



Year: 2009

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Abstract: Aims Endothelial dysfunction and plaque formation are features of atherosclerosis. Inhibition of L-type calcium channels or HMG-CoA pathway improves endothelial function and reduces plaque size. Thus, we investigated in stable coronary artery disease (CAD) the effects of a calcium antagonist on coronary endothelial function and plaque size. Methods and results In 454 patients undergoing PCI, acetylcholine (10–6 to 10–4 M) was infused in a coronary segment without significant CAD. Changes in coronary diameter were measured and an intravascular ultrasound examination (IVUS) was performed. On top of statin therapy, patients were randomized in a double-blind fashion to placebo or nifedipine GITS 30-60 mg/day and followed for 18-24 months. Blood pressure was lower on nifedipine than on placebo by 5.8/2.1 mmHg ($P < 0.001$) as was total and LDL cholesterol (4.8 mg/dL; $P = 0.495$), while HDL was higher (3.6 mg/dL; $P = 0.026$). In the most constricting segment, nifedipine reduced vasoconstriction to acetylcholine (14.0% vs. placebo 7.7%; $P < 0.0088$). The percentage change in plaque volume with nifedipine and placebo, respectively, was 1.0 and 1.9%, ns. Conclusion The ENCORE II trial demonstrates in a multi-centre setting that calcium channel blockade with nifedipine for up to 2 years improves coronary endothelial function on top of statin treatment, but did not show an effect of nifedipine on plaque volume

DOI: <https://doi.org/10.1093/eurheartj/ehp151>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-154275>

Journal Article

Published Version

Originally published at:

Lüscher, T F; Pieper, M; Tendera, M; Vrolix, M; Rutsch, W; van den Branden, F; Gil, R; Bischoff, K-O; Haude, M; Fischer, D; Meinertz, T; Münzel, Thomas (2009). A randomized placebo-controlled study on the effect of nifedipine on coronary endothelial function and plaque formation in patients with coronary artery disease: the ENCORE II study. *European Heart Journal*, 30(13):1590-1597.

DOI: <https://doi.org/10.1093/eurheartj/ehp151>

A randomized placebo-controlled study on the effect of nifedipine on coronary endothelial function and plaque formation in patients with coronary artery disease: the ENCORE II study†

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Received 17 July 2007; revised 16 March 2009; accepted 17 March 2009; online publish-ahead-of-print 27 May 2009

See page 1556 for the editorial comment on this article (doi:10.1093/eurheartj/ehp238)

Aims

Endothelial dysfunction and plaque formation are features of atherosclerosis. Inhibition of L-type calcium channels or HMG-CoA pathway improves endothelial function and reduces plaque size. Thus, we investigated in stable coronary artery disease (CAD) the effects of a calcium antagonist on coronary endothelial function and plaque size.

Methods and results

In 454 patients undergoing PCI, acetylcholine (10^{-6} to 10^{-4} M) was infused in a coronary segment without significant CAD. Changes in coronary diameter were measured and an intravascular ultrasound examination (IVUS) was performed. On top of statin therapy, patients were randomized in a double-blind fashion to placebo or nifedipine GITS 30–60 mg/day and followed for 18–24 months.

Blood pressure was lower on nifedipine than on placebo by 5.8/2.1 mmHg ($P < 0.001$) as was total and LDL cholesterol (4.8 mg/dL; $P = 0.495$), while HDL was higher (3.6 mg/dL; $P = 0.026$). In the most constricting segment, nifedipine reduced vasoconstriction to acetylcholine (14.0% vs. placebo 7.7%; $P < 0.0088$). The percentage change in plaque volume with nifedipine and placebo, respectively, was 1.0 and 1.9%, ns.

Conclusion

The ENCORE II trial demonstrates in a multi-centre setting that calcium channel blockade with nifedipine for up to 2 years improves coronary endothelial function on top of statin treatment, but did not show an effect of nifedipine on plaque volume.

Keywords

Acetylcholine • Angiography • Endothelium • IVUS • Plaque

Introduction

Coronary artery disease (CAD) is associated with functional and structural vascular changes leading to ischaemia and/or plaque

rupture.^{1,2} Functional changes of coronary arteries precede lesion formation and become more pronounced with disease progression.^{3–5} Typically, release of endothelial nitric oxide (NO) that mediates coronary vasomotion is reduced and, in turn, adherence

† This manuscript was assessed and accepted for publication by the previous Editorial Office based in Leuven.

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of monocytes and platelets and subsequently smooth muscle cell migration and proliferation are increased.^{2,3} Endothelial dysfunction occurs as a 'response to injury' to oxidized low-density lipoproteins,^{2,6} hypertension,^{7–9} increased blood glucose,^{10,11} and oxygen-derived free radicals.¹²

Treatment modalities able to reverse endothelial dysfunction might have great clinical implications. Several targets have been considered, among them the renin–angiotensin system that inactivates NO via stimulation of NADPH-oxidase and thus the production of superoxide.¹³ ACE-inhibitors improve endothelial function in the brachial¹⁴ and the coronary circulation.^{15,16} Inhibition of HMG-coenzyme reductase not only reduces cholesterol, but also leads to prenylation and geranylation of proteins involved in the regulation of nitric oxide.¹⁷ In the forearm, circulation statins improve endothelial function in hypercholesterolaemia.¹⁸ In the coronary circulation, the effects of statins are controversial at least after 6 months of treatment^{16,19} suggesting that in patients with stable CAD more time may be required to improve NO-mediated vasomotion.

Calcium channel blockers may reduce oxidative stress and improve NO release independent of their effects on L-type calcium channels.^{20,21} In previous studies, nifedipine improved endothelial function after 6 months;¹⁶ however, the impact on atherosclerotic plaque formation is uncertain.

We, therefore, investigated in patients with stable CAD undergoing a percutaneous intervention (PCI) the effects of long-acting nifedipine on coronary endothelial function and plaque formation over 18–24 months on top of standard therapy, including a statin.²²

Methods

Patients

The ENCORE II (Evaluation of Nifedipine on Coronary Endothelial Function) Study was a randomized, double-blind, placebo-controlled study investigating the effect of nifedipine GITS 30 mg/day increased to 60 mg/day on endothelial vasomotion and atherosclerotic burden in patients undergoing coronary angiography with or without PCI. Inclusion criteria were: legal age, left coronary artery segment with $\leq 40\%$ area stenosis (index artery), and no vasodilation of index artery upon acetylcholine infusion. Patients with at least one segment of the index artery without vasodilation on acetylcholine (visual inspection) were eligible. Main exclusion criteria were: myocardial infarction (MI) within 2 weeks or unstable angina (Braunwald class IIIb troponin positive), stroke, peripheral revascularization or major surgery within 3 months, uncontrolled diabetes, symptomatic hypotension or uncontrolled hypertension, left ventricular ejection fraction $< 40\%$, creatinine $> 200 \mu\text{mol/L}$, transaminases greater than three times ULN, history of liver or gastrointestinal diseases, and calcium channel blocker treatment for > 2 months prior to inclusion. ACE-inhibitors or ARBs used > 2 months were continued, otherwise they were not allowed. Participating sites had approval for the study from their Institutional Review Board or Ethics Committee and patients provided written informed consent.

The first patient entered the study in June 1999 and last patient's last visit took place in January 2004.

Interventions

Before intervention, cardiovascular drugs were withheld for 24 h (short acting nitrates for at least 3 h). After coronary angiography and/or PCI, an infusion catheter was positioned in a proximal

segment of the left anterior descending or circumflex coronary artery with luminal narrowing $< 40\%$. Acetylcholine (Miochol, Ciba Vision, Basel, Switzerland) was infused at 2 mL/min for 3 min in the following order: (1–3) acetylcholine 0.36, 3.6, and 18 $\mu\text{g/ml}$; (4) isotonic saline; and finally (5) a bolus of 250 μg nitroglycerine was injected. At the end of each infusion, heart rate and blood pressure were recorded and angiography performed with non-ionic contrast medium. Finally, an IVUS examination was made.

Planned treatment duration was 18–24 months. At the end of the treatment, patients underwent catheterization after withdrawal of study medication for 2–3 days and other cardiovascular drugs for 24 h as at the baseline study. The X-ray tube and catheter were placed in identical positions and the IVUS catheter was placed at the same anatomical landmarks as at baseline. The protocol was then repeated in the index artery.

Patients were seen in the clinic after 2 weeks, 1, 6, 12, and 18 months. Ambulatory blood pressures were taken with a sphygmomanometer. Clinical chemistry and haematology were analysed centrally (Institute for Clinical Chemistry, University Hospital of Freiburg, Germany). Cholesterol was determined enzymatically (CHOD-PAP method, Roche Diagnostics, Mannheim, Germany). HDL-cholesterol was determined with a homogenous HDL-C assay (Roche Diagnostics, Mannheim, Germany), and LDL-cholesterol was calculated using the Friedewald formula.

Study outcomes

The primary endpoint was the effect of nifedipine compared with placebo on acetylcholine-induced coronary vascular response at the highest dose of acetylcholine applied both at baseline and follow-up. The secondary endpoint was the effect of nifedipine compared with placebo on the percentage change in plaque volume as assessed by intravascular ultrasound.

Treatments and randomization

The protocol was designed as a randomized, double-blind, double dummy study with three treatment arms: cerivastatin 0.2 mg/day, cerivastatin 0.8 mg/day, or cerivastatin 0.8 mg/day plus nifedipine GITS 30–60 mg/day.²² After 294 patients had been randomized, cerivastatin was withdrawn from the market due to untoward effects.^{23,24} The study was therefore modified to continue to investigate the effects of nifedipine GITS 30–60 mg/day vs. placebo on top of lipid lowering therapy with a statin according to current guidelines.^{25,26} Thus, patients randomized before withdrawal of cerivastatin who gave informed consent to continue their participation were continued in their previous treatment arm minus cerivastatin—resulting in a 2:1 distribution of the patients on placebo and nifedipine, respectively. New patients were therefore randomized to placebo or nifedipine on a 1:2 ratio in order to get balanced samples in the two treatment arms. One hundred and twenty-three of the originally enrolled patients (42% of randomized patients) re-entered the study. Another 149 patients were randomized after restart leading to an evaluable population of 226 patients. The exposure time to nifedipine averaged 622 days (SD: ± 82 days; range: 488–853 days).

A pre-prepared randomization list for each centre was generated by sponsor's statistician with block size 6 and no stratifications. Patients were assigned to next free medication box for their random allocation to treatment group.

The randomization list was adapted for the re-design of the study. Patients randomized into the original study and who wanted to continue kept their allocation number without breaking the randomization code.

Assessment of coronary artery diameter

Angiograms were analysed at a core lab (Cardiology, Medical School, Hannover, Germany). Readers were blinded to patients' identity and to treatment arm. In the index artery 2–7 (mean 3) segments distal to the infusion catheter were measured using CMS edge-detection algorithm (MEDIS, Leiden, The Netherlands²⁷). Each segment was identified by anatomical landmarks according to AHA guidelines to facilitate identification at follow-up. Mean diameters were measured at baseline and after each acetylcholine and nitroglycerine infusion. Coronary responses were expressed as percent change from baseline of the acetylcholine-induced change in mean lumen diameter. The pre-defined target segment for the main comparison was the one with the most pronounced vasoconstriction at any acetylcholine dose at baseline.

IVUS imaging procedure

The IVUS study was performed after acetylcholine infusions. Intracoronary nitroglycerine (0.1–0.2 mg) was given and the IVUS catheter positioned in the target vessel. Two different IVUS systems were used: 2.9 F 30 MHz Ultra Cross, Scimed, Boston Scientific, Sunnyvale, CA, USA or a 2.9 F 30 MHz Vision Five-64 F/X Endosonics, Rancho Cordova, CA, USA. In each patient, the same catheter type was used at baseline and follow-up. The position of the IVUS catheter

was controlled with fluoroscopy. The imaging transducer of the IVUS catheter was placed distal to a major side branch of the target vessel and the position was documented. Anatomic orientation was also guided by spoken comments during the IVUS study. IVUS pullback was mechanically done at 0.5 mm/s. Images, electrocardiogram, and comments during the pullback were recorded on S-VHS videotape.

At follow-up, IVUS examinations were repeated to accurately match the coronary segment recorded at baseline.

Analysis of IVUS images

The tapes were analysed by the core laboratory (D.F.) at Hannover Medical School, Hannover, Germany according to the clinical expert consensus documentation standards for the acquisition, measurement, and reporting of IVUS of the American College of Cardiology.²⁸ Experienced investigators blinded to patients' identity and treatment allocation reviewed baseline and follow-up images. Calibration was performed with grid marks encoded in the images. One image per second was analysed. Using computerized planimetry (TapeMeasure, Indec Inc., Mountain View, CA, USA), borders of the vessel lumen and of the external elastic membrane (EEM) were identified and cross-sectional area (CSA) measured. Atheroma CSA was calculated by EEM CSA minus lumen CSA for each image. Total atheroma volume was computed as the

Table 1 Enrolment and flow of patients in the ENCORE II study

		302 patients enrolled	
a. Initial study: cerivastatin 0.2 mg vs. cerivastatin 0.8 mg vs. cerivastatin+nifedipine		294 Randomized (1:1:1)	
		Ceriv. 0.2 mg	Ceriv. 0.8 mg
b.			
c.	Randomized	104	97
d.	Treated	102	94
e.	Completed	3	3
f.	Premature drop-out		
g.	Not completed and not continued in re-designed study part		
h.	Continued in re-designed study part		
		Cerivastatin	Cerivast 0.8 + Nifedipine
		201	93
		196	93
		6	5
		26	12
		82	35
		82	41
		152 patients enrolled	
		Placebo	Nifedipine
		82	41
		149 Randomized (1:2)	
		50	99
		50	98
o. Total treated in this study part (k. + n.)		132	139
p. Completed in this study part		114	115
q. Totals for study			
r. Randomized		251	192
s. Treated (d. + n.)		246	191
t. Total treated, potentially reportable for efficacy (e. + o.)		138	144
u. Total completed		120	120
v. Not evaluable for efficacy		8	6
x. Evaluable Patients		112	114
y. Premature drop-outs (s. – (u. + g.))		44	36
Adverse events		16	7
Death		2	1
Consent withdrawn		20	20
Non-compliance		5	5
Other reasons		1	3

average atheroma CSA from all analysed slices of the segment multiplied by segment length. From this, total lumen volume and total EEM volume were derived. The secondary efficacy parameter, percent change in atheroma volume after 18–24 months of treatment, was defined as:

$$\% \text{Change}_{\text{VolAtheroma}} = 100 \frac{(\text{Vol}_{\text{Atheroma, Follow-Up}} - \text{Vol}_{\text{Atheroma, Baseline}})}{\text{Vol}_{\text{Atheroma, Baseline}}}$$

Statistical analysis

All patients with a readable baseline and follow-up acetylcholine and/or IVUS study were eligible for the analysis of primary and/or secondary outcome parameters.

A sample size of 60 evaluable patients per treatment group was estimated to have a 90% power to detect a mean difference of 12 percentage points change in acetylcholine-induced vasoconstriction using a two-tailed *t*-test with a 0.05 significance level assuming a within-group SD of 20%. Analyses of coronary vasomotion were done by ANCOVA with treatment and centres as fixed effects and the baseline measurement as covariate. The IVUS data were analysed using the Mann–Whitney test. The between-centre effect was insignificant in all of the performed statistical analyses of outcomes. The group comparison of vital signs and lipid values during treatment were done by *t*-test. Analyses were performed with SAS, version 9.1. If not otherwise stated data are presented as mean \pm SD for intragroup statistics and as mean difference \pm SEM for intergroup statistics.

Results

Patient characteristics

Informed consent was given by 454 patients, 443 were randomized and 437 entered study treatment (Table 1). Patients who entered placebo and who withdrew prematurely are overrepresented. This is due to the fact that patients entered before the interruption were randomized to placebo or nifedipine in a 2:1 ratio and not all of these gave consent to their continuation in the study after restart. A total of 226 patients were evaluable for the intention-to-treat analysis of changes in endothelial function and/or changes in plaque volume. Reasons for non-evaluability are given in Table 1. The two treatment groups were well matched at baseline (Table 2). Also, demographics of patients enrolled into the original protocol did not differ significantly from those enrolled after restart (data not shown).

Mean values over the treatment period for blood pressure and lipids are given in Table 3. In the nifedipine group, blood pressure was lower compared with placebo, whereas heart rate did not differ. In the nifedipine arm, total cholesterol was lower, HDL cholesterol higher, and LDL cholesterol lower compared with placebo. Blood pressure and lipids during follow-up did not differ in patients enrolled into the original protocol from those in patients enrolled after restart (data not shown).

Acetylcholine test

At baseline and at follow-up, angiograms from 427 and 214 patients, respectively, were readable. Not all patients received all three doses of acetylcholine due to early occlusion of the artery at low doses. Thus, 398 (93%) and 192 (88%) patients at baseline

Table 2 Patient demographics at baseline

	Nifedipine	Placebo
<i>n</i>	114	112
Gender: males (%)	92 (81%)	93 (83%)
Age (\pm SD)	59.1 (\pm 8.8)	57.4 (\pm 8.8)
Weight (\pm SD)	79.0 (\pm 10.8)	81.0 (\pm 11.5)
BMI	27.3 (\pm 3.4)	27.4 (\pm 3.4)
Smoking		
Present	20 (17.5%)	29 (25.7%)
Past	59 (51.8%)	54 (47.8%)
Never	35 (30.7%)	28 (24.8%)
Stopped during study	3 (2.6%)	3 (2.6%)
Alcohol consumption		
Abstinent	28 (24.6%)	34 (30.1%)
Light	80 (70.2%)	72 (63.7%)
Moderate	6 (5.3%)	7 (6.2%)
Syst BP, mmHg (\pm SD)	133 (\pm 19)	132 (\pm 18)
Diast BP, mmHg (\pm SD)	77 (\pm 10)	78 (\pm 10)
HR	67 (\pm 11)	67 (\pm 10)
Total cholesterol, mg/dL (\pm SD)	192.3 (\pm 13.6)	199.9 (\pm 11.3)
HDL-Chol., mg/dL (\pm SD)	39.3 (\pm 13.6)	37.6 (\pm 11.3)
LDL-Chol., mg/dL (\pm SD)	118.8 (\pm 33)	124.6 (\pm 38)
Triglycerides, mg/dL (\pm SD)	159 (\pm 94)	162 (\pm 95)
Prior coronary interventions, <i>n</i> (%)	48 (42.1%)	28 (25.0%)

and follow-up, respectively, got the lowest and the medium dose of acetylcholine while 311 (72%) and 173 (83%), respectively, received all three doses of acetylcholine. In the most constricting coronary segment, acetylcholine at the highest dose that was dispensed at baseline and at follow-up in a patient evoked an average reduction of vessel lumen diameter of $23.4 \pm 16.2\%$ in the nifedipine group and $24.0 \pm 18.1\%$ in the placebo group at baseline. There was no difference between groups ($P = 0.2038$).

At follow-up, the change from baseline of the acetylcholine induced change in mean luminal diameter at the highest dose of acetylcholine that was infused in a patient at baseline and at follow-up averaged $13.9 \pm 16.5\%$ on nifedipine and $7.7 \pm 18\%$ on placebo. The difference between groups was 6.3% (95% CI: 1.6–10.9, $P = 0.0088$; Figure 1 and Table 4).

Intravascular ultrasound

At baseline, mean plaque volume in the target artery of patients with evaluable IVUS was 140 (101) mm³ (*n*: 97) in the nifedipine arm and 157 (101) mm³ (*n*: 96) in the placebo group with no significant difference between groups ($P = 0.168$).

Neither the difference in absolute nor relative changes between treatments was significant ($P = 0.84$ and 0.66 , respectively; Tables 5 and 6).

Adverse events

During acetylcholine infusion, transient ECG changes were reported in five (1.1%) patients. In five (1.1%) patients, diffuse

Table 3 Blood pressure and lipids: mean values during the follow-up period

	Nifedipine	Placebo	Difference (95% CI)	P
Systolic BP, mm Hg (\pm SD)	129.5 (17.0)	135.3 (18.2)	-5.8 (-10.4 to -1.2)	P = 0.014
Diastolic BP, mm Hg (\pm SD)	78.5 (9.3)	80.6 (10.3)	-2.1 (-4.7 to 0.5)	P = 0.109
Total cholesterol, mg/dL (\pm SD)	183.3 (38.0)	187.1 (41.3)	-3.8 (-14.2 to 6.6)	P = 0.472
HDL-cholesterol, mg/dL (\pm SD)	44.4 (14.6)	40.8 (11.3)	3.6 (-0.2 to 7.0)	P = 0.040
LDL-cholesterol, mg/dL (\pm SD)	104.3 (30.6)	109.1 (33.8)	-4.8 (-13.3 to 3.7)	P = 0.233

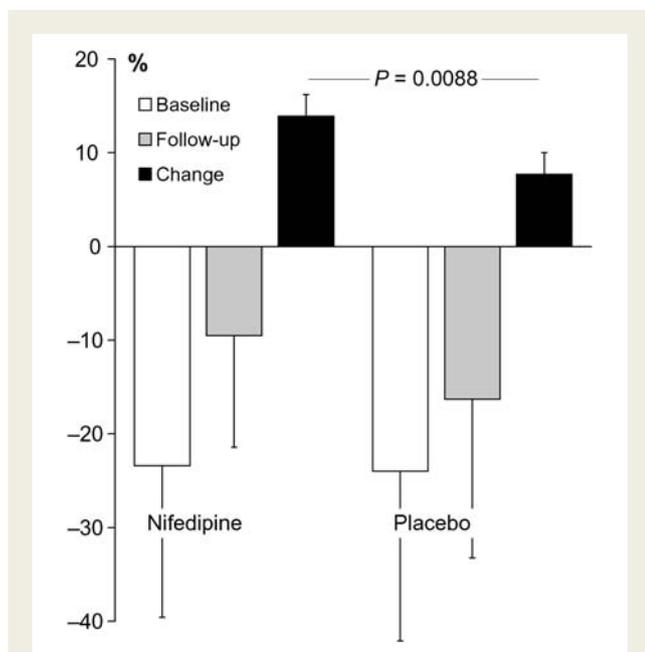


Figure 1 Change in coronary vasomotion after acetylcholine infusion. The percent change in mean lumen diameter at the highest comparable dose of acetylcholine at baseline and follow-up (mean \pm SD) and the percent change in response (mean \pm SE).

coronary vasoconstriction with marked haemodynamic consequences, requiring resuscitation in one patient, occurred. One patient suffered an MI possibly related to acetylcholine.

Five patients died during the screening procedures or study participation. One patient with acute coronary syndrome died in cardiac arrest in the catheterization laboratory, possibly related to acetylcholine. One patient died the day after an uneventful intervention, probably due to CAD. Two patients died 5–10 days after the baseline catheterization while on cerivastatin 0.2 mg/day, one suddenly and the other of unknown reason. One patient died of an unrelated neoplasm.

Peripheral oedema occurred in 20 patients (10.5%) on nifedipine compared with three patients (1.2%) on placebo, causing premature withdrawal of three patients on nifedipine and one on placebo.

An increase above five times ULN was noted for creatinine phosphokinase (CPK) in four (1.7%) patients on placebo and in four (2.3%) on nifedipine, for SGOT and/or SGPT in two patients, one in each group.

A 75-year-old female developed rhabdomyolysis after 3 weeks on cerivastatin 0.8 mg/day. Medication was stopped and the patient recovered without sequelae.

Discussion

In this multi-centre trial, we assessed the long-term effects of the calcium channel blocker nifedipine on endothelial function and plaque volume in a coronary segment with angiographically minimal disease and a vasoconstrictor response to acetylcholine. Nifedipine lowered blood pressure and had minor effects on lipids, but markedly improved coronary endothelial function with only a small effect on plaque progression.

In ENCORE I,¹⁶ the so far largest clinical trial investigating endothelial dysfunction in CAD with 250 patients, we have previously found a pronounced effect of the L-type calcium channel antagonist nifedipine on coronary endothelial function after 6 months, while the HMG-coenzyme reductase inhibitor cerivastatin had only marginal effects. The latter finding was in line with CARATS that used simvastatin in the same patient population.¹⁹ However, when considering not just the most constricting segment but all analysed coronary arteries, there was a significant effect of the combination of nifedipine and cerivastatin compared with placebo in the ENCORE I trial.¹⁶ Thus, it appeared that in contrast to studies in the forearm circulation of patients with hypercholesterolaemia,¹⁸ coronary endothelial dysfunction is more difficult to reverse and/or may require longer treatment periods than in other vascular beds with little atherosclerosis. Furthermore, based on ENCORE I and CARATS it remained unclear whether improving endothelial dysfunction would translate into a reduced atherosclerotic burden in the coronary circulation.

To that end ENCORE II was designed. Originally, the study involved three groups of patients, i.e. (i) a low statin dose group (cerivastatin 0.2 mg/day), (ii) a high statin dose group (cerivastatin 0.8 mg/day), and (iii) a group treated with a combination of high dose cerivastatin and nifedipine.²² The withdrawal of cerivastatin from the market^{23,24} forced a redesign of the trial after almost 300 patients had been randomized. It was decided that patients who consented to continued participation after the redesign would be restarted on either nifedipine or placebo on top of a statin according to current guidelines.^{25,26} Finally, a robust patient population exposed to the study drug for a prolonged period of time, ranging from 488 to 853 days was available for final analysis.

Table 4 Baseline and changes in coronary vasomotion after acetylcholine infusion

	Baseline (mean \pm SD)	Follow-up (mean \pm SD)	Change (95% CI)	P-value for difference between groups
Placebo	-24.0 (18.1)	-16.3 (17.0)	7.7 (4.2,11.1)	0.0088
Nifedipine	-23.4 ((16.2)	-9.5 (11.9)	13.9 (10.7,17.1)	

Table 5 Baseline and absolute change in total atheroma volume (mm³)

	Baseline (mean \pm SD)	Follow-up (mean \pm SD)	Change, mm ³ (95% CI)	P-value for difference between groups
Placebo	157 (101)	157 (99)	-0.5 (-7.3, 6.4)	0.84
Nifedipine	140 (101)	140 (101)	0.5 (-6.5, 7.5)	

Table 6 Percent change in total atheroma volume

	Change, % (95% CI)	P-value for difference between groups
Placebo	3.2 (-1.9, 8.3)	0.66
Nifedipine	5.0 (-1.3, 11.2)	

On nifedipine, blood pressure was lower than on placebo by 5.8/2.1 mmHg, very much in line with the ACTION trial.²⁹ Obviously, this change may in part account for the reduction in acetylcholin-induced vasoconstriction.³⁰ LDL cholesterol was lower and HDL higher on nifedipine compared to placebo. As this effect on lipids was not observed in other studies with nifedipine, it could be a play of chance. It is unlikely that these changes contributed to the improvement in vasomotion since much larger changes in lipids observed in CARAT¹⁹ and in ENCORE I¹⁶ did not lead to a significant improvement in vasomotion. Other laboratory parameters were not affected by the treatment.

The primary efficacy parameter of ENCORE II was the percent difference in the change of mean luminal diameter in response to acetylcholine after 2 years on placebo or nifedipine. The target segment was the most constricting coronary segment at baseline. Study drugs were discontinued before the follow-up study to assure that long-term and not short-term effects were analysed. Coronary vasoconstriction induced by acetylcholine averaged 25% at baseline, in line with ENCORE I.¹⁶ When compared with placebo, nifedipine led to a robust 18% reduction of the paradoxical vasoconstriction to acetylcholine no matter whether all patients or only those enrolled after restart were considered. Thus, these results confirm the shorter ENCORE I trial and demonstrate that a controlled release formulation of nifedipine persistently improves coronary endothelial function up to 2 years. In the ENCORE studies nifedipine in the controlled release form, GITS, was used. They provide a plasma level with little variation over 24–36 h as long as the GITS is present in

the GI-tract. However, as soon as the GITS is either empty or has left the bowel nifedipine is cleared from the plasma with the normal half life of about 2 h.³¹ That is, since the effects on vasomotion in our study were measured 48–72 h after last intake of study medication, the chronic L-type channel blockade appears to favourably affect the biology of diseased human coronary arteries. The improvement in vasomotion may be important for the anti-ischaemic effects of calcium blockers and their ability to reduce hospitalizations for CAD.²⁹

The secondary efficacy parameter of the ENCORE II trial was the percentage change in atheroma volume after 2 years assessed by intracoronary ultrasound.²⁸ At baseline, atheroma volume in the target artery in all patients averaged 148 mm³. This is less than in other trials on atherosclerotic lesions.³² Indeed, in ENCORE II we investigated a target artery with less than 40% stenosis. With nifedipine progression was less (1.0%) compared with placebo (1.9%) but the difference was not statistically significant. These results are in line with those seen with amlodipine in CAMELOT.³³ These findings confirm that coronary atherosclerosis is essentially progressive in nature. Thus, although in INTACT³⁴ which used angiographic criteria nifedipine led to a reduction in new coronary lesions, ENCORE II as well as CAMELOT using a more sensitive technique allowing for quantitative measurement of plaque volume suggest that clinically anti-atherosclerotic effects of calcium blockers are less pronounced than under experimental conditions.^{35,36} A third efficacy parameter of interest would have been flow reserve as an index of microvascular dysfunction. It was measured with the flow wire in the ENCORE I study, but we could not detect any change in the flow reserve after 6 months treatment despite substantial reduction in lipid levels and a marked effect of nifedipine on vasomotion (unpublished). For this reason, we did not do it in the present study.

In summary, the ENCORE II study confirmed pronounced endothelial dysfunction as assessed by acetylcholine in a large patient population with stable CAD and demonstrates that the L-type calcium channel blocker nifedipine in a long-acting formulation is able to persistently improve the functional abnormality but with no significant effect on the progression of plaque volume.

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Funding

This study was supported by a grant from Bayer Health Care AG, Leverkusen, Germany. Funding to pay the Open Access publication charges for this article was provided by Bayer Schering Pharma AG.

Conflict of interest: T.F.L. has received honoraria from Bayer Healthcare, Wuppertal.

References

- Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;**362**:801–809.
- Lüscher TF, Vanhoutte PM. *The Endothelium: Modulator of Cardiovascular Function*. Boca Raton: CRC Press; 1990. pp. 1–250.
- Lüscher TF, Noll G. The endothelium in coronary vascular control. In: Braunwald E (ed.), *Heart Disease, Update 3*. Philadelphia: Saunders; 1995. pp. 1–10.
- Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986;**315**:1046–1051.
- Schachinger V, Zeiher AM. Quantitative assessment of coronary vasoreactivity in humans in vivo. Importance of baseline vasomotor tone in atherosclerosis. *Circulation* 1995;**92**:2087–2094.
- Tanner FC, Noll G, Boulanger CM, Lüscher TF. Oxidized low density lipoproteins inhibit relaxation of porcine coronary arteries. Role of scavenger receptor and endothelium-derived nitric oxide. *Circulation* 1991;**83**:2012–2020.
- Lüscher TF, Raji L, Vanhoutte PM. Endothelium-dependent vascular responses in normotensive and hypertensive Dahl rats. *Hypertension* 1987;**9**:157–163.
- Linder L, Kiowski W, Bühler FR, Lüscher TF. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo. Blunted response in essential hypertension. *Circulation* 1990;**81**:1762–1767.
- Panza JA, Quyyumi AA, Brush JE Jr, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990;**323**:22–27.
- Cosentino F, Hishikawa K, Katusic ZS, Lüscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation* 1997;**96**:25–28.
- Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993;**88**:2510–2516.
- Mügge A, Ellwell JH, Peterson TE, Hofmeyer TG, Heistad DD, Harrison DG. Chronic treatment with polyethylene glycolated superoxide dismutase partially restores endothelium-dependent vascular relaxations in cholesterol-fed rabbits. *Circ Res* 1991;**69**:1293–1300.
- Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griending KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 1996;**97**:1916–1923.
- Mullen MJ, Clarkson P, Donald AE, Thomson H, Thorne SA, Powe AJ, Furuno T, Bull T, Deanfield JE. Effect of enalapril on endothelial function in young insulin-dependent diabetic patients: a randomized, double-blind study. *J Am Coll Cardiol* 1998;**31**:1330–1335.
- Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Lüscher TF, Klibaner MI, Haber HE, Uprichard AC, Pepine CJ, Pitt B. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study. *Circulation* 1996;**94**:258–265. Erratum in *Circulation* 1996;**94**:1490.3.
- The ENCORE Investigators. Effect of nifedipine and cerivastatin on coronary endothelial function in patients with coronary artery disease. The ENCORE I Study (Evaluation of Nifedipine and Cerivastatin On Recovery of coronary Endothelial function). *Circulation* 2003;**107**:422–428.
- Yang Z, Kozai T, van der Loo B, Viswambharan H, Lachat M, Turina MI, Malinski T, Lüscher TF. HMG-CoA reductase inhibition improves endothelial cell function and inhibits smooth muscle cell proliferation in human saphenous veins. *J Am Coll Cardiol* 2000;**36**:1691–1697.
- Stroes ES, Koomans HA, de Bruin TW, Rabelink TJ. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. *Lancet* 1995;**346**:467–471.
- Vita JA, Yeung AC, Winniford M, Hodgson JM, Treasure CB, Klein JL, Werns S, Kern M, Plotkin D, Shih WJ, Mitchel Y, Ganz P. Effect of cholesterol-lowering therapy on coronary endothelial vasomotor function in patients with coronary artery disease. *Circulation* 2000;**102**:846–851.
- Tschudi MR, Criscione L, Novosel D, Pfeiffer K, Lüscher TF. Antihypertensive therapy augments endothelium-dependent relaxations in coronary arteries of spontaneously hypertensive rats. *Circulation* 1994;**89**:2212–2218.
- Taddei S, Virdis A, Ghiadoni L, Magagna A, Favilla S, Pompella A, Salvetti A. Restoration of nitric oxide availability after calcium antagonist treatment in essential hypertension. *Hypertension* 2001;**37**:943–948.
- Sutsch G, Buchi M, Zeiher AM, Meinertz T, Hugenholz PG, Jenni R, Rafflenbeul W, Drexler H, Haussmann D, Noll G, Quitzau K, Lüscher TF. Effects of calcium antagonism and HMG-coenzyme reductase inhibition on endothelial function and atherosclerosis: rationale and outline of the ENCORE trials. *Eur Heart J* 1999;(Suppl. M):M27–M32.
- Bruno-Joyce J, Dugas JM, MacCausland OE. Cerivastatin and gemfibrozil-associated rhabdomyolysis. *Ann Pharmacother* 2001;**35**:1016–1019.
- Tomlinson B, Lan IW. Combination therapy with cerivastatin and gemfibrozil causing rhabdomyolysis: is the interaction predictable? *Am J Med* 2001;**110**:669–670.

25. Wood D, De Backer G, Faergeman O, Graham I, Mancía G, Pyörälä K. Prevention of coronary heart disease in clinical practice. Recommendations of the Second Joint Task Force of European and other Societies on coronary prevention. *Eur Heart J* 1998;**19**:1434–1503.
26. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001;**285**:2486–2497.
27. Reiber JHC, Schiemank LR, van der Zwet PMJ, Goedhart B, Koning G, Lammertsma M, Danse M, Gerbrands JJ, Schaliq MJ, Bruschke AVG. State of the art in quantitative coronary angiography as of 1996. In: Reiber JHC, van der Wall EE (eds), *Cardiovascular Imaging*. Dordrecht: Kluwer Academic Publishers; 1996. 39–56.
28. Mintz GS, Nissen SE, Anderson WD, Bailey SR, Erbel R, Fitzgerald PJ, Pinto FJ, Rosenfield K, Siegel RJ, Tuzcu EM, Yock PG. American College of Cardiology Clinical Expert Consensus Document on Standards for Acquisition, Measurement and Reporting of Intravascular Ultrasound Studies (IVUS). A report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. *J Am Coll Cardiol* 2001;**37**:1478–1492.
29. Poole-Wilson PA, Lubsen J, Kirwan BA, van Dalen FJ, Wagener G, Danchin N, Just H, Fox KA, Pocock SJ, Clayton TC, Motro M, Parker JD, Bourassa MG, Dart AM, Hildebrandt P, Hjalmarson A, Kragten JA, Molhoek GP, Otterstad JE, Seabra-Gomes R, Soler-Soler J, Weber S, [ACTION] investigators. A Coronary disease Trial Investigating Outcome with Nifedipine gastrointestinal therapeutic system. Effect of long-acting nifedipine on mortality and cardiovascular morbidity in patients with stable angina requiring treatment (ACTION trial): randomised controlled trial. *Lancet* 2004;**364**:849–857.
30. Sudano I, Viridis A, Taddei S, Spieker L, Corti R, Noll G, Salvetti A, Luscher TF. Chronic treatment with long-acting nifedipine reduces vasoconstriction to endothelin-1 in essential hypertension. *Hypertension* 2007;**49**:285–290.
31. Elliott HL, Elawad M, Wilkinson R, Singh SP. Persistence of antihypertensive efficacy after missed doses: comparison of amlodipine and nifedipine gastrointestinal therapeutic system. *J Hypertens* 2002;**20**:333–338.
32. Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, Crowe T, Howard G, Cooper CJ, Brodie B, Grines CL, DeMaria AN, for the REVERSAL Investigators. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis. A randomized controlled trial. *JAMA* 2004;**291**:1071–1080.
33. Nissen SE, Tuzcu EM, Libby P, Thompson PD, Gali M, Garza D, Berman L, Shi H, Buebendorf E, Topol EJ, for the CAMELOT Investigators. Effect of antihypertensive agents on cardiovascular events in patients with coronary disease and normal blood pressure. A Randomized Controlled Trial. *JAMA* 2004;**292**:2217–2226.
34. Lichtlen PR, Hugenholtz PG, Rafflenbeul W, Hecker H, Jost S, Deckers JW. Retardation of angiographic progression of coronary artery disease by nifedipine. Results of the international nifedipine trial on antiatherosclerotic therapy. INTACT Group Investigators. *Lancet* 1990;**335**:1109–1113.
35. Yang Z, Luscher TF, Noll G. Calcium antagonists differently inhibit proliferation of human coronary smooth muscle cells in response to pulsatile stretch and platelet-derived growth factor. (Brief communication). *Circulation* 1993;**88**:832–836.
36. Roth M, Eickelberg O, Kohler E, Erne P, Block LH. Ca²⁺ channel blockers modulate metabolism of collagens within the extracellular matrix. *Proc Natl Acad Sci USA* 1996;**93**:5478–5482.