

Cardiovascular Biology and Cell Signalling

High-dose atorvastatin in peripheral arterial disease (PAD): Effect on endothelial function, intima-media-thickness and local progression of PAD

An open randomized controlled pilot trial

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Summary

Beneficial effects of aggressive lipid-lowering with high-dose atorvastatin (80 mg/day) have been demonstrated in patients with coronary and cerebrovascular disease. The impact of such a therapy in patients with peripheral arterial disease (PAD) is less known so far. Here we studied the effects of high-dose atorvastatin on brachial artery endothelial function, common carotid intima-media thickness (IMT) and local progression of PAD in these patients. One hundred of 500 patients screened with documented PAD were randomly assigned to receive 80 mg of atorvastatin daily for six months or to continue on conventional medical treatment. Ninety-six percent of patients in the control group were on standard statin treatment. High resolution B-mode ultrasonography was used to study brachial artery flow-mediated dilation (FMD), IMT and ankle-brachial index (ABI) at baseline and at six months. FMD and IMT at baseline and at six months were 4.1 (0.06–8.6) versus 5.0 (0.76 vs. 8.1) %, $p=0.96$,

and 0.76 (0.66–0.82) versus 0.73 (0.63–0.81) mm, $p=0.41$, respectively, in the atorvastatin group, and 2.66 (–1.9 – 6.9) versus 3.65 (0.0–8.6) %, $p=0.02$, and 0.78 (0.71–0.90) versus 0.77 (0.70–0.90) mm, $p=0.48$, in the control group. ABI at baseline and at six months was not different in either group. LDL cholesterol was reduced from 2.53 (2.21–3.28) to 1.86 (1.38–2.29) mM ($p<0.0001$) in the atorvastatin group, whereas levels remained stable in the control group [2.38 (1.94–3.16) vs. 2.33 (1.82–2.84) mM, $p=0.61$]. Major adverse cardiovascular events occurred in 2.1% in the atorvastatin group and 1.9% in the control group ($p=0.61$). In conclusion, in this pilot trial aggressive lipid-lowering with 80 mg of atorvastatin daily for six months had no effect on brachial artery FMD in patients with PAD. IMT and ABI were also similar in patients with and without high-dose atorvastatin at six months.

Keywords

Atherosclerosis, clinical studies, stenosis, nitric oxide/NO

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Introduction

Beneficial effects of an aggressive lipid-lowering therapy with high-dose atorvastatin (80 mg/day) have previously been demonstrated in patients with coronary heart disease. In the MIRACLE Study this therapy reduced the frequency of recurrent ischemic cardiovascular events in patients with acute coronary syndromes within the first 16 weeks (1). Furthermore, intense

statin therapy has been shown to have an effect both in the primary (2) and secondary prevention of stroke (3).

The effects of an aggressive lipid-lowering therapy in patients with peripheral arterial disease (PAD) have not been extensively investigated so far. Although cardiovascular morbidity and mortality are increased in patients with PAD, with myocardial infarction and stroke being the main causes of death (4, 5), there are, to date, only little data concerning these effects on

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the local progression of PAD and on major adverse cardiovascular events (MACE) in this patient group.

The mechanisms by which statins may achieve those effects involve, apart from and independent of their lipid-lowering properties, pleiotropic effects (6) such as the improvement of endothelial function (7), modulation of inflammation (8), reduction of common carotid intima-media thickness (IMT) (9), and reduction of oxidative stress (10).

Endothelial dysfunction, assessed non-invasively by brachial artery flow-mediated dilation (FMD), represents an early precursor of atherosclerosis. Both in patients with cardiovascular risk factors and in patients with either ischemic heart disease or PAD FMD has been shown to be reduced (11–14). Furthermore, it is an independent predictor of cardiovascular risk in patients with PAD (15, 16). IMT is also known as an established indicator for early generalized atherosclerosis (17, 18) and has been demonstrated to be a strong predictor for both cardiovascular and cerebrovascular events (19, 20). Changes in IMT can easily be visualized by B-mode ultrasonography (21) which is commonly used in interventional studies of lipid-lowering therapies.

Therefore, we wanted to test the hypothesis that, in patients with PAD, intense lipid-lowering therapy with high-dose atorvastatin (80 mg/day), administered over a period of six months, would improve FMD. In addition, effects on IMT, local progression of PAD and the occurrence of MACE were recorded up to six months.

Methods

Study design

This open, prospective single-center trial had a parallel group design in which patients were randomized to one of two treatment arms. Group 1 received, in addition to the current medication, 80 mg atorvastatin per day for six months. Any other lipid-lowering drug in group 1 was discontinued and replaced by high-dose atorvastatin without washout period. Group 2 (controls) received standard statin treatment according to the National Cholesterol Education Program (NCEP) guidelines (22). LDL cholesterol level was targeted to levels < 100 mg/dl.

The randomization code was developed using a computer random number generator to select random permuted blocks.

Patients

Patients with angiographically or sonographically documented PAD of the lower extremities, with or without a history of peripheral vascular intervention or vascular surgery, were eligible and were consecutively recruited from PAD patients who had regular consultation at our clinic of angiology. The severity of PAD at the time of enrollment was classified according to Rutherford. Only patients with Rutherford category 0 to 3 (corresponding to Fontaine stages I and II) were eligible.

Exclusion criteria were non-atherosclerotic vascular disease, pregnancy, life expectancy < six months, active liver disease (cirrhosis, hepatitis), and known intolerance against atorvastatin.

The study protocol was approved by the local ethics committee, and all patients gave written informed consent.

At baseline patients' age, sex, current medication and vascular risk factors (diabetes, defined as fasting serum glucose ≥ 7 mM or use of oral antidiabetics or insulin; hyperlipidemia, defined as use of lipid-lowering drugs or total cholesterol > 5.17 mM and/or triglycerides > 2.26 mM; hypertension, defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg; current smoking) were recorded. The patients were asked about manifestations of coronary heart disease (prior angina, acute coronary syndromes, myocardial infarction, percutaneous coronary intervention, aorto-coronary bypass graft) and cerebrovascular events (prior transient ischemic attack, stroke, carotid stenting or carotid endarterectomy). Furthermore, a careful clinical examination was done including palpation of peripheral pulses and auscultation of bruits. Non-invasive pulse volume recordings and ankle systolic pressure measurements for calculation of the ankle-brachial index (ABI) at rest were performed, too.

Laboratory analyses

The following laboratory measurements were done by using established standard methods: liver function tests, creatine kinase (CK), C-reactive protein (CRP), D-dimer, fibrinogen, blood glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and lipoprotein a.

Carotid and brachial artery ultrasound

FMD of the brachial artery and IMT of the common carotid artery (CCA) were determined by high-resolution B-Mode ultrasound.

Measurements of FMD were done according to Celermajer et al. (11). In brief, FMD was assessed on the right brachial artery with the use of a 7.5-MHz linear transducer after the patients lay quietly for 10 minutes (min). We scanned the brachial artery in longitudinal section 2–15 cm above the elbow. The clearest picture of the anterior and posterior intimal layers was obtained, the skin was marked at the position of the transducer which was kept like that throughout the study. The diameter and the peak velocity of the brachial artery at rest and during reactive hyperemia was measured; reactive hyperemia was induced by a pneumatic cuff, placed around the forearm, being inflated to 250 mmHg for 4.5 min, and then released. After recovery of the vessel for 10–15 min and 4 min after subsequent sublingual administration of nitroglycerin 0.8 mg, the diameter and peak systolic velocity were determined again to assess endothelium-independent vessel reaction.

To assess IMT, a 7.5 MHz linear transducer was used to measure the distance between two echogenic zones that correspond to the boundaries between lumen/intima and media/adventitia. According to the meta-analysis by Kanter et al. (23) combined measurements of the near and far wall might enhance precision without loss of validity. Furthermore, variability of IMT measurements is lowest when determining the mean thickness in more than one direction (23). Therefore, IMT was defined as the mean of a total of 24 measurements of the near and far wall on both the left and right CCA recorded from an anterolateral, mediolateral and posterolateral position. All measurements were taken 1.5–2 cm proximal to the bifurcation and were ECG-triggered (on the R-wave). Measurements were always done by the same experienced ultrasonographer who was blinded to treatment assignment for the duration of the study.

Follow-up and endpoints

In the atorvastatin group liver function tests as well as measurement of the CK were done at 2, 6, and 12 weeks, and the patients were asked about the occurrence of side effects (myalgia, constipation, flatulence, diarrhea, abdominal pain, nausea, dyspepsia, headache, insomnia) of the atorvastatin therapy.

At six months, FMD, IMT, ABI and routine laboratory measurements were done in both groups. Furthermore, a history was taken with special regard to the symptoms of PAD and the occurrence of vascular events during follow up, and a clinical examination (palpation of pulses, auscultation of bruits) as well as non-invasive pulse volume recordings and ABI measurements at rest were performed. Progression of PAD was defined as drop in ABI by ≥ 0.1 with or without worsening of clinical symptoms. The occurrence of MACE (defined as non-fatal myocardial infarction, coronary revascularization, coronary death, non-fatal or fatal stroke or any vascular death) was also recorded at six months.

The primary endpoint was brachial artery FMD at six months. IMT at six months was a secondary endpoint.

Sample size calculation and statistical analysis

A sample size of 47 patients per group was calculated to detect a 40% difference in FMD between the two treatment groups with 80% power and significance at the 5% level (two sided). To compensate for drop outs, an overall of 100 patients were included in the study.

Statistical analysis was performed using Statview 5.0. All analyses were performed on an intention-to-treat basis and involved all patients who were randomly assigned. Continuous variables were summarized as medians and interquartile range and categorical variables as counts and percentages. Comparison between the two treatment groups were done using a Mann-Whitney test for continuous variables and using a chi-square test or a Fisher's exact test for categorical variables. For the main endpoints of the study (FMD, IMT and ABI at 6 months), which were approximately normally distributed as checked using boxplots, a multivariate analysis has been carried out with the measurement at six months as the response, the treatment group as factor and the baseline values and age as covariates in a multiple regression model. This allowed us to compare both treatment groups at six months for individuals having the same baseline value and the same age. This also allowed us to adjust for the slight age and baseline imbalances between the groups in spite of the randomisation. P-values lower than 0.05 were considered as statistically significant.

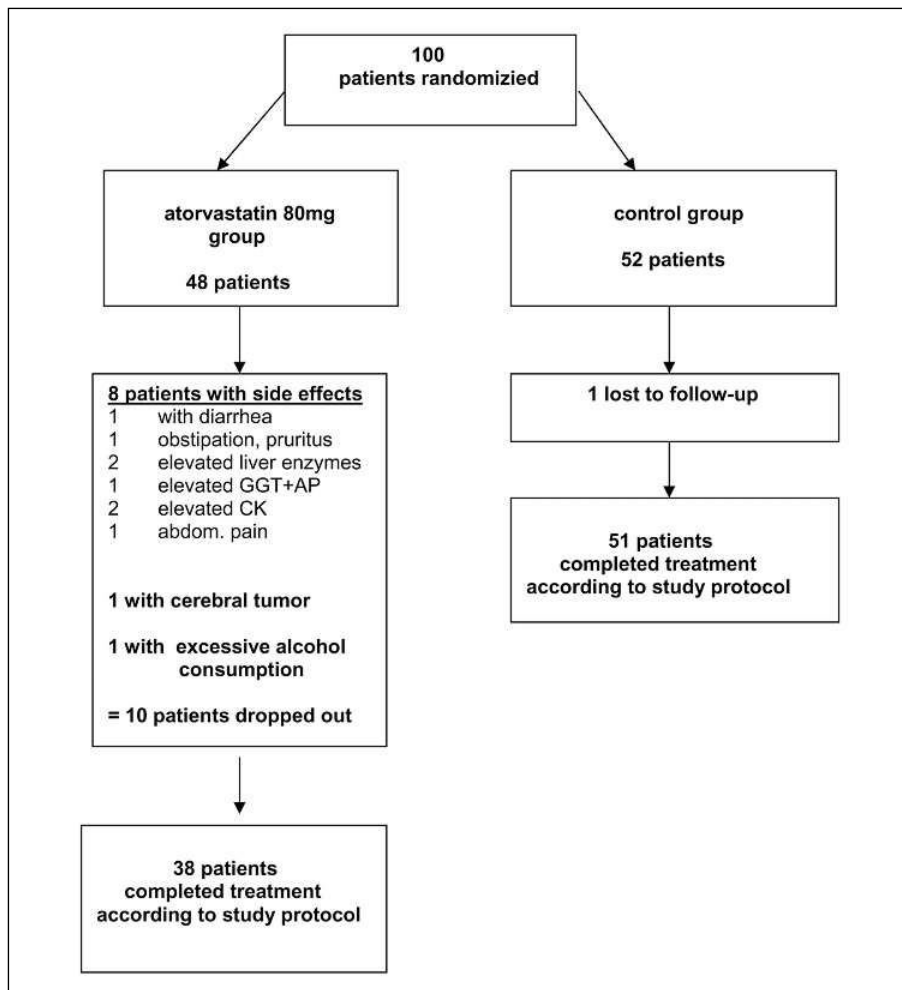


Figure 1: Flow of participants throughout the study.

Results

Of 500 patients screened, 103 did not meet the entry criteria, 200 refused to participate, 33 were excluded because of liver disease (increase in liver function tests, known cirrhosis or hepatitis), 17 because of life expectancy < six months, 25 because of known intolerance against atorvastatin and 22 because of non-atherosclerotic vascular disease.

Of the 100 patients enrolled (78 men, 22 women), 48 patients were randomized to high-dose atorvastatin and 52 to conventional medical treatment. The flow of the participants throughout the study is shown in Figure 1.

One patient in the control group was lost to follow-up. Three patients in the atorvastatin group discontinued the drug because of clinical symptoms (diarrhea N=1, abdominal pain N=1, obstipation N=1), and one because of excessive alcohol consumption; five patients developed elevated liver enzymes (N=3; 2 patients with ALT > 3x upper limit of normal) or elevated CK (N=2, CK > 10x upper limit of normal).

One patient developed a cerebral tumor, and therefore his study participation was terminated by the investigators.

In Table 1 baseline characteristics by treatment group are shown. Patients in the atorvastatin group were slightly younger than those in the control group, but there were no differences between the groups with respect to vascular risk factors, lipid status, concomitant medication and severity of PAD (Rutherford classification, ABI).

In the control group, 50 patients (96.1%) were on long-term statin therapy during the initial visit. The following statins were used: simvastatin 10 mg in one patient (1.9%), 20 mg in six patients (11.5%) and 40 mg in two patients (3.8%); pravastatin 10 mg in two patients (3.8%), 20 mg in six patients (11.5%) and 40 mg in seven patients (13.5%); atorvastatin 10 mg in two patients (3.8%), 20 mg in 18 patients (34.6%) and 40 mg in three patients (5.8%); fluvastatin 20 mg in one patient (1.9%), and rosuvastatin 20 mg in one patient (1.9%). In two patients statin therapy was initiated.

FMD

FMD of the brachial artery was not significantly different between the groups at baseline [atorvastatin vs. control group: 4.1 (0.1–8.6) vs. 2.7 (–1.9–6.9)%, $p=0.18$] and at six months [5.0 (0.8–8.1) vs. 3.7 (0.0–8.6)%, $p=0.74$] (Fig. 2). In the multivariate analysis, FMD at six months was on average 1.4% lower for the atorvastatin group compared to the control group (adjusted for baseline FMD and age), this difference being not significant ($p=0.44$).

IMT

IMT was significantly lower in the atorvastatin group than in the control group at baseline [0.76 (0.66–0.82) vs. 0.78 (0.71–0.90) mm, $p=0.046$] and at six months [0.73 (0.63–0.81) vs. 0.77 (0.70–0.90) mm, $p=0.031$] (Fig. 3). But in the multivariate analysis, IMT at six months was on average only 0.01 mm lower for the atorvastatin group compared to the control group (adjusted for baseline IMT and age), this difference being no longer significant ($p=0.58$).

Table 1: Baseline characteristics of the patients by group.

	Atorvastatin (N=48)	Control (N= 52)	P
Age, years	63 (58–72)	71 (63–78)	0.0033
Gender (male)	40 (83.3%)	38 (73.1%)	0.22
BMI, kg/m ²	25.2 (23.3–27.3)	25.5 (23.9–27.5)	0.52
Smokers	17 (35.4%)	15 (28.8%)	0.48
Pack years, years	40 (20–56)	35 (12.5–50)	0.39
Diabetes mellitus	13 (27.1%)	16 (30.8%)	0.68
Blood glucose, mM	5.35 (4.7–6.0)	5.10 (4.67–6.10)	0.54
Arterial hypertension	35 (72.9%)	42 (80.8%)	0.35
Systolic BP, mmHg	140 (130–150)	142.5 (132.5–155)	0.29
Diastolic BP, mmHg	80 (70–85)	80 (70–85)	0.99
Hypercholesterolemia	34 (70.8%)	40 (76.9%)	0.49
Total cholesterol, mM	4.70 (4.30–5.20)	4.50 (4.10–5.00)	0.24
LDL cholesterol, mM	2.53 (2.21–3.28)	2.38 (1.94–3.16)	0.16
HDL cholesterol, mM	1.25 (1.01–1.54)	1.19 (0.99–1.43)	0.32
Triglycerides, mmol/l	1.67 (1.18–2.52)	1.88 (1.28–2.44)	0.64
Lipoprotein a, mg/l	166.5 (57–446.5)	166 (47–578.5)	0.68
CRP, mg/l	3.00 (1.50–7.3)	3.4 (1.65–5.50)	0.88
Fibrinogen, mg/dl	350 (325–403.75)	357.50 (325–415)	0.51
D-Dimer, mg/l	0.20 (0.20–0.40)	0.35 (0.20–0.60)	0.0054
history of peripheral vascular intervention/ surgery	37 (77%)	42 (80%)	0.83
Rutherford classification			
0	25 (52%)	31 (59.6%)	0.46
1	7 (14.5%)	8 (15.3%)	
2	8 (16.6%)	6 (11.5%)	
3	8 (16.6%)	7 (13.5%)	
Ankle-brachial index (ABI)	0.84 (0.69–1.00)	0.90 (0.70–1.00)	0.74
Coronary heart disease	18 (37.5%)	22 (42.2%)	0.62
history of ischemic stroke or TIA	8 (16.7%)	15 (28.8%)	0.15
Aspirin	30 (62.5%)	33 (63.5%)	0.92
Clopidogrel	5 (10.4%)	4 (7.7%)	0.63
Aspirin and clopidogrel	6 (12.5%)	10 (19.2%)	0.36
ACE inhibitor	9 (18.8%)	16 (30.8%)	0.17
Beta-blocker	16 (33.3%)	27 (51.9%)	0.06
Angiotensin II receptor antagonist	17 (35.4%)	18 (34.6%)	0.93
Vitamin K antagonist	10 (20.8%)	9 (17.3%)	0.65

Patients with and without progression of the IMT during follow-up, expressed as % change from baseline, were similar in the atorvastatin group and in the control group (Fig. 3B).

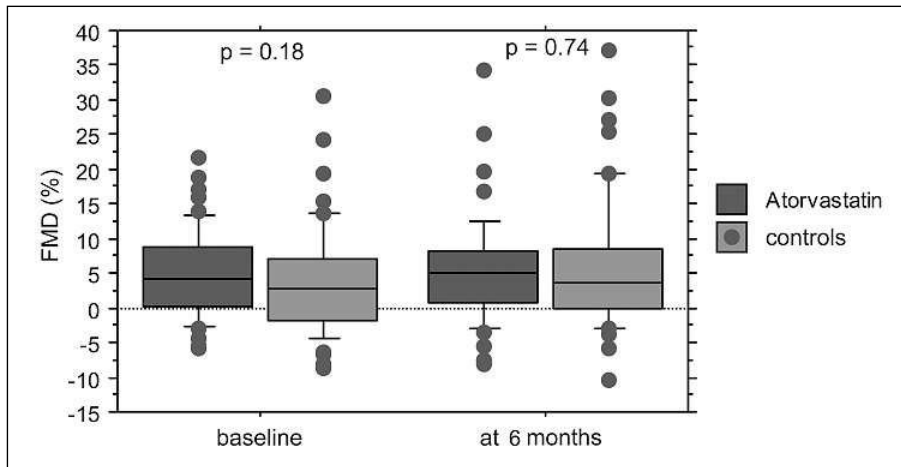


Figure 2: Flow-mediated dilation (FMD) at baseline and at six months by groups. Boxes show medians (25th to 75th percentile); the grey dots represent all observations <10th or > 90th percentile, respectively.

ABI and reintervention

ABI was not different between the groups at baseline (Table 1; Fig. 4) and at six months [0.89 (0.70–1.10) vs. 0.90 (0.77–1.00)%, $p=0.79$] (Fig. 4). In the multivariate analysis, ABI at six months was on average 0.04 higher for the atorvastatin group compared to the control group (adjusted for baseline FMD and age), this difference being not significant ($p=0.28$).

Twelve patients in the control group and eight patients in the atorvastatin group (23% vs. 16.6%, $p=0.42$) developed a drop in ABI by at least 0.1 with or without worsening of clinical symp-

toms. Of these, nine patients in the control group and eight in the atorvastatin group underwent revascularization procedures.

Laboratory findings

At six months' follow-up, total cholesterol, LDL-cholesterol and triglycerides were lower in the atorvastatin group than in the control group (Table 2). No differences were seen in CRP, D-dimer, fibrinogen and lipoprotein a between the two groups.

Further, there were also no differences between baseline values and values at six months in CRP, D-dimer, fibrinogen and lipoprotein a in either group (all $p>0.05$).

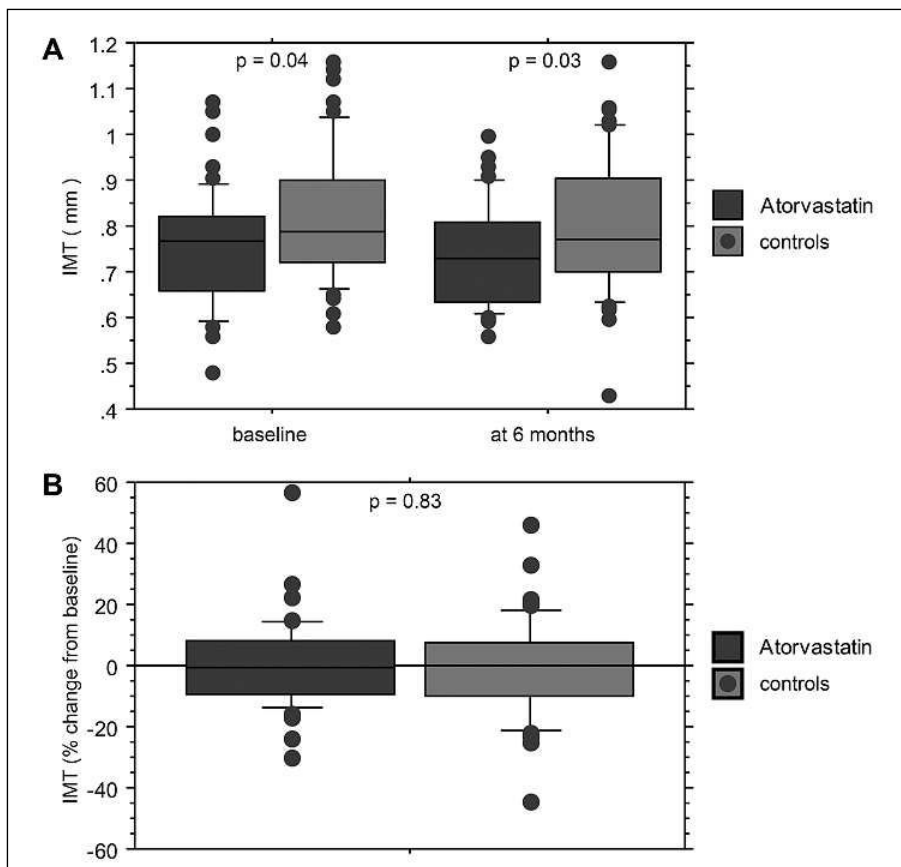
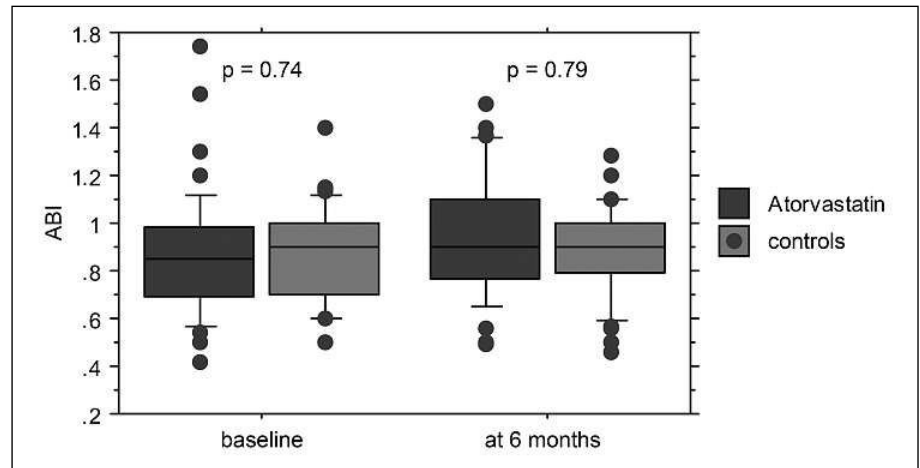


Figure 3: Intima-media thickness (IMT). A) IMT at baseline and at 6 months by groups. Boxes show medians (25th to 75th percentile); the grey dots represent all observations <10th or > 90th percentile, respectively. B) Patients with and without progression of intima-media thickness (IMT), expressed as % change from baseline, by groups. Boxes show medians (25th to 75th percentile); the blue dots represent all observations <10th or > 90th percentile, respectively.

Figure 4: Ankle-brachial index (ABI) at baseline and at six months by groups. Boxes show medians (25th to 75th percentile); the blue dots represent all observations <10th or > 90th percentile, respectively.



MACE

Myocardial infarction occurred in one patient in the atorvastatin group and in one patient in the control group (2.1 vs. 1.9%, $p = 0.61$). There were no strokes and no vascular deaths in either group.

Discussion

In this open, randomized controlled trial aggressive lipid-lowering with atorvastatin 80 mg daily for six months had no effect on brachial artery endothelial function in patients with PAD.

Atorvastatin is known as a potent drug for lipid-lowering therapy with many pleiotropic properties. One of these properties is a protective effect on endothelial function. Data in recent studies have shown that therapy with statins may improve endothelial dysfunction both in patients with type 1 diabetes (24) and in patients with familial hypercholesterolemia (25). Treatment with 80 mg of atorvastatin in healthy smokers with normal cholesterol levels normalized endothelial function after four weeks (26). As data on high-dose atorvastatin treatment in PAD patients are not available so far, the aim of our study was to investigate the effect of such a therapy on FMD in this high-risk patients in a randomized controlled setting. Our finding that we did not observe a significant effect of high-dose atorvastatin on FMD in our PAD patients is in contrast to findings of the effects of high-dose atorvastatin in other patient cohorts. Several potential reasons for the negative findings in our study have to be addressed: First, patients referred to a tertiary center might represent a negative selection with more advanced (generalized) disease; of all patients included in our study, 80% had a history of a peripheral revascularization procedure (interventional procedure and/or peripheral vascular surgery), a percentage which is extremely high (in contrast, in the Heart Protection Study, only 40% of the PAD patients had a previous revascularization procedure (27). The severity of the disease might also be reflected by the low baseline values of FMD (3–4%), which have been shown to be associated with an adverse outcome (16). Data of the ASAP study (28) suggest a strong effect of atorvastatin on endothelial function at an early stage, whereas at later stages of progressed atherosclerosis, which was the clinical situation in our patients, effects might be much less obvious. Furthermore, it has to be pointed out that 96% of the patients in our control group were

on low-dose statins as part of standard medical treatment, making it much more difficult to detect presumed effects in the treatment group. Finally, nearly one third of the patients in our treatment group were diabetics. It is known that intensive lipid-lowering by 80 mg atorvastatin is unable to restore endothelial function in type 2 diabetic patients (29).

An important and crucial mechanism by which statins exert their pleiotropic effects is the upregulation of endothelial nitric oxide synthase (eNOS) (30), increased NO bioavailability (31), and prevention of NO scavenging by reactive oxygen species (ROS) (32). It is well known that advanced stages of atherosclerosis are associated with an increased production of free radicals, and that an excess production of the ROS will scavenge and hence inactivate NO. The positive effect of statins exerted via the eNOS pathway will then, at least partly, be blunted.

IMT of the common carotid artery and the ABI after six months treatment were not different between patients receiving high-dose atorvastatin compared with controls receiving conventional medical therapy. In the ASAP study Smilde et al. could demonstrate a significant reduction of IMT of the carotid artery in patients with heterozygous familial hypercholesterolemia after two years' treatment with high-dose atorvastatin (80 mg per day) (28). Furthermore, data of the ARBITER study showed a significant regression of IMT of the carotid artery after more

Table 2: Laboratory values at six months by treatment group.

	Atorvastatin (n=48)	Control (n=51)	P
Total cholesterol, mM	3.85 (3.40–4.35)	4.50 (3.92–5.10)	0.0006
LDL cholesterol, mM	1.86 (1.38–2.29)	2.33 (1.82–2.85)	0.0028
HDL cholesterol, mM	1.25 (1.10–1.49)	1.23 (0.96–1.41)	0.28
Triglycerides, mM	1.43 (1.07–1.93)	2.01 (1.47–2.47)	0.0097
Blood glucose, mM	5.60 (4.97–7.20)	5.80 (5.07–8.05)	0.35
Lipoprotein a, mg/l	135 (44–285)	148 (40.7–667.3)	0.51
C-reactive protein, mg/l	2.20 (1.15–5.35)	3.00 (1.62–6.55)	0.23
Fibrinogen, mg/dl	350 (311.25–410)	360 (315–397.50)	0.96
D-dimer, mg/l	0.20 (0.20–0.40)	0.30 (0.20–0.47)	0.21

than two years of treatment with atorvastatin 80 mg per day (33). However, in both studies particularly patients with coronary heart disease only, not PAD, were included.

In our study follow-up was only six months. This might have been a potential reason why we were unable to observe an effect of high-dose atorvastatin therapy on IMT. On the other hand, however, in contrast to our results, a previous pilot study showed a significant reduction in carotid IMT already after eight weeks of treatment with only 20 mg atorvastatin/day in patients with PAD (9). Our data on high-dose atorvastatin treatment in patients with PAD will add to the controversy of the potential additional benefit of intensive lipid-lowering treatment with atorvastatin on the progression of coronary atherosclerosis as elucidated by a recent multicenter trial (34). From our current data, a certain new controversy may arise with respect to the effects of high-dose atorvastatin in patients with PAD.

At first glance, our results are contradictory to those of two recently published large trials demonstrating improved outcome and reduced peripheral vascular events in PAD patients using statins (27, 35). Data from the Heart Protection Study about the benefits of cholesterol-lowering with statin therapy in patients with PAD revealed that medication with 40 mg simvastatin daily reduced vascular events both in participants with PAD and in those without pre-existing PAD (27). Unlike this study, which was only done retrospectively, we used a daily 80 mg dose of atorvastatin in a randomized setting. Feringa et al. (35) prospectively studied in a not randomized trial more than 1,300 patients with PAD. In this study, statin dosage was not fixed but adjusted and converted to a percentage value of maximum recommended therapeutic dose. To the best of our knowledge, our study is the only prospectively randomized controlled trial investigating the effects of high-dose atorvastatin in patients with PAD. Although we recorded and reported MACE in our trial, these were, unlike those trials, not an endpoint in our study. Therefore, we definitely do not want to conclude from our data that high-dose atorvastatin may not be clinically beneficial in PAD patients, as our study was not designed to answer this question. We are aware of the fact that our results cannot challenge the data from the two

mentioned large scale clinical trials.

In our investigation, we were also unable to show any effect of atorvastatin 80 mg/day on CRP and fibrinogen. Indeed, in clinical trials in patients with coronary artery disease, aggressive lipid-lowering with atorvastatin diminished CRP (36, 37). In the study performed by Ridker on patients with acute coronary syndromes, baseline median CRP was 2 mg/l (8). The rather high levels of CRP in our patients (atorvastatin 80 mg group: 3 mg/l vs. control group: 3.4 mg/l) suggest an advanced stage of atherosclerosis present in the majority of our patients. Fibrinogen was reported to be reduced in patients with coronary artery disease when atorvastatin (10 mg/day) was given for three months only (38). Interestingly, atorvastatin at doses between 10 to 40 mg/day was recently shown to diminish soluble Fas, a circulating marker of inflammation secreted by cells implicated in atherosclerotic lesions, whereas atorvastatin 80 mg/day had no significant effect on sFas reduction (39). Therefore, it is conceivable that the effects of atorvastatin on inflammatory markers may be dose-dependently modifiable.

Taken together, we failed to demonstrate an effect of atorvastatin 80 mg per day on FMD in PAD patients at six months. However, this does not mean that high-dose atorvastatin might not reduce the risk of vascular events in this time interval, as our study was not designed to answer this question. The impact of a high-dose regimen on the pleiotropic effects will particularly be needed to be clarified in the future. Certainly, more profound mechanistic insights into the complex relationship between lipid-lowering therapy, atherosclerosis and plaque burden are still needed.

Study limitations

The following limitations have to be addressed: the study was not blinded, and statin treatment in the control group was not standardized. A large, randomized, double-blind study comparing high-dose atorvastatin with a standard dose group would be needed to clarify whether intense atorvastatin is superior in PAD patients with respect to clinical endpoints.

References

- Schwarz GG, Anders GO, Ezekowitz MD, et al. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: The MIRACLE Study: A Randomized Controlled Trial. *J Am Med Assoc* 2001; 285: 1711–1718.
- Amarenco P, Labreuche J, Lavallée P, et al. Statins in stroke prevention and carotid atherosclerosis: systematic review and meta-analysis. *Stroke* 2004; 35: 2902–2909.
- Amarenco P, Bogousslavsky J, Callahan A, for the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) Investigators. High-dose atorvastatin after stroke or transient ischemic attack. *N Engl J Med* 2006; 355: 549–559.
- Bainton D, Sweetnam P, Baker I, et al. Peripheral vascular disease: Consequence for survival and association with risk factors in the Speedwell prospective heart disease study. *Br Heart J* 1994; 72: 128–132.
- Dormandy JA, Murray GD. The fate of the claudicant - a prospective study of 1969 claudicants. *Eur J Vasc Surg* 1991; 5: 131–133.
- Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arterioscler Thromb Vasc Biol* 2001; 21: 1712–1719.
- Wolfrum S, Jensen KS, Liao JK. Endothelium-dependent effects of statins. *Arterioscler Thromb Vasc Biol* 2003; 23: 729–736.
- Ridker PM, Rifai N, Pfeffer MA, et al. Long-term effects of pravastatin on plasma concentrations of C-reactive protein. The Cholesterol and Recurrent Events (CARE) investigators. *Circulation* 1999; 100: 230–235.
- Davis M, Atwal AS, Nair DR, et al. The effect of short-term lipid-lowering with atorvastatin on carotid artery intima media thickness in patients with peripheral vascular disease: a pilot study. *Curr Med Res Opin* 2000; 16: 198–204.
- Ejiri J, Inoue N, Tsukube T, et al. Oxidative Stress in the pathogenesis of thoracic aortic aneurysm: protective role of statin and angiotensin II type 1 receptor blocker. *Cardiovasc Res* 2003; 59: 988–996.
- Celermajer DS, Adams MR, Clarkson P, et al. Passive smoking and impaired endothelium-dependent arterial dilation in healthy young adults. *N Engl J Med* 1996; 334: 150–154.
- Balletshofer BM, Rittig K, Enderle MD, et al. Endothelial dysfunction is detectable in young normotensive degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation* 2000; 101: 1780–1784.
- Neunteufl TH, Katzenschlager R, Hassan A, et al. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis* 1997; 129: 111–118.
- Yataco AR, Corretti MC, Gardner AW, et al. Endothelial reactivity and cardiac risk factors in older patients with peripheral arterial disease. *Am J Cardiol* 1999; 83: 754–758.
- Brevetti G, Silvestro A, Schiano V, et al. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: Additive value of flow-mediated dilation to ankle-brachial pressure index. *Circulation* 2003; 108: 2093–2098.

16. Gokce N, Keaney JF, Jr, Hunter LM, et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol* 2003; 41: 1769–1775.
17. Bots ML, Hofman A, Grobbee DE. Increased common carotid intima media thickness. Adaptive response or a reflection of atherosclerosis? Findings from the Rotterdam Study. *Stroke* 1997; 28: 2442–2447.
18. Ebrahim S, Papacosta O, Whincup P, et al. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. *Stroke* 1999; 30: 841–850.
19. Bots ML, Hoes AW, Hofman A, et al DE. Cross-sectionally assessed carotid intima-media thickness relates to long-term risk of stroke, coronary heart disease and death as estimated by available risk functions. *J Intern Med* 1999; 245: 269–276.
20. O'Leary DH, Polak JF, Kronmal RA, et al. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group (see comments). *N Engl J Med* 1999; 340: 14–22.
21. Kastelein JJP, de Groot E, Sankatsing R. Atherosclerosis measured by B-mode ultrasonography: effect of statin therapy on disease progression. *Am J Med* 2004; 116: 31S–36S.
22. 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). *J Am Med Assoc* 2001; 285: 2486–2497.
23. Kanters SDJM, Algra A, van Leeuwen MS, et al. Reproducibility of In Vivo Carotid Intima-Media Thickness Measurements: A Review. *Stroke* 1997; 28: 665–671.
24. Dogra GK, Watts GF, Chan DC, et al. Statin therapy improves brachial artery vasodilator function in patients with type 1 diabetes and microalbuminuria. *Diabet Med* 2005; 22: 239–242.
25. Alonso R, Mata P, De Andres R, et al. Sustained long-term improvement of arterial endothelial function in heterozygous familial hypercholesterolemia patients treated with simvastatin. *Atherosclerosis* 2001; 157: 423–429.
26. Agewall S, Hernberg A. Atorvastatin normalizes endothelial function in healthy smokers. *Clin Sci* 2006; 111: 87–91.
27. Heart Protection Study Collaborative Group. Randomized trial of the effects of cholesterol-lowering with simvastatin on peripheral vascular and other major vascular outcomes in 20,536 people with peripheral arterial disease and other high-risk conditions. *J Vasc Surg* 2007; 45: 645–654.
28. Smilde TJ, Wissen S, Wollersheim H, et al. Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolemia (ASAP): a prospective, randomised, double-blind trial. *Lancet* 2001; 357: 577–581.
29. van Etten RW, de Koning EJP, Honing ML, et al. Intensive lipid lowering by statin therapy does not improve vasoreactivity in patients with type 2 diabetes. *Arterioscler Thromb Vasc Biol* 2002; 22: 799–804.
30. Laufs U, La Fata V, Plutzky J, et al. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 1998; 97: 1129–1135.
31. Kalinowski L, Dobrucki LW, Brovkovich V, et al. Increased nitric oxide bioavailability in endothelial cells contributes to the pleiotropic effect of cerivastatin. *Circulation* 2002; 105: 933–938.
32. Wassmann S, Laufs U, Baumer AT, et al. Inhibition of geranylgeranylation reduces angiotensin II-mediated free radical production in vascular smooth muscle cells: involvement of angiotensin AT1 receptor expression and Rac1 GTPase. *Mol Pharmacol* 2001; 59: 646–654.
33. Taylor AJ, Kent SM, Flaherty PJ, et al. ARBITER: Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol: a randomized trial comparing the effects of atorvastatin and pravastatin on carotid intima medial thickness. *Circulation* 2002; 106: 2055–2060.
34. Schmermund A, Achenbach S, Budde T, et al. Effect of intensive versus standard lipid-lowering treatment with atorvastatin on the progression of calcified coronary atherosclerosis over 12 months. *Circulation* 2006; 113: 427–437.
35. Feringa HHH, Karagiannis SE, van Waninge VH, et al. The effect of intensified lipid-lowering therapy on long-term prognosis in patients with peripheral arterial disease. *J Vasc Surg* 2007; 45: 936–943.
36. Ridker PM, Cannon CP, Morrow D, et al. Pravastatin or atorvastatin evaluation and infection therapy – thrombolysis in myocardial infarction 22 (Prove it – TIMI 22) investigators. *N Engl J Med* 2005; 352: 20–28.
37. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Reversal of atherosclerosis with aggressive lipid lowering (Reversal) investigators. *N Engl J Med* 2005; 352: 29–38.
38. Tekin A, Tekin G, Güzelsoy D, et al. Effects of atorvastatin (10 mg) on hemostatic and inflammatory parameters in hyperlipidemic patients with angiographically proven coronary artery disease. *Am J Cardiol* 2004; 93: 206–209.
39. Blanco-Colio LM, Martin-Ventura JL, de Teresa E, et al., on behalf of the ACTFAST investigators. Increased soluble Fas plasma levels in subjects at high cardiovascular risk. Atorvastatin on inflammatory markers (AIM) study, a substudy of ACTFAST. *Arterioscler Thromb Vasc Biol* 2007; 27: 168–174.