Intramural and Pericapillary Distribution of Lipids in Gingival Tissue of Patients with Carbohydrate-Induced Hyperglyceridemia

By John L. Cornog, Jr., M.D., William T. Fitts, Jr., M.D., and Peter T. Kuo, M.D.

SUMMARY

Gingival biopsy was performed on 16 patients with carbohydrate-induced hyperglyceridemia, in the attempt to discover histological abnormalities in the capillaries. For comparison, similar studies were also made on four patients with essential familial hypercholesteremia, on five whose hyperglyceridemia had been kept under control by a low carbohydrate diet for 6 to 26 months, and on eight healthy, normolipemic subjects. Variable amounts of Oil Red O-stainable lipid material were demonstrated both in and around the capillary wall in 13 of the 16 untreated hyperglyceridemic patients. Little or no such lipid-staining material was demonstrated in the biopsy material obtained from the remaining groups of subjects so studied. Microangiopathy of the pre-diabetic and diabetic type characterized by capillary basement membrane thickening was not demonstrated in any of the hyperlipemic patients in this series. Evidence is presented to support the concept that a sustained chylomicronemia is the primary factor in the production of the intramural and pericapillary collection of Oil Red O-staining lipids in these hyperglyceridemic patients.

Additional Indexing Words:
Microangiopathy  Thickening of capillary basement membrane  Metabolic changes  Chylomicronemia  Electrophoresis

This study was undertaken (1) to determine the possible presence of microangiopathy similar to the type described in patients with either pre-diabetes or clinically established diabetes mellitus,1–3 and (2) to study the distribution of very low-density lipoproteins4 or “particulate fat”5,6 in the gingival capillaries of patients with carbohydrate-induced hyperglyceridemia (types III, IV, and V hyperlipoproteinemia). Although we have failed to demonstrate a significant thickening of the basement membrane of gingival capillaries, application of Oil Red O stain to the biopsied material obtained from these patients has shown increases in stainable lipids both in and around the walls of capillaries as compared with the amounts found in normal controls. The data suggest that the intramural and pericapillary collections of lipids are dependent upon the presence of a severe degree of hyperglyceridemia, associated with a high concentration of chylomicrons or “primary” fat particles.5,6
Methods

Gingival specimens were obtained for comparative microscopic study of 16 patients with carbohydrate-induced hyperglyceridemias (types III, IV, and V hyperlipoproteinemia) prior to the initiation of any therapeutic program, four patients with type II disease, five with carbohydrate-induced hyperglyceridemias whose hyperlipidemic state had been kept under satisfactory control for 6 to 26 months by the use of a sugar-restrictive low-carbohydrate diet, and eight young, healthy, normolipemic college students who served as controls. One additional patient, a 22-year-old man (D. G.) with severe hyperlipidemia, eruptive xanthomata, obesity, and symptoms suggestive of coronary artery disease, was admitted to the Clinical Research Center of the hospital for controlled metabolic and serial gum biopsy studies. Gingival specimens were taken: (1) soon after his first admission, while he was on an ad libitum diet with the high fat content of the average American diet, (2) at the end of a 3 to 4-week period of fat-free diet, in which the original daily caloric intake was rigidly maintained to prevent loss of weight, (3) after returning the patient to an ad libitum diet for about 4 weeks, and (4) at the end of a second 3 to 4-week period of fat-free (>10 g fat/day) diet.

The gum was chosen as the site for biopsy because of the vascularity of this tissue, ease and convenience of surgery, minimal discomfort to the subject, and the previous demonstration of diabetic microangiopathy in this location. In addition to the conventional hematoxylin and eosin stain, tissues from most of the biopsy specimens were also stained with the PAS stain according to the method of McManus. Frozen sections made from material fixed in Bouin's solution were stained with Oil Red O in propylene glycol and by the Schultz method for demonstration of cholesterol. To study the capillary basement membrane, tissue for electron microscopy from the most severely hyperglyceridemic patients was prepared according to the technique described by Sabatini and associates. The same technique has been used for successful demonstration of microangiopathy in renal biopsies of pregnant diabetic women in the laboratory.

The amount of lipid collection at a given site as revealed by the Oil Red O stain was estimated under the light microscope and graded on a scale of 0 to 4 by one of us (J. C.) who at the time of final grading did not know which slides came from the controls and which from the patients and was also unaware of the changes in the nutritional and metabolic states of patients which were accomplished from time to time by dietary manipulations. Definition of the 0 to 4 grades is as follows: 0.0, no detectable Oil Red O staining material; 0.5, sparsely scattered lipid droplets detected by high-power examination, not present in every field; 1.0, one or more droplets visualized in each high-power field, scattered widely along the sectioned capillaries; 2.0, course of capillary fairly well outlined by intramural droplets; few in pericapillary tissue; 3.0, capillary completely outlined by lipid droplets, pericapillary droplets present; 4.0, maximal deposition of intramural and pericapillary lipid droplets. To evaluate the possibility that the pericapillary and intramural collections of lipids might merely represent "spillage" of lipids from the hyperlipidemic blood stream, all biopsy specimens were taken from normolipemic controls at the height of alimentary lipemia, and blood smears of hyperglyceridemic patients were prepared and stained with Oil Red O.

Classification of hyperlipidemia was made on the basis of the following studies while the patients were maintained on ad libitum diets. All blood specimens for biochemical analyses were taken after a 12 to 14-hour fast: (1) Simultaneous measurements of serum cholesterol, phospholipid, and triglyceride were made to obtain a serum lipid pattern for use in the differential diagnosis of hyperlipidemia. (2) Plasma lipoprotein separation was accomplished by paper electrophoresis according to the technique described by Lees and Hatch to obtain characteristic patterns for the diagnosis of hyperlipoproteinemias. (3) The correctness of the diagnosis was checked by the therapeutic response obtained from a patient following the use of a sugar-restrictive, low-carbohydrate diet.

Results

Examples of the plasma lipoprotein patterns obtained in the normolipemic controls, in severely hyperglyceridemic patients, in moderately hyperglyceridemic patients, and in patients with essential familial hypercholesterolemia are shown in figure 1a, b, c, and d, respectively. Variable degrees of chylomicroemina (fig. 1b) were demonstrated in the patients with markedly elevated serum triglyceride levels (>800 mg%).

A fasting blood sugar above the normal range was found in only three of the 16 patients with types III, IV, or V hyperlipoproteinemia. Six patients in this series of 16 showed abnormal blood sugar curves on an oral glucose tolerance test.
creased in untreated hyperglyceridemic patients as compared with normolipemic controls (fig. 2) and patients whose hyperlipidemia had been kept under satisfactory control for more than 6 months prior to biopsy. A few finely divided pericapillary lipid droplets with a score of 0.5 in the 0 to 4 grading system were demonstrated in some of the "normal" controls. In contrast, examples of increased lipid droplet collection, with scores of 3 and 4, in biopsies obtained from hyperglyceridemic patients are shown in figure 3a and b. In these histological studies, care is necessary to distinguish true pericapillary lipid collections from foci of periarteriolar adipose tissue, and areas of inflammation or hemorrhage, all of which are capable of producing false positive readings.

The mean scores of intramural and pericapillary lipid collections and their standard deviations together with mean serum triglyceride and cholesterol concentrations of the control subjects and untreated hyperglyceridemic, treated hyperglyceridemic, and hypercholesteremic groups of patients are presented in table 1. The eight normolipemic subjects had a mean score of 0.5 ± 0.0 while the 16 untreated (types III, IV, or V) patients had a mean score of 2.0 ± 1.23. Thirteen of the 16 patients scored between 1 and 4. The five hyperglyceridemic patients whose hyperlipidemia was controlled by sugar-restrictive low-carbohydrate diets for 6 to 26 months had a low mean score of 0.3 ± 0.27, which is virtually the same as that of the control subjects.

Table 1

<table>
<thead>
<tr>
<th>Serum Lipids and Gingival Capillary Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject</strong></td>
</tr>
<tr>
<td>Young healthy males</td>
</tr>
<tr>
<td>Familial hypercholesteremia</td>
</tr>
<tr>
<td>Hyperglyceridemia, CHO-induced</td>
</tr>
<tr>
<td>Hyperglyceridemia, CHO-induced and treated</td>
</tr>
</tbody>
</table>

Untreated hyperglyceridemia vs. controls  
Untreated vs. treated hyperglyceridemia  
Hypercholesteremia vs. controls

\[ t = 3.39; P = 0.01 \]
\[ t = 3.00; P = 0.01 \]
\[ t = 1.56; P = N.S. \]

*The average of two or more determinations on each subject is used.

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Table 2
Effect of Diets upon Plasma Lipoproteins, Lipids, and Gingival Capillary Lipid Deposit in Patient D. G.

<table>
<thead>
<tr>
<th>Dietary types</th>
<th>No. of feeding periods</th>
<th>Plasma lipoprotein pattern</th>
<th>Serum triglycerides (range, mg%)</th>
<th>Gingival vessel lipids (0-4 scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad lib</td>
<td>2</td>
<td>Heavy pre-β, trailing, and chylomicron</td>
<td>3,200-4,770</td>
<td>3+ -4+</td>
</tr>
<tr>
<td>Fat-free</td>
<td>2</td>
<td>Heavy pre-β and trailing</td>
<td>4,030-4,980</td>
<td>0-0.5+</td>
</tr>
</tbody>
</table>

The mean score attained by patients with familial hypercholesterolemia also did not differ significantly from that of the controls. Although four of the 16 untreated hyperglyceridemic patients did score as low as some of the normolipemic controls, the differences between the mean score of the untreated group and that of (1) the treated hyperglyceridemic patients, and (2) the normal controls were both significant at the 1% level of probability. Among the untreated hyperglyceridemic patients with moderate degrees of lipemia (serum triglyceride below 800 mg%), there was no apparent correlation between the score for pericapillary lipids and the age of the patient, or the serum triglyceride level.

Correlative study of serial gum biopsies from patient D.G. with clinical data revealed the following: (1) From 3 to 4+ lipid droplets were found in and around the gingival capillaries when the patient was consuming ad libitum diets which had the usually high fat content of the American diet, and when chylomicronemia was demonstrated by lipoprotein electrophoresis (fig. 4b). (2) Only a trace of lipid-stainable material was demonstrable in and around the capillary sites at the end of the two fat-free feeding periods when the chylomicronemia was completely replaced by lipoproteins of endogenous origin (fig. 4c). Correlation of the changing plasma lipoprotein pattern with capillary lipid deposits is presented in table 2.

The Schultz stain for cholesterol was uniformly negative in all biopsies. Staining of prepared blood smears with the Oil Red O technique used for tissue study gave positive results only in a patient whose serum triglycerides were markedly elevated (over 3,000 mg%). Studies of the basement membrane of gingival capillaries with PAS stain and by electron microscopy have failed to demonstrate the abnormalities described in patients with pre-diabetes and diabetes mellitus.

Discussion

Evidence of abnormal glucose metabolism has been demonstrated in patients with carbohydrate-induced hyperlipidemia (types III, IV, and V).7, 14, 15 The study suggests that in contrast to true diabetes, carbohydrate-induced hyperglyceridemia is not particularly conducive to the production of microangiopathy in gingival capillaries.

The continual filtration of lipoproteins from the circulating blood stream through the vessel wall into the perivascular tissue is a well-recognized physiological phenomenon. According to Courtice and Garlick,16 factors such as capillary filtration pressure, the molecular size of lipoprotein species, and the integrity of capillary endothelium all interact to determine the amount of lipoprotein filtered through the capillary wall.

In the absence of demonstrable inflammation or changes in capillary filtration pressure, the size of the lipoprotein molecule and particulate fat may be the most important factor influencing the accumulation of histologically demonstrable lipids. Our data appear to indicate persistent chylomicronemia as the most likely primary factor in producing the intramural and pericapillary collection of lipid droplets. Support of this concept has come primarily from the controlled metabolic studies made on patient D.G. In this patient the appearance and subsidence of chylomicronemia were manipulated at will by the use of

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Figure 2

Typical example of gingival biopsy from a normolipemic control, which was read as 0.0. Oil Red O stain on frozen section, × 100.

Figure 3 (Below)

(Left panel) Maximum (4+) pericapillary lipid droplet accumulation in gingival biopsy from carbohydrate-induced hyperglyceridemic patient, × 250. (Right panel) Similar biopsy graded as 3+, × 280. Oil Red O stain on frozen section.
experimental diets. The positive and negative gum biopsy findings were correlated with the presence or absence of chylomicronemia as demonstrated by analysis of the plasma lipoproteins (fig. 4). Failure to demonstrate pericapillary lipid during alimentary chylomicronemia of the controls can probably be explained by the transient occurrence of mild chylomicronemia in contrast to the prolonged hyperchylomicronemia in D.G.

The fact that the pericapillary lipids were found to stain intensely with Oil Red O with its known affinity for triglycerides and negatively with the Schultz stain for cholesterol may be taken as an additional point in favor of the contention that chylomicrons are the primary source of the lipid deposits in the perivascular space. Since chylomicrons are made up mainly of triglycerides with only small amounts of cholesterol and phospholipid,\textsuperscript{17} it can be expected that they would stain intensely with Oil Red O and negatively with the Schultz stain for cholesterol.

Previous studies indicate that the body triglyceride pools are greatly expanded in patients with carbohydrate-induced hyperglyceridemia.\textsuperscript{15, 18} In patients with enlarged body pools variable degrees of chylomicronemia can be produced by imposition of dietary fat loads on clearing mechanisms already burdened by endogenous glyceride.\textsuperscript{17} Thus, moderately severe degrees of chylomicronemia are almost always demonstrable in patients with severe carbohydrate-induced hyperglyceridemia (serum triglyceride $>800$ mg%). On the other hand, in patients with more moderate elevations of serum triglycerides, the degree of the associated chylomicronemia may be quite variable. This observation may serve to explain the relatively poor correlation between moderate levels of hyperglyceridemia and the quantity of stainable pericapillary lipids.

By light or electron microscopy, the chylomicrons are found to vary from about 0.1 to 5.0 $\mu$m in diameter. It has been suggested that in vivo they should not exceed 1.0 $\mu$m.\textsuperscript{17} It can be expected that these relatively large chylomicrons would filter through the capillary endothelium with some difficulty; and the demonstration of these lipid particles in the pericapillary space might appear to contradict theoretical expectations. On the other hand, the lipoprotein species of small molecular size may be filtered easily through the capillary endothelium but would also tend to diffuse rapidly through the pericapillary tissues to be absorbed into the lymphatic circulation. Therefore, despite the fact that chylomicrons are filtered through the capillary wall at a relatively slow rate and thus may not be demonstrable in the pericapillary site in the transient alimentary lipemia of normal controls, their “stagnation” in the tissue interstices of patients with sustained hyperglyceridemia may allow them to accumulate in sufficient numbers to permit detection on histological examination.

It is perhaps reasonable to assume that heavy collections of macromolecular lipid substances in and around the capillaries may in time exert an adverse effect upon either the function or the morphology of the capillaries.
However, definitive information on these possible changes is beyond the scope of this investigation.

References
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