Aggressive Lipid Lowering Does Not Improve Endothelial Function in Type 2 Diabetes

The Diabetes Atorvastatin Lipid Intervention (DALI) Study: a randomized, double-blind, placebo-controlled trial

FRANCINE V. VAN VENROOIJ, MD, MSC, PHD[1,2]
MARCEL A. VAN DE REE, MD, PHD[3]
MICHIEL L. BOTS, MD, PHD[1]
RONALD P. STOLK, MD, PHD[1]
MENNO V. HUISMAN, MD, PHD[3]
J. D. BANGA, MD, PHD[2]
ON BEHALF OF THE DALI STUDY GROUP

OBJECTIVE — Endothelial dysfunction is considered an important early marker of atherosclerosis and cardiovascular risk and is currently used as a surrogate end point for cardiovascular risk in clinical trials. Type 2 diabetic patients show a characteristic dyslipidemia. Aggressive lipid lowering might be an effective method to improve endothelial function in these patients.

RESEARCH DESIGN AND METHODS — A randomized, double-blind, placebo-controlled trial was completed to study the effect of 30 weeks' administration of atorvastatin 10 mg and 80 mg on endothelial function, as assessed by B-mode ultrasound of the brachial artery, in 133 patients with type 2 diabetes without a history of cardiovascular disease.

RESULTS — Patients with diabetes and diabetic dyslipidemia had considerable endothelium-dependent and endothelium-independent dysfunction; mean flow-mediated vasodilation (SD) was 3.16% (3.56), and mean response on sublingual nitroglycerin was 6.58% (6.04). Despite substantial lowering of all atherogenic lipid parameters, no improvement of endothelium-dependent vasodilatation was found ($P > 0.8$).

CONCLUSIONS — We observed considerable baseline endothelium-dependent and endothelium-independent dysfunction in patients with diabetes and diabetic dyslipidemia without a history of cardiovascular disease. Aggressive lipid lowering by administration of atorvastatin, resulting in substantial improvement of the lipid profile, did not reverse endothelial dysfunction.

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Dyslipidemia is an important modifiable cardiovascular risk factor in subjects with type 2 diabetes. Diabetic dyslipidemia is characterized by both quantitative and qualitative changes in lipoproteins, such as increased triglycerides and triglyceride-rich remnants, low HDL_2 cholesterol, and increased small dense LDL cholesterol (1). The use of HMG-CoA reductase inhibitors, as suggested by post hoc subgroup analyses, may have substantial benefits for subjects with diabetes with multiple cardiovascular risk factors in reducing cardiovascular risk (2–4).

Endothelial dysfunction has already been observed in the prediabetic state (5,6). Endothelial dysfunction is considered an important early marker of atherosclerosis and cardiovascular risk (7) and is currently used as a surrogate end point for cardiovascular risk in clinical trials. Impairment of endothelial function has been shown to predict future cardiac events (8,9). Endothelial function can be restored by immediate and long-term cholesterol-lowering treatment in dyslipidemic nondiabetic subjects (10–15). Improvement of endothelial function may be a possible mechanism attributing to the risk reduction that is suggested in post hoc subgroup analyses of patients with diabetes in cholesterol-lowering trials.

Atorvastatin is a powerful (16) HMG-CoA reductase inhibitor, proven to be safe (17,18) and effective in reducing total cholesterol and triglycerides and in improving endothelial function in nondiabetic patients with hypercholesterolemia and primary hypertriglyceridemia (19–22).

There is a lack of data regarding the effect of statins on endothelial dysfunction in patients with diabetes. The aim of the present study was to examine the effect of atorvastatin 10 mg (A10) versus 80 mg (A80) versus placebo on the endothelial function in patients with diabetes and dyslipidemia.

RESEARCH DESIGN AND METHODS

Patients
The study on endothelial function comprised a subset of the 217 patients with diabetes enrolled in the Diabetes Atorvastatin Lipid Intervention (DALI) study.

DALI is a double-blind, randomized, placebo-controlled, multi-center study, conducted in the Netherlands, designed to evaluate the effect of A10 versus A80 on lipid metabolism, endothelial function, coagulation, and inflammatory factors in men and women with diabetes. The protocol and eligibility criteria have been described in detail elsewhere (23). Briefly, men and women aged 45–75 years with $HbA_{1c} \leq 10\%$ were eligible. Lipid inclusion criteria were total cholesterol between 4.0 and 8.0 mmol/l and fasting triglycerides between 1.5 and 6.0 mmol/l. The DALI study had three recruiting centers: Leiden, Rotterdam, and Utrecht. All 145 patients participating in the DALI study at the University Hospitals of Leiden and Utrecht entered the endothelial function substudy. In 12 subjects, endothelial function at baseline could not be measured or evaluated accurately. The study protocol was approved by the Ethical Committees of the participating centers and written informed consent was obtained from all subjects.

Flow-mediated vasodilation

Ultrasonography was performed at the University Medical Center Utrecht and the Leiden University Medical Center. Sonographers followed a detailed training program before the start of the study to ensure standardization of the ultrasound measurements. During the study, continuous feedback was given to ensure ongoing high-quality imaging. Patients were in fasting state, did not use tobacco on the morning of the evaluation, and rested in the supine position for 10 min before the study. All drugs were withheld on the morning of the test. Standard electrocardiograph leads were attached. A 7.5-MHz linear-array ultrasound transducer connected to an Acuson Aspen (Utrecht) or Aloka SD1400 (Leiden) was used to visualize the brachial artery longitudinally just above the antecubital fossa. During the measurement, the transducer was held in the same position using external fixation. When a satisfactory optimal image of the brachial artery was obtained, three baseline images were frozen on the R-wave of the electrocardiogram. A pneumatic blood pressure cuff positioned below the elbow was inflated to 20 mmHg above systolic blood pressure for a 4-min period to induce ischemia. The cuff was then released, and R-wave triggered artery images were frozen and recorded ev-

ery 15 s for 5 min (reactive hyperemic period). After 10 min of rest, a new set of three baseline images was obtained, followed by administration of 0.4 mg sublingual nitroglycerin (NTG) spray and imaging for an additional 5 min as described above. All images were recorded on S-VHS tape for standardized, blinded, off-line analyses in Utrecht after the last visit of the last patient, using software developed by the Wallenberg Institute of Cardiovascular Research and the Chalmers University of Technology (Gothenburg, Sweden) (24).

Measurement of the endothelial function of the brachial artery was performed at baseline and after 30 weeks. Endothelium-dependent vasodilation (flow-mediated vasodilation [FMD]) is expressed as ([maximal lumen diameter after ischemia minus lumen diameter at baseline]/lumen diameter at baseline). The endothelium-independent vasodilation (NTG) is expressed as ([maximal lumen diameter after NTG minus lumen diameter at baseline]/lumen diameter at baseline). Using this methodology and analysis, reproducibility studies with repeat readings of images from videotapes showed that mean (SE) baseline lumen diameter at first reading was 4.99 mm (0.33) and 4.87 mm (0.31) at the second reading. For postischemia lumen diameter measurements, these values were 5.12 mm (0.30) and 5.07 mm (0.31), respectively. The reliability coefficients, i.e., the proportion of

variation in the measurement that can be attributed to differences in patients, were 99% for the baseline lumen diameter measurements and 99% for the postischemia measurements. The mean endothelium-dependent vasodilation was 2.8% (1.6) at the first reading and 4.2% (0.8) at the second reading, with a reliability coefficient of 67%. For the endothelium-independent vasodilation, the values were 5.9% (1.6), 8.0% (1.8), and 66%, respectively.

Laboratory measurements

After an overnight fast for a minimum of 12 h, blood was drawn for lipid profiles. Standard lipid variables (LDL cholesterol, HDL cholesterol, triglycerides, HbA_{1c}, and glucose) were measured at baseline and at the end of the study. These variables were quantified as described (23).

Sample size determination

Based on earlier experience with this technique at our institution (25), we assumed a standard deviation of 5%. With a sample size of 50 subjects in each group, a two-sided α of 5%, and a power of 80%, an absolute difference in FMD of $\geq 2.8\%$ could be detected. This reflects a treatment effect of 35%.

Statistical analysis

All data were analyzed by intention to treat. Mean differences between the study groups were analyzed using ANCOVA,

Table 1—Baseline characteristics of the 133 type 2 diabetic patients in whom endothelial function was measured in the DAL1 study

	Placebo	A10	A80
n	44	46	43
Male sex (%)	50	67	58
Age (years)	59 ± 8	61 ± 7	61 ± 8
Duration of diabetes (years)	10.1 ± 6.3	11.6 ± 6.6	12.8 ± 8.7
Insulin use (%)	59	56	58
BMI (kg/m^2)	32.5 ± 6.4	30.0 ± 3.8	30.6 ± 3.8
Waist-to-hip ratio	0.99 ± 0.08	1.00 ± 0.06	1.01 ± 0.09
Hypertension (%)	59	54	70
Blood pressure (mmHg)	144 ± 21/86 ± 9	146 ± 18/87 ± 11	147 ± 17/87 ± 9
Current smoking (%)	23	20	23
Fasting glucose (mmol/l)	10.5 ± 4.1	10.5 ± 3.1	11.1 ± 2.8
HbA$_{1c}$ (%)	8.4 ± 1.2	8.1 ± 1.2	8.6 ± 1.2
Total cholesterol (mmol/l)	6.0 ± 0.8	5.8 ± 0.9	6.1 ± 0.9
LDL cholesterol (mmol/l)	3.7 ± 0.9	3.7 ± 0.9	3.7 ± 1.0
Triglycerides (mmol/l)	2.75 ± 1.06	2.47 ± 0.82	2.81 ± 1.14
HDL cholesterol (mmol/l)	1.04 ± 0.2	1.06 ± 0.3	1.04 ± 0.2

Continuous data are expressed as means ± SD unless otherwise indicated.

Table 2—*FMD at baseline and at end of the DAL1 study*

	Placebo	A10	A80
n	44	46	43
Diameter (mm)			
Baseline	4.88 ± 0.13	4.89 ± 0.10	4.77 ± 0.09
30 weeks	4.81 ± 0.11	4.87 ± 0.09	4.89 ± 0.09
FMD (%)			
Baseline	2.88 ± 0.65	3.41 ± 0.56	3.18 ± 0.35
30 weeks	3.41 ± 0.46	3.20 ± 0.48	3.10 ± 0.42
NTG (%)			
Baseline	6.90 ± 1.0	6.80 ± 0.89	6.01 ± 0.88
30 weeks	7.74 ± 0.53	6.87 ± 0.68	6.59 ± 0.86

Data are means ± SE.

adjusted for baseline levels and study location. Intervention effects were further adjusted for additional potential confounders using ANCOVA. Additional explanatory analysis was used to evaluate whether indicators of diabetes severity modified the effect of atorvastatin on endothelial function using multiplicative interaction terms in a linear regression model. Analyses were performed by SPSS for Windows (release 9.0).

RESULTS

The baseline characteristics of the subset of 133 patients who entered the FMD protocol are shown in Table 1. Patients randomized to placebo had higher BMI. In all three groups, the compliance with the study medication was >95%. Eight patients (6%) withdrew before the end of the study because of adverse events ($n = 4$), personal reasons ($n = 3$), or protocol violation ($n = 1$). This was equally distributed across the three groups. In three patients, the final FMD measurement could not be evaluated.

Endothelium-dependent vasodilation was similar among all groups at baseline (Table 2). Both standard and aggressive therapy with atorvastatin did not affect FMD. After 30 weeks, the mean FMD was 3.20 ± 0.48 and 3.10 ± 0.42% in A10 and A80, respectively, versus 3.41 ± 0.46% in the placebo group ($P > 0.8$) (Table 2, Fig. 1). Similar results were seen for endothelium-independent vasodilation (NTG) (Table 2).

The effect of atorvastatin did not differ across groups according to sex, age, smoking, antihypertensive therapies (ACE inhibitors, β-blockers, and calcium antagonists), hypoglycemic treatment (insulin, metformin, sulfonylurea derivatives, or combination therapy), lipid variables, or parameters of glycemic control.

No significant associations were found between endothelial function and lipid variables at baseline. Atorvastatin treatment effectively reduced all atherogenic lipid parameters and increased HDL cholesterol. After 30 weeks, LDL cholesterol (SD) was reduced to 2.0 mmol/l (0.5) (−46%; $P < 0.001$) in A10 and 1.8 mmol/l (1.0) (−51%; $P < 0.001$) in A80 (A80 versus A10; $P < 0.01$). Fasting triglyceride levels were reduced to 1.76 mmol/l (0.74) (−29%; $P < 0.001$) in A10 and 1.61 mmol/l (1.0) (−43%; $P < 0.001$) in A80. HDL cholesterol was increased to 1.12 mmol/l (0.3) in A10 and 1.10 mmol/l (0.3) in A80 (both +6%; $P < 0.005$). The effects of atorvastatin treatment on lipid variables have been described extensively elsewhere (23).

CONCLUSIONS

In this randomized, double-blind, placebo-controlled study, we did not find evidence for improvement of flow-mediated and NTG-induced vasodilation by standard and aggressive cholesterol lowering with atorvastatin in patients with diabetes and diabetic dyslipidemia. These results were observed despite marked improvement of the lipid profile after treatment with A10 and A80. Furthermore, in this study, endothelial function seemed not to be associated with any traditional lipid variable, in contrast to studies of nondiabetic subjects (11,26,27).

In nondiabetic subjects, HMG-CoA reductase inhibitors were able to improve endothelial function (13,28). In contrast, it is not clear whether lowering cholesterol may improve endothelial function in patients with diabetes. To our knowledge,

the only report available is from Sheu et al. (29), who observed that endothelial dysfunction, measured using the same noninvasive method, was not reversed by 24 weeks of administration of 10 mg simvastatin in an open study of 21 patients with diabetes and hypercholesterolemia. Our findings in this double-blind, placebo-controlled trial with standard and aggressive lipid lowering in 133 patients are in accordance with these published results of Sheu et al. Recently, van de Ree et al. (30), using 40 mg simvastatin, showed no improvement of endothelial function measured by plethysmography in patients with diabetes without other risk factors for cardiovascular disease.

Both FMD (flow-induced response) and NTG (NTG-induced response) at baseline were low (3.16 and 6.58%, respectively) in our study compared with published comparable data from our clinic in nondiabetic, healthy, postmenopausal Dutch women (4.5 and 10.5%, respectively) (25). This is in accordance with other studies reporting impaired endothelium-dependent endothelial function and impaired smooth muscle cell function in patients with diabetes (31–33). This study confirms that endothelial function in patients with diabetes is compromised, even in subjects without history of a cardiovascular event.

Endothelial dysfunction and insulin resistance may be associated in a very early stage, as reviewed by Baron (34). Endothelial dysfunction is already detectable in young normotensive first-degree relatives of subjects with diabetes and subjects with gestational diabetes (35,36). Petrie et al. (37) observed this association between endothelial dysfunction and insulin resistance in young, apparently healthy men. In our study, the average duration of diabetes was 11.5 years, and the mean HbA$_{1c}$ was 8.4%. Persistent hyperglycemia and hyperinsulinemia may have contributed to the loss of endothelial function. These results may point to a pathophysiological limitation of endothelial function measurements, i.e., subjects with diabetes apparently have substantial and possibly irreversible loss of endothelial function that may not be ameliorated with statin treatment as observed in nondiabetic subjects. Conflicting data concerning the effect of improved glycemic control on endothelial function have been published. Insulin and glibenclamide therapy improved en-
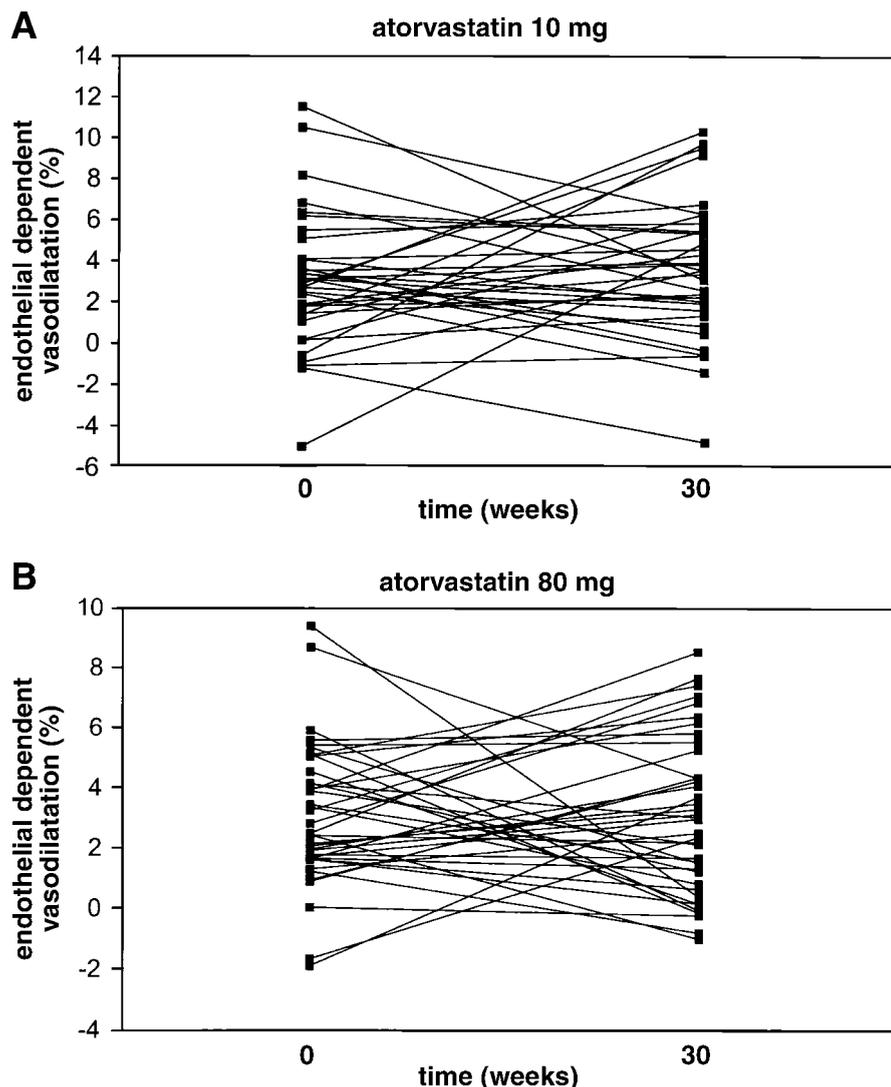
Figure 1—*Endothelial-dependent vasodilation in the DALI study at baseline and after 30 weeks of atorvastatin treatment.*

of 67%), which to some extent, may have attenuated our findings. The study was powered on a sample size of 50 patients per group and a standard deviation of 5%. A total of 133 patients entered this study, 122 of which had a final measurement. However, the variability was less than expected. There was no trend toward improvement of endothelial function, which rules out a type II error. In addition, a posterior power calculation indicated that with 122 patients, equally distributed in the three groups, and a two-sided α of 0.05, the trial had a power of 83% to detect an a priori set difference of 2.8% in FMD. Alternatively, with 122 patients, we were adequately powered to detect at least a difference of 2.7% in FMD. Therefore, these findings seem not to be hampered by insufficient statistical power.

These results suggest that one should be careful with the extrapolation of the effects of statins on endothelial function in nondiabetic patients to patients with diabetes at this moment. Multiple risk factor intervention may be needed to improve endothelial function. Furthermore, the relevance of endothelial function improvement as a therapeutic target of statins, especially in patients with diabetes, is a matter of debate (42).

In conclusion, in this double-blind, placebo-controlled, randomized study, we observed considerable endothelium-dependent and endothelium-independent dysfunction in type 2 diabetic patients with diabetic dyslipidemia without a history of cardiovascular disease. Aggressive lipid lowering by atorvastatin, resulting in substantial improvement of the lipid profile, did not reverse endothelial dysfunction.

dothelial function measured by plethysmography (38,39). However, intensive glycemic control did not improve endothelial function as assessed by FMD measurement in patients with diabetes and previously poorly regulated glycemic control (40). Similarly, ACE inhibitor treatment has shown highly disparate effects on endothelial dysfunction in patients with diabetes as well, in contrast to nondiabetic subjects in whom endothelial dysfunction can be improved by ACE inhibition (41). In type 2 diabetic patients, dyslipidemia, hyperinsulinemia, and hyperglycemia all contribute to oxidative stress that may inactivate nitric oxide and impair endothelial function and thus interfere with physiologic vessel wall properties. Moreover, the impaired NTG response suggests the presence of structural vessel wall changes.

Possible limitations of the study are differences at baseline between the placebo and atorvastatin groups. The patients randomized to the A80 group may have had more advanced disease due to a combination of longer duration of diabetes, higher prevalence of hypertension, and worse glycemic control. However, adjustment for BMI, duration of diabetes, hypertension, and glycemic control did not affect the results, nor did more detailed adjustments for type of antihypertensive and hypoglycemic treatment. The reproducibility of the endothelial function test is moderate (reliability coefficient

APPENDIX

The DALI Study Group (in alphabetical order)

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

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