

The Effect of Statin Therapy on Endothelial Function in Type 2 Diabetes Without Manifest Cardiovascular Disease

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OBJECTIVE — Cardiovascular disease (CVD) is the most important cause of mortality in patients with type 2 diabetes and is preceded by endothelial dysfunction. Flow-mediated dilation (FMD) is a noninvasive technique for measuring endothelial dysfunction. We aimed to determine the effect of long-term statin therapy versus placebo on FMD in patients with type 2 diabetes without manifest CVD.

RESEARCH DESIGN AND METHODS — A randomized, placebo-controlled, double-blind trial was performed with 250 type 2 diabetic patients. Patients were given 0.4 mg cerivastatin or placebo daily. In August 2001, when cerivastatin was withdrawn from the market, the 0.4 mg cerivastatin was replaced by 20 mg simvastatin, without deblinding the study. The primary end point was the change in FMD, measured by B-mode ultrasound, after 2 years.

RESULTS — Determinants of baseline FMD were diabetes duration, common carotid intima-media thickness, and brachial artery diameter. FMD at baseline was 1.51% in the placebo group and 1.66% in the statin group and did not change significantly after 2 years.

CONCLUSIONS — The 2-year statin therapy had no effect on FMD in type 2 diabetes. Statin-induced improvement of cardiovascular risk in patients with type 2 diabetes may be mediated through mechanisms other than increased nitric oxide availability.

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Cardiovascular disease (CVD) is the most important cause of mortality in patients with type 2 diabetes (1). Endothelial dysfunction precedes the development of atherosclerotic plaques and is believed to be reversible (2). Nitric oxide (NO) is a key molecule in this process. It modulates blood flow and vascular permeability, limits inflammation and coag-

ulation, and diminishes vascular smooth muscle cell proliferation and migration. Type 2 diabetes is associated with endothelial dysfunction; the underlying mechanisms are complex and related to hyperglycemia (sorbitol, hexosamine, protein kinase C, and advanced glycation end product pathways) and insulin resistance, resulting in mitochondrial super-

oxide overproduction and thus decreased NO availability (3). Regarding insulin, its vasodilatory capacity is at least in part NO dependent (4,5), thus explaining how insulin resistance might be related to endothelial dysfunction.

Flow-mediated dilation (FMD) of the brachial artery is a noninvasive technique for measuring endothelial function. FMD of the brachial artery has been shown to be the result of endothelium-derived NO release (6) and is related to coronary vasoreactivity (7). FMD has proven to be predictive for the presence of coronary artery disease (8,9), for future cardiovascular events (10–12), and for postoperative cardiovascular events (13) in high-risk populations. Improvement in FMD predicts a favorable cardiovascular outcome in postmenopausal hypertensive women (2). However, in patients at lower risk, FMD was not independently associated with outcome (14). FMD is impaired in patients with type 2 diabetes with reported FMD values of 4.47–12.3% in control subjects vs. 2.96–6.1% in type 2 diabetic patients in cross-sectional studies (15–22).

Hydroxymethylglutaryl-CoA reductase inhibitors (statins) have been shown to reverse endothelial dysfunction in hypercholesterolemic nondiabetic patients, possibly through upregulation of endothelial NO synthase expression (23–25), resulting in increased NO production. Statins also inhibit superoxide production (25), thereby reducing NO breakdown. The net effect is an increase in NO availability, theoretically within days after starting statin therapy. This may explain the rapid improvement in endothelial dysfunction observed in several studies in nondiabetic subjects (26). In patients with type 2 diabetes, the results of studies with short-term statin therapy are, however, contradictory with respect to FMD. We therefore conducted a randomized, placebo-controlled trial to evaluate the effect of 2 years of statin therapy on endothelial function in patients with type 2 diabetes without CVD.

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Abbreviations: CCA, common carotid artery; CVD, cardiovascular disease; FMD, flow-mediated dilation; IMT, intima-media thickness; NMD, nitroglycerin-mediated dilation.

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

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RESEARCH DESIGN AND METHODS

The study design and baseline characteristics of the original patient population have been described elsewhere (27). Briefly, 250 patients aged 30–80 years, without CVD and with type 2 diabetes for ≥ 1 year, were included in this randomized, double-blind clinical trial. Patients were given 0.4 mg cerivastatin (Bayer, Mijdrecht, the Netherlands) or placebo daily for 2 years. After the withdrawal of cerivastatin from the market, the 0.4-mg cerivastatin dose was replaced by 20 mg simvastatin (Merck Sharp & Dome, Haarlem, the Netherlands) without debinding the study. Only patients who completed the study were included in the present analysis. There were no significant differences in demographic or lipid parameters between the full cohort ($n = 250$) and the patients in this study ($n = 182$), except for race, since more whites than nonwhites completed the study (data not shown). Eligible patients gave their written informed consent. The study was performed at the Leyenburg Hospital, the Hague. The study was approved by the hospital's medical ethics committee.

The primary end point of the study was the change in FMD between baseline and 24 months. Secondary end points were the change in absolute diameter ($D_{\max} - D$), the time to peak (t_{\max}), the change in nitroglycerin-mediated dilation (NMD), and the FMD-to-NMD ratio. Comparisons between standard measurements for FMD at 1 min after cuff deflation and for NMD at 3, 4, or 5 min after nitroglycerin administration and real maximum values obtained by beat-to-beat analysis were analyzed as exploratory end points.

Patients returned to the study site after a 12-h fast at 3, 6, 12, 18, and 24 months when blinded lipid and safety measurements (creatinine kinase and alanine aminotransferase) were performed. Ultrasound measurements were performed at baseline and 24 months. A 2-year follow-up for clinical events was performed for all 250 patients.

Ultrasound measurements

Ultrasound imaging was performed with an Acuson Aspen scanner with a linear array 7.5 MHz probe. All images were recorded digitally for offline, blinded analysis by an independent core laboratory (Heart Core, Leiden, the Netherlands).

Table 1—Baseline characteristics

	Placebo	Statin
<i>n</i>	79	103
Male sex	38 (48)	52 (51)
Age (years)	59 \pm 10	59 \pm 11
Race		
White	60 (76)	72 (70)
Indo-Asian	10 (13)	21 (20)
Other	9 (11)	10 (10)
BMI (kg/m ²)	31.2 \pm 6.0	30.5 \pm 5.4
Waist-to-hip ratio	1.00 \pm 0.09	0.98 \pm 0.08
Current smoker	19 (24)	27 (26)
Hypertension	46 (58)	49 (48)
Diabetes duration (years)	9 \pm 8	8 \pm 7
Insulin use	45 (57)	51 (50)
A1C (%)	7.68 \pm 1.31	7.50 \pm 0.98
Microalbuminuria*	12 (15)	21 (20)
CCA IMT (mm)	0.780 \pm 0.129	0.763 \pm 0.124

Data are means \pm SD or *n* (%). *Defined for men as >2.5 g/mol creatine and for women as >3.5 g/mol creatine.

During the study, all measurements were performed by the same two certified ultrasonographers.

Fasting subjects were examined in the supine position. Heart rate was continuously monitored by three-lead electrocardiogram. Mean common carotid intima-media thickness (IMT) was measured as reported earlier (27). Briefly, the left and right distal 1.0 cm of the common carotid arteries, near and far walls, were examined longitudinally in the angle resulting in an optimal and maximal IMT (while avoiding plaques). For each segment, three R-wave triggered images were stored. Mean IMT was measured when possible over the entire 1 cm of the vessel segment. Mean common carotid artery (CCA) IMT was obtained by averaging the mean IMTs of the far and near wall, both left and right.

For FMD, the right arm was placed in extension in the elbow, hand in supination, with wrist and elbow supported by foam cushions. An optimal longitudinal image of the brachial artery at, or just above, the elbow was established and kept stable using a specially designed fixative. To obtain clearer images, a water bag was placed between the transducer and the skin. At baseline, 15 consecutive R-wave-triggered beats were stored. A cuff placed just distally from the elbow was inflated to 50 mmHg above systolic blood pressure (up to a maximum of 230 mmHg) for 4 min. After deflation, R-wave-frozen images were recorded for

every beat during a 5-min period. After a 10-min rest, another 15 R-wave-triggered beats were stored. Subsequently, two puffs of nitroglycerin (0.8 mg) spray were given sublingually, upon which R-wave-frozen images were again recorded for every beat during a 5-min period.

Lumen diameter (D) was defined as the distance between the media-adventitia interfaces of far and near wall. Using an automated contour detection system, D was measured semiautomatically by placing a cursor on the media-adventitia interfaces. FMD was defined as the percentage increase in brachial artery diameter within 30–120 s after ischemia ($(D_{\max} - D)/D$). NMD is defined as the percentage increase within 5 min after nitroglycerin.

Earlier studies in our institute reported reliability coefficients of 99, 99, and 67% for baseline diameter, peak diameter, and FMD, respectively (28). In a recent report on variability of FMD (using a continuous method like we did) in type 2 diabetes, coefficients of variation for baseline diameter, peak diameter, and FMD were 2.7, 2.5, and 29.7%, respectively (29).

Laboratory investigations

All laboratory measurements were performed at the Department of Clinical Chemistry and Hematology of the Leyenburg Hospital, according to International Standards Organization 15189 standard

procedures. Blood samples were collected from the subjects after a 12-h fast. EDTA tubes were used for the determination of HbA_{1c} (A1C). Liver enzymes and lipids were measured in serum. A urine sample was collected for the determination of the albumin-to-creatinine ratio. Serum or plasma was isolated by centrifugation at 1,700g (2,900 rpm) for 5 min.

Serum levels of total cholesterol and triglycerides were measured by enzymatic methods on a Synchron LX20 analyzer (Beckman Coulter, Brea, CA). LDL cholesterol was calculated according to the Friedewald formula. If triglycerides were >4.5 mmol/l, LDL cholesterol was measured directly with the use of a reagent kit (Genzyme Diagnostics). HDL cholesterol levels were determined after dextran sulfate-magnesium precipitation of apolipoprotein-B-containing lipoproteins. Creatine kinase and alanine aminotransferase were measured by an enzymatic rate method on a Synchron LX20 multichannel chemistry analyzer, according to International Federation of Clinical Chemistry methods. A1C was measured by high-performance liquid chromatography on a Variant II (BioRad). For the urine sample, a Jaffé rate method was used for the measurement of creatinine on a Synchron LX20 analyzer, whereas albumin was measured by rate nephelometry.

Statistical analysis

The number needed to detect a difference in FMD of 2% after 2 years (expected SD 4%) with a power of 80% ($\alpha = 0.05$) was 63 patients in each group. The primary treatment comparison was between placebo and statin therapy in patients completing the study, as on-treatment analysis. Changes from baseline within each treatment group were analyzed using Student's paired *t* test. Comparisons of the effects between the treatment groups were performed using Student's independent samples *t* test. Stepwise regression techniques were used to investigate the effect on baseline FMD and on changes in FMD of baseline characteristics, carotid IMT, and duration of cerivastatin versus simvastatin use. To test the equivalence of 0.4 mg cerivastatin and 20 mg simvastatin, LDL levels before and after the switch to simvastatin were compared using Student's paired *t* test. Correlation between changes in FMD and changes in lipid levels were evaluated by calculating Pearson's correlation coefficient.

Comparison between beat-to-beat analysis and standard methods was performed using the using Student's paired *t* test and Bland Altman analysis (30).

Analyses were performed using SPSS 11.0 for Windows software. All analyses were two sided, with a level of significance of $\alpha = 0.05$.

RESULTS — The characteristics of the study population are given in Table 1. No statistical differences between the groups were observed.

LDL cholesterol was 3.44 ± 0.71 mmol/l at baseline and 2.58 ± 0.95 mmol/l at 2 years (-25% , $P < 0.001$) in the statin group and 3.55 ± 0.71 mmol/l at baseline and 3.78 ± 0.81 mmol/l at 2 years (8% , $P = 0.003$) in the placebo group ($P < 0.001$). HDL cholesterol was 1.23 ± 0.39 mmol/l at baseline and 1.20 ± 0.36 mmol/l at 2 years in the statin group and 1.21 ± 0.37 mmol/l at baseline and 1.22 ± 0.38 mmol/l at 2 years in the placebo group. Triglycerides were 1.88 ± 0.79 mmol/l at baseline and 1.72 ± 1.22 mmol/l at 2 years in the statin group and 1.82 ± 0.97 mmol/l at baseline and 1.60 ± 1.38 mmol/l at 2 years in the placebo group. Changes in HDL cholesterol and triglycerides were not significantly different compared with baseline or the placebo group. Average LDL cholesterol levels were higher after the switch to simvastatin (2.34 mmol/l before vs. 2.56 mmol/l after the switch, $P < 0.001$).

FMD measurements

Baseline FMD was not significantly different between the groups. Baseline FMD in the group of 182 patients who completed the study was not significantly different from baseline FMD in the dropouts (data not shown). For the 182 patients who completed the study, FMD in the placebo group was 1.51% at baseline and 1.59% at 2 years ($P = 0.78$); in the statin group, it was 1.66% at baseline and 2.10% at 2 years ($P = 0.10$) (Table 2). There was no significant difference between the change in FMD in the statin group and the placebo group (mean difference 0.36% [95% CI -0.42 to 1.13%]; $P = 0.37$). We performed an intention-to-treat analysis for the whole group of 250 patients by using the method of "last observation carried forward" for missing values: FMD in the placebo group was 1.69% at baseline and 1.75% at 2 years ($P = 0.78$); in the statin group it was 1.65% at baseline and 2.02%

Table 2—Parameters for endothelial function

	Placebo			Statin		
	Baseline	2 years	P value*	Baseline	2 years	P value†
n	79			103		
Primary end point						
FMD (%)	1.51 ± 1.73	1.59 ± 1.84	0.78	1.66 ± 1.75	2.10 ± 2.20	0.10
Secondary end point						
Brachial artery diameter (D) (mm)	4.77 ± 0.55	4.82 ± 0.58	0.22	4.67 ± 0.70	4.67 ± 0.69	0.97
D _{max} - D (mm)	0.07 ± 0.08	0.08 ± 0.09	0.61	0.08 ± 0.08	0.09 ± 0.10	0.10
t _{max} (s)	65 ± 30	64 ± 29	0.78	64 ± 28	61 ± 26	0.37
NMD (%)	10.24 ± 4.40	10.28 ± 4.32	0.94	10.98 ± 5.73	10.27 ± 4.56	0.13
FMD-to-NMD ratio	0.14 ± 0.19	0.18 ± 0.22	0.31	0.19 ± 0.30	0.23 ± 0.26	0.36

Data are means ±SD or means (95% CI). *For change within the placebo group; †for change within the statin group.

at 2 years ($P = 0.10$). There was no significant difference between the change in FMD in the statin group and the placebo group (mean difference 0.32% [−0.89 to 0.26%]; $P = 0.28$). There was also no significant difference between the changes in absolute increase in diameter after ischemia, t_{max} , NMD, and the FMD-to-NMD ratio.

Determinants for baseline FMD were age ($r = -0.145$; $P = 0.055$), systolic blood pressure ($r = -0.192$; $P = 0.011$), diabetes duration ($r = -0.160$; $P = 0.034$), and baseline brachial artery diameter ($r = -0.582$; $P < 0.001$). Baseline CCA IMT as a continuous variable was not a determinant of baseline FMD. However, when split into quartiles, FMD at baseline was significantly lower in the highest CCA IMT quartile compared with the three lower CCA IMT quartiles (0.94 vs. 1.77%; $P = 0.006$). When included into a regression model, only the highest quartile CCA IMT, diabetes duration, and baseline brachial artery diameter remained significant determinants and together explained 11% of the variance in baseline FMD.

Baseline FMD and changes in FMD were not correlated with LDL cholesterol or any other lipid parameter. Baseline FMD and changes in FMD were also not related to sex, race, insulin use, antihypertensive medication, A1C, anthropometric parameters, and smoking habits. Changes in FMD were not related to baseline CCA IMT. Changes in FMD were negatively correlated to changes in CCA IMT in the placebo group ($r = -0.259$; $P = 0.029$). Thus, an increase in CCA IMT in the placebo group during follow-up was associated with a decrease in FMD. This could not be observed in the statin group.

The effect of the two statins used was analyzed by correcting the change in FMD for duration of cerivastatin treatment (range 6–23 months). This did not change the results.

D_{max} , FMD, and NMD as determined by beat-to-beat analysis were significantly higher compared with values obtained at fixed times. The extent of these differences was not related to absolute values. However, standard deviations of the baseline values and 95% CIs of the changes after 2 years were not lower in the beat-to-beat analysis (data not shown). When the analysis was repeated with fixed times values as an outcome measure, results did not change.

Table 3—Intervention studies on the effect of statins on FMD in patients with type 2 diabetes without CVD

Author (ref.)	Statin-treated group (n)	Design	D_{max} method	Inflation (mmHg)	Cuff	Statin dose (mg)	LDL decrease (%)	Follow-up (weeks)	D	FMD ₀	FMD _f	P value	NMD ₀	NMD _f
Sheu et al. (21)	21	Nonrandomized trial	NA	200	NA	Simvastatin 10	36	24	4.71	6.1	7.7	NS	14.5	13.3
Sheu et al. (32)	6	Nonrandomized trial	NA	200	NA	Simvastatin 20–40	>2.1 mmol/l	12	NA	4.4	8.2	0.173†	NA	NA
	6						<2.1 mmol/l	12	NA	5.6	13.6	<0.028†	NA	NA
Tsunekawa et al. (33)	14	Randomized controlled, open-label trial	At 60 s	250	Forearm	Cerivastatin 0.15	2	0.5	NA	4.0*	8.5*	<0.05	7.0*	7.4*
	8						21	14	NA	4.0*	8.5*	<0.05	6.5*	6.8*
Ventooij et al. (28)	46	Randomized controlled, double-blind trial	Per 15 s	20 >BP _{sys}	Forearm	Atorvastatin 10	46	30	4.89	3.41	3.20	>0.8	6.80	6.87
	43					Atorvastatin 80	51	30	4.77	3.18	3.10	>0.8	6.01	6.59
Cerullo et al. (16)	30	Cross-over, randomized controlled, double-blind trial	45–90 s beat-to-beat analysis	300	Forearm	Simvastatin 40	3	0.5	NA	4.8	7.3	<0.001	NA	NA
	30						28	14	NA	4.9	9.2	<0.05	NA	NA
Economides et al. (31)	19	Randomized controlled, double-blind trial	NA	50 >BP _{sys}	Forearm	Atorvastatin 20	41	14	3.7	4.2	5.6	0.07†	12.5	11.9
Present study	103	Randomized controlled, double-blind trial	30–120 s beat-to-beat analysis	50 >BP _{sys}	Forearm	Cerivastatin 0.4, simvastatin 20	25	104	4.67	1.66	2.10	0.37	10.98	10.27

P value compares changes in FMD between statin and placebo. *Estimated from figure; †for comparison of FMD at follow-up (FMD_f) with FMD at baseline (FMD₀). BP_{sys}, systolic blood pressure; NA, not available; NMD₀, NMD at baseline; NMD_f, NMD at follow-up.

CONCLUSIONS— Patients with type 2 diabetes have a high risk of cardiovascular events, and endothelial dysfunction can be viewed as an early sign of atherosclerosis. No long-term, blinded, placebo-controlled trials on the effect of statin therapy on endothelial function in type 2 diabetes have been reported. The present study shows that in our patient group endothelial dysfunction is not reversible with medium-dose statin therapy.

Several earlier studies have been performed to evaluate the effect of statin therapy on FMD in patients with type 2 diabetes. In Table 3, these studies are summarized. In a randomized study, van Venrooij et al. (28) did not find an effect of 30 weeks of atorvastatin (10 or 80 mg) versus placebo on FMD. Ceriello et al. (16) reported an improvement in FMD after simvastatin 40 mg given for only 3–6 days. Recently, Economides et al. (31) reported a nonsignificant improvement in FMD after 12 weeks of atorvastatin 20 mg. The other studies are not randomized trials or open-label trials (21,32,33).

There are several explanations for the discrepancy in the results of these studies. All studies have included patients without CVD, and age, diabetes duration, and A1C seem quite comparable. However, FMD methodology was not always clearly defined. First, the way D_{\max} is determined is critical. Simply measuring once 1 min after cuff deflation or measuring every 15 s can result in underestimation of FMD; however, with outliers, it can result in overestimation of FMD. Beat-to-beat analysis results in a more precise estimate of D_{\max} but did not lead to lower CIs in the present study.

Second, several authors do not mention their baseline lumen diameters, which is an established determinant of FMD (34). If lumen diameter is defined as the distance between the intima-lumen interfaces instead of media-adventitia interfaces of the vessel wall, lumen diameter decreases and FMD increases.

Third, some authors do not mention whether the cuff is placed around the forearm or upper arm. This is a critical issue because the latter location results in a higher FMD. Baseline FMD in our patients was low in comparison to the diabetic populations in the intervention studies mentioned, but comparable to another Dutch study, the Hoorn study

(FMD 2.96%) (22) and to a cross-sectional study (FMD 1.9%) in diabetic patients with microalbuminuria (35). In our study, with long-term statin therapy, more patients per treatment arm were included than in any other study and we used the beat-to-beat analysis for optimal precision. Moreover, given the CI of the mean difference in FMD change between placebo and statin, there is a 95% certainty that there is no treatment effect greater than an absolute difference in FMD of 1.13%.

There is much debate whether statin-induced improvement of endothelial function is mediated through a change in lipid profile, so-called pleiotropic effects, or both. In the present study, we found no relation between (changes in) lipid profile and (changes in) FMD. There is also much discussion about possible differing pleiotropic effects between the different statins (36). In our study, because of unforeseen circumstances, two different statins were used and we found no difference in effect on FMD between the statins.

Until recently, the value of statin therapy in diabetic patients was not clear in the setting of primary prevention. However, a recent meta-analysis (37) and the Collaborative Atorvastatin Diabetes Study trial (38), in which diabetic patients with at least one additional cardiovascular risk factor were included, reported marked cardiovascular risk reduction. We also found a reduced cardiovascular event rate in the statin-treated group in the present study population as reported before (27). Event reduction, on the one hand, and no difference in FMD, on the other, imply that statin-induced risk reduction in type 2 diabetes is either not mediated through restoration of endothelial dependent dilation or that FMD is not a proper test to detect changes in endothelial dysfunction in type 2 diabetic patients. The latter possibility is less likely because forearm blood flow measured by venous occlusion plethysmography, another parameter for endothelial function, also showed no improvement after statin therapy in diabetic patients (39,40). Other interventions in patients with recent-onset type 2 diabetes have resulted in an improvement in FMD (41), indicating that FMD is not simply irreversibly impaired in type 2 diabetes. Moreover, diabetes duration, carotid IMT, and vessel diameter together only explain 11% of the variance in FMD, indicating that irreversible diabetic vessel

wall changes may not have an important impact on FMD in this population.

Therefore, we conclude that statin-induced cardiovascular risk reduction in type 2 diabetes is probably not mediated through improved NO availability. Other mechanisms, such as suppression of inflammatory response, improvement of plaque stability, and reduced thrombogenic potential of the endothelial cell (42) are possible alternative explanations for the beneficial effect of statin therapy in diabetic subjects. Our results imply that in patients with type 2 diabetes, FMD is not a proper intermediate end point for statin studies. Until now, data on the prognostic value of FMD for future cardiovascular events in patients with type 2 diabetes are lacking.

We feel that the present study adds strongly to the evidence that medium-dose statin therapy has no effect on FMD in type 2 diabetic subjects without manifest CVD. FMD is impaired in diabetes of longer duration and with higher carotid IMT. Beat-to-beat analysis gives a more precise estimate of D_{\max} but did not lead to lower CIs in the present study. In patients with type 2 diabetes, statin-induced improvement of cardiovascular risk may be mediated through mechanisms other than increased NO availability.

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