

# HMG CoA reductase inhibitors are related to improved systemic endothelial function in coronary artery disease

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## Abstract

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (statins) may enhance vascular endothelial function independent of their cholesterol lowering effect. To test this hypothesis, we surveyed two groups of patients (age  $55 \pm 7$ , mean  $\pm$  SD) with coronary artery disease that were matched for age, blood pressure and serum lipid levels. Group 1 comprised 23 men without lipid-lowering medication and Group 2 included 22 patients with ongoing HMG CoA reductase inhibitor medication. Flow-mediated (endothelium-dependent) arterial dilatation (FMD) and nitrate-mediated (smooth muscle dependent) dilatation (NMD) were measured in the brachial artery using high resolution ultrasound. FMD was considerably higher in group 2 ( $4.3 \pm 2.6$  vs.  $2.6 \pm 2.8\%$ ;  $P < 0.05$ ). In multivariate regression model, statin use was the only significant ( $P < 0.05$ ) predictor of FMD. In all subjects, FMD correlated with statin dose ( $P < 0.05$  for trend). NMD was non-significantly higher in group 2 ( $11.4 \pm 5.0$  vs.  $9.0 \pm 4.2\%$ ,  $P = 0.08$ ). We conclude that patients with established coronary artery disease on HMG CoA reductase inhibitor therapy have better vascular endothelial function than similar patients without the medication. These data provide further support for the idea that HMG CoA reductase inhibitors enhance endothelial function independent of their lipid-lowering effects. This may suggest that these drugs could be beneficial in secondary prevention of coronary artery disease regardless of the serum cholesterol concentration. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** Endothelial function; HMG CoA reductase inhibitors; Serum cholesterol

## 1. Introduction

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, or statins, are widely used in clinical practice for lowering of blood lipids. Large interventional studies have clearly shown that these drugs reduce cardiovascular events and mortality [1–3]. According to quantitative angiographic assessments, statin therapy improves topographical morphology of coronary arteries rather slowly and only to a small extent [4], however the beneficial effects on vascular endothelial function can be observed relatively soon

after the initiation of the treatment [5]. Serum low-density lipoprotein cholesterol concentration (LDL-C) has been shown to be inversely associated with vascular endothelial function [6]. A clinical study by O'Driscoll et al. [5], however, showed that the beneficial effect on endothelial function induced by HMG CoA reductase inhibitors was not correlated with the decrease in serum total cholesterol levels, and that the improvement in endothelial function seemed to continue even without further decrease in cholesterol concentration. This suggests that the effects of HMG CoA reductase inhibitors on vascular endothelium may not be solely a consequence of the reduction in circulating LDL-cholesterol concentration. Experimental studies are in line with these clinical findings, showing that pravastatin-treated

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cynomolgus monkeys have a better endothelial function than control monkeys, even when there are no differences in plasma lipid levels [7].

Therefore, to test the hypothesis that the use of HMG CoA reductase inhibitors is associated with improved endothelial function regardless of serum lipid profile, we measured endothelial function in two matched groups of patients with stenosis in 1–3 major coronary arteries. One group had been on stable dose of HMG CoA reductase inhibitors at least for 3 months and the other group was without lipid lowering therapy.

## 2. Methods

### 2.1. Subjects

We studied 55 unselected men (age 33–66) who had been admitted for diagnostic coronary angiography. Patients without significant coronary artery disease (stenosis of > 50% in one of the three major coronary arteries) were excluded. Group 1 included patients without HMG CoA reductase inhibitor and group 2 patients with ongoing HMG CoA reductase inhibitor therapy that had been maintained at least 3 months prior to the study. Groups were matched for age, blood pressure, body mass index and serum lipoprotein levels (Table 1). A total of 45 subjects (23 + 22) were included into the final analysis. Group 2, on statin therapy, had significantly more advanced coronary artery disease compared to group 1, with the mean number of stenosed vessels  $2.0 \pm 0.9$  vs.  $2.5 \pm 0.5$  ( $P = 0.011$ ). Three patients in group 1 and two patients in group 2 had diabetes mellitus ( $P = \text{NS}$ ). The use of medications other than statins was similar in both groups (Table 2).

In group 2 with statin therapy, the daily lipid lowering regimens were: simvastatin 10 mg (10 patients), simvastatin 20 mg (5 patients), lovastatin 20 mg (2 patients), lovastatin 40 mg (3 patients), fluvastatin 20 mg (1 patient) and pravastatin 40 mg (1 patient). All subjects gave their written informed consent. The study was conducted according to the guidelines of the Declaration of Helsinki and the study protocol had been approved by the ethics committee of Turku University and Turku University Central Hospital.

### 2.2. Coronary angiography

Coronary angiography was performed using the standard percutaneous transfemoral technique. Cineangiograms were obtained on a Philips Optimus M 200 biplane angiographic equipment (Philips Medical Systems Division, Eindhoven, The Netherlands) with an Acad 4100C biplane digital system (Acad Laboratories, Milipitas, CA). Angiograms were recorded on both 35 mm cine film and on  $512 \times 512$  matrix digital cine. The left coronary artery was routinely visualised on 4–6 projections and the right coronary artery on 2–4 projections, respectively. All angiograms were made and interpreted by an experienced diagnostic and interventional cardiac radiologist (J.M.). The stenosis measurements were based on visual and/or digital (quantitative coronary angiogram) quantification of the luminal diameters. A diameter stenosis of > 50% was interpreted as significant.

### 2.3. Ultrasound studies

All subjects underwent an ultrasound study for measurement of brachial artery reactivity. All studies were performed using an Acuson 128XP/10 mainframe (Acu-

Table 1  
Characteristics of subjects<sup>a</sup>

	Group 1 statins (–)	Group 2 Statins (+)	P-value
Number of subjects	23	22	—
Age (years)	$56 \pm 6$	$54 \pm 8$	0.42
Height (cm)	$176 \pm 6$	$177 \pm 5$	0.46
Weight (kg)	$88 \pm 10$	$88 \pm 9$	0.97
Body mass index (kg/m <sup>2</sup> )	$28 \pm 3$	$28 \pm 3$	0.70
Systolic blood pressure (mmHg)	$146 \pm 22$	$143 \pm 23$	0.67
Diastolic blood pressure (mmHg)	$81 \pm 10$	$78 \pm 10$	0.28
Total cholesterol (mmol/l)	$4.8 \pm 0.8$	$4.7 \pm 0.8$	0.84
HDL-C (mmol/l)	$0.94 \pm 0.4$	$0.88 \pm 0.3$	0.53
LDL-C (mmol/l)	$3.0 \pm 0.8$	$3.0 \pm 0.7$	0.93
Triglycerides (mmol/l)	$2.0 \pm 1.1$	$1.9 \pm 0.8$	0.85
Apolipoprotein A-1 (g/l)	$1.18 \pm 0.24$	$1.14 \pm 0.25$	0.55
Apolipoprotein B (g/l)	$1.01 \pm 0.22$	$1.06 \pm 0.16$	0.42
Lipoprotein (a) (mg/dl) <sup>b</sup>	120 (135)	209 (133)	0.10

<sup>a</sup> HDL-C  $\pm$  high-density lipoprotein cholesterol; LDL-C  $\pm$  low-density lipoprotein cholesterol.

<sup>b</sup> Mean (Median).

Table 2  
Number of subjects in study groups using other medications<sup>a</sup>

	Group 1 (n = 23) statins (–)	Group 2, (n = 22) statins (+)	P-value
ACE inhibitors	7	9	0.46
Aspirin	16	20	0.14
Beta-blockers	16	20	0.14
Long-acting nitrates	18	15	0.45

<sup>a</sup> ACE ± angiotensin converting enzyme.

son, Mountain View, CA) with a 7.0 MHz linear array transducer. Ultrasound studies were analysed unaware of angiographic results.

### 2.3.1. Arterial physiology testing

Brachial artery diameter was measured from B-mode ultrasound images. In all studies, scans were obtained at rest, during reactive hyperaemia, again at rest, and after sublingual nitrate. The subjects lay quietly for > 10 min before the first scan. The brachial artery was scanned in longitudinal section approx. 5 cm above the elbow. Depth and gain settings were set to optimise images of the lumen/arterial wall interface, images were magnified using a resolution box function and the operating parameters were not changed during any study. When a satisfactory transducer position was found, the position was marked on the skin, and the arm remained in the same position throughout the study. A resting scan was recorded, and arterial flow velocity was measured using a Doppler signal. Increased flow was then induced by inflation of a pneumatic tourniquet placed around the forearm (distal to the scanned part of the artery) to a pressure of 250 mmHg for 4.5 min, followed by release. A second scan was taken continuously for 15 s before and 90 s after cuff deflation, including a repeat flow velocity recording for the first 15 s after the cuff was released. Sublingual nitroglycerine in standard anti-anginal doses (isosorbide dinitrate spray 2.5 mg) was then administered and the last scan was acquired 4 min later.

Vessel diameter was measured in every case by independent observers who were 'blinded' to the subject's clinical details and stage of the experiment. The arterial diameter was measured at a fixed distance from an anatomic marker (e.g. a fascial plane) using ultrasonic calipers. Measurements were taken from the anterior to the posterior 'm' line at end-diastole, incident with the R-wave on a continuously recorded ECG. The 'm' line represents the edge of the media-adventitia interface in the ultrasound image of the arterial wall. For the reactive hyperaemia scan, diameter measurements were taken approx. 60 s after cuff deflation. Four cardiac cycles were analysed for each scan, and the measurements were averaged. The vessel diameter in scans after reactive hyperaemia and nitroglycerine administration

was expressed as the percentage relative to the average diameter of the artery in the baseline scan (100%). This method has been previously shown to be accurate and reproducible for measurement of small changes in arterial diameter [8]. The interobserver variation of mean FMD measurements in our laboratory was  $0.63 \pm 0.45\%$  (range 0.14–1.63%; CV,  $8.6 \pm 6.4\%$ ) and the intraobserver variation of two consecutive FMD measurements was  $0.48 \pm 0.43\%$  (range 0.07–1.34%; CV,  $6.2 \pm 4.4\%$ ).

### 2.4. Serum lipoproteins

Venous blood specimens were drawn from an antecubital vein of a sitting subject after an overnight fast. Serum total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride concentrations were measured using standard enzymatic methods (Boehringer Mannheim GmbH, Mannheim, Germany) with a fully automated analyser (Hitachi 704; Hitachi Ltd., Tokyo). HDL-C concentration was measured after polyethyleneglycol (final concentration 10%) precipitation [9]. Low-density lipoprotein cholesterol (LDL-C) concentration was calculated using Friedewald's equation [10]. Apolipoprotein A-I (apo A-I) and apolipoprotein B (apo B) were measured by immunonephelometric method (Behring BNA). The serum level of lipoprotein (a) was analysed using a commercially available solid phase two-site immunoradiometric assay kit (Mercodia Apo(a) RIA, Mercodia AB, Uppsala, Sweden).

### 2.5. Statistical methods

Results are expressed as mean  $\pm$  SD, unless stated otherwise. Data on serum triglycerides and lipoprotein (a) were skewed towards high values and were included as their logarithms in the analyses. Comparisons between the groups were conducted by student's *t*-test,  $\chi^2$  or Fisher's Exact, as appropriate. Univariate associations between study variables were analysed by calculating the Pearson's correlation coefficients. Multivariate analysis for FMD was done using stepwise multivariate linear regression technique. The following explanatory variables were included in the analysis: age,

body mass index, systolic and diastolic blood pressure, total cholesterol and HDL-C, log-transformed triglycerides, apolipoproteins, statin therapy group and the number of diseased coronary vessels. All statistical analyses were performed using statistical analysis system, SAS [11].

### 3. Results

The characteristics of the subjects are shown in Table 2. There were no significant differences in age, body mass index, serum lipoproteins or blood pressure between the groups (Table 1). The severity of coronary artery disease expressed as the number of diseased coronary vessels was greater in patients with statin therapy than in patients without the medication ( $2.0 \pm 0.9$  vs.  $2.5 \pm 0.5$ ,  $P = 0.011$ ).

The mean brachial artery diameter at rest ( $4.9 \pm 0.5$  vs.  $5.0 \pm 0.5$  mm;  $P = 0.49$ ), and the increase in blood flow during reactive hyperaemia ( $688 \pm 214$  vs.  $606 \pm 244\%$ ;  $P = 0.26$ ) were similar in groups 1 and 2, respectively. FMD was considerably higher in group 2 than in group 1 ( $4.3 \pm 2.6$  vs.  $2.6 \pm 2.8\%$ ,  $P = 0.04$ ) (Fig. 1). Nitrate mediated dilatation tended to be higher in group 2 ( $11.4 \pm 5.0$  and  $9.0 \pm 4.2\%$ ,  $P = 0.08$ ). Patients with high dose statin therapy (simvastatin 20 mg, lovastatin 40 mg or pravastatin 40 mg,  $n = 9$ ) tended to have higher FMD than patients with statin therapy with lower doses (Fig. 2).

In all subjects pooled together, FMD showed no significant univariate associations with serum lipoproteins. In the multivariate regression model including all subjects, the only significant explanatory variable for FMD was the group variable for statin therapy ( $P = 0.04$ ).

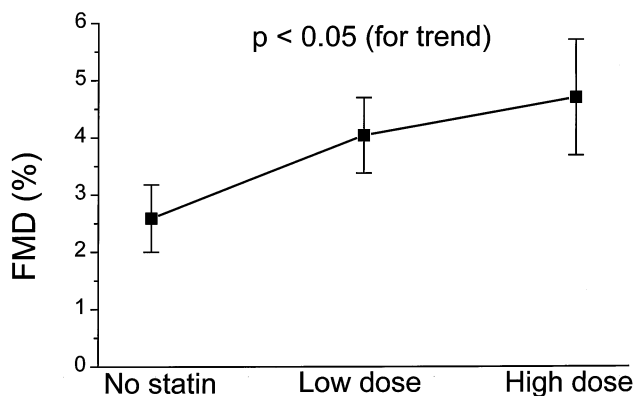


Fig. 1. Flow mediated dilatation (FMD) in patients with coronary artery disease with (statin+) or without (statin-) HMG CoA reductase inhibitor medication. The figure shows the actual values, the mean values and the 95% confidential intervals.

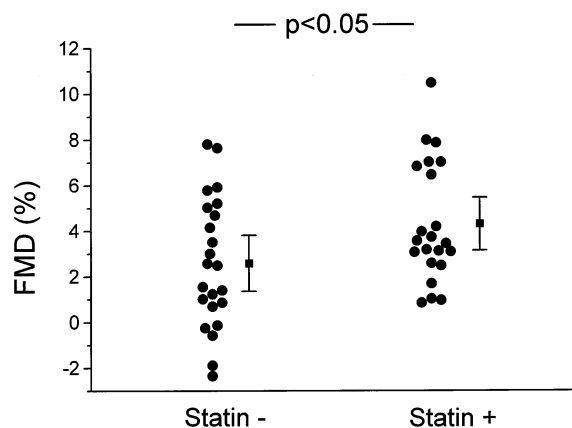


Fig. 2. Flow mediated dilatation (FMD) by statin dosage. High dose group includes simvastatin 20 mg, lovastatin 40 mg and pravastatin 40 mg. Values are mean  $\pm$  SEM.

### 4. Discussion

The present study showed that patients with established coronary artery disease who are on statin therapy have better vascular endothelial function than patients without this medication, although there were no differences in serum lipid profile between the study groups. This finding supports the idea that some of the beneficial effects of HMG CoA reductase inhibitors on vascular endothelium may be mediated via some other mechanism(s) than cholesterol lowering alone [12].

There is experimental evidence that statins have effects on the immune function [13,14], macrophage cholesterol metabolism [15], cell proliferation [16,17] and LDL oxidation [18,19] that are independent of changes in plasma LDL concentration. Giroux et al. [18] demonstrated that simvastatin, in similar concentrations that are observed in human plasma after a single oral 40 mg dose, inhibits the superoxide formation of the macrophages by 36%. Aviram et al. [19] have reported that lovastatin may act as an antioxidant by inhibiting LDL oxidation. Therefore, experimental studies suggest that the beneficial effects of HMG CoA reductase inhibitors on endothelial function may be mediated through the inhibition of macrophage superoxide production and oxidative modification of LDL. Oxidatively modified LDL (ox-LDL) is a more potent inhibitor of endothelium-dependent vasorelaxation than native LDL [20–22]. Ox-LDL has been found to reduce the effect of endothelial nitric oxide by decreasing its production [23] and by increasing its inactivation [24]. Thus, the inhibition of LDL oxidation by statins may offer one mechanistic explanation for the observed effect on vascular endothelium. Furthermore, a recent study demonstrated that HMG CoA reductase inhibitors may act also directly on vascular endothelium by upregulating the endothelial nitric oxide synthase [25].

We used a noninvasive ultrasound method to study vascular endothelial function. This method measures flow-dependent dilatation of the brachial artery, which is mediated by the excretion of nitric oxide from endothelial cells induced by shear stress [26]. Previous studies have indicated that the method is accurate [8] and significantly related to angiographically-determined extent of coronary atherosclerosis and coronary endothelial physiology [27–29].

In the present study, the group of patients with ongoing statin therapy had more severe coronary artery disease compared to the group without statins, but nevertheless significantly better endothelial function. This suggests that endothelial dysfunction associated with coronary artery disease is potentially reversible, as shown previously with smoke-induced endothelial dysfunction [30,31]. Furthermore, the result may suggest that for normocholesterolaemic patients with established coronary artery disease, the correct indication for statin therapy might be the presence of endothelial dysfunction, not lipid-lowering per se. The noninvasive ultrasound method for testing vascular endothelial function may be a potential candidate for means to evaluate whether normocholesterolaemic patients with coronary artery disease would benefit from statin therapy, although the method is currently not recommended for routine clinical practice in individuals [32].

We examined the relationships between statin therapy, lipid profile and arterial endothelial function in a cross-sectional setting. A more ideal approach would be prospective study of subjects before and after therapeutic interventions. The study included a relatively small amount of participants and only males. Nevertheless, by using a typical case-control approach with well matched patients, we were able to show that statin therapy is associated with improved endothelial function independently of serum lipoprotein levels.

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