet, for four to ten months. Similar blood lipid and PPLA studies were made on each of the two patients with fat-induced hyperglyceridemia before and after the low-fat diet treatment.

Two normolipemic medical students and two patients with "mixed" hyperglyceridemia were fasted for 60 hours to discover the effect of prolonged starvation upon their PPLA. Repeated PPLA studies were made on patient E.G. with low plasma beta-lipoprotein for more than two years.

The methods of Abell et al. and Zilversmit and Davis were used for serum cholesterol and triglyceride determinations. PPLA was assayed according to the method developed by Fredrickson et al.

Results

POST-HEPARIN PLASMA LIPOLYTIC ACTIVITIES (PPLA) OF THE SEVERAL GROUPS OF SUBJECTS STUDIED

The PPLA levels and serum cholesterol and triglyceride concentrations of 30 young healthy subjects, 24 hyperglyceridemic patients (including 2 fat-induced type and 22 "mixed" form), 2 hypo-beta-lipoproteinemic brothers, one a-beta-lipo-proteinemic girl, and 8 patients with disturbances of intestinal fat digestion and absorption are tabulated by groups in table 1 for comparison. The data indicate that while consuming a self-chosen diet with high fat intake: (1) All patients with "mixed" hyperglyceridemia were capable of elaborating levels of PPLA similar to the group of healthy subjects studied as controls. (2) Patients with fat-induced hyperglyceridemia had the highest serum triglyceride readings, and had very low PPLA levels. (3) The hypolipidemic patients all had low PPLA levels. (4) A normal PPLA value was found in each of the hyperlipidemic patients who presented clinical evidence of atherosclerosis, and all of whom were in the "mixed" hyperglyceridemia group.

EFFECT OF DIET UPON THE PPLA AND PLASMA LIPIDS OF PATIENTS WITH "MIXED" HYPERGLYCERIDEMA

Changes induced in the PPLA levels and in the plasma cholesterol and triglyceride concentrations of six patients with "mixed" hyperglyceridemia, by the use of different dietary programs, are summarized in table 2. Rice and fruit diet caused a sharp reduction of the PPLA levels for all six patients, and a moderate lowering of the plasma cholesterol and triglyceride concentrations in four of them. In the first six to eight weeks of low calorie diet containing 40 to 50 g fat per day the patients lost 6.4 to 13.1 kg body wt. The weights of these patients were then stabilized at their respective low levels throughout the four to ten months of observation. The dietary program was effective in keeping the plasma cholesterol and triglyceride concentrations of these hyperglyceridemic patients close to nor-
TABLE 2
Effect of Diet upon Plasma Post-Heparin Lipolytic Activity and Lipid Concentrations in Patients with "Mixed Type" of Hyperglyceridemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dietary intake per day</th>
<th>Post-heparin lipolytic activity</th>
<th>Total cholesterol mg/100 ml</th>
<th>Triglyceride mg/100 ml</th>
<th>Weight change from self-chosen diet period kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.A.</td>
<td>Fats</td>
<td>(0.41, 0.44)</td>
<td>304, 355</td>
<td>496, 544</td>
<td></td>
</tr>
<tr>
<td>J.C.</td>
<td>&lt; 100 g, calories</td>
<td>0.43</td>
<td>595, 780</td>
<td>910, 1,340</td>
<td></td>
</tr>
<tr>
<td>E.R.</td>
<td>ad</td>
<td>(0.32, 0.36)</td>
<td>372, 424</td>
<td>484, 580</td>
<td></td>
</tr>
<tr>
<td>R.B.</td>
<td>&lt; 2500</td>
<td>0.41</td>
<td>471, 413</td>
<td>725, 569</td>
<td></td>
</tr>
<tr>
<td>A.B.</td>
<td></td>
<td>(0.45, 0.43)</td>
<td>316, 354</td>
<td>480, 566</td>
<td></td>
</tr>
<tr>
<td>W.B.</td>
<td></td>
<td>(0.34, 0.32)</td>
<td>515, 353</td>
<td>1,225, 710</td>
<td></td>
</tr>
<tr>
<td>H.A.</td>
<td>Rice</td>
<td>0.19</td>
<td>288 - 291</td>
<td>341 - 517</td>
<td>-1.3</td>
</tr>
<tr>
<td>J.C.</td>
<td>diet</td>
<td>0.28</td>
<td>382 - 424</td>
<td>467 - 589</td>
<td>-1.5</td>
</tr>
<tr>
<td>E.R.</td>
<td>ad</td>
<td>0.20</td>
<td>283 - 343</td>
<td>298 - 384</td>
<td>-2.3</td>
</tr>
<tr>
<td>R.B.</td>
<td>lib.</td>
<td>0.18</td>
<td>355 - 453</td>
<td>443 - 547</td>
<td>+1.7</td>
</tr>
<tr>
<td>A.B.</td>
<td></td>
<td>0.30</td>
<td>301 - 361</td>
<td>400 - 640</td>
<td>+2.1</td>
</tr>
<tr>
<td>W.B.</td>
<td></td>
<td>0.19</td>
<td>322 - 360</td>
<td>498 - 643</td>
<td>-2.3</td>
</tr>
<tr>
<td>H.A.</td>
<td>40-50 g</td>
<td>0.28</td>
<td>152 - 204</td>
<td>119 - 185</td>
<td>-10.9</td>
</tr>
<tr>
<td>J.R.</td>
<td>fat,</td>
<td>0.33</td>
<td>158 - 254</td>
<td>83 - 158</td>
<td>-13.1</td>
</tr>
<tr>
<td>E.R.</td>
<td>calories</td>
<td>0.25</td>
<td>184 - 249</td>
<td>80 - 209</td>
<td>-8.2</td>
</tr>
<tr>
<td>R.B.</td>
<td>1800-2000</td>
<td>0.24</td>
<td>173 - 265</td>
<td>81 - 122</td>
<td>-7.3</td>
</tr>
<tr>
<td>A.B.</td>
<td></td>
<td>0.33</td>
<td>200 - 267</td>
<td>170 - 198</td>
<td>-6.4</td>
</tr>
<tr>
<td>W.B.</td>
<td></td>
<td>0.24</td>
<td>220 - 253</td>
<td>100 - 163</td>
<td>-7.3</td>
</tr>
</tbody>
</table>

*Mean value.
†Range of values observed.

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mal ranges, and was effective also in maintaining their PPLA at relatively low levels. The effects of dietary fat and caloric intake upon the PPLA levels of these six patients are presented graphically in figure 1.

Repeated PPLA determinations were made on two healthy normolipemic subjects on self-selected diets during the course of six and eight weeks respectively. Only minor fluctuations in their respective plasma enzyme levels were observed, confirming the experience of Fredrickson et al.16 that the biological and technical variations of test results are small. Two medical students and two "mixed" type hyperglyceridemic patients were fasted for 60 hours, and their PPLA levels were checked at 12 to 24 hourly intervals. Their plasma enzyme activities did not change significantly during the period of fast.

PPLA OF PATIENTS WITH FAT-INDUCED HYPERGLYCEMERIDEMIA

Table 3 shows the plasma lipid concentrations and PPLA of two patients and some members of their families. These patients presented acute abdominal pain, eruptive xanthomata, and milky plasma. A thick layer of fat "creamed out" at the top of the cloudy plasma following centrifugation of the blood specimen. G. F. responded rapidly to a diet containing less than 10 g of fat per day which was isocaloric to her self-selected diet. Her hyperglyceridemia cleared completely in three weeks. The 4-year-old child, D. C., also responded well to a less rigidly controlled low-fat diet. The PPLA values of both patients were low both before and after the use of low-fat diets. As shown in table 3, all the immediate members of the boy's (D. C.) family have normal plasma lipid concentrations, but generally low PPLA levels. Only two members of the G. F. family were studied. One brother of patient G. F. had normal blood lipids and a low PPLA value.

PPLA OF PATIENTS WITH LOW OR NO BETA-LIPOPROTEINS

The results obtained from members of the G. family with low beta-lipoproteins are tabulated in table 4. PPLA values of patient E. G., of his brother F. G., and one of his two sisters, were low. E. G. had severe steatorrhea and his PPLA reading was the lowest among members of the G. family. The 15-year-old girl, C. R., with no beta-lipoproteins, acanthocytosis, neuromuscular disturbance, and severely impaired intestinal fat absorption, also had a very low PPLA value.

Repeated PPLA determinations were made on patient E. G. for 26 months. The graph in

TABLE 3

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Cholesterol (mg/100 ml)</th>
<th>Triglyceride (mg/100 ml)</th>
<th>Lipolytic activity (μeq FFA/min/ml*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. C., male</td>
<td>4</td>
<td>490</td>
<td>6,800</td>
<td>0.11†</td>
</tr>
<tr>
<td>Low-fat diet</td>
<td></td>
<td>308</td>
<td>204</td>
<td>0.16</td>
</tr>
<tr>
<td>Low-fat diet</td>
<td></td>
<td>243</td>
<td>166</td>
<td>0.15</td>
</tr>
<tr>
<td>W. C., father</td>
<td>37</td>
<td>178</td>
<td>61</td>
<td>0.33</td>
</tr>
<tr>
<td>F. C., mother</td>
<td>36</td>
<td>196</td>
<td>121</td>
<td>0.22</td>
</tr>
<tr>
<td>G. C., sister</td>
<td>7</td>
<td>139</td>
<td>44</td>
<td>0.20</td>
</tr>
<tr>
<td>D. C., twin sister</td>
<td>4</td>
<td>149</td>
<td>56</td>
<td>0.20</td>
</tr>
<tr>
<td>G. F., female</td>
<td>16</td>
<td>265</td>
<td>2,475</td>
<td>0.09, 0.10</td>
</tr>
<tr>
<td>Low-fat diet</td>
<td></td>
<td>144</td>
<td>80</td>
<td>0.09</td>
</tr>
<tr>
<td>L. F., brother</td>
<td>38</td>
<td>173</td>
<td>73</td>
<td>0.21</td>
</tr>
<tr>
<td>J. F., sister</td>
<td>20</td>
<td>148</td>
<td>68</td>
<td>0.38</td>
</tr>
</tbody>
</table>

*Lipolytic activity is expressed in μeq of free fatty acid (FFA) released per min/ml of plasma.
†Enzyme activities less than the lower limit of our normal range are underlined.
TABLE 4

Post-Heparin Plasma Lipolytic Activities and Lipid Levels in Family with Low and No Beta-Lipoproteinemia

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>Lipolytic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. family</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.G., mother</td>
<td>72</td>
<td>230</td>
<td>99</td>
<td>0.37</td>
</tr>
<tr>
<td>E.G., patient</td>
<td>42</td>
<td>113</td>
<td>35</td>
<td>0.12*</td>
</tr>
<tr>
<td>F.G., brother</td>
<td>47</td>
<td>149</td>
<td>69</td>
<td>0.18</td>
</tr>
<tr>
<td>R.H., sister</td>
<td>44</td>
<td>157</td>
<td>72</td>
<td>0.35</td>
</tr>
<tr>
<td>B.H., sister</td>
<td>38</td>
<td>145</td>
<td>61</td>
<td>0.24</td>
</tr>
<tr>
<td>C.R., female</td>
<td>15</td>
<td>33</td>
<td>15</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* Lipolytic activity is expressed in μeq of free fatty acid (FFA) released per min/ml of plasma.

† Enzyme activities less than our lower normal range of 0.28 unit are underlined.

Figure 2 shows a slow rise of his PPLA when his steatorrhea was controlled by gluten-free diet and when his general nutrition improved. This patient did not improve further during a period of mental depression. After recovering from the mental disturbance, he gained more weight and his PPLA increased to the lower limits of the normal range.

PPLA OF PATIENTS WITH DISTURBANCES OF INTESTINAL FAT DIGESTION OR ABSORPTION

Moderately depressed PPLA levels ranging from 0.18 to 0.27 units (table 1) were observed in eight patients with disturbances of fat absorption. This group included five patients with cystic fibrosis of the pancreas, two patients with idiopathic steatorrhea, and one patient with chronic pancreatitis and pancreatic insufficiency. The degree of PPLA depression seen in these patients was generally related to the severity of the steatorrhea from which they suffered.

Discussion

The differentiation of primary hyperlipidemias into fairly distinct types by their clinical and laboratory characteristics and by the development of a standardized PPLA assay have facilitated the interpretation of PPLA studies made on patients with elevated plasma lipid values. It is difficult to exclude individuals with latent atherosclerosis from the normal control group. It is difficult, too, to maintain every person in this group on exactly the same diet, in order to permit a fair comparison of their PPLA with those of the diseased groups. For these reasons, repeated PPLA determinations were made on several hyperlipidemic and hypolipidemic patients over long periods to ascertain the effects of fat and calories upon their PPLA levels.

Two main types of PPLA response were demonstrated in hyperglyceridemic patients. (1) On a self-selected diet, estimated to contain over 100 g of fat per day, patients with "mixed" type of hyperglyceridemia were found to have normal PPLA. Consistent with the current belief that these hyperlipidemic patients are prone to develop arterial disease, most of the patients in this group showed clinical evidence of either coronary or peripheral arterial disease or both. (2) In contrast to these patients, low PPLA levels were observed in two fat-induced hyperglyceridemic patients and in several of their normolipemic family members. Such patients undoubtedly belong to the type of hyperglyceridemia with decreased activity of plasma "lipoprotein lipase" reported by Havel and Gordon, Ahrens et al., Fredrickson et al., and Furman and Robinson. A recent review of familial hyperglyceridemia by Fredrickson revealed that in none of the 43 cases with this form of hyperglyceridemia was clinical evidence of vascular disease found.
In a recent report, Yoshitshi et al.\textsuperscript{24} stated that the peak PPLA values, observed by them in a small number of patients with coronary artery disease following heparin injection, were the same as those of normal controls, but that the rate of PPLA "inactivation" or disappearance from the blood was significantly faster in the diseased than in the healthy subjects. These observations cannot be compared directly with the present study because these authors did not report concentrations of blood lipids for their patients. In the present study only peak PPLA levels were determined. In developing the PPLA assay Fredrickson et al.\textsuperscript{16} found that in human beings, their PPLA rose to peak values 9 to 10 minutes after intravenous heparin injection.

Although numerous investigators have presented evidence that implicates the lipoprotein lipase system as the major pathway for the clearance of alimentary lipemia,\textsuperscript{25-28} yet the exact role of PPLA in the production of clinical hyperglyceridemia still remains uncertain. It has been shown that: (a) Hyperglyceridemia may be maintained in many patients chiefly by active endogenous lipogenesis from nonfat calories.\textsuperscript{11, 16, 17} (b) Some apparently healthy individuals in the families of patients with fat-induced hyperglyceridemia may have low PPLA values but do not develop hyperglyceridemia. (c) Successful control of the hyperlipidemia in hyperglyceridemic and atherosclerotic patients by diet was not accompanied by an increase but actually by a decrease of their PPLA levels. These findings do not support the view that a deficiency of PPLA is the basic defect in the production of the common "mixed" and carbohydrate-induced types of hyperglyceridemia.

The most important finding in this study is perhaps the demonstration that reduced PPLA levels can be maintained in hyperglyceridemic and atherosclerotic patients by moderate restrictions of dietary fats and of total calories. A reduced absorption or utilization of fats and other food calories probably accounts for the suppression of PPLA levels in patients with malabsorption syndrome,\textsuperscript{29} in patients with deficient beta-lipoprotein formation, and in those with severe grades of dietary fat restriction.\textsuperscript{10, 30}

The lipoprotein lipase is believed to be located on the wall of the blood vessels before

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Post-heparin plasma lipolytic activity of hypo-beta-lipoproteinemic patient, E. G. Enzyme activity is expressed in \(\mu\)eq of free fatty acid released per min/ml plasma. Studies were made during periods of severe steatorrhea, during control of steatorrhea with six-pound gain of weight, and during another eight-pound gain of weight in the last period.}
\end{figure}
its release into the blood after heparin injection.\textsuperscript{81} At this site the enzyme hydrolyzes triglyceride into monoglyceride, diglyceride, and free fatty acid. These products of lipolysis may function in the presence of plasma albumin to “disperse” particulate fat and lipoproteins in a manner analogous to the preparation of lipids for absorption in the intestinal tract, and so may facilitate the transport of plasma lipids into body tissues which may include the arterial wall itself. Thus it is reasonable to speculate that, in conditions with low PPLA, the passage of plasma cholesterol and other lipids into the arterial wall may be significantly decreased. This diminished entry of lipids into the arterial wall may well lessen the likelihood and the rate of atheroma formation, even though the plasma lipid concentration is high, as in fat-induced hyperglyceridemia. The importance of the physical state of the particulate fat in the plasma in contrast to its actual concentration in atherogenesis is beginning to attract the attention of investigators in the field of lipid metabolism.\textsuperscript{82-85}

**Summary**

1. The role of post-heparin plasma lipolytic activity (PPLA) in the production of hyperglyceridemia was investigated in patients with high concentrations of plasma triglycerides produced in two ways, viz. fat-induced and “mixed” (both fat and carbohydrate-induced). While on a self-chosen diet, the PPLA levels of patients with “mixed” type of hyperglyceridemia differed little from those of young healthy subjects. PPLA levels of these patients were promptly lowered by rice diet, despite the persistence of hyperglyceridemia. Normal serum lipid concentrations and low PPLA levels were induced and maintained in these patients by reducing moderately their intake of both dietary fat and calories.

2. PPLA levels were reduced in the fat-induced hyperglyceridemic patients both before and after the use of low fat diet to reduce their plasma triglyceride concentrations.

3. PPLA was also low in patients with low and absent beta-lipoproteins, and in those with disturbance of intestinal fat digestion and absorption.

**Acknowledgment**

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