The effect of statin therapy on vessel wall properties in type 2 diabetes without manifest cardiovascular disease

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Chapter 4

The effect of statin therapy on endothelial function in type 2 diabetes mellitus without manifest cardiovascular disease

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Diabetes Care 2005; 28: 1668-1674

ABSTRACT

Objective

Cardiovascular disease is the most important cause of mortality in patients with type 2 diabetes mellitus (DM2) and is preceded by endothelial dysfunction. Flow Mediated Dilation (FMD) is a non-invasive technique for measuring endothelial dysfunction. We aimed to determine the effect of long-term statin therapy versus placebo on FMD in patients with DM2 without manifest cardiovascular disease.

Research Design and Methods

A randomized, placebo-controlled, double-blind trial was performed in 250 patients with DM2. Patients were given 0.4 mg cerivastatin or placebo daily. In August 2001, when cerivastatin was withdrawn from the market, 0.4 mg cerivastatin was replaced by 20 mg simvastatin, without deblinding the study. The primary endpoint 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 (IMT) 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 two years.

Conclusions

There was no effect of 2 years' statin therapy on FMD in DM2. Statin-induced improvement of cardiovascular risk in patients with DM2 may be mediated through mechanisms other than increased nitric oxide availability.

INTRODUCTION

Cardiovascular disease (CVD) is the most important cause of mortality in patients with type 2 diabetes mellitus (DM2) [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, it limits inflammation and coagulation and diminishes vascular smooth muscle cell proliferation and migration. DM2 is associated with endothelial dysfunction, the underlying mechanisms are complex and related to hyperglycemia (sorbitol, hexosamin, Protein Kinase C, and Advanced Glycemic Endproducts pathways) and insulin resistance, resulting in mitochondrial superoxide 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 non-invasive 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 favourable 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 DM2 with FMD values reported from 4.47-12.3 % in controls versus 2.96-6.1% in DM2 patients in cross-sectional studies[15-22].

HMG-coenzyme A reductase inhibitors (statins) have been shown to reverse endothelial dysfunction in hypercholesterolemic non-diabetic patients, possibly through upregulation of endothelial Nitric Oxide 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 non-diabetics[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' statin therapy on endothelial function in patients with DM2 without CVD.

RESEARCH DESIGN AND METHODS

Subjects and design

The study design and baseline characteristics of the original patient population have been described elsewhere [27]. Briefly, 250 patients with DM2 for at least one year, aged 30-80 years,

without CVD were included in this randomized, double-blind, clinical trial. Patients were given 0.4 mg cerivastatin (Bayer B.V., Mijdrecht , The Netherlands) or placebo daily for 2 years. After the withdrawal of cerivastatin from the market, 0.4 mg cerivastatin was replaced by 20 mg simvastatin (Merck Sharp & Dome, Haarlem, the Netherlands), without deblinding 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, as more Caucasians than non-Caucasians 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.

Study Objectives

The primary endpoint of the study was the change in FMD between 24 months and baseline. Secondary endpoints 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(FMD/NMD). Comparisons between standard measurements for FMD at 1 minute after cuff deflation and for NMD at 3,4 or 5 minutes after Nitroglycerin administration and real maximum values obtained by beat-to-beat analysis were analyzed as an exploratory endpoint.

Follow-up

Patients returned to the study site after a 12 hours fast at 3, 6, 12, 18 and 24 months when blinded lipid and safety measurements (creatinin kinase, ALT) were performed. Ultrasound measurements were performed at baseline and 24 months. Two years 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 off-line, blinded, analysis by an independent core laboratory, Heart Core, Leiden, the Netherlands. 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 ECG. Mean common carotid artery Intima-Media Thickness (CCA 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. CCA IMT was obtained by averaging the mean IMT's of far and near wall, left and right.

For FMD the right arm was placed in extension in the elbow, hand in supination, 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 mm Hg above systolic blood pressure (up to a maximum of 230 mm Hg) for four minutes. After deflation, R-wave frozen images were recorded for every beat, during 5 minutes. After 10 minutes rest again 15 R-wave triggered beats were stored. Subsequently two puffs of nitroglycerin (0.8 mg) spray were given sublingually, upon which again R-wave frozen images were recorded for every beat during 5 minutes.

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 semi-automatically by placing a cursor on the media-adventitia interfaces. FMD was defined as the percentage increase in brachial artery diameter within 30 to 120 seconds after ischemia ((D_{max}-D)/ D). NMD is defined as the percentage increase within five minutes 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 DM2, CVs 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 ISO 15189 standard procedures. Blood samples were collected from the subjects after a 12 hour fast. EDTA tubes were used for the determination of HbA1c. Liver enzymes and lipids were measured in serum. A urine sample was collected for the determination of the albumin-to-creatinin ratio. Serum or plasma was isolated by centrifugation at 1700 g (2900 rpm) for 5 minutes.

Serum levels of total cholesterol and triglycerides were measured by enzymatic methods on a Synchron LX20-analyzer (Beckman Coulter, Brea, USA). 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. Creatinin kinase and ALT were measured by an enzymatic rate method on a Synchron LX20 multichannel chemistry analyzer, according to IFCC-methods. HbA1c was measured by HPLC on a Variant II (BioRad, USA). For the urine sample, a Jaffé rate method was used for the measurement of creatinine on a Synchron LX20-analyzer, while albumin was measured by rate nephelometry.

Statistical analysis

The number of patients needed to detect a difference in FMD of 2% after 2 years (expected SD 4%) with a power of 80 % (α = 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 cerivastatin 0.4 mg and simvastatin 20 mg, 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 coefficients. Comparison between beat-to-beat analysis and standard methods was performed using the Student's paired t-test and Bland Altman analysis[30].

Analyses were performed using SPSS 11.0 for Windows software. All analyses were 2-sided, with a level of significance of α = 0.05.

RESULTS

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

Table 1. Baseline Characteristics of 182 patients

	Placebo (n=79)	Statin (n=103)
Male sex	38 (48)	52 (51)
Age (years)	59 ± 10	59 ± 11
Ethnicity		
Caucasian	60 (76)	72 (70)
Asian-Indian	10 (13)	21 (20)
Other	9 (11)	10 (10)
BMI (kg/m^2)	31.2 ± 6.0	30.5 ± 5.4
Waist-to-hip ratio	1.00 ± 0.09	0.98 ± 0.08
Current smoker	19 (24)	27 (26)
Hypertension	46 (58)	49 (48)
Diabetes duration (years)	9 ± 8	8 ± 7
Insulin use	45 (57)	51 (50)
HbA1c (%)	7.68 ± 1.31	7.50 ± 0.98
Microalbuminuria *	12 (15)	21 (20)
CCA IMT (mm)	0.780 ± 0.129	0.763 ± 0.124

Data are means ± SD or n (%).

* Men, > 2.5 g/mol creatinine;women > 3.5 g/mol creatinine

CCA IMT: Intima-media Thickness of the common carotid artery.

Lipids

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 before versus 2.56 mmol/l after the switch, p < 0.001).

FMD

Baseline FMD was not significantly different between the groups. Baseline FMD as in the group of 182 patients who completed the study was not significantly different from baseline FMD in the drop-outs (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

Table 2. Parameters for endothelial function of 182 patients

	Baseline	2 years	Mean change [95% CI]	p
Placebo (n= 79)				
Primary endpoint				
FMD (%)	1.51 ± 1.73	1.59 ± 1.84	0.08 [-0.50 to 0.66]	0.78
Secondary endpoints				
D (mm)	4.77 ± 0.55	4.82 ± 0.58	0.05 [-0.03 to 0.12]	0.22
D_{max}-D (mm)	0.07 ± 0.08	0.08 ± 0.09	0.01 [-0.02 to 0.03]	0.61
T_{max} (sec)	65 ± 30	64 ± 29	-1 [-11 to 8]	0.78
NMD (%)	10.24 ± 4.40	10.28 ± 4.32	0.04 [-0.87 to 0.94]	0.94
FMD/NMD	0.14 ± 0.19	0.18 ± 0.22	0.04 [-0.03 to 0.10]	0.31
Statin (n= 103)				
Primary endpoint				
FMD (%)	1.66 ± 1.75	2.10 ± 2.20	0.44 [-0.08 to 0.96]	0.10
Secondary endpoints				
D(mm)	4.67 ± 0.70	4.67 ± 0.69	0.00 [-0.08 to 0.08]	0.97
D_{max}-D(mm)	0.08 ± 0.08	0.09 ± 0.10	0.02 [0.00 to 0.04]	0.10
T_{max} (sec)	64 ± 28	61 ± 26	-3 [-10 to 4]	0.37
NMD (%)	10.98 ± 5.73	10.27 ± 4.56	-0.71[-1.64 to 0.22]	0.13
FMD/NMD	0.19 ± 0.30	0.23 ± 0.26	0.04 [-0.04 to 0.11]	0.36

Data are means ± SD or means [95 % CI]

Mean change = mean change from baseline to 2 years

D: brachial artery diameter; D_{max}: maximal brachial artery diameter after ischemia;

T_{max}: time to reach maximal brachial artery diameter

(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 % 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 % [95% CI –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 % versus 1.77 %, p=0.006). When included into a regression model, only 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, anti-hypertensive medication, HbA1c, 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 to 23 months). This did not change the results.

The 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 confidence intervals of the changes after two years were not lower in the beat-to-beat analysis (data not shown). When repeating the analysis with fixed times values as an outcome measure, results did not change.

CONCLUSIONS

Patients with DM2 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 DM2 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 DM2. In Table 3 these studies are summarized. In a randomized study, van Venrooy et al. [28] did not find an effect of 30 weeks 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 three to six days. Recently, Economides et al. reported a non-significant improvement in FMD after 12 weeks atorvastatin 20 mg [31]. 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 HbA1c seem quite comparable. However, FMD methodology was not always clearly defined. First, the way D_{max} is determined is critical. Simply measuring once, one minute after cuff deflation, or measuring every 15 seconds, can result in underestimation, but in case of outliers, also in overestimation of FMD. Beat-to-beat analysis results in a more precise estimate of D_{max}, but did not lead to lower confidence intervals 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 confidence interval 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, through 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 have been 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 CARDS trial, in which diabetic patients with at least one additional cardiovascular risk factor were included[38], 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

Table 3. Intervention studies on the effect of statins on FMD in patients with DM2 without CVD

Author	N	Design	Dmax *method*	Inflation *mmHg*	Cuff	Statin dose *mg*	LDL↓ %	F-up *weeks*	D *mm*	FMD_{bl} %	$FMD_{f\text{-}up}$ %	p	NMD_{bl} %	$NMD_{f\text{-}up}$ %
Sheu[21]	21	non-rand	NA	200	NA	simva 10	36	24	4.71	6.1	7.7	NS	14.5	13.3
Sheu[32]	6	non-rand	NA	200	NA	simva 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[33]	14	RCT open	at 60 s.	250	forearm	ceriva 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*	7.5*
Venrooij[28]	46	RCT db	per 15 s.	20>BP_{sys}	forearm	atorva 10	46	30	4.89	3.41	3.20	>0.8	6.80	6.87
	43					atorva 80	51	30	4.77	3.18	3.10	>0.8	6.01	6.59
Ceriello[16]	30	cross-over RCT db	45-90 s. b-t-b	300	forearm	simva 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[31]	19	RCT db	NA	50>BP_{sys}	forearm	atorva20	41	14	3.7	4.2	5.6	0.07†	12.5	11.9
present study	103	RCT db	30-120 s. b-t-b	50>BP_{sys}	forearm	ceriva 0.4 / simva 20	25	104	4.67	1.66	2.10	0.37	10.98	10.27

N = number of patients in the statin treated group; NA = data not available

non-rand = non-randomized trial; RCT = randomized controlled trial; db = double blind; b-t-b = beat-to-beat analysis

BP_{sys} = systolic blood pressure; LDL↓ % = percentage decrease in LDL cholesterol; F-up = Follow-up

D = lumendiameter; FMD_{bl} = FMD at baseline; $FMD_{f\text{-}up}$ = FMD at follow-up; NMD_{bl} = NMD at baseline; $NMD_{f\text{-}up}$ = NMD at follow-up

p = p-value for comparison of changes in FMD between statin and placebo; † p-value for comparison of FMD at follow-up with FMD at baseline

* = estimated from figure

reduction on the one hand and no difference in FMD on the other, imply that statin-induced risk reduction in DM2 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 DM2 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 DM2 have resulted in an improvement in FMD[41], indicating that FMD is not simply irreversibly impaired in DM2. 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 DM2 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 DM2, FMD is not a proper intermediate endpoint for statin studies. Until now, data on the prognostic value of FMD for future cardiovascular events in patients with DM2 are lacking.

We feel that the present study adds strongly to the evidence that medium-dose statin therapy has no effect on FMD in DM2 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 confidence intervals in the present study. In patients with DM2, statin-induced improvement of cardiovascular risk may be mediated through mechanisms other than increased NO availability.

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