Effects of Lovastatin on the Levels, Structure, and Atherogenicity of VLDL in Patients With Moderate Hypertriglyceridemia

Sandra H. Gianturco, William A. Bradley, Shuichi Nozaki, Gloria L. Vega, and Scott M. Grundy

The purpose of this study was to determine whether lovastatin treatment reduced very low density lipoprotein (VLDL) abnormalities in hypertriglyceridemia patients. Lovastatin reduced plasma triglyceride levels and the levels of total VLDL, intermediate density lipoprotein (IDL), and low density lipoprotein (LDL) cholesterol. The numbers of VLDL particles of S1, 100–400 and S2, 60–100 but not S2, 20–60 particles were reduced by lovastatin, as was the amount of cholesteryl ester per particle. All VLDL subspecies bound to the LDL receptor of cultured human fibroblasts with similar, high affinities on both placebo and lovastatin, but VLDL S1, 100–400 and VLDL S2, 60–100 caused less suppression of 3-hydroxy-3-methyl glutaryl coenzyme A reductase activity after lovastatin therapy, indicating reduced LDL receptor-mediated cholesterol delivery.

Hypertriglyceridemia is considered a risk factor for coronary heart disease (CHD) in Europe, although this association remains controversial in the United States, as recently reviewed. There is growing evidence, however, that hypertriglyceridemia may be associated with increased risk for CHD. The mechanism(s) for a connection between hypertriglyceridemia and CHD has not been determined with certainty, but two general possibilities must be considered. These two potential mechanisms are not mutually exclusive, and both may play a role in CHD. First, triglyceride-rich lipoproteins (TGRLPs) could be directly atherogenic, and abnormally high levels could promote the development of coronary atherosclerosis. Alternatively, the metabolic consequences of hypertriglyceridemia, i.e., low levels of high density lipoprotein (HDL), the presence of small, dense low density lipoprotein (LDL), or a tendency for thrombogenesis, could account for the increased risk for CHD. Although the latter, indirect mechanism is favored by many investigations, data are accumulating that implicate a direct atherogenic role of TGRLPs.

Since TGRLPs represent a heterogeneous group of particles, it might be expected that some lipoproteins in this class are more atherogenic than others. Although there are no established ways to differentiate between TGRLPs that do or do not promote atherogenesis, those that promote lipid accumulation in cells may be the best candidates. Previous studies from our laboratory indicate that very low density lipoproteins (VLDLs) from hypertriglyceridemic plasma are more likely to promote cellular lipid uptake than those from normolipidemic plasma.

Two types of cells are useful for in vitro tests of lipid accumulation by TGRLPs. These are fibroblasts, which express LDL receptors, and macrophages, which express a variety of “scavenger” receptors. VLDLs of S1, 20–400 from hypertriglyceridemic plasma bind to LDL receptors of fibroblasts and deliver cholesterol to these cells. In contrast, in normotriglyceridemic plasma, only VLDLs of S1, <60 bind to the LDL receptor and deliver cholesterol; VLDLs of S1, >60 fail to bind or deliver cholesterol. In addition, the large VLDLs (S1, >100) of some hypertriglyceridemic patients appear to bind to macrophage receptors. Presumably, the VLDLs that bind have been modified in the circulation in a way to recognize macrophage receptors. Studies in our laboratory have recently identified a specific receptor on macrophages that recognizes and internalizes modified VLDL. The abnormal VLDL particles thus
could be particularly atherogenic through their ability to transform macrophages into foam cells.

The current study was carried out to determine whether a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, lovastatin, would reduce concentrations of potentially atherogenic VLDL particles. If so, this action could be one mechanism whereby HMG-CoA reductase inhibitors reduce the risk for CHD.

Methods

Patients

Twelve male subjects were recruited from the Lipid Clinic of the Veterans Affairs Medical Center, Dallas, Tex. Their ages ranged from 35 to 68 years (mean±SEM, 50±3 years) and body mass indexes from 24.5 to 33.8 kg/m² (mean, 29.1±0.8 kg/m²). All of the subjects had elevated plasma triglyceride and cholesterol levels, with elevations in VLDL, intermediate density lipoprotein (IDL), and LDL cholesterol and decreased HDL cholesterol levels (Table 1). Triglyceride values ranged from 300 mg/dL to 764 mg/dL. Family histories were not sufficient to draw any conclusions about the presence or absence of familial combined hyperlipidemia (FCHL). None of the patients had diabetes mellitus or disorders of the gastrointestinal tract, liver, kidneys, or endocrine systems. Subjects with a history of CHD were not excluded unless they had clinically significant cardiopulmonary dysfunction. Nine patients had CHD, as documented either by a history of myocardial infarction or coronary artery bypass graft surgery at least 6 months preceding entrance into the study. None had sustained a myocardial infarction or coronary artery surgery, and none had taken lipid-lowering drugs during the 6-month period preceding recruitment. Six patients with CHD were taking β-adrenergic blocking agents at the time of the study, and these drugs may have accentuated the hyperlipidemia; their medication remained the same throughout the study. All subjects gave written, informed consent, and the protocol had been approved by the appropriate institutional review board. For normal control subjects, healthy volunteers were recruited from Southwestern Medical Center. They were not taking any hypolipidemic agents or drugs known to affect levels of plasma lipids or lipoproteins. All normal subjects were middle-aged and nonobese.

Treatment

Lovastatin, 40 mg twice daily, was compared with placebo, which was indistinguishable from the active drug, in a single-blind protocol. Placebo was given for 8 weeks followed bylovastatin for 8 weeks. Treatment was not randomized, since the lipid levels of hypertriglyceridemic patients are sometimes slow to return to pretreatment levels after drug treatment and a steady state might not have been reestablished within the 8-week period. The subjects consumed diets throughout the study that resembled a "typical" American diet as used previously for studies of lovastatin effects as described.13–17 Plasma lipids and lipoproteins were measured five times during weeks 7 and 8 of the placebo/drug phases to obtain average values for each subject. Plasma for lipoprotein isolation was obtained in the last week of each treatment period. The study was coded and blinded to the laboratory for cell studies but not to the clinical staff at the Veterans Affairs Medical Center, where the drug trial was conducted.

Lipoprotein Analyses

For chemical analyses, VLDL, IDL, LDL, and HDL were isolated from plasma by sequential ultracentrifugation, and VLDL was subfractionated by cumulative flotation.18,19 Cholesterol (free and total), triglycerides, and phospholipids were determined enzymatically.20 Cholesteryl ester mass was estimated by multiplying the difference between free and total cholesterol by 1.68 to account for the esterified fatty acid. Total protein was determined by the method of Markwell, as previously detailed.21,22 Apolipoprotein (apo) B was measured after precipitation of the protein with isopropanol.23–28

For cell studies, plasma was obtained from fasting subjects with normal lipid values for isolation of lipoprotein-deficient serum (LPDS).26 The d<1.019 g/mL fraction of plasma from patients was isolated in a single spin begun on the same day that plasma was obtained. Immediately after ultracentrifugation, the d<1.019 g/mL fraction was removed after tube slicing; inhibitors were added and samples shipped on wet ice by overnight delivery from Dallas. All samples were coded before shipment from Dallas; neither the treatment phase nor the pair status of the samples was known by the investigator involved in cell studies until those studies were complete. Lipoprotein subfractions for cell studies were isolated by cumulative flotation.18,19 from the d<1.019 g/mL fraction, which contained 1 mM EDTA, 1 mM NaN₃, 10 μM phenylmethylsulfonyl fluoride (Sigma), and 50 units of Trasylol per milliliter, as previously detailed for VLDL₃ (S₁₀₀–₄₀₀), VLDL₁ (S₁₅–₆₀), and VLDL₀ (S₂₀–₆₀).19 After VLDL₀ was removed, IDL was visible as a distinct band midway in the gradient and was removed by aspiration. For cell studies, total protein contents of the lipoproteins were obtained by a modification27 of the method of Lowry et al.28

<table>
<thead>
<tr>
<th>TABLE 1. Lipid and Lipoprotein Cholesterol Levels in Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Placebo</td>
</tr>
<tr>
<td>Lovastatin</td>
</tr>
<tr>
<td>Normal</td>
</tr>
</tbody>
</table>

VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein. All measurements are in milligrams per deciliter and are means of values from 12 subjects after 6 weeks of treatment with placebo or lovastatin (determined five times during weeks 7 and 8 for each subject). Paired t tests were used to determine significance of differences between placebo and drug phases.

*p<0.0001, t p<0.01, 4p<0.001.
Fibroblast Studies

Monolayer cultures of normal human newborn foreskin fibroblasts were grown and maintained as described previously.\(^7,8\) For experiments, approximately \(5 \times 10^4\) cells (third to 10th passage) were seeded into dishes (60 x 15 mm) with 5 mL complete medium containing 10\% Nu-Serum (Collaborative Research, Inc., Bedford, Mass.). When the cells were approximately 75\% confluent (2-3 days), the cells were washed with saline, and the medium was replaced with 2 mL of medium containing 5\% human LPDS\(^8\) for 24 hours to induce LDL receptor activity. Indicated quantities of lipoproteins were added to duplicate dishes and incubated at 37\°C for 5 hours. HMG-CoA reductase activity, determined as described,\(^7,8,10,19\) was used as an intracellular end point for assessing receptor-mediated cholesterol delivery to the cells. The effects of several levels of normal LDL on HMG-CoA reductase are included as a control in each experiment. For the reductase assay, the medium was removed, the cell monolayers washed twice at room temperature with 2 mL saline, and the cells scraped with a rubber policeman into 2 mL of 0.15 M NaCl, 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7.4. The cells were sedimented by centrifugation, the supernatant discarded, and the cell pellets stored at \(-80\°C\). The cells were then extracted and assayed for HMG-CoA reductase activity, following the methods of Brown et al,\(^8\) except that ether extraction was omitted.\(^7,8,19\)

Reductase Assay Procedure

Cell pellets were thawed rapidly and incubated for 10 minutes at 37\°C with 0.1 mL of 50 mM K\(_2\)HPO\(_4\), pH 7.4, containing 5 mM dithiothreitol, 1 mM EDTA, 0.14 M KCl, and 0.25\% Zwittergent-13 (Calbiochem-Behring Corp.). After centrifugation at room temperature, the clear supernatants were assayed for HMG-CoA reductase activity in duplicate. Cell extracts (20 \(\mu\)L) were incubated at 37\°C in a final volume of 35 \(\mu\)L with 3 mM nicotinamide adenine dinucleotide phosphate, 22 mM glucose-6-phosphate, 14 mM Tris-HCl (pH 7.5), 5 mM dithiothreitol, 0.15 units glucose-6-phosphate dehydrogenase (type X, sulfate free; Sigma), and 43 \(\mu\)M DL-3-hydroxy-3-methyl-[3\(^{-14}\)C]glutaryl-CoA. The extraction buffer was incubated as a control for background. After 2 hours, 10 \(\mu\)L of 2.5N HCl containing 60 mM mevalonolactone as carrier was added. After 20 minutes at 37\°C, 15 \(\mu\)L of the acidified reaction mixture was streaked on plastic-backed, silica thin-layer chromatography plates (Eastman). The chromatograms were developed in acetone/benzene (1:1, vol/vol) until the solvent front moved to the top of the plate. To calculate the number of picomoles of mevalonate formed during the incubations, the fraction of total radioactivity (residual substrate plus product) that appeared in the mevalonolactone region (adjusted for background) was multiplied by the number of picomoles of HMG-CoA initially present in the assay. Cell protein content of the extracts was determined by the method of Bradford.\(^8\)

Binding Studies

Lipoproteins were iodinated by a modification of the iodine monochloride method of McFarlane.\(^30\) Free iodine was removed by gel filtration and extensive dialysis. Samples were dialyzed and filtered (0.45-\(\mu\)m Millipore) immediately before use; specific activities ranged from 42 to 200 cpm per nanogram of protein. Less than 10\% of the label was extractable into organic solvent. Fibroblasts were preincubated in lipoprotein-deficient medium for 36 hours before the binding studies. Binding of \(125\)I-lipoproteins was measured as the cell-associated radioactivity in duplicate dishes after incubation of precooled cells with \(125\)I-lipoprotein alone or with excess unlabelled LDL for 3 hours at 4\°C, followed by extensive washing with chilled albumin-containing buffer\(^31\); each value was corrected by subtracting the amount “bound” in control dishes that contained no cells. Each iodinated lipoprotein was tested at six different concentrations in duplicate ranging from 0.5 to 15 \(\mu\)g protein per millilitre in the absence or presence of LDL at 250 \(\mu\)g protein per millilitre. Specific binding was calculated by subtracting the amount of \(125\)I-lipoprotein bound by cells in the presence of excess unlabelled LDL (these plots were linear) from the amount bound in the absence of unlabelled LDL (curvilinear); the specific binding curves were analyzed by the method of Scatchard to calculate the dissociation constant (\(K_d\)) of binding, as previously reported.\(^22\)

Macrophage Studies

The murine macrophage cell line P388D, was obtained from the Salk Institute Cell Repository and was cultured in RPMI-1640 (high glucose) supplemented with 10\% fetal bovine serum (GIBCO), 100 \(\mu\)g/mL penicillin, and 100 units/mL streptomycin.\(^33\) Cells were maintained in 100-mm dishes in a humidified incubator (5\% CO\(_2\)) at 37\°C.

For triglyceride accumulation studies, approximately 3.7\times 10^5 cells/dish were seeded into dishes (60 x 15 mm) in 2 mL complete medium. Cultures were refed 24 hours later with complete medium, and on the second day the medium was removed. After washing twice with saline, duplicate dishes of cells (and no cells, for blanks) were incubated in 2 mL serum-free RPMI-1640 containing the indicated quantities of lipoproteins for 4 hours at 37\°C and washed extensively with ice-cold, albumin-containing buffer as in the binding studies. The lipids were then extracted with hexane/isopropanol, 3:2 (vol/vol). The solvent was evaporated under nitrogen, and triglyceride mass was determined using an enzymatic kit (Boehringer Mannheim, catalog No. 701912) after the lipids were resolubilized in 0.5 mL of the kit reagent containing additional Triton X-100 (10 \(\mu\)L Triton X-100 per milliliter of reagent).\(^33\)

Statistics

Probability values for drug/placebo were determined by paired \(t\) tests and probability values between patients and normal subjects by unpaired \(t\) tests, as indicated in each table.

Results

Lipid and Lipoprotein Cholesterol Levels

Twelve hypertriglyceridemic patients were treated with lovastatin and placebo; total cholesterol and triglyceride levels fell by 29\% and 28\%, respectively (Table 1). Cholesterol in the major apoB-containing...
After lovastatin treatment, the amount of cholesteryl ester per particle was reduced in VLDL, and, to a lesser extent, in VLDL subfractions. Changes in VLDL subfractions were calculated to identify potential abnormalities that might be corrected by lovastatin therapy. The greatest changes were seen in VLDL₃; plasma concentrations of each constituent fell by over 50%, and cholesteryl ester declined the most, by 75%. For VLDL₂, all constituents showed a reduction on lovastatin therapy, but only the decreases in triglycerides and cholesteryl esters were statistically significant. There were no significant changes in VLDL₁ constituents on lovastatin compared with placebo, although there was a trend toward lower cholesteryl ester levels. Despite lovastatin therapy, all the subfractions remained elevated compared with those of normal control subjects.

The average percent compositions of VLDL subfractions were calculated to identify potential abnormalities in the patients’ particle compositions that might be corrected by lovastatin therapy. After the placebo period, patients' VLDL subfractions, particularly VLDL₃, were enriched in cholesteryl ester relative to comparable subfractions from normal subjects. After lovastatin treatment, the amount of cholesteryl ester per particle was reduced in VLDL₁ and, to a lesser extent, in VLDL₂ and VLDL₃, so that the percent compositions of the VLDL subfractions were near normal after drug therapy.

ApoB levels in each subfraction were measured to quantify the relative numbers of particles within the VLDL subfractions in patients during placebo versus drug therapy. ApoB levels in all of the patients' VLDL subfractions were elevated relative to those of normal control subjects during both treatment phases (Table 4). If one assumes that each VLDL particle contains one apoB molecule, there were, on the average, 8.8-fold more VLDL₁ particles in patients after the placebo period than in normal subjects; this was reduced to 3.4-fold above normal after lovastatin therapy. VLDL₂ levels were 5.8-fold elevated after the placebo period; VLDL₂ levels were reduced but not normalized by lovastatin, with a 4.1-fold elevation after lovastatin relative to normal subjects. VLDL₃ particle numbers, as indicated by apoB molarities, were hardly affected by lovastatin therapy and remained about threefold elevated relative to those of normal control subjects.

The molar ratio of cholesteryl ester to apoB in each subfraction, as presented in Table 4, represents the average number of cholesteryl ester molecules per VLDL particle, again assuming one apoB molecule per VLDL particle. The cholesteryl ester/apoB molar ratios also indicate that the VLDL subfractions, particularly VLDL₃, were enriched in cholesteryl ester during the placebo period and were near normal in cholesteryl ester content after lovastatin treatment (Table 4). For VLDL₁, there was a 34% average decrease in the number of cholesteryl ester molecules per VLDL particle after lovastatin treatment, from an average of 4,291 to 2,841 per VLDL₁, whereas normal VLDL₁ averaged

### Table 2. Concentrations of VLDL Components in Plasma

<table>
<thead>
<tr>
<th>Fraction/period</th>
<th>Protein</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
<th>Cholesteryl ester</th>
<th>Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL₁ P</td>
<td>9.8±3.5</td>
<td>133.6±48.3</td>
<td>10.6±4.9</td>
<td>14.2±13.4</td>
<td>27.9±11.2</td>
</tr>
<tr>
<td>D</td>
<td>4.5±2.4†</td>
<td>59.4±31.3†</td>
<td>4.1±2.4†</td>
<td>3.6±4.1†</td>
<td>12.4±6.3†</td>
</tr>
<tr>
<td>%Δ</td>
<td>−54†</td>
<td>−56†</td>
<td>−61</td>
<td>−75†</td>
<td>−42</td>
</tr>
<tr>
<td>VLDL₂ P</td>
<td>11.4±3.4</td>
<td>89.5±25.7</td>
<td>9.5±2.8</td>
<td>13.1±5.6</td>
<td>27.1±7.9</td>
</tr>
<tr>
<td>D</td>
<td>8.9±2.9</td>
<td>71.1±23.8†</td>
<td>6.6±2.5</td>
<td>8.6±6.7†</td>
<td>23.8±14.5</td>
</tr>
<tr>
<td>%Δ</td>
<td>−22</td>
<td>−21</td>
<td>−30</td>
<td>−34</td>
<td>−12</td>
</tr>
<tr>
<td>VLDL₃ P</td>
<td>22.9±13.2</td>
<td>100.3±60.2</td>
<td>15.3±8.3</td>
<td>34.7±23.0</td>
<td>44.4±26.3</td>
</tr>
<tr>
<td>D</td>
<td>22.3±8.9</td>
<td>102.9±43.4</td>
<td>15.1±5.7</td>
<td>25.7±13.0</td>
<td>48.3±30.0</td>
</tr>
<tr>
<td>%Δ</td>
<td>−3</td>
<td>+3</td>
<td>−1</td>
<td>−26</td>
<td>+9</td>
</tr>
<tr>
<td>VLDL₄ N</td>
<td>1.5±1.3‖</td>
<td>19.1±16.6‖§</td>
<td>1.4±1.2‖§</td>
<td>1.0±1.0‖</td>
<td>3.5±3.1‖§</td>
</tr>
<tr>
<td>VLDL₂ N</td>
<td>2.4±1.6‡∥</td>
<td>19.3±12.9‡∥§</td>
<td>2.0±1.3‡∥§</td>
<td>1.9±1.5‡∥§</td>
<td>5.5±3.8‡∥§</td>
</tr>
<tr>
<td>VLDL₃ N</td>
<td>6.5±4.0‖∥</td>
<td>29.2±17.3‖∥</td>
<td>5.0±3.0‖∥</td>
<td>8.9±7.9‖∥</td>
<td>12.5±7.9‖∥</td>
</tr>
</tbody>
</table>

VLDL, very low density lipoprotein. Values are mean±SD in milligrams per deciliter.

* N, normolipidemic subjects (12 men, five women), with a mean age of 40±3 years; P, placebo phase; D, drug phase.

Probability values for drug/placebo were determined by paired t tests, probability values between patients and normal subjects by unpaired t tests.

† p<0.05, drug vs. placebo; ‡ p<0.001, placebo vs. normal; §§ p<0.01 drug vs. normal; ‖ p<0.01, placebo vs. normal; ∥ p<0.001, drug vs. normal.
but not after lovastatin therapy. The effective than IDL in suppressing reductase activity on
jects remained hypertriglyceridemic after therapy rather
ble 6). Lipoproteins isolated during the placebo period
were significantly more
activity than were lipoproteins isolated after lovastatin
 treatment. VLDL, and VLDL
2
9
27
- after lovastatin is consistent with the fact that the sub-
removal of these particles in vivo. The retention of the
or lovastatin periods. During the placebo period but not
hypertriglyceridemic VLDL, and hypertriglyceridemic
affinity binding to the LDL receptor is characteristic of
receptor-mediated cholesterol delivery to the cells (Ta-
the lovastatin treatment periods as a measure of LDL
in cultured human fibroblasts after both the placebo and
hypertriglyceridemic VLDL, and VLDL
2
). Of note, lovastatin therapy did not decrease the ability of
of binding of
d
binding to the LDL receptor, and the
K
s
of binding
2
VLDL subclasses and IDL. Although
less consistent in VLDL
3
and IDL subspecies. Although
changed little and two in-
more, and two were unchanged). It should be empha-
sized that VLDL
3
and IDL from normal subjects sup-
pression reductase to a similar extent to that of VLDL
3
three suppressed less, three
more, and two were unchanged). It should be empha-
suppression in this subclass. The response in IDL was
idal LDL receptor with a similar average reduction as
noted in VLDL
1
. The effects of lovastatin therapy were
less consistent in VLDL
1
and IDL subspecies. Although
subjects' VLDL
s
were less suppressive during lovastatin therapy, three changed little and two in-
creased, resulting in a small average decrease (–5%) in
suppression in this subclass. The response in IDL was
similar to that of VLDL
2
three suppressed less, three
more, and two were unchanged). It should be empha-
suppression reductase to an extent
5,9; i.e., these VLDL
3
and IDL appear normal in this respect.
Triglyceride Accumulation
VLDL subclasses from seven subjects were tested for
their abilities to cause rapid accumulation of triglycer-
ide in murine P388D
1
macrophages after both the placebo and the lovastatin periods (Table 7), under
conditions in which triglyceride accumulation is medi-
ated primarily by a distinct macrophage receptor for
abnormal TGRLPs.12,33 VLDL
1
caused little to no cel-
lular triglyceride accumulation, whether from placebo or
lovastatin periods. During the placebo period but not
after lovastatin therapy, VLDL
1
and VLDL
2
caused significantly greater average triglyceride accumulation than did VLDL
1
(p<0.05). Although the difference between placebo and lovastatin in VLDL
1
2
3
mg/dL)

<table>
<thead>
<tr>
<th>Fraction/p</th>
<th>Total mass</th>
<th>Protein</th>
<th>TG</th>
<th>Chol</th>
<th>Chol ester</th>
<th>Phospholipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>period*</td>
<td>(mg/dL)†</td>
<td>(%)‡</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
</tbody>
</table>
| VLDL
1
   P      | 196.1       | 5.00    | 68.2 | 5.41 | 7.23       | 14.2         |
|         | 84.0        | 5.36    | 70.7 | 4.88 | 4.29       | 14.8         |
|         | 26.5        | 5.66    | 72.1 | 5.28 | 3.77       | 13.2         |
| VLDL
2
   P      | 150.6       | 7.57    | 59.4 | 6.31 | 8.70       | 18.0         |
|         | 119         | 7.48    | 59.8 | 5.55 | 7.23       | 20.0         |
|         | 31.1        | 7.72    | 62.1 | 6.43 | 6.11       | 17.7         |
| VLDL
3
   P      | 217.6       | 10.5    | 46.1 | 7.03 | 15.9       | 20.4         |
|         | 214         | 10.4    | 48.0 | 7.04 | 12.0       | 22.5         |
|         | 62.1        | 10.5    | 47.0 | 8.1  | 14.3       | 20.1         |

TABLE 3. Average Percentage Compositions of VLDL Subclasses After Placebo and Lovastatin Treatment Periods

VLDL, very low density lipoprotein; TG, triglyceride; chol, cholesterol.
*P, placebo phase; D, drug phase; N, normal subjects.
†Values are expressed as total milligrams per deciliter, are calculated from average serum concentrations given in
Table 2, and represent the sum of the protein, triglyceride, unesterified cholesterol, esterified cholesterol, and
phospholipid contents in each fraction of plasma.
‡Percentages are calculated from the average total mass and average mass of each component in each fraction as
given in Table 2.

2,655 molecules of cholesteryl ester per particle. Similar
trends were seen in VLDL
2
and VLDL
3
, although less
pronounced than in VLDL
1
.

Interactions of VLDL Subclasses and IDL With the LDL Receptor
VLDL subclasses and IDL from eight patients on
placebo and lovastatin were tested for binding to the
LDL receptor of cultured human fibroblasts (Table 5). All lipoproteins demonstrated high-affinity, specific
binding to the LDL receptor, and the K
s
of binding of
each subclass were similar whether from the placebo or
lovastatin treatment period. The affinities of the VLDL
subspecies were higher than those of IDL, the latter
being similar to that of LDL, i.e., approximately 2.8 nM.32
Of note, lovastatin therapy did not decrease the ability
of any of the lipoproteins from hypertriglyceridemic pa-
tients to bind to LDL receptors, and so lovastatin treat-
ment would not impede hepatic LDL receptor-mediated
removal of these particles in vivo. The retention of the
abnormal, high-affinity binding of VLDL and VLDL
after lovastatin is consistent with the fact that the sub-
jects remained hypertriglyceridemic after therapy rather
than achieving normal plasma triglyceride levels (high-
affinity binding to the LDL receptor is characteristic of
hypertriglyceridemic VLDL
1
, and hypertriglyceridemic
VLDL
2
but not of normal VLDL
1
and VLDL
3
).
Table 4. Levels (Concentration) of ApoB and Cholesteryl Ester in VLDL Subspecies

<table>
<thead>
<tr>
<th>Fraction/period*</th>
<th>ApoB</th>
<th>Molar ratio of CE/apoB$</th>
<th>CE†</th>
<th>ApoB‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL₁ P</td>
<td>14.2±13.4</td>
<td>2.6±1.1</td>
<td>8.8</td>
<td>4,291</td>
</tr>
<tr>
<td></td>
<td>(218)</td>
<td>(0.0508)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>3.6±4.1</td>
<td>1.0±0.55</td>
<td>3.4</td>
<td>2,841</td>
</tr>
<tr>
<td></td>
<td>(55.4)</td>
<td>(0.0195)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1.0±1.0</td>
<td>0.30±0.227**</td>
<td>1.0</td>
<td>2,655</td>
</tr>
<tr>
<td></td>
<td>(15.4)</td>
<td>(0.0058)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VLDL₂

<table>
<thead>
<tr>
<th>Fraction/period*</th>
<th>ApoB</th>
<th>Molar ratio of CE/apoB$</th>
<th>CE†</th>
<th>ApoB‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>13.1±5.6</td>
<td>4.3±1.4</td>
<td>5.8</td>
<td>2,393</td>
</tr>
<tr>
<td></td>
<td>(201)</td>
<td>(0.084)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>8.6±6.7</td>
<td>3.1±1.21†</td>
<td>4.1</td>
<td>2,182</td>
</tr>
<tr>
<td></td>
<td>(132)</td>
<td>(0.0605)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1.9±1.5</td>
<td>0.75±0.54**</td>
<td>1.0</td>
<td>2,000</td>
</tr>
<tr>
<td></td>
<td>(29.2)</td>
<td>(0.0146)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VLDL₃

<table>
<thead>
<tr>
<th>Fraction/period*</th>
<th>ApoB</th>
<th>Molar ratio of CE/apoB$</th>
<th>CE†</th>
<th>ApoB‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>34.7±23.0</td>
<td>11.6±6.9</td>
<td>3.2</td>
<td>2,352</td>
</tr>
<tr>
<td></td>
<td>(534)</td>
<td>(0.227)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>25.7±13.0</td>
<td>11.1±4.1#</td>
<td>3.1</td>
<td>1,820</td>
</tr>
<tr>
<td></td>
<td>(395)</td>
<td>(0.217)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>8.9±7.9</td>
<td>3.6±2.5**</td>
<td>1.0</td>
<td>1,949</td>
</tr>
<tr>
<td></td>
<td>(137)</td>
<td>(0.0703)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Apo, apolipoprotein; VLDL, very low density lipoprotein; CE, cholesteryl ester.

*P, placebo phase; D, drug phase; N, normal subjects.
†CE molarities of each VLDL subspecies were calculated from Table 2 average plasma values by using a molecular weight of 650 for CE.
‡ApoB molarities were calculated from average mass values determined as described in "Methods," using a molecular weight of 512,000 for apoB.
§In nanomoles of CE per nanomole of apoB. If one assumes one apoB molecule per VLDL particle, this ratio gives the average number of molecules of CE per VLDL particle.

Table 5. $K_0$ of LDL Receptor Binding of Lipoproteins After Placebo Versus Lovastatin Period*

<table>
<thead>
<tr>
<th>Code</th>
<th>VLDL₁</th>
<th>VLDL₂</th>
<th>VLDL₃</th>
<th>IDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=8)</td>
<td>0.89±0.42†</td>
<td>0.86±0.50†</td>
<td>1.40±0.76§</td>
<td>2.70±0.86§</td>
</tr>
<tr>
<td>Lovastatin (n=8)</td>
<td>0.73±0.26†</td>
<td>0.84±0.41**</td>
<td>1.46±0.81†</td>
<td>3.44±1.48§</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein.

*Disassociation constants ($K_a$) of binding to the LDL receptor were determined in cultured normal human skin fibroblasts in standard 4°C direct-binding studies as described in "Methods" and as previously published. $K_a$ were derived by Scatchard analysis of 4°C specific-binding curves and are expressed as nanomolar concentrations. There were no statistically significant ($p<0.05$) differences between $K_a$ of comparable subclasses during placebo versus lovastatin periods by paired t tests.

$K_a$ of binding (nM)*

Table 6. Gianturco et al

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Discussion

This study demonstrates that lovastatin reduces plasma triglyceride levels and the levels of total VLDL, IDL, and LDL cholesterol (Table 4). This reduction is presumably due to enhanced LDL receptor-mediated uptake of the lipoproteins, since lovastatin is known to increase hepatic LDL receptor levels. Subfractionation of their total VLDL and quantification of their apoB levels demonstrated that the numbers of VLDL₁ and VLDL₂ but not of VLDL₃ particles were significantly reduced (Table 4). It is not apparent from this study why VLDL₃ particle numbers were not affected by treatment, but it is known that VLDL₃s are kinetically heterogeneous and that this heterogeneity is related to size. 34-36 In both normal and hypertriglyceridemic subjects, the VLDL₃ density subclass contains at least two metabolically distinct particle populations. 35 One of these is derived from large triglyceride-rich VLDL₁, S₃ >60, and its turnover within the interval S₁ 12–100 is slow, with little reaching LDL (S₁ 0–12). The other particle within the VLDL₃ subclass is rapidly turned over into LDL and represents a major precursor. 35

VLDL₃ is also the subclass in which LDL receptor binding can be mediated by either apoE or apoB in hypertriglyceridemic subjects, 32,37 further indicating metabolic heterogeneity and at least two subpopulations within this subclass. Although the average $K_a$ of binding of VLDL₃ to the LDL receptor did not change after treatment, as measured in vitro (Table 5), there may be changes in the metabolism of this subclass in vivo (i.e., increased transport into this subclass from VLDL₁ and VLDL₂ or direct synthesis) that compensate for any enhanced hepatic clearance due to increased LDL receptor number induced by the drug, resulting in little overall change in the VLDL₃ particle number.
TABLE 6. Suppression of HMG-CoA Reductase Activity by Lipoproteins From Placebo Versus Lovastatin Treatment Periods

<table>
<thead>
<tr>
<th>Period</th>
<th>VLDL$_3$ (µg/mL)</th>
<th>VLDL$_3$ (µg/mL)</th>
<th>VLDL$_2$ (µg/mL)</th>
<th>IDL (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>15</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Placebo (n=8)</td>
<td>54.6±27.4</td>
<td>62.7±25.5</td>
<td>43.1±30.7</td>
<td>63.6±25.3</td>
</tr>
<tr>
<td>Drug</td>
<td>34.9±23.6†</td>
<td>45.1±24.3†</td>
<td>38.2±21.4†</td>
<td>45.3±21.6†</td>
</tr>
<tr>
<td>Average* change (%)</td>
<td>−36.1</td>
<td>−28.1</td>
<td>−11.4</td>
<td>−28.8</td>
</tr>
<tr>
<td>p</td>
<td>0.073</td>
<td>0.088</td>
<td>NS</td>
<td>0.071</td>
</tr>
</tbody>
</table>

HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein. Values are mean±SD. Each lipoprotein was isolated after the placebo and drug-treatment phases and incubated for 5 hours at 8 and 15 µg protein per mL with cultured normal human skin fibroblasts that had been preincubated in lipoprotein-deficient serum to induce LDL receptor activity. The cells were harvested and assayed for HMG-CoA reductase activity as described in “Methods.” Since lipoproteins from the different treatment periods were tested in separate experiments, each value was expressed as the average percent suppression from duplicate dishes; variation between duplicates was ≤5%. Normal LDL at 10 µg/mL suppressed reductase activity by 86.3±7.7% (mean±SD of 12 experiments). Zero percent suppression is the unsuppressed HMG-CoA reductase activity of cells not exposed to lipoproteins. *Average percent change from placebo to lovastatin in extent of reductase suppression caused by each lipoprotein at the indicated concentration. †p<0.02, VLDL vs. IDL; NS, not significant.

Increased levels of VLDL$_3$ and IDL as seen in FCHL are associated with increased risk for CHD. In the present study, we were unable to measure a significant change in VLDL$_3$ particle number or properties. Lack of a change in any of the parameters measured in this study does not necessarily mean that there were no changes in the atherogenicity of a subpopulation within this range that may (or may not) reduce the progression of disease. Although VLDL$_3$ and IDL are associated with increased risk, this does not mean this is a causal relation. There may be a direct causal relation. Alternatively, their levels may reflect a precursor that is causative or, as suggested for LDL, that VLDL$_3$ and IDL as seen in FCHL may require some modification such as oxidation before they become atherogenic. Further studies both in vivo and in vitro may be able to distinguish among these possibilities.

In addition to lowering total VLDL particle number, lovastatin treatment altered the composition of the particles by decreasing the amount of cholesteryl ester carried by each particle (Tables 2–4). Normalization of plasma triglyceride levels with bezafibrate or nico- tinic acid also normalizes VLDL cholesteryl ester content in hypertriglyceridemic subjects. If hypertriglyceridemic VLDLs are atherogenic and if cholesterol is the most atherogenic component of a lipoprotein, then a reduction in the number of VLDL$_3$ and VLDL$_2$ particles coupled with a reduction in the cholesterol ester content of all particles could be considered as antiatherogenic effects of treatment.

Since VLDL$_1$ and VLDL$_2$ from subjects with normal fasting triglyceride levels do not bind to the LDL receptor, we anticipated that if lovastatin lowered plasma triglyceride levels, then these VLDL subspecies might have a reduced affinity for the LDL receptor (higher $K_d$) or not bind at all, as seen in hypertriglyceridemic subjects whose plasma triglycerides were normalized with bezafibrate. All VLDL subspecies bound to the LDL receptor with similar, high affinities on both placebo and lovastatin (Table 5), however, even

TABLE 7. Effects of VLDL Subclasses on Triglyceride Accumulation in Macrophages

<table>
<thead>
<tr>
<th>Patient code (P/D)</th>
<th>VLDL$_1$</th>
<th>VLDL$_2$</th>
<th>VLDL$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>D</td>
<td>P</td>
</tr>
<tr>
<td>024/025</td>
<td>22.0</td>
<td>9.2</td>
<td>14.4</td>
</tr>
<tr>
<td>013/014</td>
<td>25.7</td>
<td>15.1</td>
<td>12.4</td>
</tr>
<tr>
<td>011/010</td>
<td>5.4</td>
<td>4.0</td>
<td>2.6</td>
</tr>
<tr>
<td>022/023</td>
<td>6.9</td>
<td>−0.3</td>
<td>7.3</td>
</tr>
<tr>
<td>019/018</td>
<td>−1.5</td>
<td>9.6</td>
<td>3.0</td>
</tr>
<tr>
<td>007/008</td>
<td>10.4</td>
<td>12.1</td>
<td>10.7</td>
</tr>
<tr>
<td>017/016</td>
<td>13.4</td>
<td>5.1</td>
<td>5.6</td>
</tr>
</tbody>
</table>

P, placebo; D, drug. Lipoproteins were isolated as described in “Methods” and tested for their effects on cellular triglyceride levels in a 4-hour 37°C incubation of P388D$_5$ macrophages with each lipoprotein at 40 µg/mL, as described in “Methods.” Each value, expressed as micrograms triglyceride per milligram of cell protein, represents the average of values from duplicate dishes minus the average value obtained from control dishes incubated in the absence of lipoproteins to normalize experimental results, since basal levels of triglyceride in control cells varied from experiment to experiment. The average variation between duplicate dishes was 10.2%. Positive values represent triglyceride accumulation; negative values indicate the amount by which cellular triglyceride content was lower than that obtained in control cells incubated without lipoprotein.

*Within subclasses, P vs. D not significantly different; †p<0.05, compared with VLDL$_3$ placebo; ND, not determined.
though plasma triglyceride levels were lowered by treatment with lovastatin (Table 1). Retention of binding activity is consistent with the still-elevated plasma triglyceride levels that remained after lovastatin treatment; i.e., the patients remained moderately hypertriglyceridemic. Since LDL receptors are expressed primarily in the liver in vivo, binding of VLDL subclasses and IDL to the LDL receptor after lovastatin treatment provides a receptor-mediated mechanism for the efficient uptake and disposal of potentially atherogenic lipoproteins. Receptor-mediated removal of VLDL also reduces the LDL precursor pool and therefore diminishes production of LDL. Since binding of hypertriglyceridemic VLDL1 and hypertriglyceridemic VLDL2 to the LDL receptor is known to be mediated by a conformation of apoE that is absent in normal VLDL1 and VLDL2,10,32,37 these results indicate that the VLDL retained sufficient apoE for LDL receptor binding after lovastatin treatment, although apoE levels were not measured directly in this study. Based on studies in which apoE levels in hypertriglyceridemic VLDL1 were related to the binding constants for the LDL receptor, hypertriglyceridemic VLDL1 containing 3–6 moles of apoE per mole of VLDL1, have $K_{d}$ similar to that of LDL (2–4 nM), whereas normal VLDL1, which contain 1–2 moles apoE per mole of VLDL, do not bind to the LDL receptor at all.9,32 $K_{d}$ ≤1 nM occur in hypertriglyceridemic VLDL1, containing ≥6 moles apoE per mole of hypertriglyceridemic VLDL1 (S.H. Gianturco and W.A. Bradley, unpublished observations).

Even though binding to the LDL receptor was not diminished by lovastatin, the abilities of the VLDL1 and VLDL2 subclasses to suppress HMG-CoA reductase activity via the LDL receptor pathway were, in general, diminished after lovastatin therapy (Table 6), indicating reduced LDL receptor-mediated cholesterol delivery. The average decrease in reductase suppression by VLDL after lovastatin was 32% and was most likely due to the similar percent decrease (34%) in cholesterol ester content of VLDL after lovastatin. There were no significant changes in VLDL3 and IDL suppression after treatment. There were no statistically significant differences among subclasses during the placebo period. After lovastatin, however, VLDL1 and VLDL2 were less suppressive than VLDL3 or IDL, consistent with their decreased cholesterol ester content.

Decreased LDL receptor-mediated cholesterol delivery, because of reduced cholesterol ester content per VLDL particle, could be considered beneficial in two ways. First, hepatic uptake of particles containing less total cholesterol lessens the reduction of hepatic LDL receptors that would occur with the cholesterol ester–enriched VLDL from the placebo period. Active hepatic LDL receptor–mediated removal of these potentially atherogenic particles could account in part for the lower plasma levels of VLDL1 and VLDL2 observed after lovastatin treatment. Second, the VLDL1 and VLDL2 after treatment would deliver less cholesterol to peripheral arterial cells if the lipoproteins were to be internalized by these cells via the LDL receptor. The 34% decrease in cholesterol ester per VLDL after the lovastatin period was not a trivial amount. This is an average decrease of 1,450 molecules of cholesterol ester for each VLDL1 particle after lovastatin therapy, a remarkable decrease when one considers that one average LDL particle contains only 1,310 total cholesterol ester molecules per particle.43

The occurrence of foam cells in hypertriglyceridemic subjects44 suggests that TGRLPs can induce foam cell formation in vivo, since most plasma triglycerides are found in TGRLPs. Although macrophage-derived arterial foam cells are engorged with cholesterol ester, initial receptor-mediated uptake of TGRLPs in vitro results in accumulation of more triglyceride than cholesterol ester, reflecting the composition of the particle.11,33 Severely diabetic subjects with fasting chylomicrons develop macrophage-derived foam cells in eruptive xanthomas that are filled with more triglyceride than cholesterol ester,45 as seen in vitro.11,33 After insulin therapy, the chylomicrons disappear and foam cell triglyceride is rapidly lost, leaving cholesterol ester as the predominant lipid, until with time and continued treatment, this too is resolved,45 suggesting that the TGRLPs caused foam cell formation. The faster loss of triglyceride from xanthoma foam cells is probably due to the >100-fold greater hydrolysis of macrophage triglycerides than cholesterol esters.46 A similar sequence might occur in developing arterial foam cells in hypertriglyceridemic subjects after receptor-mediated uptake of VLDL: initial triglyceride and cholesterol ester engorgement (triglyceride > cholesterol ester) followed by selective hydrolysis and removal of triglyceride, leaving cholesterol ester as the predominant lipid.

Chylomicrons and large VLDL subspecies ($S_{100}$–400) from certain hypertriglyceridemic subjects cause rapid, receptor-mediated lipid accumulation in macrophages.11,12,33 By contrast, VLDLs from normal subjects do not induce lipid accumulation in macrophages under identical conditions,11,33 even when incubated with peritoneal macrophages for 24 hours.11 Moreover, normal VLDLs do not bind to the macrophage plasma membrane proteins that are likely receptor candidates.12

The predominant lipid that accumulates intracellularly after receptor-mediated uptake of hypertriglyceridemic VLDL is triglyceride, reflecting the predominant lipid in the lipoprotein.11,33 Although hypertriglyceridemic VLDL can also induce cholesterol ester accumulation in P388D1 and J774 macrophages, Triglyceride accumulation in P388D1 macrophages after short-term incubations with VLDL under conditions in which lipoprotein lipase plays no role can be used as a sensitive and amplifying intracellular end point indicating uptake of VLDL via the macrophage receptor for abnormal TGRLPs,12,33 as HMG-CoA reductase suppression and acyl cholesterol acyl transferase activation have been used as sensitive and amplifying intracellular end points indicating lipoprotein uptake via the LDL receptor48 and uptake of $\beta$-VLDL with the LDL receptor–related protein.49 Recognizing that cholesterol and not triglyceride is the predominant lipid in atheroma, we nevertheless used triglyceride rather than cholesterol accumulation in 4-hour incubations as a more sensitive measure of VLDL uptake via the macrophage receptor for abnormal TGRLP12,23 or LDL receptor–mediated uptake. Only TGRLPs that
bind to the candidate macrophage receptor protein (a membrane binding protein of ~190 kD in P388D1 macrophages) cause triglyceride engorgement in 4-hour incubations under these conditions. By contrast, longer incubations in many types of macrophages can result in significant triglyceride accumulation as a result of macrophage lipoprotein lipase activity. Other macrophage lines that secrete lipoprotein lipase or express high levels of LDL receptors, such as J774 cells, are not suitable for studies of uptake by the macrophage TGLLP receptor because these additional mechanisms lead to triglyceride and cholesterol accumulation.

Lovastatin caused a general decrease (34% mean decrease) in the ability of the VLDL1 subspecies to induce rapid triglyceride accumulation in macrophages. Both VLDL1 and VLDL2 caused significantly more triglyceride accumulation than did VLDL1 during the placebo phase (p<0.05). After drug treatment, mean differences among subclasses were not statistically significant, consistent with the trend in the largest subclasses toward a decreased ability to cause macrophage triglyceride accumulation. Because of the limited number of subjects tested and because VLDLs from only two of the seven subjects were active inducers of triglyceride accumulation, the mean differences in drug versus placebo in VLDL1 did not achieve statistical significance. In the two subjects whose VLDL1 and VLDL2 caused marked macrophage triglyceride accumulation after the placebo period, lovastatin significantly decreased the ability of the VLDL1 to cause triglyceride accumulation by approximately 50% (p<0.05) (Table 7), suggesting thatlovastatin can decrease cellular uptake of VLDL1 and VLDL2 by the distinct macrophage receptor for abnormal TGLLPs without diminishing binding to the LDL receptor. Additional studies in a number of subjects whose VLDLs are active inducers of triglyceride accumulation, when not treated, are required to determine if this potentially desirable effect of treatment (diminished macrophage VLDL uptake) is general. Such studies would also help to identify the binding determinants for the receptor.

The binding determinants for the distinct macrophage receptor are not completely understood, but apoE is not required for this interaction. A good candidate for the binding determinant is one or more domains of apoB that are not expressed in normal VLDL or LDL but can be expressed in chylomicrons, certain hypertriglyceridemic VLDL1s, and after proteolysis of VLDL111,12,23. It is possible that subtle changes in the lipid composition of triglyceride-rich particles can induce apoprotein conformational changes that permit or prevent binding to the macrophage receptor.

Some types of hypertriglyceridemia such as FCHL have appeared to confer greater CHD risk than other types, such as familial hypercholesterolemia, although the absence of CHD risk in the latter or in subjects with types 4 and 5 hyperlipidemia is controversial. The hypertriglyceridemic subjects in our early studies11,12,23 were CHD patients and had type 4 or 5 hyperlipoprotein profiles, all of which caused triglyceride accumulation under similar11 or identical12,23 conditions as used in this study.

In the current study of subjects with mixed hyperlipidemia, the two subjects whose VLDL1 and VLDL2 were most active in causing macrophage triglyceride accumulations had no apparent clinical evidence of CHD, although one was only 35 years old. Only one subject’s VLDL1 failed to induce triglyceride accumulation on placebo; he also had no clinical evidence of CHD. The other four subjects, whose VLDL1 on placebo caused modest triglyceride accumulation, had CHD. Although this test with fasting VLDL appears to be an accurate indicator of binding to the macrophage receptor for abnormal TGRLPs, it may not be sufficient to distinguish persons at increased risk for CHD from those not at risk. Current studies are under way to test the hypothesis that use of postprandial rather than fasting TGRLPs may correlate better with increased risk for CHD. Preliminary studies are consistent with this hypothesis.

In summary, this study demonstrates that lovastatin 1) lowers plasma VLDL1 and VLDL2 but not VLDL3 levels; 2) changes the distribution of particles to a more normal pattern; 3) decreases the choleseryl ester content of the VLDL subspecies; 4) does not affect binding of any of the VLDL subspecies or IDL to the LDL receptor; 5) reduces LDL receptor-mediated cholesterol delivery to cells by VLDL1 and VLDL2; and 6) can reduce the ability of VLDL1 to cause rapid, receptor-mediated lipid accumulation in macrophages. Thus, lovastatin may be of potential benefit in decreasing the atherosclerotic complications of hypertriglyceridemia.

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S H Gianturco, W A Bradley, S Nozaki, G L Vega and S M Grundy

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