Ciprofibrate Therapy Improves Endothelial Function and Reduces Postprandial Lipemia and Oxidative Stress in Type 2 Diabetes Mellitus

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Background—Exaggerated postprandial lipemia (PPL) is a factor in atherogenesis, involving endothelial dysfunction and enhanced oxidative stress. We examined the effect of ciprofibrate therapy on these parameters in type 2 diabetes mellitus.

Methods and Results—Twenty patients entered a 3-month, double-blind, placebo-controlled study. Each subject was studied fasting and after a fatty meal, at baseline, and after 3 months of treatment. Glucose and lipid profiles were measured over an 8-hour postprandial period. Endothelial function (flow-mediated endothelium-dependent vasodilation [FMD]) and oxidative stress (electron paramagnetic resonance spectroscopy) were measured after fasting and 4 hours postprandially. At baseline, both groups exhibited similar PPL and deterioration in endothelial function. After ciprofibrate, fasting and postprandial FMD values were significantly higher (from 3.8 ± 1.8% and 1.8 ± 1.3% to 4.8 ± 1.1% and 3.4 ± 1.1%; P < 0.05). This was mirrored by a fall in fasting and postprandial triglycerides (3.1 ± 2.1 and 6.6 ± 4.1 mmol/L to 1.5 ± 0.8 and 2.8 ± 1.3 mmol/L, P < 0.05). Fasting and postprandial HDL cholesterol was also elevated (0.9 ± 0.1 and 0.8 ± 0.1 mmol/L and 1.2 ± 0.2 and 1.2 ± 0.1 mmol/L, P < 0.05). There were no changes in total or LDL cholesterol. Fasting and postprandial triglyceride enrichment of all lipoproteins was attenuated, with cholesterol depletion of VLDL and enrichment of HDL. There were similar postprandial increases in oxidative stress in both groups at baseline, which was significantly attenuated by ciprofibrate (0.3 ± 0.6 versus 1.5 ± 1.1 U, P < 0.05).

Conclusions—This study demonstrates that fibrate therapy improves fasting and postprandial endothelial function in type 2 diabetes. Attenuation of PPL and the associated oxidative stress, with increased HDL cholesterol levels, may be important. (Circulation. 2000;101:1773-1779.)

Key Words: diabetes mellitus ■ lipemia ■ endothelium ■ stress

Cardiovascular disease is the most common complication of type 2 diabetes. Many established risk factors for atherosclerosis are characteristic of type 2 diabetes. The so-called metabolic syndrome includes a dyslipidemia characterized by hypertriglyceridemia, reduced HDL-cholesterol (HDL-C), and abnormal postprandial lipemia (PPL). Recent evidence suggests that glyceremia is not the major determinant of coronary heart disease (CHD) in type 2 diabetes, with fasting hypertriglyceridemia being an independent predictor of CHD in type 2 diabetes. Furthermore, fasting hypertriglyceridemia (elevated triglyceride [TG]-rich VLDL) in nondiabetic subjects with normal total and LDL cholesterol levels is associated with endothelial dysfunction (ED). PPL represents the state of absorption during which TG metabolism is under challenge. Because many factors involved in postprandial lipid metabolism are insulin sensitive, there is potential for abnormalities of PPL to arise in type 2 diabetes. PPL in type 2 diabetes consists of prolonged and exaggerated excursions in plasma TG with subsequent lipoprotein TG enrichment. A significant relationship between PPL and coronary atherosclerosis has been described in subjects with and without type 2 diabetes. After a fatty meal, nondiabetic subjects demonstrate transient ED, which can be attenuated by the antioxidant vitamins C and E. No dysfunction is noted after a low-fat meal. Thus, lipoprotein TG enrichment during PPL results in an atherogenic lipoprotein profile, putatively causing enhanced oxidative stress and ED.

Studies have consistently demonstrated the presence of ED in type 2 diabetes. These, however, were conducted in...
Fibrates are a widely used class of lipid-regulating agents, exerting a variety of effects on lipid and lipoprotein metabolism, particularly attenuation of PPL and reduction in TG-rich VLDL. We therefore studied the effect of ciprofibrate therapy on endothelial function, oxidative stress, and PPL in type 2 diabetes in a double-blind, placebo-controlled study.

Methods

Subjects
Twenty type 2 diabetic patients (mean age, 47.5 years; age range, 35 to 53 years; 11 men, 9 women; diagnosis based on WHO criteria) with a mean HbA1C of 8.5% were recruited. All subjects were normotensive nonsmokers with fasting total cholesterol <6.5 mmol/L. Each had a normal resting ECG and no personal or family history of premature vascular disease. A family history of premature vascular disease was defined as the absence of symptomatic macrovascular disease in a first-degree relative before the age of 65 years. All subjects were not taking aspirin, lipid-lowering agents, or supplemental vitamins. Female subjects were premenopausal and were studied at approximately the same time in their menstrual cycles (follicular phase). Written consent was obtained from all subjects, with local ethics committee approval.

Study Protocol
Studies were begun after a 12-hour overnight fast. After 30 minutes of supine rest, venous blood was drawn for measurement of total cholesterol, LDL-C, HDL-C, plasma TG, insulin, glucose, and glycosylated HbA1C. 5 mL venous blood was also drawn to measure venous free radicals. Each subject was given a fatty meal containing 80 g saturated fat, and lipid profiles and plasma glucose were repeated every 2 hours over the following 8 hours. Endothelial function and oxidative stress were assessed while the subjects were fasting and during peak lipemia. Each patient was randomized to 3-month treatment with either ciprofibrate 100 mg once daily or matched placebo, after which the above investigations were repeated.

Endothelial Function Assessment
Changes in brachial artery diameter in response to reactive hyperemia (FMD) and sublingual NTG (400 μg) (endothelium-independent dilatation) were measured with an ultrasonic vessel wall-tracking system (resolution, ±3 μm) as previously described and validated. Vasodilatation data are presented as percentage change from resting vessel diameter.

Biochemical Measurements
Total cholesterol and TG concentrations were measured enzymatically. HDL-C concentrations were measured after precipitation of apolipoprotein B with phosphotungstate/magnesium. LDL-C was calculated after lipoprotein separation. Plasma glucose was measured by a hexokinase-based technique. HbA1C via enzyme immunoassay, and plasma insulin by a commercial radioimmunoassay (INS-RIA-100, Medgenix Diagnostics).

Lipoprotein Separation
Blood was collected into EDTA tubes, and the plasma was prepared by centrifugation at 20 000g for 20 minutes. Chylomicrons were removed by centrifugation at 20 000g for 20 minutes. Then, 2.4 mL chylomicron-free plasma was placed in a tube containing 0.6 mL iodixanol (Liposep) and centrifuged. Gradient fractions were collected by tube puncture into a multiwell plate and analyzed for cholesterol and TG (with commercial diagnostic kits). By plotting cholesterol and TG profiles of gradients, we identified the fractions containing HDL, LDL, and VLDL and confirmed them by agarose gel electrophoresis of the appropriate fractions. Total cholesterol and TG in each lipoprotein class were calculated by summation of the amounts of these lipids in those fractions corresponding to each lipoprotein class. Total concentrations of HDL and LDL may also be calculated with this methodology.

Free Radical Measurement
Ex vivo spin trapping was used to measure free radicals in venous blood. Reactive oxygen species induce lipid peroxidation, producing secondary lipid radicals, which are detectable by EPR spectroscopy when spin trapped. Then, 2.5 mL venous blood was taken directly in sealed glass tubes containing 1 mL of the spin trap, α-phenyl-α-tert-butyl nitrore (PBN) (0.125 mol/L). After centrifugation, the PBN adduct was extracted from plasma supernatant with toluene, dried under nitrogen gas, and reconstituted in degassed chloroform. EPR spectra were recorded on a Varian E104 spectrometer operating at 9.1 GHz at 10-MW power, 1-G modulation, 0.25-second time constant, and 100-G scan range. EPR spectral parameters were obtained from data acquisition and processing with EPR computational software. EPR spectral peak heights were taken as a good correlation of spin-adduct concentration after confirmation of peak-to-peak line width conformity and double integration on selected samples and expressed in arbitrary units. Analysis of the EPR spectra from spin-trapped radicals suggests that alkoxyl radicals (coupling constants, an=13.9 G, aβH=2.2 G) and carboxyl radicals (aN=14.1 G, aβH=4.0 G) are trapped, which is in agreement with previous studies.

Statistical Analysis
Conventional methods were used for calculating mean, SD, and checks for normal distribution. Group differences in continuous variables were determined by a 2-tailed t test. Statistical significance for differences in continuous variables between groups was tested by 1-way ANOVA. As a measure of plasma glucose and the total amounts of lipid and lipoprotein present in plasma during PPL, AUCs were calculated for plasma concentrations without subtraction of baseline values. This measure of AUC was used because the principle aim of this study was to investigate the effect of PPL on fasting measures of endothelial function and oxidative stress. Multiple regression analysis was used to study the independent relationship between variables with logarithmic transformation of skewed data. Statistical significance was inferred at P<0.05.
Results

Patient Characteristics
There were no differences in blood pressure or body mass index between groups at baseline and after treatment (Table 1).

Biochemistry
There were no differences in total, LDL cholesterol, insulin, glucose, and HbA1C between groups at baseline and after treatment (Table 2). There was a significant reduction in fasting plasma TG and increase in HDL-C after ciprofibrate. Table 3 illustrates the fasting VLDL, LDL, and HDL compositional changes after placebo and ciprofibrate.

Postprandial Lipemia
Area under the curve (AUC) for postprandial plasma TG (mmol·L⁻¹·8 h⁻¹) was similar in both groups at baseline (199.8±98 for ciprofibrate, 188.6±110 for placebo). There was a significant reduction in postprandial hypertriglyceridemia after ciprofibrate (Figure 1), resulting in reduced plasma TG AUC (199.8±98 at baseline, 78.9±21.6 at 3 months; P<0.05). No changes were noted after placebo (Figure 2).

AUC for TG content of HDL, LDL, and VLDL was initially similar in both groups (Table 3). After ciprofibrate, postprandial lipoprotein TG enrichment was significantly reduced (Table 4). There was no significant increase in glucose between fasting and 4-hour levels. Postprandial glycemia (AUC glucose) was not changed by treatment.

Vascular Data
At baseline, fasting and postprandial flow-mediated endothelium-dependent vasodilatation (FMD) was similar in both groups, with a significant reduction in postprandial FMD (Figure 3). Fasting FMD significantly improved in the ciprofibrate group (3.8±1.8% versus 4.8±1.1%, P<0.05). Similarly, postprandial FMD improved significantly (1.8±1.3% versus 3.4±1.1%, P<0.05). There were no changes after placebo (Figure 3). Nitroglycerin (NTG)-mediated vasodilatation was similar in both groups, with no changes after treatment (Figure 4). Fasting and postprandial resting arterial diameter and resting and hyperemic blood flow were similar in both groups before and after treatment.

Free Radical Data
Both groups initially exhibited similar measures of fasting and postprandial oxidative stress (fasting: 2.33±1.1 for ciprofibrate, 2.4±1.8 for placebo; postprandial: 3.85±2.5 for ciprofibrate, 3.7±2.9 for placebo; Figure 5). After treatment, fasting oxidative stress was similar in both groups with no change from baseline (2.27±1.3 for ciprofibrate, 2.36±1.5 for placebo). Ciprofibrate attenuated the postprandial excursion in oxidative stress (2.57±1.9 versus 3.89±2.5, P<0.05).

Correlation Between PPL, Endothelial Function, and Oxidative Stress
Fasting FMD correlated inversely with LDL cholesterol (LDL-C) (r=−0.55, P=0.03 for ciprofibrate; r=−0.52, P=0.03 for placebo).

Table 2. Fasting Biochemistry in Both Groups at Baseline and After Treatment

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ciprofibrate</td>
<td>Placebo</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>11.1±3.3</td>
<td>10.1±4.1</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.9±1.1</td>
<td>5.6±1.4</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.57±0.6</td>
<td>3.46±0.9</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.03±0.2</td>
<td>1.09±0.2</td>
</tr>
<tr>
<td>Insulin, IU/L</td>
<td>32.1±17</td>
<td>31.9±10.8</td>
</tr>
<tr>
<td>HbA1C, %</td>
<td>8.3±1.3</td>
<td>8.01±1.5</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>2.8±2.1</td>
<td>2.8±1.7</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*P<0.05 vs baseline.

Figure 1. PPL in ciprofibrate group at baseline and after 3 months of treatment. *P>0.05 for pretreatment vs posttreatment.

Figure 2. PPL in placebo group at baseline and after 3 months of treatment.
P=0.02 for placebo) and TG content of VLDL (r=−0.51, P=0.03 for ciprofibrate; r=−0.50, P=0.04 for placebo). HDL-C correlated positively with FMD (r=0.52, P=0.03 for ciprofibrate; r=0.55, P=0.02 for placebo). The postprandial reduction in FMD correlated with postprandial TG enrichment of VLDL (r=0.52, P=0.04 for ciprofibrate; r=0.54, P=0.03 for placebo) and LDL (r=0.55, P=0.03 for ciprofibrate; r=0.52, P=0.04 for placebo) and inversely with 4-hour HDL-C (r=−0.57, P=0.02 for ciprofibrate; r=−0.54, P=0.03 for placebo). Fasting oxidative stress demonstrated no significant correlations. The postprandial increase in oxidative stress correlated with postprandial TG enrichment of VLDL (AUC for VLDL TG content) (r=0.51, P=0.04 for ciprofibrate; r=0.53, P=0.03 for placebo). After treatment, there were no significant correlations (at the 95% confidence level) between the fasting and postprandial improvement in endothelial function and any changes in the measured metabolic parameters as a result of ciprofibrate therapy.

**Discussion**

This study demonstrates an association between exaggerated PPL with the production of atherogenic lipoproteins, enhanced oxidative stress, and augmented ED in type 2 diabetes. Fasting endothelial function correlated inversely with LDL-C levels and TG content of VLDL and positively with HDL-C levels. The postprandial deterioration in endothelial function correlated inversely with HDL-C and positively with the postprandial TG enrichment of VLDL and LDL. Postprandial TG enrichment of VLDL was the only parameter to correlate with postprandial oxidative stress. There was also a trend toward reduced postprandial NTG responsiveness, which has been previously described in other studies examining the effects on of transient hypertriglyceridemia on endothelial function. This effect may due partly to increased free fatty acid levels, which may downregulate smooth muscle responsiveness to nitric oxide (NO).

Associations between endothelial function, LDL-C, and HDL-C in type 2 diabetes have been previously described. This study demonstrates a direct association between TG-rich lipoproteins and ED, both fasting and postprandial in type 2 diabetes. Furthermore, we report an association between TG-rich VLDL and enhanced oxidative stress in type 2 diabetes.

Lipoprotein analysis from the Monitored Atherosclerosis Regression Study (MARS) demonstrated the importance of TG-rich VLDL and IDL as predictors of atherosclerotic disease progression. TG-rich VLDL particles preferentially undergo endocytosis by receptors on macrophages to form foam cells. Furthermore, lipolytic products of TG-rich VLDL are toxic to endothelial cells and macrophages. TG-rich apolipoprotein B–containing particles have also been isolated in excess from atherosclerotic plaques. Increased free fatty acid fluxes, particularly during PPL, may potentate the effects of TG-rich VLDL on endothelial function, because VLDL causes enhanced endothelial toxicity when FFA/albumin ratios are elevated.

TG enrichment of VLDL also affects LDL metabolism, with increased synthesis of atherogenic small, dense LDL particles, which are toxic to endothelial cells and exhibit enhanced pro-oxidant potential.

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**TABLE 3. Fasting Cholesterol and TG Distribution Among Major Lipoproteins in Both Groups at Baseline and After Treatment**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Ciprofibrate (Baseline)</th>
<th>Placebo (Baseline)</th>
<th>Ciprofibrate (After Treatment)</th>
<th>Placebo (After Treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL TG content, μmol/mL</td>
<td>2.54±0.98</td>
<td>2.61±1.14</td>
<td>1.46±0.55*</td>
<td>2.53±1.06</td>
</tr>
<tr>
<td>VLDL-C content, μmol/mL</td>
<td>2.02±1.29</td>
<td>2.07±1.19</td>
<td>1.08±0.65*</td>
<td>2.09±1.05</td>
</tr>
<tr>
<td>LDL TG content, μmol/mL</td>
<td>0.99±0.39</td>
<td>0.94±0.51</td>
<td>0.40±0.24*</td>
<td>0.92±0.36</td>
</tr>
<tr>
<td>LDL-C content, μmol/mL</td>
<td>4.28±1.20</td>
<td>4.39±1.21</td>
<td>4.31±1.27</td>
<td>4.43±1.29</td>
</tr>
<tr>
<td>HDL TG content, μmol/mL</td>
<td>0.59±0.49</td>
<td>0.55±0.41</td>
<td>0.13±0.11*</td>
<td>0.51±0.44</td>
</tr>
<tr>
<td>HDL-C content, μmol/mL</td>
<td>1.09±0.7</td>
<td>1.16±0.9</td>
<td>1.79±0.22†</td>
<td>1.18±0.65</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.05, baseline vs postciprofibrate TG and cholesterol content.
†P<0.05, posttreatment vs baseline cholesterol content.

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**TABLE 4. Postprandial AUC for TG content of Major Lipoproteins in Both Groups**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>AUC for VLDL TG Content, μmol·mL⁻¹·8 h⁻¹</th>
<th>AUC for LDL TG Content, μmol·mL⁻¹·8 h⁻¹</th>
<th>AUC for HDL TG Content, μmol·mL⁻¹·8 h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofibrate group (baseline)</td>
<td>1.66±0.35</td>
<td>1.19±0.45</td>
<td>1.07±0.31</td>
</tr>
<tr>
<td>Placebo group (baseline)</td>
<td>1.72±0.41</td>
<td>1.25±0.30</td>
<td>1.03±0.48</td>
</tr>
<tr>
<td>Ciprofibrate group (3 mo)</td>
<td>1.19±0.21*</td>
<td>0.76±0.48*</td>
<td>0.88±0.21*</td>
</tr>
<tr>
<td>Placebo group (3 mo)</td>
<td>1.79±0.57</td>
<td>1.21±0.22</td>
<td>1.07±0.39</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.05, TG content reduction in ciprofibrate group after 3 months.
During PPL, there was TG enrichment of LDL and HDL particles. Hydrolysis of TG in these TG-rich LDL particles contributes further to the production of smaller, denser particles. TG-rich, cholesterol-depleted HDL particles, produced partly as a result of enhanced neutral lipid exchange with TG-rich VLDL, demonstrate decreased endothelium-protective properties, including reduced antioxidant properties.38 Not only are these particles produced in excess during PPL in type 2 diabetes, there is also reduced particle catabolism as a result of impaired lipoprotein lipase function5 and defective particle clearance as a result of increased particle competition for receptor-mediated endocytosis.29 Functional lipoprotein lipase is required to produce such an atherogenic lipoprotein profile and thus mediate the effects of hypertriglyceridemia on endothelial function, because in hypertriglyceridemic patients with lipoprotein lipase deficiency, endothelial function is preserved.39

Type 2 diabetes is associated with enhanced oxidative stress, 40 representing a state of disequilibrium between free radical production and antioxidant defenses. This study supports an association between transient hypertriglyceridemia and the production of TG-rich VLDL particles during PPL in type 2 diabetes with increased oxidative stress. The precise mechanisms accounting for this observation remain speculative and may involve a variety of complex changes in lipoprotein metabolism.38 Enhanced oxidative stress has a variety of important effects in atherogenesis, including lipoprotein oxidation, particularly LDL oxidation41; this may be of particular relevance in type 2 diabetes. Oxidized LDL has both pro-oxidant properties and enhanced endothelial toxicity.42 Enhanced oxidative stress may also directly induce ED by decreasing synthesis and release of NO by endothelial cells and by inactivating NO in the subendothelial space.43 Furthermore, the alkoxyl radicals detected by electron paramagnetic resonance (EPR) spectroscopy have been shown to directly interact with NO.44 Thus, our observation of deteriorating endothelial function associated with PPL in type 2 diabetes may result from a combination of dyslipidemia and oxidative stress.

After ciprofibrate, fasting and postprandial endothelial function significantly improved, coupled with a reduction in postprandial oxidative stress. Fasting plasma TG and postprandial AUC for TG were also reduced, whereas HDL-C levels were increased. There were also modest but nonsignificant reductions in total and LDL cholesterol, plasma insulin, and HbA1C. Furthermore, there was TG depletion of all lipoproteins, with cholesterol enrichment of HDL and cholesterol depletion of VLDL.
The nonsignificant reduction in fasting insulin and HbA1C may reflect improved insulin sensitivity, and because this positively relates to endothelial NO synthesis, it could potentially account for some of the observed change in endothelial function. However, improved fasting endothelial function correlates most strongly with reduced fasting plasma TG and TG depletion of VLDL and HDL (r = 0.54, 0.46, and 0.48, respectively). Moreover, the improvement in postprandial endothelial function correlates most strongly with attenuation of postprandial hypertriglyceridemia and lipoprotein TG enrichment. Additionally, the changes in postprandial oxidative stress correlate most strongly with attenuation of VLDL TG enrichment. These correlations were not significant at the 95% confidence level, which may be due to the relatively small sample size and small absolute differences rather than to a true lack of biological significance.

Fibrates cause decreased production and enhanced catabolism of TG-rich VLDL. Subsequently, there is reduced neutral lipid exchange, resulting in TG depletion of LDL and HDL with cholesterol enrichment of increased concentrations of HDL. This results in production of larger, less dense particles with reduced atherogenic potential, which may be of even greater significance during PPL.

The mechanisms by which ciprofibrate improves endothelial function and oxidative stress during PPL remain speculative. Attenuating the magnitude and duration of the exposure of the endothelium to atherogenic lipoproteins may be of benefit, with reduced TG-rich VLDL particles and increased HDL-C levels being of particular importance.

In summary, we confirm that PPL in type 2 diabetes results in ED and enhanced oxidative stress. Ciprofibrate therapy, by attenuating PPL and modifying an atherogenic lipoprotein profile, leads to significant improvement in fasting and postprandial endothelial function and attenuated postprandial oxidative stress.

Study Limitations
This study evaluates the effect of ciprofibrate therapy on potential atherogenic mechanisms in type 2 diabetes. A hypertriglyceridemic nondiabetic control group may have provided some interesting data. However, hypertriglyceridemia is heterogeneous with different clinical phenotypes, is associated with different degrees of cardiovascular risk, is a cardinal feature of the insulin resistance syndrome, and may be considered a marker of impaired glucose tolerance. Indeed, a significant progression from hypertriglyceridemia to overt type 2 diabetes mellitus is well recognized. Additionally, the basic metabolic disorders accounting for hypertriglyceridemia—namely, reduced lipoprotein lipase activity, increased circulating fasting and postprandial free fatty acids, and enhanced neutral lipid exchange—are also typical of the dyslipidemia of type 2 diabetes. Thus, the inclusion of such a control group would do little to address the original questions posed.

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