Cardiovascular disease is the major cause of death in diabetic patients, and the risk of cardiovascular morbidity and mortality is significantly higher in subjects with diabetes compared with nondiabetic subjects. Hypercholesterolemia, hypertension, and other traditional risk factors of atherosclerosis are also important in diabetic patients, but they do not explain the excess risk in diabetes. Hypertriglyceridemia, low HDL cholesterol (HDL-C), and a preponderance of small LDL particles are typical features of dyslipidemia in subjects with type 2 diabetes. Several cross-sectional and prospective studies have linked small, dense LDL to atherosclerosis, low HDL cholesterol, hypertension, and other traditional risk factors of cardiovascular disease. However, small LDL particles are associated with other metabolic disturbances, and their significance in atherogenesis is still under debate.

In the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT), treatment with gemfibrozil significantly reduced recurrence of CAD events without a significant reduction in serum LDL cholesterol (LDL-C) concentration. Further analyses have suggested that although the beneficial effects of gemfibrozil in VA-HIT were significantly associated with an increase in HDL-C, only a portion of this benefit could be explained by changes in total HDL-C concentration or other traditional lipid parameters. The results of the Diabetes Atherosclerosis Intervention Study (DAIS) showed...
that fenofibrate treatment is associated with significantly less progression of focal CAD (40% less progression of minimum lumen diameter versus placebo treatment, $P=0.029$; 42% less progression in percentage diameter stenosis versus placebo treatment, $P=0.02$) and improved the plasma lipid profile in subjects with type 2 diabetes.\(^8\) Fenofibrate causes a shift toward larger, more buoyant LDL particles,\(^9,10\) which is a potentially antiatherogenic change. Therefore, we examined whether changes in LDL particle size may contribute to the favorable effect of fenofibrate on CAD progression in DAIS participants.

### Methods

Subject inclusion and exclusion criteria have been described.\(^11\) Briefly, patients were men and women with type 2 diabetes, 40 to 65 years old, with or without previous coronary intervention (coronary artery bypass grafting or percutaneous transluminal coronary angioplasty). The lipid entry criteria were a total cholesterol/HDL-C ratio of $\geq 4$, plus either LDL-C $3.5$ to $4.5$ mmol/L and triglyceride concentration of $\leq 5.2$ mmol/L, or a triglyceride concentration of $1.7$ to $5.2$ mmol/L and LDL-C $\leq 4.5$ mmol/L. Each physician was allowed to adjust the glucose-lowering treatment to optimize metabolic control in the individual participant. Eligible patients were randomly assigned to micronized fenofibrate or placebo for 3 years.\(^8\) The average time between baseline and final angiograms was 39.6 months. Progression of coronary artery atherosclerosis was analyzed by quantitative angiograms as described.\(^8\) The study was approved by local ethics committees, and all participants gave their informed consent.

Of the 418 patients randomized, 405 (207 placebo, 198 fenofibrate) patients had LDL size measurements at baseline and either at the end of the study or after $\geq 1$ year of treatment. Of the 405 patients, 379 had a final angiogram. For the 26 (12 placebo, 14 fenofibrate) patients with a missing final angiogram, \(\leq\) worst-case imputed angiographic parameters were used as described to permit analysis by intention to treat.\(^8\) Two subjects in the fenofibrate group and 4 subjects in the placebo group were excluded from analyses that required on-treatment plasma apolipoprotein B (apoB) levels because of missing data.

### Biochemical Analyses

Plasma total and HDL-C, total triglycerides, and apoB were measured as described.\(^13\) LDL-C was determined by the Friedewald formula.\(^13\) LDL peak particle diameter (LDL size) was measured from serum samples with polycrylamide gradient gel electrophoresis as described,\(^14\) with slight modifications. Specifically, gels were stained with Coomassie brilliant blue protein stain instead of Sudan black lipid stain. All available values during the treatment period were used to calculate mean on-treatment lipid levels. For the LDL size, only the baseline and 1 on-treatment value (obtained at the end of the study and referred to as \"final LDL size\") were available. Because plasma triglyceride level is the major determinant of LDL size and the effect of fenofibrate on triglyceride levels was stable during the whole treatment period, it is likely that the final LDL size is a good measure of mean on-treatment LDL size.

### Statistical Analyses

The between-groups comparison of on-treatment lipid parameters and final LDL size was performed by use of ANCOVA with baseline value, center, sex, and history of previous coronary intervention as covariates. Pearson’s correlation coefficients were used to analyze associations between absolute changes in LDL size and other lipoprotein parameters and associations between mean on-treatment lipids or final LDL size and absolute changes in angiographic variables. Absolute changes in percentage diameter stenosis are presented according to tertiles of final LDL size and mean on-treatment lipid parameters for illustrative purposes (Figure).\(^2\) and probability values are provided for multivariate regression analysis on changes in percentage diameter stenosis versus on-treatment lipid parameters and final LDL size, which were used as continuous variables. Plasma triglycerides were logarithmically transformed before the analysis. Results are given as mean values; all statistical analyses were done with SAS software (version 8.1). A probability value of $P<0.05$ was considered statistically significant.

### Results

Baseline characteristics are shown in Table 1. No significant differences in age, body mass index, supine systolic and diastolic blood pressure, fasting plasma glucose, and HbA1c.

#### Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Total (n=405)</th>
<th>Fenofibrate (n=198)</th>
<th>Placebo (n=207)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>298/107</td>
<td>144/54</td>
<td>154/53</td>
</tr>
<tr>
<td>Age, y</td>
<td>56.8±5.9</td>
<td>57.3±5.7</td>
<td>56.3±6.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.8±3.2</td>
<td>28.9±3.2</td>
<td>28.7±3.2</td>
</tr>
<tr>
<td>SBP, mm Hg*</td>
<td>140.4±18.1</td>
<td>140.3±18.8</td>
<td>140.4±17.5</td>
</tr>
<tr>
<td>DBP, mm Hg*</td>
<td>81.7±8.7</td>
<td>82.2±8.8</td>
<td>81.3±8.6</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>8.79±2.45</td>
<td>8.54±2.24</td>
<td>9.03±2.61</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.5±1.2</td>
<td>7.5±1.1</td>
<td>7.5±1.3</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; SBP, systolic blood pressure; and DBP, diastolic blood pressure.

*For SBP and DBP: $n=400$ (all), $n=205$ (placebo), $n=195$ (fenofibrate).
were observed between the placebo and fenofibrate groups. Fenofibrate did not have relevant effects on blood pressure or glycemic control, as described. Baseline and mean on-treatment lipid assessments are shown in Table 2. Fenofibrate treatment significantly increased LDL particle size and HDL-C concentration and decreased plasma triglycerides, total cholesterol, LDL-C, and apoB concentrations compared with placebo. The absolute change in LDL size in placebo was related to the plasma triglyceride concentration achieved by fenofibrate treatment. In subjects in the lowest tertile of on-treatment triglycerides (<1.275 mmol/L), the increase in LDL size was 1.19±0.93 mm, but in the highest tertile (>1.805 mmol/L), it was only 0.69±1.05 mm. In the combined group, the change in LDL size was significantly associated with changes in plasma triglycerides (r=−0.56, P<0.0001), HDL-C (r=−0.34, P<0.0001), total cholesterol (r=−0.30, P<0.0001), apoB (r=−0.31, P<0.0001), and LDL-C/apoB ratio (r=0.29, P<0.0001) but not with the change in LDL-C concentration (r=0.03). These associations were also observed when fenofibrate and placebo groups were analyzed separately (not shown).

The relationships between plasma lipoproteins and angiographic changes are shown in Table 3. In the combined group and in the fenofibrate group alone, the final LDL particle size was inversely correlated with the increase in percentage diameter stenosis and the decreases in mean and minimum lumen diameter, indicating that the greatest progression of atherosclerosis occurred in subjects with small LDL particles. On-treatment apoB, total and LDL-C, and triglyceride concentrations showed positive correlations with the angiographic changes, indicating that the greatest progression of atherosclerosis occurred in subjects with the highest levels of these lipoproteins. Interestingly, in the placebo group, on-treatment lipoproteins were not significantly associated with angiographic changes.

We performed multivariate regression analyses to analyze the combined effects of LDL particle size and lipoprotein concentrations on the progression of coronary atherosclerosis (Table 4). Both on-treatment LDL-C concentration and final LDL particle size, as well as on-treatment apoB concentration and final LDL particle size, contributed significantly to the progression of CAD in the combined group. Addition of triglyceride and HDL-C did not significantly improve the model, which is probably explained by the intercorrelations between these variables and LDL size. These associations were a result of the changes observed in the fenofibrate group alone, whereas no such associations were observed in the placebo group.

Progression of focal coronary atherosclerosis (increase in percentage diameter stenosis) in the combined group is illustrated in the Figure. The 405 subjects were divided into tertiles of mean on-treatment lipoproteins. The greatest deterioration was observed in patients with the highest LDL-C, apoB, and triglyceride levels. Importantly, LDL size modulated the progression of CAD so that subjects with small LDL particles had more progression of atherosclerosis than those with large LDL particles. Again, the effect of LDL size and other lipoproteins was because of the fenofibrate group alone and was not observed in the placebo group (not shown).

**Discussion**

The Diabetes Atherosclerosis Intervention Study was the first intervention trial that examined whether the correction of moderate dyslipidemia by fenofibrate reduces the progression of CAD in subjects with type 2 diabetes. The main result was that the subjects receiving fenofibrate had significantly less progression of focal atherosclerosis than the subjects receiving placebo. We have now investigated the effect of fenofibrate on LDL particle size and other components of diabetic dyslipidemia and their effect on CAD progression. Fenofibrate significantly increased LDL size and HDL-C concentration and decreased plasma triglyceride, total and LDL-C, and apoB concentrations. The on-treatment lipid values explained a significant but relatively small proportion of the changes in the coronary angiograms.

Two smaller angiography trials have examined the associations of small, dense LDL and progression of coronary atherosclerosis in subjects without diabetes. In the Familial Atherosclerosis Treatment Study, colestipol-lovastatin and colestipol-niacin treatments decreased hepatic lipase activity and increased LDL buoyancy. The increase in LDL buoyancy explained 37% of the variance in the change in coronary stenosis. In contrast, in the Bezafrilate Coronary Athero-
sclerosis Intervention Trial, on-trial LDL size was not correlated with the progression of atherosclerosis in coronary angiograms, whereas on-trial HDL, cholesterol and apoB explained a small proportion of the changes. In the present study, LDL size and plasma lipoprotein concentrations showed a statistically significant association with the progression of coronary atherosclerosis. In addition, we found support for the combined effect of small LDL particle size and other dyslipidemias on the progression of atherosclerosis. Notably, especially in subjects with low LDL-C or apoB levels, a preponderance of small, dense LDL particles increased the progression of coronary atherosclerosis. These results indicate that both the quality and the quantity of LDL modulate the development of atherosclerosis. Thus, focusing only on LDL-C concentration as a therapeutic target may be misleading in these subjects. Potential mechanisms for increased atherogenicity of small, dense LDL include enhanced inflow into the arterial wall, increased binding affinity to arterial wall proteoglycans, decreased binding affinity to the LDL receptor compared with mid-sized LDL particles, and increased susceptibility to oxidative modification.

Case-control studies with clinical end points have suggested that small LDL size is a risk factor for clinical CAD before but not after adjustment for other lipid variables. In the Québec Cardiovascular Study, a prospective follow-up study of 2057 men, Lamarche et al recently showed that small LDL particles increase the probability of developing ischemic heart disease. The effect of small LDL was independent of other lipid and nonlipid risk factors and additive to the effects of high LDL-C and apoB level. To the best of our knowledge, our study shows for the first time that increasing LDL particle size and decreasing LDL-C and apoB levels by fenofibrate provides antiatherogenic benefit in subjects with type 2 diabetes and therefore extends the previous data. Further analysis of the Québec Cardiovascular Study data showed that the calculated cholesterol concentration in small LDL particles was superior to the LDL particle size in predicting the ischemic heart disease risk. Because we used protein and not lipid staining in measuring LDL size, we could not conduct a similar analysis. However, our finding that small LDL peak particle diameter (indicating increased proportion of small LDL particles) and LDL-C concentration have a combined effect in predicting the progression of coronary atherosclerosis supports the idea that both the amount and the quality of LDL-C are important risk factors for atherosclerosis. Interestingly, in the Cholesterol and Recurrent Events (CARE) trial, large LDL particle size at baseline was associated with increased risk of recurrence of coronary events in the placebo group but not in the pravastatin group. The participants of the CARE trial were survivors of myocardial infarction, but it is uncertain whether this could have an influence on the result.

We used quantitative coronary angiography to measure progression of atherosclerosis. The method has good reproducibility, and it was the accepted standard for the assessment of progression of CAD when DAIS was initiated. The weaknesses of angiography include the fact that it visualizes only vessel lumen diameter and not the size of the atheroma in the vessel wall and that vascular remodeling decreases its accuracy in measuring the progression of coronary atherosclerosis. It is therefore likely that the actual associations between plasma lipoproteins and progression of atherosclerosis are stronger than those found by us and others. The associations between plasma lipoprotein levels and progression of CAD were observed only in subjects assigned to fenofibrate but not in those assigned to placebo, which diluted the findings in the combined group. The data imply that significant changes in plasma lipoproteins must be

### TABLE 3. Univariate Correlation Analyses Between On-Treatment Lipid Parameters and Progression of Coronary Atherosclerosis in Total, Fenofibrate, and Placebo Groups

<table>
<thead>
<tr>
<th></th>
<th>Decrease in Mean Lumen Diameter</th>
<th>Decrease in Minimum Lumen Diameter</th>
<th>Increase in Percentage Diameter Stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n=405)</td>
<td>Fenofibrate (n=198)</td>
<td>Placebo (n=207)</td>
</tr>
<tr>
<td>LDL size</td>
<td>-0.10†</td>
<td>-0.21†</td>
<td>0.00</td>
</tr>
<tr>
<td>ApoB*</td>
<td>0.15†</td>
<td>0.24§</td>
<td>0.05</td>
</tr>
<tr>
<td>TC</td>
<td>0.10</td>
<td>0.23‡</td>
<td>-0.05</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.11†</td>
<td>0.20‡</td>
<td>0.03</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.13§</td>
<td>-0.12</td>
<td>-0.13</td>
</tr>
<tr>
<td>TG</td>
<td>0.10‡</td>
<td>0.22‡</td>
<td>-0.02</td>
</tr>
<tr>
<td>LDL-C/apoB†</td>
<td>-0.05</td>
<td>-0.10</td>
<td>-0.02</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 2. Values represent Pearson correlation coefficients.

#### TABLE 4. Multivariate Regression Analysis of the Change in Percentage Diameter Stenosis in Total (n=405), Fenofibrate (n=198), and Placebo (n=207) Groups

<table>
<thead>
<tr>
<th></th>
<th>Model R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (n=405)</td>
</tr>
<tr>
<td>LDL size and LDL-C</td>
<td>0.039§</td>
</tr>
<tr>
<td>LDL size and apoB*</td>
<td>0.041§</td>
</tr>
<tr>
<td>LDL size and HDL-C</td>
<td>0.026‡</td>
</tr>
<tr>
<td>LDL size and TG</td>
<td>0.027†</td>
</tr>
<tr>
<td>LDL size, LDL-C, apoB, HDL-C, TG*</td>
<td>0.044‡</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 2.

†P<0.05, †P<0.01, §P<0.001.
obtained to decrease the progression of atherosclerosis. In a recent follow-up study, LDL-C and C-reactive protein, a marker of inflammation, had additive power in predicting future cardiovascular events.28 Fibrates have antiinflammatory and plaque-stabilizing effects in addition to the lipid-lowering effect.29 It is possible that these nonlipid actions increase the clinical benefit of fibrates even beyond the decreased progression of atherosclerosis that was detected in DAIS. Our preliminary data suggest, however, that fenofibrate did not significantly influence serum C-reactive protein levels (Hamsten et al, unpublished data). This implies that lipoprotein levels are more important than markers of inflammation in predicting the progression of atherosclerosis measured with angiography. The results of larger trials, such as the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial,30 have to be awaited before the clinical benefit of the nonlipid actions of fibrates can be determined.

We conclude that treatment with fenofibrate has a favorable effect on the plasma lipid profile and reduces the progression of coronary atherosclerosis. Changes in LDL size and plasma lipid levels account for at least part of the beneficial action of fenofibrate. Small LDL particles increase the atherogenicity of hyperlipidemia in subjects with type 2 diabetes.

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References

Relationships Between Low-Density Lipoprotein Particle Size, Plasma Lipoproteins, and Progression of Coronary Artery Disease: The Diabetes Atherosclerosis Intervention Study (DAIS)

Juha Vakkilainen, George Steiner, Jean-Claude Ansquer, Francois Aubin, Stephanie Rattier, Christelle Foucher, Anders Hamsten and Marja-Riitta Taskinen
on behalf of the DAIS Group

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