Atorvastatin Decreases Apolipoprotein C-III in Apolipoprotein B-Containing Lipoprotein and HDL in Type 2 Diabetes

A potential mechanism to lower plasma triglycerides

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OBJECTIVE — Apolipoprotein (apo)C-III is a constituent of HDL (HDL apoC-III) and of apoB-containing lipoproteins (LpB:C-III). It slows the clearance of triglyceride-rich lipoproteins (TRLs) by inhibition of the activity of the enzyme lipoprotein lipase (LPL) and by interference with lipoprotein binding to cell-surface receptors. Elevated plasma LpB:C-III is an independent risk factor for cardiovascular disease. We studied the effect of atorvastatin on plasma LpB:C-III and HDL apoC-III.

RESEARCH DESIGN AND METHODS — We studied the effect of 30 weeks’ treatment with 10 and 80 mg atorvastatin on plasma apoC-III levels in a randomized, double-blind, placebo-controlled trial involving 217 patients with type 2 diabetes and fasting plasma triglycerides between 1.5 and 6.0 mmol/l.

RESULTS — Baseline levels of total plasma apoC-III, HDL apoC-III, and LpB:C-III were 41.5 ± 10.0, 17.7 ± 5.5, and 23.8 ± 7.7 mg/l, respectively. Plasma apoC-III was strongly correlated with plasma triglycerides (r = 0.74, P < 0.001). Atorvastatin 10- and 80-mg treatment significantly decreased plasma apoC-III (atorvastatin 10 mg, 21%, and 80 mg, 27%), HDL apoC-III (atorvastatin 10 mg, 22%, and 80 mg, 28%), and LpB:C-III (atorvastatin 10 mg, 23%, and 80 mg, 28%; all P < 0.001). The decrease in plasma apoC-III, mainly in LpB:C-III, strongly correlated with a decrease in triglycerides (atorvastatin 10 mg, r = 0.70, and 80 mg, r = 0.78; P < 0.001). Atorvastatin treatment also leads to a reduction in the HDL apoC-III–to–HDL cholesterol and HDL apoC-III–to–apoA-I ratios, indicating a change in the number of apoC-III per HDL particle (atorvastatin 10 mg, −21%, and 80 mg, −31%; P < 0.001).

CONCLUSIONS — Atorvastatin treatment resulted in a significant dose-dependent reduction in plasma apoC-III, HDL apoC-III, and LpB:C-III levels in patients with type 2 diabetes. These data indicate a potentially important antitherogenic effect of statin treatment and may explain (part of) the triglyceride-lowering effect of atorvastatin.
increased plasma levels of apoC-III, with a fourfold larger proportion of apoC-III present in VLDL-sized particles compared with normolipidemic individuals (17–20). In contrast, patients with a genetic deficiency in apoC-III had low levels of plasma VLDL because of an enhanced fractional catabolic rate and a rapid conversion of VLDL into intermediate-density lipoprotein and LDL (21). ApoC-III in apoB-containing lipoproteins (LpB:C-III) mainly reflects apoC-III present on triglyceride-rich particles and may directly affect LPL activity and receptor binding. HDL functions as a sink for surplus apoC-III in plasma, and a high level may indirectly affect triglyceride clearance (22).

A number of clinical studies have revealed that total apoC-III as well as apoC-III present on TRLs (LpB:C-III) are risk indicators for CVD (23,24). In the Monitored Atherosclerosis Regression Study (MARS) (25), apoC-III, particularly in apoB-containing lipoproteins, was a major risk factor for the severity of CVD in patients with mild and moderate atherosclerosis. In the Cholesterol and Recurrent Events (CARE) study, LpB:C-III was strongly and independently associated with the risk of CVD (26). However, the concentration of apoC-III in HDL (HDL apoC-III) was the major predictor of global coronary atherosclerosis progression in subjects treated with niacin-colestipol (Cholesterol Lowering Atherosclerosis Study [CLAS]) (27). The important role of apoC-III in lipoprotein metabolism and as a risk factor for CVD means that treatments aimed at affecting apoC-III levels are of great interest. Statins may lower plasma apoC-III. In 27 patients with primary hypertriglyceridemia, 20–40 mg atorvastatin for 4 weeks reduced plasma apoC-III by 18–30% (28). In 305 patients with primary hypercholesterolemia (29), both atorvastatin and pravastatin reduced LpB:C-III by ~30%. In contrast, in patients with coronary artery bypass graft, lovastatin therapy lowered the concentrations of apoB-containing lipoproteins (LDL), but not plasma apoC-III or LpB:C-III (19).

Atorvastatin is a powerful HMG-CoA reductase inhibitor proven to effectively reduce total plasma cholesterol and triglyceride levels. We showed (30) that atorvastatin (10 vs. 80 mg daily for 30 weeks) lowered plasma triglycerides by 25–35% in subjects with type 2 diabetes. The mechanism leading to this decrease was unclear. LPL activity in postheparin plasma was not affected by the treatment. Additionally, it has been shown (31,32) that atorvastatin treatment resulted in an increase in fractional catabolic rate of apoB-containing lipoproteins but no change in the apoB production rate.

In the present study, we established the effect of high and low levels of atorvastatin treatment on plasma apoC-III in patients with type 2 diabetes, and, secondly, we attempted to determine whether atorvastatin results in a decrease of both LpB:C-III and HDL apoC-III levels as a potential mechanism to lower plasma triglycerides.

### RESEARCH DESIGN AND METHODS

This study is part of the Diabetes Atorvastatin Lipid Intervention (DALI) study. DALI is a randomized, double-blind, placebo-controlled, multicenter study conducted in the Netherlands (30). Briefly, 217 patients, aged 45–75 years, with type 2 diabetes for at least 1 year and an HbA1c ≤10%, participating in the DALI study were randomized to placebo, atorvastatin 10 mg, or atorvastatin 80 mg for 30 weeks to evaluate the effect of treatment on lipid metabolism. Type 2 diabetes was defined according to the American Diabetes Association classification (33). Inclusion criteria were fasting plasma triglycerides between 1.5 and 6.0 mmol/l, total cholesterol between 4.0 and 8.0 mmol/l, and no history of CVD. Patients were recruited in Leiden, Rotterdam, and Utrecht. The ethics committees of the participating centers approved the study protocol, and all procedures followed were in accordance with institutional guidelines. The primary end point was the percentage decrease in plasma triglyceride levels after atorvastatin treatment, as described elsewhere (30). The secondary end point was changes in specialized lipid parameters. Written informed consent was obtained from all subjects.

### Analytical methods

Blood samples were drawn after a fasting period of 12 h at baseline and after 30 weeks’ treatment at the end of the study. Plasma was prepared by immediate centrifugation, and samples were stored at −80°C for further analyses. Cholesterol and triglycerides were determined by the enzymatic colorimetric method on a Hitachi 911 automatic analyzer (Boehringer Mannheim, Mannheim, Germany) and apoB and apoA-I by automated immunoturbidimetric assays (Tina-quant; Roche Diagnostics, Mannheim, Germany). Plasma HDL cholesterol was measured by a direct enzymatic HDL cholesterol method based on polyethylene glycol-modified enzymes on a Hitachi 911 autoanalyzer (Roche Diagnostics, Mannheim, Germany). LDL cholesterol was estimated by the Friedewald formula (34). Fasting plasma glucose was determined on a Hitachi 917 analyzer using an ultraviolet hexokinase method (cat. no. 18,766,899; Boehringer Mannheim). HbA1c was determined by high-performance liquid chromatography, using the Bio-Rad Variant
HDL apoC-III to HDL cholesterol
ApoA-I (mg/l)
HDL apoC-III to apoAI (mg/mg)
HDL cholesterol (mmol/l)
†
HDL apoC-III (mg/l)
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teins with the use of a specific precipitation of the apoB-containing lipopro-
determined in the supernatant after precipitation with a commercial available electroimmuno-
Plasma apoC-III was analyzed with an immunochemical method (35) representing an estimate of the amount of apoC-III per HDL particle, correlated much stronger with plasma triglycerides (r = 0.68, P < 0.001) than HDL apoC-III (Fig. 2). After log transformation of the plasma triglyceride levels, similar correlations were found (data not shown). Post-
heparin LPL activity only showed a weak inverse correlation with plasma triglycerides (r = −0.13, P = 0.05) and not with apoC-III.

HbA1c and plasma glucose levels remained unchanged after atorvastatin treatment. Plasma apoC-III levels were significantly decreased after 10-mg (21%) and 80-mg (27%; both P < 0.05) atorvastatin treatment compared with placebo (Table 2). After 30 weeks’ treatment, both 10 mg and 80 mg atorvastatin decreased the HDL apoC-III and LpB:C-III fractions to a similar extent (21–23 and 27–28%, respectively). Because the LpB:C-III fraction contained the major part of the total apoC-III, atorvastatin decreased the absolute amount of apoC-III in the LpB:C-III fraction more than in the HDL apoC-III fraction.

Earlier, we reported (30) that 10 mg and 80 mg atorvastatin decreased plasma triglycerides in the DALI population by 25 and 35%, respectively (both P < 0.001). The decrease in total plasma apoC-III strongly correlated with the decrease in plasma triglycerides in both the

Table 2 — Plasma apoC-III levels at baseline and after 30 weeks of atorvastatin

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>10 mg</th>
<th>80 mg</th>
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<tbody>
<tr>
<td>n</td>
<td>71</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Total apoC-III (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>41.3 ± 0.9</td>
<td>39.8 ± 9.5</td>
<td>43.1 ± 9.9</td>
</tr>
<tr>
<td>30 weeks</td>
<td>41.2 ± 12.0</td>
<td>31.8 ± 10.6*</td>
<td>31.1 ± 11.3*</td>
</tr>
<tr>
<td>Change (%)</td>
<td>0</td>
<td>−21.1</td>
<td>−27.2</td>
</tr>
<tr>
<td>HDL apoC-III (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>17.6 ± 5.2</td>
<td>16.7 ± 5.3</td>
<td>18.4 ± 6.0</td>
</tr>
<tr>
<td>30 weeks</td>
<td>17.6 ± 8.0</td>
<td>14.1 ± 5.2†</td>
<td>13.2 ± 5.8**</td>
</tr>
<tr>
<td>Change (%)</td>
<td>0</td>
<td>−22.2</td>
<td>−28.3</td>
</tr>
<tr>
<td>LpB:C-III (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>23.6 ± 6.8</td>
<td>23.1 ± 7.3</td>
<td>24.7 ± 8.6</td>
</tr>
<tr>
<td>30 weeks</td>
<td>23.6 ± 7.7</td>
<td>17.7 ± 7.8*</td>
<td>17.9 ± 8.7*</td>
</tr>
<tr>
<td>Change (%)</td>
<td>0</td>
<td>−23.4</td>
<td>−27.5</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.05 ± 0.21</td>
<td>1.05 ± 0.26</td>
<td>1.04 ± 0.24</td>
</tr>
<tr>
<td>30 weeks</td>
<td>1.03 ± 0.22</td>
<td>1.11 ± 0.31</td>
<td>1.09 ± 0.28</td>
</tr>
<tr>
<td>Change (%)</td>
<td>−2.0</td>
<td>5.7</td>
<td>4.8</td>
</tr>
<tr>
<td>ApoA-I (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.41 ± 0.19</td>
<td>1.39 ± 0.20</td>
<td>1.40 ± 0.21</td>
</tr>
<tr>
<td>30 weeks</td>
<td>1.37 ± 0.18</td>
<td>1.38 ± 0.20</td>
<td>1.34 ± 0.20</td>
</tr>
<tr>
<td>Change (%)</td>
<td>−2.8</td>
<td>−0.7</td>
<td>−4.3</td>
</tr>
<tr>
<td>HDL apoC-III to apoAI (mg/mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>9.11 ± 2.97</td>
<td>8.83 ± 2.71</td>
<td>9.76 ± 3.53</td>
</tr>
<tr>
<td>30 weeks</td>
<td>9.89 ± 6.05</td>
<td>7.48 ± 2.29</td>
<td>7.44 ± 3.42***</td>
</tr>
<tr>
<td>Change (%)</td>
<td>8.8</td>
<td>−18</td>
<td>−31</td>
</tr>
<tr>
<td>HDL apoC-III to HDL cholesterol (mg/mmol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>17.1 ± 5.4</td>
<td>16.5 ± 5.2</td>
<td>18.3 ± 6.1</td>
</tr>
<tr>
<td>30 weeks</td>
<td>18.2 ± 11.4</td>
<td>13.1 ± 4.1</td>
<td>12.5 ± 5.8***</td>
</tr>
<tr>
<td>Change (%)</td>
<td>6.4</td>
<td>−20.7</td>
<td>−31.7</td>
</tr>
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</table>

Data are means ± SD. Test for difference among the three groups, adjusted for baseline value: *P < 0.001; †P = 0.005. Test for difference versus 10 mg atorvastatin, adjusted for baseline: ‡P = 0.05.

Atorvastatin and type 2 diabetes

Postheparin plasma LPL activity
Postheparin LPL activity was measured using an immunochemical method (35) in plasma collected 20 min after contralateral intravenous administration of heparin (50 IU/kg body wt; Leo Pharmaceutical Products, Weesp, the Netherlands). Postheparin LPL was also measured in a normolipidemic control population of 103 male and female volun-
teers, aged 45–75 years, without type 2 diabetes and hypertriglyceridemia.

Statistical analysis
Analyses were performed using SPSS for Windows (release 9.0). Continuous variables are presented as mean values ± SD. Pearson’s correlation coefficients were calculated for associations between different variables. Mean differences between the groups were analyzed using ANCOVA and adjusted for baseline levels and study location. P values <0.05 were considered statistically significant.

RESULTS — The baseline characteristics of 217 randomized patients in the DALI study are shown in Table 1. There were no significant differences in baseline characteristics between the placebo and atorvastatin 10-mg and 80-mg groups, except for the duration of diabetes, which was shorter in the placebo group compared with the atorvastatin 80-mg group.

Baseline levels of apoC-III were similar in the treatment groups (Table 2). The mean apoC-III level was 41.5 ± 10.0 mg/l. ApoC-III correlated significantly with plasma triglycerides (r = 0.74, P < 0.001; apoB (r = 0.24, P < 0.001), and total cholesterol (r = 0.33, P < 0.001). Dividing apoC-III levels into quartiles showed that subjects in the highest quartile (apoC-III >48.0 mg/l), compared with those in the lowest quartile (apoC-III <34 mg/l), exhibited plasma triglyceride levels twice as high (3.79 vs. 1.80 mmol/l). ApoC-III is a constituent of HDL and triglyceride-rich apoB-containing lipoproteins. The average amount of HDL apoC-III and LpB:C-III was 17.7 ± 5.5 (12.8–21.8) and 23.8 ± 7.7 mg/l (17.1–29.5), respectively. LpB:C-III correlated strongly (r = 0.76, P < 0.001) and HDL apoC-III weakly (r = 0.28, P < 0.001) with plasma triglycerides (Fig. 1). The HDL apoC-III to HDL cholesterol ratio, representing an estimate of the amount of apoC-III per HDL particle, correlated much stronger with plasma triglycerides (r = 0.68, P < 0.001) than HDL apoC-III (Fig. 2). After log transformation of the plasma triglyceride levels, similar correlations were found (data not shown). Postheparin LPL activity only showed a weak inverse correlation with plasma triglycerides (r = −0.13, P = 0.05) and not with apoC-III.

HbA1c and plasma glucose levels remained unchanged after atorvastatin treatment. Plasma apoC-III levels were significantly decreased after 10-mg (21%) and 80-mg (27%; both P < 0.05) atorvastatin treatment compared with placebo (Table 2). After 30 weeks’ treatment, both 10 mg and 80 mg atorvastatin decreased the HDL apoC-III and LpB:C-III fractions to a similar extent (21–23 and 27–28%, respectively). Because the LpB:C-III fraction contained the major part of the total apoC-III, atorvastatin decreased the absolute amount of apoC-III in the LpB:C-III fraction more than in the HDL apoC-III fraction.

Earlier, we reported (30) that 10 mg and 80 mg atorvastatin decreased plasma triglycerides in the DALI population by 25 and 35%, respectively (both P < 0.001). The decrease in total plasma apoC-III strongly correlated with the decrease in plasma triglycerides in both the
10-mg and 80-mg atorvastatin groups, respectively ($r = 0.70$ and $r = 0.78$; both $P < 0.001$). In the 80-mg atorvastatin group, the decrease in LpB:C-III correlated more strongly with the decrease in plasma triglycerides ($r = 0.80$, $P < 0.001$) than the decrease in HDL apoC-III ($r = 0.43$, $P < 0.001$). Because atorvastatin increased HDL cholesterol without an effect on apoA-I (Table 2), these data suggest that the amount of apoC-III per HDL particle was lowered by atorvastatin. To test this, we calculated the HDL apoC-III–to–HDL cholesterol and HDL apoC-III–to–apoA-I ratio. Atorvastatin 10 mg and 80 mg dose-dependently lowered both ratios by 18–21 and 31%, respectively ($P < 0.005$) (Table 2).

Figure 1—Correlation between LpB:C-III (A) and HDL apoC-III (B) with plasma triglycerides (TG) in type 2 diabetic patients at baseline.

Figure 2—Correlation between HDL apoC-III–to–HDL cholesterol ratio and plasma triglycerides (TG) at baseline ($r = 0.68$, $P < 0.001$).
**CONCLUSIONS** — We show that treatment of type 2 diabetic patients for 30 weeks with 10 mg and 80 mg atorvastatin resulted in a significant lowering of apoC-III in the non-HDL (LpB-C-III) and HDL (HDL apoC-III) fractions. High LpB-C-III was found to be associated with increased CVD risk. HDL apoC-III was a strong indicator of CVD progression after hypolipidemic treatment. Therefore, the decrease in these variables may represent an important antiatherogenic effect of statin treatment. The association of apoC-III with CVD risk probably arises from its inhibitory effect on the clearance of VLDL and intermediate-density lipoprotein.

Hypertriglyceridemia is an independent risk factor for CVD in type 2 diabetes, whereas intermediate-density lipoprotein has been shown to be highly atherogenic. LPL activity only marginally correlated with plasma triglycerides and was not a major determinant of the plasma triglyceride level in the present study. This is quite conceivable. LPL activity, determined in vitro after heparin injection, reflects the maximum available amount of enzymatically active lipase. The in situ clearance capacity, however, is also dependent on stimulatory and inhibitory factors. ApoC-III is an effective inhibitor of LPL activity and is present in TRLs and HDL. Part of apoC-III is exchangeable between these lipoproteins. After partial degradation of triglycerides in TRL by LPL, apoC-III transfers back to HDL, such that clearance of the triglyceride-rich particles may proceed uninhibited. We found that the HDL apoC-III-to-HDL cholesterol ratio (a measure of the amount apoC-III per HDL particle) correlated more strongly with plasma triglycerides than HDL apoC-III. It is conceivable that in conditions where HDL becomes “saturated” with apoC-III, it is evenly distributed over HDL and TRLs and impairs further degradation of the TRLs. This could explain the strong correlation between the HDL apoC-III-to-HDL cholesterol ratio and the plasma triglyceride level.

High apoC-III levels may result from increased apoC-III synthesis, which is triggered by increased triglyceride synthesis and secretion (43) or delayed clearance (45). Batal et al. (20) demonstrated increased apoC-III synthesis using stable isotope techniques in hypertriglyceridemic subjects. They suggested that the increased apoC-III synthesis caused impairment in apoB clearance. Our data are consistent with a mechanism in type 2 diabetic patients in which apoC-III synthesis and triglyceride synthesis are increased, resulting in elevated plasma apoC-III levels, impairment of TRL (and apoC-III) clearance, and, finally, hypertriglyceridemia. Atorvastatin treatment reduced plasma triglycerides and total plasma apoC-III levels by 21–28% in both HDLs and TRLs. The decrease in plasma triglycerides was strongly correlated with the decrease in apoC-III. Because apoC-III is a constituent of TRLs, an enhanced clearance rate of TRLs will lower plasma apoC-III and, especially, LpB-C-III. However, even though this accounts for a drop in total plasma apoC-III, it does not explain the decrease in HDL apoC-III by atorvastatin treatment. Schoonjans et al. (46) showed that simvastatin decreased apoC-III mRNA and plasma apoC-III levels in rats. Therefore, part of the lowering of apoC-III may be due to inhibition of the apoC-III synthesis by atorvastatin and may explain (part of) the triglyceride-lowering effect of atorvastatin.

After atorvastatin treatment, the HDL apoC-III-to-HDL cholesterol ratio (a measure of the amount apoC-III per HDL particle) correlated more strongly with plasma triglycerides than HDL apoC-III. It is conceivable that in conditions where HDL becomes “saturated” with apoC-III, it is evenly distributed over HDL and TRLs and impairs further degradation of the TRLs. This could explain the strong correlation between the HDL apoC-III-to-HDL cholesterol ratio and the plasma triglyceride level.

Although the proposed mechanisms have to be verified by kinetic studies, our results demonstrate that atorvastatin may lower the elevated risk for atherosclerotic disease in patients with type 2 diabetes in several ways. It effectively reduces total cholesterol and triglycerides and elevates HDL cholesterol, but, additionally, it lowers atherogenic LpB-C-III and HDL apoC-III. Therefore, measurement of apoC-III in different lipoproteins may be helpful in the assessment of CVD risk and of the efficacy of treatment strategies.

**APPENDIX**

**DALI Study Group (in alphabetical order)**
Erasmus Medical Center, Rotterdam, Department of Internal Medicine: L.L. Berk-Planken, N. Hoogerbrugge, and H. Jansen. Erasmus Medical Center, Department of Biochemistry and Clinical Chemistry: H. Jansen. Gaubius Laboratory TNO-PG Leiden: H.M.G. Princen. Leiden University Medical Center: M.V. Huisman and M.A. van de Ree. University Medical Center Utrecht, Julius Center for General Practice and Patient Oriented Research: R.P. Stolk and F.V. van Veenooij. University Center Utrecht, Division of Internal Medicine: J.D. Banga, G.M. Dallinga-Thie, and F.V. van Veenooij.

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**References**


