Gemfibrozil Reduces Postprandial Lipemia in Non–Insulin-Dependent Diabetes Mellitus

Mikko Syvánne, Helena Vuorinen-Markkola, Hannele Hilden, and Marja-Riitta Taskinen

The effect of gemfibrozil on postprandial lipoprotein metabolism was investigated in a 12-week, randomized, double-blind, placebo-controlled trial in 20 non–insulin-dependent diabetic patients with moderate hypertriglyceridemia. The patients were given a meal containing 78 g of fat and 345,000 units of vitamin A to label chylomicrons and their remnants. Plasma obtained at various times during the fat-load test was separated into six fractions by gradient-density ultracentrifugation. Gemfibrozil reduced the postprandial triglyceride response, measured as the area under the time-dependent concentration curve, on average by 32% in whole plasma, by 38% in the Svedberg flotation unit (Sₚ) 1,100–3,200 chylomicron fraction, by 36% in Sₚ 400–1,100 chylomicrons, and by 38% in the Sₚ 60–400 lipoproteins. Retinyl palmitate, a measure of intestinally derived particles, was reduced in plasma by 34%, in Sₚ 1,100–3,200 by 46%, in Sₚ 400–1,100 by 44%, and in Sₚ 60–400 by 37%. All these reductions were significant in comparison with the placebo group. Particles with Sᵥ<60 were not significantly affected. In contrast to earlier observations in healthy subjects, no significant negative correlations existed between postprandial lipemia and high density lipoprotein cholesterol or the postheparin lipoprotein lipase activity. The reduction of the potentially atherogenic chylomicron remnants may decrease the risk of atherosclerosis in non–insulin-dependent diabetes mellitus, a hypothesis that awaits testing in prospective studies.

KEY WORDS • non–insulin-dependent diabetes mellitus • chylomicrons • triglyceride-rich lipoproteins • chylomicron remnants • postprandial lipemia • lipid-lowering therapy • fibrates • gemfibrozil

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Since the suggestion by Zilversmit¹ more than 10 years ago that postprandial lipoprotein remnants may be atherogenic, several lines of evidence have emerged in support of this hypothesis. Remnants of triglyceride-rich lipoproteins (TRLs: chylomicrons and very low density lipoproteins [VLDLs]) are certainly atherogenic in fat-fed experimental animals and in humans with type III hyperlipoproteinemia.²⁻³ TRLs derived from hypertriglycerideremic humans are toxic to endothelial cells and are taken up by macrophages, resulting in foam cell formation.⁴ Moreover, recent case–control studies⁵⁻⁷ have suggested that individuals with angiographically verified coronary artery disease have elevated levels of chylomicron remnants in their postprandial plasma compared with control subjects without coronary lesions.

The risk of atherosclerotic vascular disease is markedly increased in non–insulin-dependent diabetes mellitus (NIDDM). Most of this excess morbidity cannot be explained by the conventional risk factors.⁸ In general, NIDDM patients have increased fasting levels of TRLs, notably VLDL triglyceride (TG).⁹⁻¹⁰ In nondiabetic subjects, a strong positive correlation has been shown to exist between fasting TG levels and the magnitude of the postprandial chylomicron remnant response.¹¹⁻¹² Therefore, it is rational to propose that NIDDM patients may have abnormalities in their postprandial lipoprotein metabolism. Indeed, our preliminary observations¹³ as well as those of others¹⁴ suggest that postprandial lipemia is more pronounced in NIDDM than in nondiabetic subjects.

Low levels of high density lipoproteins (HDLs) are another characteristic of the lipoprotein profile in NIDDM.⁹⁻¹⁰ Postprandial lipemia is one determinant of HDL₂ levels in healthy normolipidemic subjects,¹⁵ and low concentrations of HDL cholesterol may reflect an impaired metabolism of TRLs. The mechanisms underlying this association are not fully understood, but the two TG hydrolases, lipoprotein lipase (LPL) and hepatic lipase (HL), as well as cholesteryl ester transfer from HDL to apolipoprotein (apo) B–containing particles, are important mediators in this relation.¹⁶⁻¹⁸ There is subtle evidence that lipase activities are abnormal in NIDDM.¹⁹⁻²⁰ No studies as yet have addressed the question of whether the capacity to metabolize TRLs is a determinant of HDL levels or of coronary disease in diabetic populations.

Gemfibrozil is a lipid-regulating agent of the fibrate group.²¹ It lowers VLDL TG levels, increases HDL, and has variable effects on low density lipoproteins (LDLs), depending on lipoprotein phenotype.²²⁻²⁴ In general, few studies have tested the lipid-modifying actions of
gemfibrozil in NIDDM, but prevailing evidence suggests that its efficacy is largely comparable to that in nondiabetic individuals. Gemfibrozil has been shown to reduce postprandial lipemia in type IV hyperlipoproteinemia, but as yet the effect of gemfibrozil on postprandial lipoprotein metabolism in NIDDM has not been investigated.

The present study is a randomized, double-blind, placebo-controlled trial addressing the effect of gemfibrozil on postprandial lipid metabolism in NIDDM patients. To obtain detailed analyses of particles of various sizes, we have separated six lipoprotein fractions from postprandial plasma by density-gradient ultracentrifugation. In addition to measuring TG and cholesterol at various times, we have labeled intestinally derived lipoproteins, i.e., chylomicrons and their remnants, with retinyl palmitate (RP), which is a widely accepted marker of these particles.

Methods

Patients

Twenty patients (18 men and two women) participated in the study (Table 1). Their characteristics have been described in detail. NIDDM patients with a fasting serum TG concentration between 1.5 and 4.0 mmol/L were eligible; at the —6-week run-in visit, the mean TG level was 2.85 mmol/L. All patients were treated with oral hypoglycemic agents. In the gemfibrozil group, four patients received a sulfonylurea alone, five a combination of a sulfonylurea and metformin, and one of them was also taking a thiazide diuretic. All individuals in the gemfibrozil group received digoxin, and one patient who had suffered a myocardial infarction several years earlier, none had symptoms or signs of significant macrovascular or microvascular disease. In the placebo group, two subjects were treated for hypertension. One had nifedipine combined with metoprolol, and the other had enalapril therapy. Two individuals in the gemfibrozil group received digoxin, and one of them was also taking a thiazide diuretic. All had normal liver, kidney, and thyroid function. The patients gave their informed consent. The study protocol was approved by the Ethical Committee of the Helsinki University Central Hospital.

Table 1. Clinical Characteristics of the Patients at Baseline

<table>
<thead>
<tr>
<th></th>
<th>Gemfibrozil</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (M/F)</td>
<td>8/2</td>
<td>10/0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60±2</td>
<td>52±3</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>10±2</td>
<td>5±1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27±0.7</td>
<td>27±0.7</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.97±0.02</td>
<td>1.03±0.01</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>7.6±0.3</td>
<td>7.8±0.2</td>
</tr>
<tr>
<td>C-peptide (nmol/L)</td>
<td>0.86±0.07</td>
<td>0.98±0.11</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>9.3±0.5</td>
<td>9.0±0.6</td>
</tr>
</tbody>
</table>

HbA₁c, glycylated hemoglobin A₁c.

*p<0.05.

Study Design

Details of the study design have been reported elsewhere. Briefly, after a 6-week run-in period during which dietary instructions were given, the patients were admitted to the metabolic ward for baseline oral fat-load tests and other diagnostic procedures. The double-blind medication period lasted 12 weeks, during which the patients were randomized to receive gemfibrozil (Lopid, Parke-Davis, Pontypool, UK) 600 mg twice daily or a matching placebo. After 12 weeks, the metabolic studies were repeated.

Oral Fat-Load Test

Between 7:30 and 8 AM after an overnight fast, a plastic cannula was inserted into the patient’s antecubital vein and fasting blood samples were drawn. The fatty meal consisted of 200 mL cream (38% fat) and an egg yolk and contained a total of 78 g fat, 490 mg cholesterol, and 760 kcal energy. In addition, 345,000 IU of vitamin A was given. The meal was ingested over a 5-minute period, and it was well tolerated by the patients. Apart from the test meal, only water was allowed until the 9-hour blood sample had been drawn. Beyond that, regular hospital meals were served, i.e., a dinner at 5 PM and a light evening snack at 7:30 PM.

Blood was drawn into tubes containing EDTA before the fatty meal and 2, 3, 4, 6, 9, 12, and 24 hours thereafter. The samples were protected from light at all stages of processing. Plasma was separated promptly and kept at 4°C. Gradient-density ultracentrifugation was started within 24 hours from sampling.

Gradient-Density Ultracentrifugation

Plasma obtained at the various times during the oral fat-load test was separated by gradient-density ultracentrifugation, a modification of the method described by Lossow et al. Six fractions were isolated: large chylomicrons (chylol, Svedberg flotation units [Sf] >3,200), intermediate-size chylomicrons (chylol, S, 1,100–3,200), small chylomicrons (chylol, S, 400–1,100), large VLDL (VLDL₁, S, 60–400), small VLDL (VLDL₂, S, 20–60), and intermediate density lipoproteins (IDLs, S, 12–20). To separate the three chylomicron fractions, 2 mL of plasma, raised to a background density of 1.065 g/mL with solid sodium chloride, was placed in an ultracentrifuge tube (Ultra-Clear 14×95 mm, Beckman Inc., Palo Alto, Calif.) on top of 0.5 mL of 1.065 g/mL NaCl and carefully overlaid with a density gradient consisting of the following saline solutions: 1 mL 1.0464, 1 mL 1.0336, 2 mL 1.0271, 2 mL 1.0197, 2 mL 1.0117, and 2 mL 1.0060 g/mL. After centrifugation in a Beckman L 8-70 ultracentrifuge at 12,000 rpm (18,000g) for 41 minutes in a swinging-bucket rotor (SW 40 Ti), the chylol, fraction (0.5 mL) was removed by aspiration from the top of the tube. The chylol, and chylol, fractions (0.5 mL each) were collected similarly after centrifugations at 12,000 rpm (18,000g) for 1 hour and 12 minutes and at 20,000 rpm (50,500g) for 1 hour and 7 minutes, respectively. To separate the fractions with S, <400, 3 mL of plasma was first centrifuged in a 50.3 Ti rotor at 18,000 rpm for 30 minutes to remove the chylomicrons. Thereafter, VLDL₁ (S, 60–400), VLDL₂ (S, 20–60), and IDL (S, 12–20) were separated from 2 mL of chylomicron-free plasma essentially as detailed in previous
Quantification of Postprandial Lipemic Responses

Postprandial TG, RP, and cholesterol responses were quantified in plasma and in the lipoprotein fractions as follows. For each patient, the concentration of the measured parameter was plotted against time, and the area between the 24-hour concentration curve and zero level was determined by the trapezoidal rule. This area under the curve (AUC) measures the entire TG, RP, or cholesterol load to which the patient is exposed during the postprandial state, all of these fractions contain lipoproteins of both intestinal and hepatic origin. In six patients, TG was measured in the bottom fraction (LDL plus HDL) as well as in the six TG-rich fractions. The recovery of TG was 74±3% in the fasting samples and 81±3% in the 4-hour postprandial samples (mean±SEM).

Quantificaiton of Postprandial Lipemic Responses

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Other Procedures

In the morning of the day preceding the oral fat-load test, after an overnight fast, 100 IU of heparin per kilogram of body weight was given as an intravenous injection, and blood was drawn after 5 and 15 minutes into lithium–heparin tubes kept on ice. Plasma was promptly separated at 4°C and stored at -20°C. LPL and other lipoprotein activities were measured by an immunoneutralization assay as previously described. On a separate day during which the patients received standardized meals and their normal medicines, diurnal blood glucose and serum insulin profiles were determined from blood samples taken at 7:30 and 11:30 AM; 2, 4, and 8 PM; midnight; and 4 and 8 AM. C-peptide was determined from the fasting sample.

Insulin sensitivity was assessed at baseline and after the double-blind therapy by use of the insulin clamp technique as previously described in detail.

Lipoprotein Ultracentrifugation

Fasting serum lipoprotein fractions were separated by sequential flotation in an ultracentrifuge. Briefly, after isolation of chylomicrons from fresh fasting serum, VLDL (d<1.006), IDL (1.006<d<1.019), LDL (1.019<d<1.063), HDL1 (1.063<d<1.215), and HDL2 (1.215<d<1.210) were separated as described.

Laboratory Measurements

TG and cholesterol concentrations were determined with an automated Cobas Mira analyzer (Hoffman–La Roche, Basel, Switzerland) by enzymatic methods (Hoffman–La Roche kits 0722138 and 0715166, respectively). Blood glucose, serum C-peptide and insulin, and glycosylated hemoglobin (HbA1c; reference range, 4.0–6.0%) were measured as described previously.

RP assays were carried out by reverse-phase high-performance liquid chromatography (HPLC) essentially as described by Weintraub et al. All assays were carried out under subdued light. Retinyl acetate (R-4632, Sigma Chemical Co., St. Louis, Mo.) was added to the samples as an internal standard. The samples (500 μL of plasma and 400–500 μL of lipoprotein fractions) were mixed with ethanol, hexane, and water. The hexane phase was removed and evaporated under nitrogen. The residue was dissolved in 300 μL methanol/chloroform (4:1, vol/vol), and 200 μL was injected into an HPLC column (Spherisorb ODS-2, 5 μm, Superpac cartridge, LKB, Bromma, Sweden). Methanol (100%) was used as the mobile phase at a flow rate of 2 mL/min. The effluent was monitored at 330 nm, and the peak of RP was identified by comparison with the retention time of a purified standard (R-3375, Sigma).

Apo E photyping was done from serum by isoelectric focusing.

Statistical Analyses

Statistical comparisons between the gemfibrozil and placebo groups were done by the Mann-Whitney U test. Wilcoxon’s signed rank test was used to compare pretreatment and posttreatment values separately for the gemfibrozil and placebo groups. The 95% confidence intervals (CI) for the mean difference between pretreatment and posttreatment values were calculated. Pearson’s correlation coefficients were calculated after logarithmic transformation of parameters with skewed distributions.

Results

All patients completed the study. One subject in the placebo group had grossly elevated fasting (14.5 mmol/L) and postprandial TG levels at the 12-week visit and was excluded from data analyses as an outlier. The results of the placebo group are thus based on nine patients.

The baseline fasting lipoprotein concentrations and lipase activities of the two groups were comparable (Table 2). Overall, baseline fasting plasma TG ranged from 1.6 to 6.4 mmol/L and cholesterol from 3.7 to 7.5 mmol/L. In the placebo group, no significant changes occurred over the 12-week study period, although there was a trend to lower serum total and LDL cholesterol levels (p=0.051 and p=0.086, respectively, Table 2). In the gemfibrozil group, levels of total and VLDL TG and VLDL cholesterol decreased, whereas those of HDL cholesterol increased significantly. Total and LDL cholesterol levels showed a nonsignificant rise; however, together with an opposite change in the placebo group, this resulted in significantly higher LDL cholesterol levels in the gemfibrozil group compared with the placebo group at 12 weeks as reported in detail elsewhere (Table 2).

Postheparin plasma LPL and HL activities increased significantly in the gemfibrozil-treated patients (Table 2). The mean increase was 17% (95% CI, 7–27) for LPL and 19% (5–32) for HL. At 12 weeks, however, there
was no significant difference between the gemfibrozil and placebo groups in lipase activities.

**Postprandial Lipoprotein Responses**

At baseline, no major differences were observed between the gemfibrozil and placebo groups in any of the various postprandial responses measured (Tables 3 and 4). No significant changes were found in these parameters in the placebo group over the 12-week study period. In contrast, the gemfibrozil-treated group showed major reductions in lipoprotein concentrations measured after the fatty meal.

**Triglycerides.** Gemfibrozil significantly reduced plasma TG concentrations in the fasting state and at all times after the fatty meal (Figure 1A). Of note, the TG-lowering effect persisted until the late hours of the postprandial period, consistent with improved removal of TG-rich remnant particles. The mean reduction in plasma TG response, quantified as mean change of AUCs, was 32%. The postprandial TG increment, reflected by the AUIC, was also markedly diminished after gemfibrozil. Compared with the placebo group, TG levels were significantly lowered by gemfibrozil in the lipoprotein fractions chylo, chylomicron fraction S,>3,200; chylc, Sf 1,100-3,200; chylc, Sf 400-1,100; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein. Values are AUCs (lipase activities), mean±SEM.

Lipoprotein fractions were separated by ultracentrifugation. These data were available for nine patients in each group.

\*p<0.05, \*p<0.01 different from baseline, Wilcoxon’s test.

\*p<0.01 different from gemfibrozil group at 12 weeks, Mann-Whitney U test.

**Table 2.** Cholesterol and Triglyceride Concentrations and Postheparin Plasma Lipase Activities in Gemfibrozil- and Placebo-Treated Patients at Baseline and After 12 Weeks of Therapy

<table>
<thead>
<tr>
<th>Lipoprotein Fraction</th>
<th>Gemfibrozil (Baseline)</th>
<th>Gemfibrozil (12 Weeks)</th>
<th>Placebo (Baseline)</th>
<th>Placebo (12 Weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total chol</td>
<td>5.8±0.4</td>
<td>6.0±0.3</td>
<td>5.5±0.3</td>
<td>5.1±0.3</td>
</tr>
<tr>
<td>VLDL chol</td>
<td>0.97±0.15</td>
<td>0.51±0.06∗</td>
<td>1.09±0.19</td>
<td>1.17±0.24‡</td>
</tr>
<tr>
<td>HDL chol</td>
<td>3.3±0.3</td>
<td>3.9±0.3</td>
<td>3.1±0.3</td>
<td>2.6±0.3‡</td>
</tr>
<tr>
<td>LDL chol</td>
<td>1.15±0.07</td>
<td>1.25±0.07∗</td>
<td>1.07±0.08</td>
<td>1.10±0.08</td>
</tr>
<tr>
<td>HDL₂ chol</td>
<td>0.76±0.06</td>
<td>0.57±0.05</td>
<td>0.48±0.04</td>
<td>0.51±0.07</td>
</tr>
<tr>
<td>HDL₃ chol</td>
<td>0.54±0.03</td>
<td>0.63±0.04∗</td>
<td>0.55±0.05</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>Lipoprotein lipase</td>
<td>19±1.5</td>
<td>22±1.5∗</td>
<td>21±2.3</td>
<td>23±4.5</td>
</tr>
<tr>
<td>Hepatic lipase</td>
<td>31±5.0</td>
<td>36±5.0†</td>
<td>46±8.0</td>
<td>43±9.4</td>
</tr>
</tbody>
</table>

TG, triglyceride; VLDL, very low density lipoprotein; chol, cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein. Values are mmol/L (chol and TG) or μmol free fatty acid • hr⁻¹ • mL⁻¹ (lipase activities), mean±SEM.

%Change (CI) denotes mean percent change from baseline (95% confidence interval of the mean).

\*p<0.05, \*p<0.01 different from baseline, Wilcoxon’s test.

\*p<0.01 different from gemfibrozil group at 12 weeks, Mann-Whitney U test.

**Table 3.** Triglyceride Responses Quantified as Areas Under Time-Dependent Concentration Curves (AUC) or Concentration Curves Above Fasting Levels (AUIC) After Oral Fat Load

<table>
<thead>
<tr>
<th>Lipoprotein Fraction</th>
<th>Gemfibrozil (Baseline)</th>
<th>Gemfibrozil (12 Weeks)</th>
<th>Placebo (Baseline)</th>
<th>Placebo (12 Weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, AUC</td>
<td>82±8</td>
<td>51±3∗</td>
<td>-32 (-49, -14)</td>
<td>88±11</td>
</tr>
<tr>
<td>Chylo₁, AUC</td>
<td>2.4±0.4</td>
<td>1.8±0.3∗</td>
<td>-17 (-44, +11)</td>
<td>2.4±0.5</td>
</tr>
<tr>
<td>Chylo₂, AUC</td>
<td>2.0±0.3</td>
<td>1.2±0.1†</td>
<td>-38 (-56, -20)</td>
<td>2.3±0.4</td>
</tr>
<tr>
<td>Chylo₃, AUC</td>
<td>4.4±0.8</td>
<td>2.2±0.3*</td>
<td>-36 (-68, -4)</td>
<td>5.5±1.0</td>
</tr>
<tr>
<td>VLDL₁, AUC</td>
<td>29±4</td>
<td>14±1*</td>
<td>-38 (-65, -13)</td>
<td>26±3</td>
</tr>
<tr>
<td>VLDL₂, AUC</td>
<td>9.2±0.7</td>
<td>6.6±0.6*</td>
<td>-22 (-41, -5)</td>
<td>7.9±1.0</td>
</tr>
<tr>
<td>IDL, AUC</td>
<td>2.6±0.3</td>
<td>2.4±0.3</td>
<td>-5 (-27, +17)</td>
<td>2.2±0.4</td>
</tr>
<tr>
<td>Plasma, AUIC</td>
<td>18±3</td>
<td>8.6±2.2†</td>
<td>-60 (-113, -8)</td>
<td>18±2</td>
</tr>
<tr>
<td>Chylo₁, AUIC</td>
<td>2.5±0.4</td>
<td>1.4±0.2*</td>
<td>-37 (-56, -17)</td>
<td>3.0±0.3</td>
</tr>
<tr>
<td>VLDL₁, AUIC</td>
<td>4.9±2.0</td>
<td>3.9±0.8</td>
<td>-40 (-108, +29)</td>
<td>4.4±1.5</td>
</tr>
</tbody>
</table>

Chylo₁, chylomicron fraction S,>3,200; chylo₂, 400-1,100; chylo₃, 1,100-3,200; VLDL₁, very low density lipoprotein fraction S, 60-400; VLDL₂, 20-60; IDL, intermediate density lipoproteins, S, 12-20. Values are AUCs or AUICs (see “Methods” for definitions), mmol/L • hr, mean±SEM. Chylo and chyl AUCs are virtually identical to AUCs because of zero fasting concentrations. VLDL₂ and IDL AUICs were not calculated because the postprandial responses are not incremental. % Change (CI) denotes mean percent change from baseline (95% confidence interval of the mean).

\*p<0.05, \*p<0.01 different from baseline, Wilcoxon’s test.

\*p<0.05, §p<0.01 different from gemfibrozil group at 12 weeks, Mann-Whitney U test.
TABLE 4. Retinyl Palmitate Responses, Quantified as Areas Under Time-Dependent Concentration Curves (AUC), After Oral Fat Load

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Baseline</th>
<th>12 Weeks</th>
<th>% Change (CI)</th>
<th>Baseline</th>
<th>12 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>221 ± 27</td>
<td>142 ± 16†</td>
<td>-34 (-43, -24)</td>
<td>217 ± 29</td>
<td>231 ± 29‡</td>
</tr>
<tr>
<td>Chylo₁</td>
<td>7.3 ± 2.2</td>
<td>5.7 ± 1.7</td>
<td>+15 (-49, +79)</td>
<td>4.4 ± 1.2</td>
<td>6.6 ± 1.6</td>
</tr>
<tr>
<td>Chylo₂</td>
<td>21 ± 6</td>
<td>9.2 ± 2.9†</td>
<td>-46 (-66, -25)</td>
<td>15 ± 3</td>
<td>22 ± 6 $</td>
</tr>
<tr>
<td>Chylo₃</td>
<td>48 ± 9</td>
<td>24 ± 4†</td>
<td>-44 (-60, -28)</td>
<td>46 ± 7</td>
<td>50 ± 8‡</td>
</tr>
<tr>
<td>VLDL₁</td>
<td>36 ± 7</td>
<td>20 ± 3*</td>
<td>-37 (-54, -20)</td>
<td>25 ± 2</td>
<td>46 ± 10‡</td>
</tr>
<tr>
<td>VLDL₂</td>
<td>9.6 ± 1.4</td>
<td>8.1 ± 1.1</td>
<td>-8.9 (-38, +21)</td>
<td>7.6 ± 0.7</td>
<td>8.1 ± 1.8</td>
</tr>
<tr>
<td>IDL</td>
<td>2.6 ± 0.4</td>
<td>3.4 ± 0.3</td>
<td>+43 (-12, +97)</td>
<td>2.3 ± 0.5</td>
<td>2.8 ± 0.4</td>
</tr>
</tbody>
</table>

Chylo₁, chylomicron fraction S₉>3,200; chylo₂, S ₁,100–3,200; chylo₃, S 400–1,100; VLDL₁, very low density lipoprotein fraction S ₉, 60–400; VLDL₂, S ₂0–60; IDL, intermediate density lipoproteins, S ₁2–20. Values are AUCs (see "Methods" for definitions), μmol/L · hr, mean ± SEM. % Change (CI) denotes mean percent change from baseline (95% confidence interval of the mean).

* p<0.05, † p<0.01 different from baseline, Wilcoxon's test.

Gemfibrozil had no effect on these fractions.

To control for the small differences between the gemfibrozil and placebo groups with respect to age, duration of diabetes, and apo E phenotype distribution, these variables were included in an analysis of covariance. The between-group differences in postprandial TG and RP responses were not affected by these adjustments.

Cholesterol. Plasma cholesterol levels during the postprandial period were not influenced by gemfibrozil (data not shown). The chylomicron fractions contained minute amounts of cholesterol, which were further diminished after gemfibrozil in parallel to changes in TG concentrations. In VLDL₁ as well, cholesterol and TG changes were similar.

Lipoprotein compositions. Plasma TG:RP ratio decreased during the early postprandial period to a nadir at 6 hours, consistent with removal of TG by LPL-mediated lipolysis. After 6 hours the ratio steadily increased, indicating chylomicron remnant removal into the liver occurring in parallel with continuing and possibly increased production of endogenous TG. Gemfibrozil had no effect on the TG:RP ratio in plasma or any of the lipoprotein fractions studied.

The plasma TG:cholesterol ratio was decreased in the gemfibrozil group before and at all times after the fatty meal, in accord with decreased TG and unchanged cholesterol levels. However, this ratio was not changed in any of the fractions separated by gradient-density ultracentrifugation. This observation is consistent with the similar reductions of TG and cholesterol in the fractions.

FIGURE 1. Graphs showing postprandial plasma triglyceride (panel A, gemfibrozil; panel B, placebo group) and retinyl palmitate (panel C, gemfibrozil; panel D, placebo) responses. ●, At baseline; ○, after 12 weeks of therapy. Data points are group mean values; bars are SEM.
Correlations of Postprandial Responses With Fasting Lipoproteins and Other Baseline Characteristics

Associations between fasting lipoproteins and postprandial responses were tested by calculating Pearson's correlation coefficients, using logarithmic transformations where appropriate (Figures 3A-3C). The postprandial TG response (AUC) was strongly related to fasting plasma TG \((r=0.96, p<0.001)\) and VLDL TG \((r=0.93, p<0.001)\). Plasma RP responses (AUC) also were significantly predicted by fasting TG (plasma, \(r=0.63, p<0.01\); VLDL, \(r=0.65, p<0.01\)). In contrast, the incremental response (AUIC) of plasma TG after the fatty meal was not related to fasting plasma \((r=0.04)\) or VLDL \((r=0.03)\) TG.

There was only a weak negative correlation \((r=-0.36, p=0.14)\) between fasting HDL cholesterol and postprandial lipemia measured as TG AUC. No correlation existed between HDL cholesterol and TG AUIC or RP AUC. HDL \(_2\) cholesterol and HDL \(_2\) TG concentrations were also unrelated to the measures of postprandial lipemia. In contrast, HDL \(_3\) TG showed positive and HDL \(_3\) cholesterol negative correlations with the lipemic responses. The ratio of cholesterol to TG in the HDL \(_3\) fraction was inversely correlated with RP AUCs of plasma \((r=-0.65, p<0.01)\), chylo \(_2\) \((r=-0.48, p<0.05)\), chylo \(_3\) \((r=-0.49, p<0.05)\), VLDL \(_1\) \((r=-0.64, p<0.01)\), and VLDL \(_2\) \((r=-0.54, p<0.05)\). Thus, high levels of chylomicrons and their remnants were associated with TG enrichment of HDL \(_3\) particles. Gemfibrozil did not alter the ratio of cholesterol to TG in the HDL \(_3\) fraction. As for HDL \(_2\), this ratio increased from 6.7±1.2 to 7.7±1.3 (mean±SEM) in the gemfibrozil group and decreased from 5.6±0.9 to 4.9±0.5 in the placebo group \((p=0.058 at 12 weeks between the groups)\).

We also looked at interrelations between the post-heparin plasma lipase activities and the postprandial lipemic responses. LPL showed only a modest trend toward negative correlations with incremental plasma TG (AUIC, \(r=-0.32, \text{NS}\)) and VLDL TG (AUIC, \(r=-0.36, \text{NS}\)). HL was inversely related to the IDL TG response \((r=-0.59, p<0.05)\).

Ten subjects (gemfibrozil, seven; placebo, three) had the apo E phenotype \(3/3\), and nine (gemfibrozil, three; placebo, six) had the \(4/3\) phenotype. Postprandial lipemia tended to be more pronounced in those with the \(e4\) allele, but the differences were not significant.

There was no correlation between parameters of glycemic control (HbA\(_1c\), fasting blood glucose, and mean diurnal blood glucose concentrations) and fasting or postprandial lipoprotein levels. Nor were there significant correlations between C-peptide, fasting or diurnal insulin levels, or insulin sensitivity (glucose disposal measured by the hyperinsulinemic clamp technique) on the one hand and fasting or postprandial lipid parameters on the other.

**FIGURE 2.** Graphs showing postprandial responses in lipoprotein fractions before and after gemfibrozil. ●, At baseline; ○, after 12 weeks of therapy. Panels A and D, triglyceride and retinyl palmitate profiles in the chylo \(_2\) (Svedberg flotation unit \([S_f]\) 1,100–3,200) fraction. Panels B and E, chylo \(_3\) (S\(_f\) 400–1,100). Panels C and F, VLDL \(_1\) (S\(_f\) 60–400). Chylo \(_2\) (S\(_f\) >3,200), VLDL \(_2\) (S\(_f\) 20–60), and IDL (S\(_f\) 12–20) fractions are not shown. Chylo, chylomicron; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein.
FIGURE 3. Scatterplots showing correlations of postprandial lipemia with fasting plasma triglyceride. Panel A: Postprandial triglyceride (TG) response (area under the curve [AUC], see “Methods”) vs. fasting TG, \( r=0.96, p<0.001 \). Panel B: TG incremental response (area under the incremental curve [AUIC]) vs. fasting TG, \( r=0.04, p=\text{NS} \). Panel C: Retinyl palmitate (RP) AUC vs. fasting TG, \( r=0.63, p<0.01 \).

Determinants of Response to Gemfibrozil

Absolute reductions in postprandial TG responses (baseline AUC minus 12-week AUC) in the gemfibrozil group were well predicted by pretreatment fasting plasma TG \( r=0.93, p<0.001 \) and VLDL TG \( r=0.96, p<0.001 \) levels; i.e., hypertriglyceridemic individuals who also tended to have exaggerated postprandial lipemia had the greatest reductions. There was also a trend toward correlation between baseline fasting plasma TG and the decrement of postprandial plasma RP response \( r=0.58, p=0.079 \). In contrast, baseline fasting VLDL TG was not a significant predictor of the change in incremental TG response (AUIC, \( r=0.29, \text{NS} \)). Although gemfibrozil increased the postheparin plasma LPL activity, this change was not correlated with alterations in any of the postprandial lipemic measures.

Discussion

The key finding of the present study was the marked reduction of postprandial lipemia in NIDDM patients by gemfibrozil. It was of interest to separate particles of various sizes by density-gradient ultracentrifugation in addition to measuring the lipemic response in native plasma. We found that gemfibrozil reduced particles larger than S\(_{400}\), which represent almost exclusively intestinally derived chylomicrons and their partially hydrolyzed remnants. The postprandial VLDL\(_{1}\) fraction (S\(_{60-400}\)) is a mixture of smaller chylomicron remnants and endogenous VLDL that are not separable by size or density. By using RP as a label of the intestinally derived particles, however, we could show that chylomicron remnants within this size range were also effectively reduced by gemfibrozil. In contrast, changes in the postprandial VLDL\(_{2}\) fraction (S\(_{20-60}\)) were minor, with no significant differences from the placebo group, and no effect was seen in the IDL fraction (S\(_{12-20}\)).

Two previous investigations have studied the effect of fibrates on postprandial lipid metabolism in nondiabetic subjects. Our results agree with those of Weintraub et al,\(^1\) who treated seven hypertriglyceridemic (type IV) patients with gemfibrozil. They found significantly reduced postprandial RP responses, particularly in the S\(_{1,000}\) but also in the S\(_{1,000}\) fractions. In contrast, our results only partly agree with those of Simpson et al.\(^6\) Using fenofibrate, they also observed lowered TG concentrations in the postprandial \( d<1.006 \) fraction but no reductions in RP levels. It is unclear whether these dissimilarities reflect different patient populations or differences between gemfibrozil and fenofibrate.

Of interest are the mechanisms underlying the action of gemfibrozil on postprandial lipoprotein metabolism. In general, the mode of action of fibrates is complex and only partly understood. Most authorities believe that fibrates both reduce hepatic VLDL production and enhance its catabolism.\(^20,21\) There is evidence that the increased fractional catabolic rate of VLDLs is quantitatively more important than their reduced synthesis.\(^22\) Grundy and Vega\(^21\) have emphasized the LPL-raising effect of the fibrates and suggested that it may be the most important mechanism by which these drugs promote the catabolism of VLDL. However, the present data do not indicate that increased LPL activity is the main mechanism by which gemfibrozil reduces postprandial lipemia. First, although there was a 17% increase in postheparin plasma LPL activity in the gemfibrozil group, there was no significant difference compared with the placebo-treated patients. Second, there was no correlation between the changes in the postprandial lipid responses and the increment in LPL activity. Finally, an unchanged TG:RP ratio argues against a significant enhancement of LPL-mediated TG hydrolysis of the chylomicron particles.

Although glycemic control and insulin sensitivity have multiple effects on lipoproteins in diabetes,\(^10\) the reduction of postprandial lipemia observed in this study cannot be explained by these metabolic factors because they were not affected by gemfibrozil.

One feasible mechanism that may underlie the reduction of postprandial lipoproteins during gemfibrozil...
therapy is decreased competition for catabolic sites between intestinal and hepatic lipoprotein particles. Such competition may take place at the lipolytic as well as receptor-mediated removal stages. The reduction of endogenous VLDL TG levels by fibrates, also documented in this study, would leave more catabolic sites available for the processing and removal of intestinally derived lipoprotein particles. The unchanged TG:RP ratio of postprandial lipoproteins during gemfibrozil therapy is compatible with enhanced removal of whole particles via hepatic receptors. Other, so far unknown, mechanisms can also be speculated upon. Fibrates are known to exert diverse effects on the liver, including alterations in the expression of several apoprotein genes. Thus, one can hypothesize that gemfibrozil might increase the number of chylomicron remnant receptors on hepatocytes.

Although the main focus of this study was to evaluate the effect of fibrate therapy on postprandial lipoprotein metabolism, the baseline data enabled us to explore some of the determinants of alimentary lipemia in NIDDM. Like Cohn et al., we found a highly significant positive correlation between fasting plasma TG concentrations and total postprandial TG responses (AUC, Figure 3A). This is not surprising, since a large part of the area is directly determined by the fasting TG level. Also, in accord with Groot et al., we observed that fasting plasma TG levels were moderately predictive of postprandial plasma RP responses (Figure 3C). A similar relation was reported by Weintraub et al. for fasting TG versus chylomicron RP responses in hypertriglyceridemic patients. However, in contrast to Cohn et al., Patsch et al., and Groot et al., we observed no correlation between fasting TG levels and the incremental TG responses (AUIC, Figure 3B). This discrepancy can be accounted for by differences in study populations. The three other groups studied normolipidemic individuals, whereas our patients have NIDDM and most of them have various degrees of fasting hypertriglyceridemia. On the other hand, Lewis et al. reported a significant correlation between fasting TG levels and the postprandial TG increment in a population of normotriglyceridemic patients. We recognize that our sample size is relatively small, and this may disguise subtle interrelations. Further studies are warranted to discover which metabolic disturbances modulate postprandial responses in NIDDM and in hypertriglyceridemic individuals.

One puzzling aspect is that we could not observe a significant correlation between alimentary lipemia and HDL or HDL2 cholesterol levels. This contrasts with data by Patsch and coworkers demonstrating that in healthy volunteers, significant negative correlations (Pearson coefficients on the order of −0.60 to −0.86) exist between incremental TG responses on the one hand and HDL or HDL2 (but not HDL3) on the other. However, Cohen et al. found no correlation between postprandial lipemia and HDL cholesterol in a population entirely different from ours, namely, healthy endurance-trained men. These workers suggested that the lack of correlation might have resulted partly from the narrow range of postprandial responses in their study. We do not believe that this explanation is relevant to our results because our TG AUCs varied by more than threefold (range, 48–157 mmol/L·hr) and HDL cholesterol by almost twofold (range, 0.80–1.42 mmol/L). Instead, we propose that in NIDDM, there may be a derangement in the interrelation between TRL catabolism and HDL that remains to be characterized.

Moreover, relations between alimentary lipemia and postheparin lipase activities appear to be disrupted in our NIDDM patients. According to Patsch et al., the postprandial TG increment is related inversely to LPL and positively to HL activity. We found no significant correlations between LPL and any postprandial lipemic measure, and for HL there was only a negative correlation with the IDL TG response, compatible with the putative role of HL in IDL catabolism in hyperlipidemia. In agreement, Weintraub et al. observed inverse relations between LPL activity and the postprandial chylomicron RP response and between HL activity and the nonchylomicron RP response only in normal subjects but not in hypertriglyceridemic patients.

Finally, what are the implications of our findings for the prevention of atherosclerosis in NIDDM? Over the past few years, data have accumulated to imply a role for the remnants of TRLs, including chylomicron remnants, in the pathogenesis of occlusive arterial disease and its complications. In a case–control study, the relative amount of chylomicron remnants in lipoproteins Sₚ>60 was a significant predictor of coronary artery disease. Notably, in the present study, this fraction was significantly reduced by gemfibrozil. Moreover, hypertriglyceridemic VLDLs have been found to stimulate the secretion of plasminogen activator inhibitor–1 from endothelial cells, linking TRLs to impaired fibrinolysis and a thrombosis-prone state. Against this background, we hypothesize that reducing chylomicrons and their remnants may have antiatherosclerotic and antithrombotic potential in NIDDM. This is supported by the results of the Helsinki Heart Study, in which gemfibrozil reduced cardiac events in hyperlipidemic middle-aged men, and most of this benefit was confined to individuals with hypertriglyceridemia and low HDL cholesterol, a lipoprotein pattern resembling that of NIDDM. On the other hand, the beneficial effects of gemfibrozil observed in the present study may be limited by lack of effect on the smaller particles (Sₚ<12), which constitute at least 75% of TRL particles in the fasting state and are associated with coronary artery disease and its progression. Since no prospective studies have yet been conducted to test the effect of lipid-lowering therapy on atherosclerosis in NIDDM, such studies are urgently needed.

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