Studies of Fat Lipolysis by Post-Heparin Human Plasma Lipoprotein Lipase and by Human Pancreatic Lipase

By H. Engelberg, M.D.

Many studies indicate that unsaturated fats may lower serum cholesterol whereas saturated fats of animal origin usually increase it. The mechanism of these differing actions is unexplained, however. It has also been demonstrated that alimentary lipemia is cleared largely by an enzymatic lipolytic mechanism in which heparin is involved (heparin lipoprotein lipase). In this paper differences in hydrolysis of saturated and unsaturated fats by the heparin lipolytic factor are studied.

Since the original suggestion in 1941 there have been many studies recently summarized, which indicate that relatively unsaturated oils of vegetable or marine origin may lower the serum cholesterol and low-density lipoproteins, whereas saturated animal fats usually increase the lipid values. This fact has aroused widespread interest because of its implications in the prevention of atherosclerotic disease. However, the metabolic factors underlying this differential effect of saturated and unsaturated fats are not well understood. It might be anticipated that fatty acid molecules varying in the number of hydrogen electrons attached to carbon would not behave identically in many biochemical interactions, some of which could profoundly affect serum lipid levels.

The problem has been investigated from various aspects. Both saturated and unsaturated fats are well absorbed in the human intestine. The absorption of dietary cholesterol is augmented by unsaturated fats as compared to saturated ones. Thus it is apparent that the latter do not elevate plasma cholesterol by enhancing its absorption in the intestine. No difference has been found between the 2 types of fat in their effectiveness in depressing the ability of the liver to convert acetate carbon to fatty acids. There has been disagreement among investigators who have studied the effect of various dietary fats on cholesterol synthesis in rat liver. Similar amounts of choline were required to prevent fatty livers when various fats were fed. It has been stated that the ease of or resistance to hydrolysis of the cholesterol esters of saturated and unsaturated fats varies and that this, in some manner affects lipid transport. Recently it was found that cholesterol-induced deposition of lipid in tissue cultures of human aorta cells is inhibited by linolenic acid and enhanced by stearic acid. These results, however, do not explain the action of saturated fats in elevating circulating cholesterol levels. Furthermore, analysis of atheromatous plaques did not indicate any preferential deposition of saturated fats or of the cholesterol esters of saturated fats, although the linoleic acid content of the cholesterol esters was lower and the oleic acid level in the plaques higher than in normal sera. The authors interpreted their data as support for the concept that plaques are formed by filtration and indiscriminate deposition of blood lipids in the arterial wall. No evidence was found of any difference in the take-up of saturated or unsaturated fats by rat mesenteric adipose tissue. In contrast to the previously cited studies, which do not explain the hypercholesterolemic effect of the saturated fats, recent investigations have shown an increased fecal excretion of bile acids or radioactive material fed as C14 cholesterol coincident with the feeding of vegetable oils. Further studies along these lines in rat and man have clearly shown that the substitution of unsaturated for saturated fats results in an increased bile acid secretion in the bile and a fall in plasma cholesterol, indicating an accelerated conversion of cholesterol to bile acids in the liver.
FAT LIPOLYSIS

Before this mechanism of action can be accepted as primarily responsible for the difference between the effect of animal and vegetable fats upon serum cholesterol, however, it should be realized that the level of cholesterol and bile acids in the bile may be secondary to other processes involved in fat metabolism and transport.

We have approached the problem of possible pathways whereby saturated and unsaturated fats might differ in their impact upon serum lipids by investigating their relation to the heparin lipemia-clearing system. A large body of evidence has been accumulating which indicates that alimentary lipemia is predominantly removed from the blood by an enzymatic lipolytic mechanism in which heparin plays a role. The neutral fat of the chylomicra and the larger low-density or β-lipoproteins is split into fatty acids and glycerol, the fatty acids are bound to albumin, and then they are rapidly transferred from the blood to the tissues. It has been shown that albumin binds both oleic and stearic acid adequately.24 It thus seems most unlikely that albumin would be a limiting factor in this process except when it is markedly reduced, as in nephrosis. No difference has been found in the release of heparin into the blood, or in the production of lipemia-clearing factor following the oral ingestion of animal or vegetable fats.25 Thus the 2 types of fat apparently affect this enzymatic mechanism to the same degree. However, the possibility existed that lipoproteins that contained predominantly saturated or unsaturated fats might differ in their rates of hydrolysis by the heparin lipolytic factor (lipoprotein lipase). It is known that fat substrates may vary in their susceptibility to lipolysis by the same enzyme.26, 27 The experimental results presented in this paper are the preliminary findings relative to this subject.

METHODS

Clearing, or decrease in optical density, of fat substrates incubated in vitro was not studied, since this method of investigation may be deceptive. It does not always parallel lipolysis, the formation of calcium soaps may obscure optical density changes, and nonlytic clearing occasionally occurs. Lipolysis was not followed by glycerol measurements, since partial triglyceride splitting

<table>
<thead>
<tr>
<th>Incubation time at 37 C. (min.)</th>
<th>6 ml. Post-heparin plasma</th>
<th>7 ml. Post-heparin plasma</th>
<th>3 ml. Post-heparin plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.4</td>
<td>2.0</td>
<td>.8</td>
</tr>
<tr>
<td>10</td>
<td>1.9</td>
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<td>1.7</td>
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<td>2.4</td>
<td>2.0</td>
<td>1.9</td>
</tr>
<tr>
<td>30</td>
<td>2.3</td>
<td>2.1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Maximum rate of lipolysis in mEq./L./hr.

| 3.0 | 2 | 5.4 | 3.0 | .2 | .6 | 2.4 | 1.3 | .4 | .5 |

TABLE 2.—Rate of in Vitro Lipolysis of Cream and Cottonseed Oil Emulsions by Fasting Post-Heparin Plasma in Eleven Subjects. Incubation at 37 C.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Maximum rate of free fatty acid release in mEq./L./hour fasting post-heparin plasma 2-4 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 ml. 5% Cream emulsion</td>
<td>.6</td>
</tr>
<tr>
<td>2 ml. 5% Cott. oil emulsion</td>
<td>3.0</td>
</tr>
</tbody>
</table>
TABLE 3.—Release of Unesterified Fatty Acid Upon Incubation of Citrate Eluates of Ca\textsubscript{3}(P\textsubscript{4}O\textsubscript{7})\textsubscript{2} Adsorbates of Post-Heparin Plasma with Ultra centrifugally Separated Lipoproteins and Activated Lipoproteins of Animal and Vegetable Fat Origin

<table>
<thead>
<tr>
<th>Incubation time at 37° C. (min.)</th>
<th>2 ml. Citrate eluate</th>
<th>2 ml. Citrate eluate</th>
<th>1 ml. Citrate eluate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+3 ml. 1% CaCl\textsubscript{2}</td>
<td>+3 ml. 1% CaCl\textsubscript{2}</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.2 3.5</td>
<td>0.9 1.0</td>
<td>1.2 1.1</td>
</tr>
<tr>
<td>5</td>
<td>3.0 3.3</td>
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<td>1.2 1.1</td>
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<tr>
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<td>4.2 3.4</td>
<td>1.3 1.1</td>
<td>1.4 1.1</td>
</tr>
<tr>
<td>30</td>
<td>4.7 3.9</td>
<td>1.2 1.2</td>
<td>1.5 1.4</td>
</tr>
</tbody>
</table>

Maximum rate of lipolysis in mEq./L./hr.

<table>
<thead>
<tr>
<th>2 ml. Citrate eluate</th>
<th>.2 ml. Bovine albumin</th>
<th>1 ml. Citrate eluate</th>
<th>+3 ml. 1% CaCl\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.4 1.8</td>
<td>3.2 2.7</td>
<td>2.9</td>
</tr>
<tr>
<td>10</td>
<td>1.8 1.7</td>
<td>3.4 2.6</td>
<td>3.0</td>
</tr>
<tr>
<td>20</td>
<td>1.9 1.9</td>
<td>3.4 2.7</td>
<td>3.0</td>
</tr>
<tr>
<td>30</td>
<td>2.0 2.1</td>
<td>3.6 2.8</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Maximum rate of lipolysis in mEq./L./hr.

can take place without the production of free glycerol. Accordingly the rate of lipolysis was studied by determining the release of unesterified fatty acids. In general the plan of investigation involved the incubation in vitro at 37° C. of aliquots of plasma plus the various fat substrates. One-milliliter samples were removed at stated time intervals and analyzed (usually in duplicate) for free fatty acids by Borgstrom’s method.\textsuperscript{28} The maximal release of the latter in mEq./L./hour was then calculated from the maximal rate observed in any time period, since this is a more accurate measure of enzyme activity than the total release of unesterified fatty acids. This is particularly true in this in vitro test system in which the end-products of the reaction, the unesterified fatty acids, are not removed and so inhibit further lipolysis.\textsuperscript{29} Furthermore, it has been shown, with use of post-heparin plasma, that the concentration of lipemia-clearing factor is best determined by observations of the rate of clearing rather than by the absolute amount of optical density decrease in a fixed time period.\textsuperscript{30, 31}

Since the lipolysis of various fats by the same enzyme was to be measured in all experiments, it was essential to have little or no neutral fat present in the plasma other than the various substrates that were added in vitro. Therefore, the sources of lipoprotein lipase used were testing post-heparin plasma (10 to 25 mg. intravenously), and citrate eluates of tricalcium phosphate adsorbates of post-heparin nonfasting plasma.\textsuperscript{32} Human pancreatic lipase was obtained from fresh autopsy material.*

Various types of fat substrates were used. When fasting post-heparin plasma was used as the enzyme source, 2 to 4 ml. of plasma were mixed with 2 ml. of 5 per cent emulsion in normal saline of the fats to be tested, thus providing adequate and equal amounts of fat substrate. Cream was used as the source of animal fat, since in this form it is well emulsified. It was bought in the market on the day it was to be tested as 35 per cent whipping cream, and diluted with saline to a 5 per cent solution. Commercial cottonseed oil was the usual vegetable fat used and it was prepared daily as a 5 per cent emulsion by the method of Tauber.\textsuperscript{33} Although more stable emulsions resulted from the use of this method, it was our observation that the cream emulsions were superior to any of the vegetable fat emulsions. The plasma was then incubated with the fat emulsions. In this preparation lipoproteins were formed in the tube, and adequate fatty acid acceptor (albumin) and the necessary ions were in the plasma, so that all conditions were present for lipolysis to proceed. When pancreatic lipase was used, 1 per cent calcium chloride was added to provide fatty acid acceptor, and the various neutral fat emulsions themselves were excellent neutral fat substrates. In the experiments with citrate eluates of post-heparin plasma, preformed lipoproteins had to be used as fat substrate, since there was no opportunity for lipoprotein formation in the tube such as existed when plasma itself was directly used as the enzyme source. These activated lipoproteins were prepared in vitro\textsuperscript{34} by prior incubation at 37° C. for 4 to 12 hours of cream and vegetable oil emulsions with equal volumes of fasting serum, or of infranatant serum obtained after ultracentrifugal removal of low-density lipo-

*Supplied through the cooperation of Dr. N. Friedman, and extracted by the Southern California Gland Company.
### Table 4.—Release of Unesterified Fatty Acids upon Incubation of Two Lots of Human Pancreatic Lipase with Various Fat Substrates. Values in mEq./L.

<table>
<thead>
<tr>
<th>Incubation time at 37 C. (Min.)</th>
<th>5 ml. 6%Cream emulsion</th>
<th>5 ml. 5%Cotton oil emulsion</th>
<th>5 ml. 5%Olive oil emulsion</th>
<th>5 ml. 5%Coconut oil emulsion</th>
<th>5 ml. 5%Peanut oil emulsion</th>
<th>5 ml. 5%Corn oil emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>1.5</td>
<td>1.5</td>
<td>1.6</td>
<td>1.4</td>
<td>2.4</td>
<td>1.9</td>
</tr>
<tr>
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<td>2.0</td>
<td>2.7</td>
<td>2.2</td>
<td>3.0</td>
<td>3.4</td>
</tr>
<tr>
<td>20</td>
<td>1.9</td>
<td>2.5</td>
<td>3.2</td>
<td>2.6</td>
<td>3.6</td>
<td>4.0</td>
</tr>
<tr>
<td>30</td>
<td>2.2</td>
<td>3.0</td>
<td>3.2</td>
<td>3.2</td>
<td>3.8</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Maximum rate of lipolysis in mEq./L./hr.

1.4 3.0 6.6 4.8 3.6 9.0 7.4 13.2 7.2 7.8 9.0 10.8

proteins. When used as fat substrate the "activated" lipoproteins prepared in vitro were not separated from the fasting serum with which they had been incubated. In addition low-density lipoproteins with differing neutral fat composition were separated ultracentrifugally* from serum samples obtained from the same subject after the ingestion of cream and safflower oil emulsion on different days. In the citrate eluate studies either bovine albumin or 1 per cent calcium chloride was added as fatty acid acceptor. In several experiments the in vitro activated lipoproteins were also used as fat substrate with post-heparin plasma or pancreatic lipase. The details of all experiments are found in the tables.

**Results**

The findings with aliquots of fasting post-heparin plasma samples from 3 individuals plus various lipoprotein substrates are shown in table 1. In all 3 instances the rate of lipolysis of the unsaturated triglyceride lipoproteins was more rapid than that of the saturated (cream) lipoproteins. The method of calculation of the maximum rate of lipolysis is apparent from the data. At this time no comment will be made about comparisons in the rates of lipolysis of the various unsaturated vegetable oils as other experiments have not always shown similar results. However, the findings of lesser degrees of lipolysis with cream lipoproteins have been consistent. As shown in table 2, in 9 of 11 studies with aliquots of fasting post-heparin plasma plus

*Both the infranatant serum and the ultracentrifugally separated cream and safflower oil lipoproteins were obtained through the cooperation of Mr. D. Specter of the Institute of Medical Physics, Belmont, Calif.

cream or cottonseed oil emulsions, the rate triglyceride splitting was faster when the unsaturated fat was the substrate. The results obtained with aliquots of citrate eluates of tricalcium phosphate adsorbates of nonfasting post-heparin plasma are outlined in table 3. In each of the 5 experiments the release of unesterified fatty acid was more rapid when the lipoprotein substrate was of vegetable oil origin. We have observed that when lipoproteins, either those prepared in vitro or in vivo, are over 1 week old, they may become unstable.

Tables 4 and 5 present the results of studies with human pancreatic lipase. In table 4 the fat substrates were neutral fat emulsions. The rate of hydrolysis of cream was lower than that of the vegetable oils with both batches of lipase. However, the results with the various oils as compared to one another were not consistent. As shown in table 5, with lipoprotein substrates and a larger amount of lipase, once again the saturated animal fat was split more slowly than the unsaturated vegetable fat.

Toward the conclusion of these investigations a serious complicating factor was recognized, which perhaps should have been anticipated, namely, the occasional activation of lipases contained in the fats themselves upon the addition of fatty acid acceptors. Also, rarely, there was a sudden release of fairly large amounts of free fatty acid that apparently represented a nonenzymatic disruption of triglyceride. Both these sources of error occurred much more frequently with cream
than with cottonseed oil. Table 6 shows several examples of the action of endogenous lipases in the fats. It may be that the results in patients B.M. and L.F. in table 2 were not entirely due to lipoprotein lipase activity. It is improbable that when a fat emulsion is added to plasma in vitro, the entire neutral fat content becomes incorporated into lipoproteins in a few minutes. Furthermore lipoproteins themselves may be degraded nonenzymatically.35

**DISCUSSION**

A large amount of evidence has accumulated which indicates that the heparin-lipoprotein lipase enzyme system is probably the major normally functioning pathway for the removal of alimentary neutral fat from the blood. Thus any major distinction in the activation of this mechanism by the various types of fat, or in the efficiency of lipolysis of the fats by the enzyme, would be of importance in determining the effect of the ingested fat upon serum lipid levels. In previous studies36 no difference was found in the stimulation of heparin production or of lipemia-clearing activity by animal and vegetable fats. The present data indicate that the lipolysis of unsaturated tri-glyceride lipoproteins by lipoprotein lipase is more rapid than that of the saturated variety. Since these experiments were performed in vitro, variations in the rate of absorption, in tissue takeup of fatty acids, in liver function in relation to fats and cholesterol, in bile acid or cholesterol excretion, in reticuloendothelial phagocytosis of lipid particles, played no part in the results.

The similar findings with use of human pancreatic lipase fortify the conclusion that triglycerides composed of fatty acids of essentially the same chain length, but of different degrees of unsaturation, vary in their susceptibility to lipolysis by fat-splitting enzymes. Previous workers have found that rat pancreatic lipase is more active on saturated fats than on those with a high degree of unsaturation.36 37 On the other hand, with pork pancreatic lipase, the number of unsaturated bonds (0 to 2) did not affect the speed of hydrolysis.38 Apparently species differences in pancreatic lipase selectivity do exist. This question has not been investigated in animals with use of the post-heparin enzyme.

It may be argued that the various lipoprotein substrates used in these experiments were unnatural. Thus ultracentrifugally separated low-density lipoproteins may have been altered in some way by the involved procedure. The “activated” lipoproteins were prepared in vitro, as were the lipoproteins that formed when the fat emulsions were added to plasma.

There is little reason, however, to believe that these substrates are substantially different in their differential susceptibility to hydrolysis from their circulating counterparts. Also the efficiency of the emulsifica-

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**TABLE 5.—Unesterified Fatty Acid Release upon Incubation of Human Pancreatic Lipase with Cream and Vegetable Oil Lipoproteins, Values in mEq./L.**

<table>
<thead>
<tr>
<th>Incubation time at 37 C. (min.)</th>
<th>2 ml. Pancreatic lipase</th>
<th>Incubation time at 37 C. (min.)</th>
<th>2 ml. Pancreatic lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 ml. 1% CaCl₂</td>
<td></td>
<td>3 ml. 1% CaCl₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 ml. oil lipoprot.</td>
<td>1 ml. oil lipoprot.</td>
</tr>
<tr>
<td>0</td>
<td>1.9</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>2.2</td>
<td>2.1</td>
<td>1.0</td>
</tr>
<tr>
<td>15</td>
<td>5.1</td>
<td>2.6</td>
<td>2.0</td>
</tr>
<tr>
<td>30</td>
<td>17.7</td>
<td>4.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Maximum rate of lipolysis in mEq./L./hr. 31.6 4.0 6.6 4.8

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**TABLE 6.—Release of Unesterified Fatty Acid upon Incubation of Cream and Cottonseed Oil Emulsions Plus Fatty Acid Acceptor**

<table>
<thead>
<tr>
<th>Incubation time at 37 C. (min.)</th>
<th>4 ml. Cottonseed oil emulsion + 1 ml. bov. alb.</th>
<th>4 ml. Cottonseed oil emulsion + 1 ml. human albumin</th>
<th>5 ml. Cottonseed oil emulsion + 1 ml. 1% CaCl₂</th>
<th>5 ml. Cottonseed oil emulsion + 1 ml. 1% CaCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.8</td>
<td>.6</td>
<td>.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Maximum rate of lipolysis in mEq./L./hr. 1.8 .6 .6 0
FAT LIPOLYSIS

The rate of lipolysis of vegetable fat lipoproteins by human post-heparin lipemia-clearing factor was more rapid than that of animal fat lipoproteins in nearly all experiments.

Similar results were obtained with human pancreatic lipase as the fat-splitting enzyme.

The more efficient activity of heparin lipoprotein lipase upon unsaturated fat substrates may account for the hypercholesterolemic and hyperlipoproteinemic effect of animal (saturated) fats in man.

SUMMARY IN INTERLINGUA

Le intensitate del lipolysy de lipoproteinas de grassia vegetal per human factor de clearance de lipemia post uso de heparina esseva plus grande que le intensitate del lipolysy de lipoproteinas de grassia animal in quasi omne le experimentos del presente studio.

Simile resultatos eseva obtenite con le uso de human lipase pancreatic como enzyma lipolytic.

Le plus efficace activitate de lipase de lipoproteina post heparina in substratos de grassia nonsaturate explica possibilemente le effecto hypercholesterolemic e hyperlipoproteinemic de grassias animal (saturate) in humanos.

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H. ENGELBERG

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