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 antiatherogenic effect of statin treatment and may explain (part of) the triglyceride-lowering effect of atorvastatin.

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Abbreviations: apo, apolipoprotein; CVD, cardiovascular disease; DALI, Diabetes Atorvastatin Lipid Intervention; HDL apoC-III, apoC-III in HDL; LpB:C-III, apoC-III in apoB-containing lipoprotein; LPL, lipoprotein lipase; TRL, triglyceride-rich lipoprotein.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Hypertriglyceridemia, low levels of HDL cholesterol, and preponderance of small, dense LDL particles are risk factors for cardiovascular disease (CVD) (1,2) and are part of the atherogenic lipoprotein profile often present in patients with type 2 diabetes. The initiation of the atherogenic lipoprotein profile remains to be established. Kissebah et al. (3) demonstrated that in type 2 diabetic subjects, apolipoprotein (apo)B and VLDL triglyceride secretion was enhanced, but did not necessarily lead to hypertriglyceridemia. In Pima Indians, hypertriglyceridemia may develop without increased triglyceride production due to a decreased VLDL triglyceride clearance capacity (4). Wilson et al. (5) showed that in a pedigree with type 2 diabetes, hypertriglyceridemia only developed in subjects with decreased lipoprotein lipase (LPL) activity due to a genetic defect. Therefore, delayed efficiency of clearance of triglyceride-rich lipoproteins (TRLs) may be a major cause of the development of hypertriglyceridemia in type 2 diabetes. The clearance of TRLs in situ is dependent on the presence of stimulatory and inhibitory factors. In vitro studies (6,7) have implicated apoC-III as a noncompetitive inhibitor of LPL. ApoC-III may also attenuate the clearance of TRL remnants by interfering with the hepatic lipoprotein receptors (8,9). Studies with genetically modified animals support the hypothesis that apoC-III affects the metabolism of TRLs. Transgenic mice overexpressing the human apoC-III gene developed severe hypertriglyceridemia (10) due to impaired catabolism of TRLs (11,12), whereas mice lacking apoC-III showed a very efficient clearance of VLDL particles and had enhanced selective uptake of VLDL cholesterol esters (13,14).

In healthy control subjects and hyperlipidemic patients, plasma triglycerides and apoC-III concentration are strongly correlated (15,16). Patients with elevated levels of plasma triglycerides had

Table 1 —*Baseline characteristics*

	Placebo	Atorvastatin 10 mg	Atorvastatin 80 mg
n	71	72	70
Male sex (%)	46.5	61.1	54.3
Age (years)	59 ± 8	60 ± 8	60 ± 8
BMI (kg/m^2)	32.2 ± 6.1	30.0 ± 3.8	30.4 ± 4.5
Fasting glucose (mmol/l)	10.53 ± 3.66	10.52 ± 3007	10.66 ± 2.98
HbA_{1c} (%)	8.34 ± 1.14	8.26 ± 1.16	8.42 ± 1.12
LPL (units/l)	126.8 ± 52.4	131.9 ± 52.9	129.5 ± 46.4
Triglycerides (mmol/l)	2.63 ± 0.91	2.55 ± 0.88	2.84 ± 1.12
Total cholesterol (mmol/l)	6.04 ± 0.81	5.90 ± 0.90	6.02 ± 0.90
LDL cholesterol (mmol/l)	3.80 ± 0.79	3.70 ± 0.91	3.74 ± 0.90
HDL cholesterol (mmol/l)	1.05 ± 0.21	1.05 ± 0.26	1.04 ± 0.24
ApoA-I (g/l)	1.41 ± 0.19	1.39 ± 0.20	1.40 ± 0.21
ApoB (mg/100 ml)	1.28 ± 0.19	1.22 ± 0.20	1.24 ± 0.24

Data are means ± SD.

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, second, 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 $HbA_{1c} \leq 10\%$, 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). HbA_{1c} was determined by high-performance liquid chromatography, using the Bio-Rad Variant

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

	Placebo	Atorvastatin 10 mg	Atorvastatin 80 mg
n	71	71	71
Total apoC-III (mg/l)			
Baseline	41.3 ± 9.0	39.8 ± 9.5	43.1 ± 9.9
30 weeks	41.2 ± 12.0	31.8 ± 10.6*	31.1 ± 11.3*
Change (%)	0	−21.1	−27.2
HDL apoC-III (mg/l)			
Baseline	17.6 ± 5.2	16.7 ± 5.3	18.4 ± 6.0
30 weeks	17.6 ± 8.0	14.1 ± 5.2†	13.2 ± 5.8*‡
Change (%)	0	−22.2	−28.3
LpB:C-III (mg/l)			
Baseline	23.6 ± 6.8	23.1 ± 7.3	24.7 ± 8.6
30 weeks	23.6 ± 7.7	17.7 ± 7.8*	17.9 ± 8.7*
Change (%)	0	−23.4	−27.5
HDL cholestrol (mmol/l)			
Baseline	1.05 ± 0.21	1.05 ± 0.26	1.04 ± 0.24
30 weeks	1.03 ± 0.22	1.11 ± 0.31	1.09 ± 0.28
Change (%)	−2.0	5.7	4.8
ApoA-I (mg/l)			
Baseline	1.41 ± 0.19	1.39 ± 0.20	1.40 ± 0.21
30 weeks	1.37 ± 0.18	1.38 ± 0.20	1.34 ± 0.20
Change (%)	−2.8	−0.7	−4.3
HDL apoC-III to apoAI (mg/mg)			
Baseline	9.11 ± 2.97	8.83 ± 2.71	9.76 ± 3.53
30 weeks	9.89 ± 6.05	7.48 ± 2.29	7.44 ± 3.42*‡
Change (%)	8.8	−18	−31
HDL apoC-III to HDL cholesterol (mg/mmol)			
Baseline	17.1 ± 5.4	16.5 ± 5.2	18.3 ± 6.1
30 weeks	18.2 ± 11.4	13.1 ± 4.1	12.5 ± 5.8*‡
Change (%)	6.4	−20.7	−31.7

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: ‡$P = 0.05$.

method (cat. no. 270-0003; Bio-Rad). Plasma apoC-III was analyzed with a commercially available electroimmunoassay (Hydragel LP C-III; Sebia, Issey-les-Moulineaux, France). HDL apoC-III was determined in the supernatant after precipitation of the apoB-containing lipoproteins with the use of a specific antibody. LpB:C-III is calculated by subtracting HDL apoC-III from total apoC-III.

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 volunteers, 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. 1.86 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.

HbA_{1c} 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

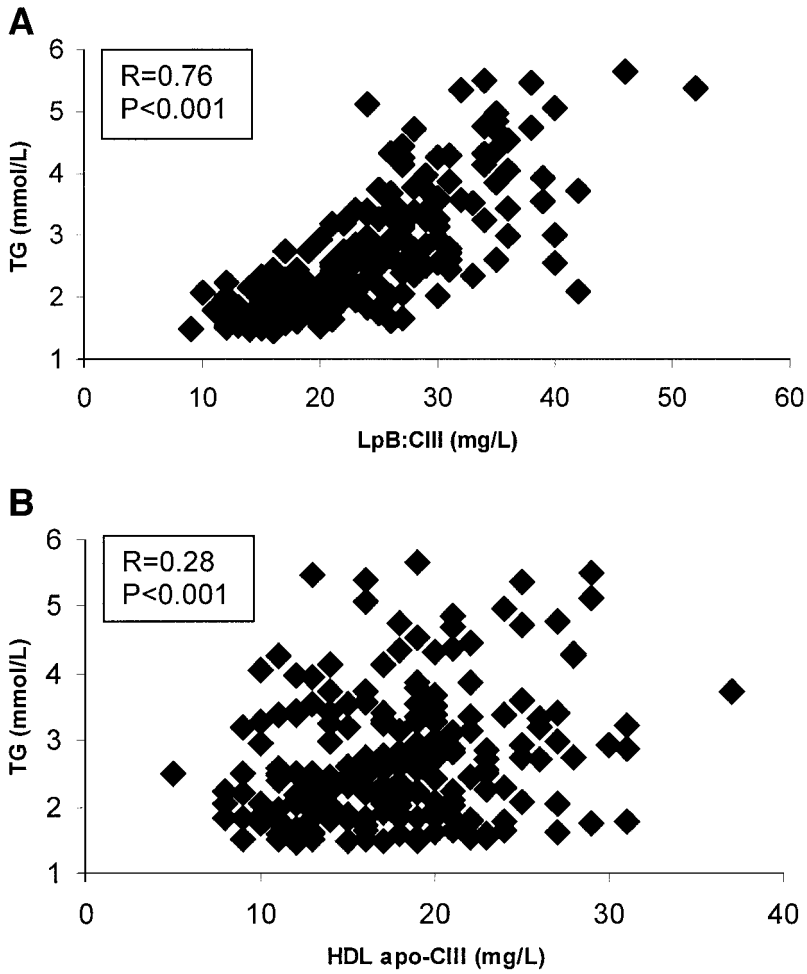

Figure 1—*Correlation between LpB:C-III* (A) *and HDL apoC-III* (B) *with plasma triglycerides (TG) in type 2 diabetic patients at baseline.*

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).

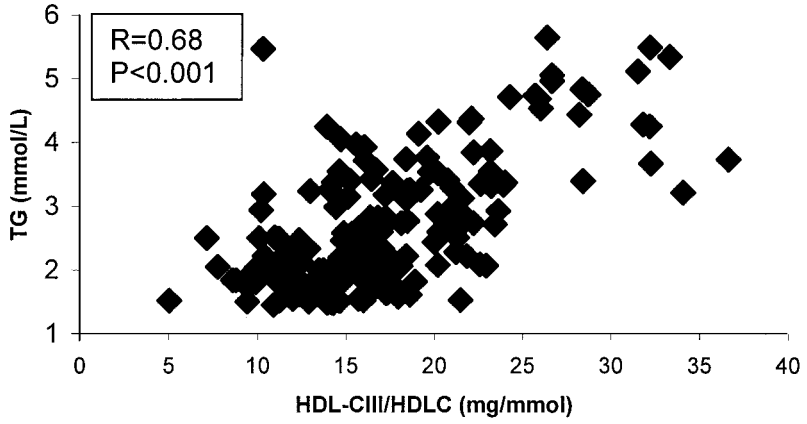

Figure 2—*Correlation between HDL apoC-III–to–HDL cholesterol ratio and plasma triglycerides (TG) at baseline* ($r = 0.68$, $P < 0.001$).

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 has been found (25,26,36,37) to be associated with increased CVD risk. HDL apoC-III was a strong indicator of CVD progression after hypolipidemic treatment (27). 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 (38), whereas intermediate-density lipoprotein (39,40) 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 (6,7) and is present in TRLs and HDL. Part of apoC-III is exchangeable between these lipoproteins (20,41). ApoC-III will be transferred from HDL to TRLs in the presence of elevated concentrations of TRLs, such that HDL apoC-III concentrations decrease (41, 42). After (partial) degradation of triglycerides in TRL by LPL, apoC-III transfers back to HDL (41,43,44), 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 and HDL apoC-III–to–apoA-I ratios were decreased, suggesting that apoC-III in HDL was no longer present in excess. Under these conditions, during (partial) degradation of triglycerides in TRLs, apoC-III may be transferred to HDLs. The relative depletion of apoC-III in the TRLs abolishes the inhibited degradation of the TRLs, such that clearance proceeds and plasma triglycerides are lowered. In this mechanism, inhibition of apoC-III synthesis by statins could play a pivotal role in the triglyceride-lowering capacity of statin treatment.

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)

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