Enhanced Lipoprotein Lipase Secretion from Human Monocyte-derived Macrophages Caused by Hypertriglyceridemic Very Low Density Lipoproteins

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We studied the effects of very low density lipoprotein (VLDL) obtained from hypertriglyceridemic subjects on the secretion of lipoprotein lipase and lipid accumulation in human monocyte-derived macrophages. The incubation of macrophages with VLDL obtained from different subjects caused different effects on the secretion of lipoprotein lipase (6.8 to 137.7 nM free fatty acid/ml/mg cell protein) and triglyceride accumulation (184 to 507 μg/ml/mg cell protein) in human monocyte-derived macrophages. VLDL from subjects with marked hypertriglyceridemia (>1000 mg/dl) had a fourfold greater effect on lipoprotein lipase activity and a twofold greater effect on cellular triglyceride accumulation when compared with the effects of VLDL from normolipidemic subjects. Both lipoprotein lipase activity and triglyceride accumulation correlated positively with plasma VLDL triglyceride levels (r=0.50 and 0.45, respectively, p<0.05). From these data, we suggest that the activity of lipoprotein lipase secreted from macrophages incubated with VLDL was dependent on triglyceride concentrations, and that the secretion of lipoprotein lipase enhanced by hypertriglyceridemic VLDL was closely related to the intracellular accumulation of triglyceride. (Arteriosclerosis 9:650–655, September/October 1989)

A n early event in the development of atherosclerosis is the accumulation of lipid-loaded "foam cells" in the subendothelial space. Recent evidence suggests that most of the foam cells are derived from monocytes/macrophages that have migrated from the circulating blood.1,2,3 These cells have the ability to take up various lipoproteins, resulting in varying degrees of accumulation of cholesteryl esters, triglycerides, or both.4

Triglyceride-rich lipoproteins, especially hypertriglyceridemic very low density lipoproteins (VLDL), are the major lipoproteins causing accumulation of triglyceride in macrophages.5–8 Both the amount and configuration of apolipoprotein (apo) E on the lipoprotein surface are thought to be important for the uptake of triglyceride-rich lipoproteins by macrophages.9 In addition to apo E, lipoprotein lipase secreted from macrophages may be involved in the process of intracellular triglyceride accumulation by supplying both free fatty acids and remnant particles produced through hydrolysis of triglyceride-rich lipoproteins.10

Macrophages not only endocytose macromolecules like lipoproteins but also synthesize and secrete many proteins. Lipoprotein lipase is one of the secretory proteins of macrophages and shows the classical characteristics of lipoprotein lipase in adipose tissue.11–14 However, the role and regulation of lipoprotein lipase secreted from macrophages are not well understood.15,16,17

Recently, we found that VLDL from normal and hypercholesterolemic rats enhance the secretion of lipoprotein lipase from rat alveolar macrophages.18 In the present study, human monocyte-derived macrophages were incubated with human VLDL to test this observation in humans. Furthermore, to prove the hypothesis that lipoprotein lipase secreted from macrophages is involved in intracellular triglyceride accumulation,10 the effects of VLDL, especially hypertriglyceridemic VLDL, on the secretion of lipoprotein lipase from human monocyte-derived macrophages were investigated. Using VLDL obtained from subjects with a wide variety of hypertriglyceridemia, we observed that VLDL with higher triglyceride concentrations causes more lipoprotein lipase secretion from human monocyte-derived macrophages as well as more accumulation of triglycerides within the cells.

Methods

Study Subjects

Ten normolipidemic subjects (having triglyceride levels of <150 mg/dl and cholesterol levels of <240 mg/dl) and 23 hyperlipidemic subjects were studied. The clinical characteristics of these patients are outlined in Table 1. Ten of the hyperlipidemic patients were free from secondary illness. Of these, two had hypercholesterolemia, four had hypertriglyceridemia, and four had both hypercholesterolemia and hypertriglyceridemia. The remaining 13 hyperlipidemic patients had noninsulin-dependent dia-

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Isolation of Lipoproteins

Blood was drawn from the study subjects into tubes containing ethylenediaminetetraacetic acid (EDTA) (1 mg/ml) after an overnight fast. The VLDL (density <1.006) were isolated by ultracentrifugation in a 40.3 rotor (Beckman Instruments, Incorporated, Palo Alto, CA) at 39 000 rpm for 18 hours and were stored at 4°C after the addition of penicillin and streptomycin (GIBCO, Grand Island, NY).

The day before the experiment, the lipoproteins were washed by recentrifugation at the same density for 20 hours. The isolated lipoproteins were extensively dialyzed three times against 5 liters of dialysis buffer containing 150 mM NaCl, 2 mM sodium phosphate, and 0.01% (wt/vol) EDTA (pH 7.4) and then against 5 liters of the dialysis buffer without EDTA. This solution was sterilized by filtration through a 0.45-μm Millex-HA filter (Millipore Corporation, Bedford, MA).

The protein concentrations of VLDL were determined by using the modified method of Lowry et al. and Kashyap et al. Cholesterol and triglyceride concentrations were measured enzymatically.

Culturing of Human Monocyte-derived Macrophages

Human monocyte-derived macrophages were prepared by culturing human peripheral monocytes, which were isolated from blood drawn from a normolipidemic healthy donor by using a Ficoll-Hypaque gradient method (Lymphoprep, Daichi Pharmaceutical Company, Tokyo, Japan). The mononuclear cells were recovered from the interface and were washed three times with phosphate-buffered saline (PBS). The washed cells were suspended in Medium 1640 from Roswell Park Memorial Institute (RPMI-1640) containing 10% (vol/vol) autologous serum. The medium was replaced every 3 to 4 days.

On the 11th day after culturing began, the medium was replaced by RPMI-1640 containing 10% (vol/vol) lipoprotein-deficient serum (LPDS) unless otherwise stated in the figure legends. At 24 hours after incubation with LPDS, the cells were washed three times with PBS and were incubated for another 18 hours with 300 μl of medium containing 10% (vol/vol) LPDS. In the pulse-chase experiments, the cells were incubated for 24 hours at 37°C (pulse) with 0.5 ml of medium containing 5 mg/ml of LPDS and VLDL. Thereafter, the cells were gently washed twice with 1 ml of prewarmed PBS. They were incubated with 0.5 ml of 2 mg/ml of LPDS for another 4 hours (chase).

The medium was removed and supplemented with a 10% solution (vol/vol) of bovine serum albumin (Sigma Chemical Company, St. Louis, MO, pH 7.4) to give a final concentration of 2% (vol/vol) bovine serum albumin. This solution was stored at −70°C until measurement of lipoprotein lipase activity. The remaining cells were used to determine the cellular lipid and protein contents. The cellular lipids were extracted with hexane-isopropanol (3:2) and were measured enzymatically as mentioned above. The cellular protein digested in 0.1 N NaOH was measured by the Lowry method.

Lipoprotein Lipase Assay

Lipoprotein lipase was measured with the method of Nilsson-Ehle and Schotz by using a stable, radioactive substrate emulsion. A 150-μl aliquot of the sample was mixed with the substrate containing [14C]-oleate (1.4 μCi/μmol; Amersham International, Buckinghamshire, England). Lipoprotein lipase activity was determined as the amounts of radioactive oleic acids liberated during incubation. The lipase activity was inhibited more than 90% by the addition of 1 M NaCl and more than 80% without serum in this assay. Lipoproteins containing less than 50 μg protein/ml in the assay did not interfere with the lipoprotein lipase assay. When 100 μg protein/ml of VLDL was added to the assay, lipoprotein lipase activity was suppressed by 20%. In the present study, 40 μg protein/ml of VLDL were incubated with macrophages.

Statistical Analysis

All results were expressed as the means±SD unless otherwise indicated. The statistical significance of the data was analyzed by Student’s t test.

Results

Characteristics of Hypertriglyceridemia

In the hypertriglyceridemic patients, the plasma concentration of triglyceride was 387±462 mg/dl, and that of

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nondiabetics</th>
<th>Diabetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>52±14</td>
<td>54±11</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162±9</td>
<td>161±8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58±10</td>
<td>59±9</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>241±90</td>
<td>261±126</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>387±462</td>
<td>582±615</td>
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The results are given as means±SD.
cholesterol was 241±90 mg/dl (Table 1). No significant differences in plasma lipid concentrations between diabetics and nondiabetics were found. The lipid and apolipoprotein composition of VLDL is indicated in Table 2. The lipid/protein ratios and apolipoprotein/apo B ratios in hypertriglyceridemic subjects (plasma triglyceride level >150 mg/dl) were greater than in normotriglyceridemic subjects.

**Cellular Lipid Contents**

When macrophages were incubated with 100 mg protein/ml of normolipidemic VLDL, the cellular contents of both triglyceride and cholesterol increased up to 24 hours, at which time they were saturated. The ratio of triglyceride accumulation to that of cholesterol was 4.8, which was similar to the lipid ratio of VLDL. When the increase in triglyceride accumulation within the cells was related to the increasing amounts of VLDL added to the medium, the curve was saturated at 100 mg protein/ml of VLDL. Based on the preliminary data, the following experiments, in which macrophages were incubated with 40 mg protein/ml of VLDL for 24 hours, were performed.

When macrophages were incubated with VLDL (40 mg/ml) from different subjects with varying plasma triglyceride concentrations, the cellular content of triglyceride varied greatly from 184 to 507 mg triglyceride/mg cell protein. The cellular triglyceride content correlated positively with triglyceride and cholesterol concentrations in both plasma and VLDL (r=0.36, 0.40, 0.41, and 0.45, respectively, p<0.05; Table 3). The relationship of triglyceride accumulation to triglyceride concentrations in VLDL is represented in Figure 1. There was no significant difference between diabetic and nondiabetic subjects in this relationship.

**Lipoprotein Lipase Secretion into Medium**

The lipoprotein lipase activity in the culture medium increased up to 100 nM free fatty acid/min/mg cell protein during 24 hours of incubation and slightly declined thereafter (Figure 2). When cultured monocytes were incubated with medium containing autologous serum and the medium was replaced daily by fresh medium, the lipoprotein lipase activity secreted during daily incubation increased up to 110 nM of free fatty acid/min/mg cell protein reaching a plateau on the 6th day of culture (Figure 3).

Next, we focused our attention on the effect of VLDL on the activity of lipoprotein lipase secreted from macrophages. Figure 4 shows the dose response of the effect of normotriglyceridemic VLDL on lipoprotein lipase activity in the culture medium. The addition of VLDL elevated the lipoprotein lipase activity in the medium in a dose-dependent manner. Lipoprotein lipase activity in the medium is the sum of both secretion and inactivation of the enzyme. When the medium containing lipoprotein lipase secreted from macrophages during a 24-hour incubation (conditioned medium) was incubated with or without VLDL (40 mg/ml) at 37°C, the degree of inactivation of lipoprotein lipase in the conditioned medium was approximately four times greater in the absence of VLDL (no VLDL, 1.9 hours, VLDL, 7.3 hours as T1/2), indicating that the inactivation of lipoprotein lipase in the medium was suppressed by the addition of VLDL. When different VLDL obtained from various hypertriglyceridemias (Experiment 1:
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Figure 2. The time-course (hour) of secretion of lipoprotein lipase by macrophages (nmol FFA (free fatty acid)/min/mg cell protein). Macrophages were pre-incubated for 24 hours with RPMI-1640 containing 10% lipoprotein-deficient serum (LPDS). The medium was removed, and the cells were washed three times with 1 ml of phosphate-buffered saline. Then, RPMI-1640 containing 10% LPDS was added. At each time point shown on the abscissa, the medium was removed for the determination of lipoprotein lipase activity. The values are the means of duplicate wells.

Figure 3. The temporal pattern (days) of secretion of lipoprotein lipase by macrophages (nmol FFA (free fatty acid)/min/mg cell protein). Monocytes from a normolipidemic, nondiabetic subject were cultured in RPMI-1640 containing 10% autologous serum. At each time point shown on the abscissa, the medium was removed, and the cells were washed three times with 1 ml of phosphate-buffered saline. Then, 0.5 ml of RPMI-1640 containing 10% autologous serum was added. After 24 hours of incubation at 37°C, lipoprotein lipase activity in the medium was determined. The values are shown as the means±one SD of quadruplicate wells.

Figure 4. The dose-response relationship between very low density lipoprotein (VLDL) (μg protein/ml) and lipoprotein lipase activity (nmol FFA (free fatty acid)/min/mg cell protein) in the incubation medium. After the cells were pre-incubated with 10% lipoprotein-deficient serum (LPDS) for 24 hours, 0.5 ml of medium containing 10% LPDS and varying concentrations of VLDL was added. After 24 hours of incubation at 37°C, lipoprotein lipase activity was determined. The values are shown as the means±one SEM of quadruplicate wells.

Figure 5. In this pulse-chase experiment, the cells were incubated with very low density lipoprotein (VLDL) (μg protein/ml) in medium containing 5 mg/ml of lipoprotein-deficient serum (LPDS) for 24 hours as shown in Figure 4. The cells were then gently washed twice with 1 ml of prewarmed phosphate-buffered saline and were incubated with 0.5 ml of medium containing 2 mg/ml of LPDS for another 4 hours. The incubation medium was removed for determination of lipoprotein lipase activity (nmol FFA (free fatty acid)/min/mg cell protein). The values are shown as the means±one SEM of quadruplicate wells.

57 to 3919 mg/dl, n=11; Experiment 2: 55 to 1108 mg/dl, n=20) at the same protein level (40 μg/ml) were incubated in the conditioned medium for 8 hours, the inactivation of lipoprotein lipase was similar. The correlation coefficients between the inactivation rate and the plasma triglyceride levels were not statistically significant.

To see whether the treatment of macrophages with VLDL induces the release of lipoprotein lipase, we carried out pulse-chase experiments. As shown in Figure 5, when the cells were pre-incubated with VLDL for 24 hours, the lipoprotein lipase activity in the chase medium was elevated in a dose-dependent manner: 51% increase at 10 μg protein/ml of VLDL (p=0.015). To see whether this stimulatory effect of VLDL on lipoprotein lipase release in the chase phase was dependent on the degree of hypertriglyceridemia, we measured the lipoprotein lipase activity secreted from macrophages that had been pre-incubated with 40 μg protein/ml of VLDL from subjects with varying plasma triglyceride levels. The lipoprotein lipase activity in the chase medium was found to correlate positively with both cellular triglyceride and cholesterol content (r=0.49 and 0.61, respectively; p<0.05, n=19; plasma triglyceride levels, 51 to 612 mg/dl). Hence, the increase of lipoprotein lipase activity by the addition of hypertriglyceridemic VLDL in the present study reflected the enhanced secretion of lipoprotein lipase in macrophages.
The VLDL from subjects with varying degrees of hypertriglyceridemia exhibited different effects on the stimulation of lipoprotein lipase secretion (6.8 to 137.7 nM free fatty acid/min/mg cell protein). The lipoprotein lipase activity in the medium correlated with various parameters, including triglycerides in VLDL and cellular triglyceride concentrations, as shown in Table 3. While many parameters strongly correlated with lipoprotein lipase activity, the correlation with cellular triglyceride contents was the greatest \( r=0.68; p<0.01 \).

The relationships of lipoprotein lipase activity to triglycerides in VLDL and cellular triglyceride concentrations are indicated in Figure 6. There was no significant difference between diabetic and nondiabetic subjects in the ability of their VLDL to enhance lipoprotein lipase secretion.

**Discussion**

Epidemiological studies have shown a significant correlation between hypertriglyceridemia and coronary heart disease.\(^{25,27,28}\) To clarify this relationship, we studied the effect of hypertriglyceridemic VLDL on lipid accumulation in human monocyte-derived macrophages, since macrophages are thought to be precursors of foam cells in atherosclerotic lesions.\(^1,2,3\) Furthermore, based on the suggestion by Lindqvist et al.\(^6\) that lipoprotein lipase secreted from macrophages plays an important role in the process of intracellular triglyceride accumulation, we attempted to show that the secretion of lipoprotein lipase from macrophages is associated with intracellular lipid accumulation when macrophages are incubated with hypertriglyceridemic VLDL.

There are two possible explanations for intracellular triglyceride accumulation: In the first, hydrolysis of VLDL triglyceride by lipoprotein lipase results in the liberation of free fatty acids into the medium. This is followed by the mobilization into macrophages, and then free fatty acids are re-esterified to triglycerides for storage in the cells. In the second explanation, the cells accumulate only triglycerides, and the cellular cholesterol could not increase. However, our finding that triglyceride and cholesterol in the cells increased suggests that whole VLDL particles are endocytosed into macrophages rather than the selective uptake of VLDL triglyceride.

Incubation of macrophages with VLDL showed intracellular triglyceride accumulation and increased activity of lipoprotein lipase in the medium. The increased activity of the enzyme was caused by the enhanced enzyme secretion, since lipoprotein lipase secretion was induced by the addition of VLDL in a dose-dependent manner in the pulse-chase experiment. The results were compatible with those from our previous study; the VLDL from rats enhanced the secretion of lipoprotein lipase from rat alveolar macrophages.\(^16\) Another factor in the increased enzyme activity was stabilization of the enzyme by the addition of VLDL.

The addition of hypertriglyceridemic VLDL to macrophages showed greater intracellular triglyceride accumulation and more activity of lipoprotein lipase in the culture medium than when normolipidemic VLDL was added. The increase in lipoprotein lipase activity was mainly due to the increase in lipoprotein lipase secretion from macrophages, which was induced by the uptake of hypertriglyceridemic VLDL, not to the stabilization of the enzyme, since hypertriglyceridemic VLDL had an effect similar to normolipidemic VLDL on the stabilization of the enzyme at the same protein level.

It is important to know how hypertriglyceridemic VLDL enhances the secretion of lipoprotein lipase. The correlation between cellular lipid contents and enzyme activity suggests that the secretion of lipoprotein lipase could be regulated through either the uptake of VLDL or the intracellular lipid accumulation. Gian turfco et al.\(^9\) noted that VLDL obtained from hypertriglyceridemic subjects are essentially different from normal VLDL. Apo E is considered to be a determinant for the increased uptake of VLDL in macrophages.\(^9,29,30,31\) This is consistent with our finding that the triglyceride accumulation correlated positively with the apo E content in VLDL (data not shown). In addition to apo E, the modification of hypertriglyceridemic VLDL by lipoprotein lipase secreted from macrophages may also affect the
interaction between cells and the VLDL as mentioned earlier. We are investigating this mechanism further.

These findings have important implications for atherogenesis. Since VLDL obtained from hypertriglyceridemic subjects causes both cholesterol and triglyceride accumulation in macrophages, hypertriglyceridemia itself could be atherogenic. Recent studies of VLDL metabolism in vivo indicate that a considerable portion of VLDL are catabolized directly through LDL receptors on the liver membrane before converting to LDL, especially in hypertriglyceridemic persons.32,33 From these studies and our present study, we suggest that VLDL, especially in hypertriglyceridemia, are effectively taken up by arterial macrophages. This is followed by atheroma formation. Lipoprotein lipase secretion stimulated by hypertriglyceridemic VLDL accelerates the process of atherogenesis.

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References

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