

# Microvascular Reactivity in Patients with Hypercholesterolemia: Effect of Lipid Lowering Treatment

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## Summary

Impaired NO-dependent vasodilation of resistance vessels is an early marker of an increased risk of atherosclerosis; utility of the examination of microcirculation, however, is far less established. We have therefore tested the hypothesis that hypercholesterolemia is associated with an impaired microvascular reactivity and that this defect is at least partially reversible by lipid-lowering treatment. Twenty-seven otherwise healthy patients with severe hypercholesterolemia (HLP) were examined at rest and then after 10 weeks of atorvastatin treatment (20 mg/day). Skin microvascular reactivity (MVR) was examined by laser-Doppler flowmetry. Baseline MVR values of the studied group were compared to healthy control subjects, HLP patients with coronary artery disease (CAD) and diabetic patients with and without diabetic retinopathy. MVR was normal in HLP subjects without CAD. On the contrary, MVR was impaired in HLP patients with CAD. There was no effect of atorvastatin on MVR, despite the profound reduction of serum lipids. MVR values did not correlate with cholesterol levels. In diabetic subjects, the MVR was substantially impaired only in patients with retinopathy. In the subjects without retinopathy, MVR was either normal (type I diabetes) or moderately impaired (type II diabetes). MVR was thus normal in HLP patients without manifest vascular disease and was not influenced by lipid lowering therapy. Impairment in the MVR was only evident in subjects with HLP and severe CAD. These results suggest that microcirculation is not involved in the early vascular dysfunction induced by HLP and that MVR rather reflects changes which appear later in the course of the atherosclerotic disease.

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## Key words

Hypercholesterolemia • Microcirculation • Cholesterol lowering • Endothelial dysfunction • Laser-Doppler

## Introduction

Conventional risk factors often fail to identify patients who are prone to premature atherosclerosis. New markers of atherosclerotic plaque development and activity are therefore sought to identify patients at the highest risk of clinical events. Endothelial dysfunction, characterized by the impaired vasodilatation capacity and excessive vasoconstriction, is an early event in

atherosclerosis lesion development (Berliner *et al.* 1990, Šejda *et al.* 2002). Endothelial dysfunction has been associated with an increased risk of clinical events (Schachinger *et al.* 2000) and it is therefore widely used as a surrogate marker of the risk of atherosclerosis.

Hyperlipidemia is the most potent factor contributing to the progression of atherosclerosis; cholesterol reduction is therefore the critical treatment in reducing the risk of acute cardiac events (Gould *et al.*

1997, Štulc and Češka 2001). Impaired endothelium-dependent vasodilation of resistance vessels has been documented in patients with hyperlipidemia and was reversible by lipid lowering (Tamai *et al.* 1997, Dupuis *et al.* 1999). On the contrary, the involvement of microvascular bed in hyperlipidemia and atherosclerosis is not well established.

Recently, a non-invasive method of laser-Doppler flowmetry became available for the examination of microcirculation. So far, the method has been mainly used for studying the microcirculatory disturbances in patients with diabetes (Morris *et al.* 1995, Škrha *et al.* 2001). On the other hand, only few studies have been done to investigate the microvascular function in patients with hyperlipidemia and/or atherosclerosis (Khan *et al.* 1999, Asberg *et al.* 2001, Haak *et al.* 2001).

We have therefore studied the effect of lipid-lowering therapy with atorvastatin on microvascular reactivity (MVR) in patients with severe hypercholesterolemia. Baseline MVR of these patients was compared to healthy control subjects, patients with severe coronary artery disease (CAD), and to diabetic patients with or without microvascular complications. In CAD patients, the effect of aggressive lipid lowering was also tested in a pilot study of the short-term treatment with LDL-apheresis.

## Subjects and Methods

### Study subjects

*Patients with hypercholesterolemia, without symptomatic CAD (HLP<sub>0</sub> group).* Eligible were subjects over 18 years old with serum total cholesterol (TC) >7.0 mmol/l after 4 weeks without any lipid-lowering medication. Patients with diabetes, malignancy, other major disease or hypertriglyceridemia (>4.5 mmol/l) were excluded.

*Patients with hypercholesterolemia and CAD (HLP<sub>CAD</sub> group).* We included patients with resistant hyperlipidemia and severe symptomatic CAD, who were not suitable for percutaneous or surgical revascularization. Patients with LDL-cholesterol >3.2 mmol/l and triglycerides <4.5 mmol/l on maximum lipid-lowering medication were eligible for the study (the maximum lipid lowering medication was either the highest tolerated dose of statin or the statin-fibrate combination). All patients had exertional angina CCS grade III-IV; they had a finding of severe and multiple stenoses in their coronary angiograms, which precluded both surgical or percutaneous revascularization.

*Patients with diabetes mellitus.* Patients with type I and type II diabetes were included (groups designated DM1 and DM2, respectively). Each group was subdivided into patients without retinopathy (DM1<sub>0</sub>, DM2<sub>0</sub>) and with incipient retinopathy (DM1<sub>RNP</sub>, DM2<sub>RNP</sub>), according to the ophthalmological findings. Smokers and subjects with proliferative retinopathy, uncontrolled hypertension or symptomatic CAD were not included.

A control group of healthy subjects consisted of non-smoking individuals without diabetes, hyperlipidemia, CAD or any other major disease. The control subjects and diabetic patients were examined earlier in our laboratory and the detailed results have been published (Škrha *et al.* 2001). Baseline demographic and laboratory characteristics of the study subjects are summarized in Table 1.

### Lipid-lowering interventions

Hypercholesterolemic patients without symptomatic CAD (HLP<sub>0</sub> group) were treated with atorvastatin, 20 mg daily for 10-weeks. The treatment was preceded by a 4-week washout period without lipid-lowering medication. The patients were examined before and at the end of the treatment period.

Patients with hypercholesterolemia and severe CAD (HLP<sub>CAD</sub> group) were included in a pilot study of the short-term treatment with LDL-apheresis. The LDL-apheresis was performed using the HELP system (apparatus Plasmatec Secura, B. Braun, Melsungen, Germany) as described elsewhere (Schuff-Werner *et al.* 1989). A total volume of 2.5-3.5 l of plasma was treated during each session. In each patient, the apheresis was performed three times with a 7-day interval between the treatments. The patients were examined at the baseline and on the days after the first and the third apheresis.

### Microvascular reactivity

Reactivity of the skin microvascular bed to thermal and ischemic stimuli was measured using laser-Doppler flowmetry after an overnight fast as described previously (Škrha *et al.* 2001). The measurements were performed after 30 min of rest at room temperature, using Periflux 4001 apparatus (Perimed, Järfälla, Sweden). The laser-Doppler probe was fixed in a heating chamber to the skin of the ventral forearm. After a 5-min stabilization period, the baseline flow was recorded at skin temperature (32 °C) which was maintained by the heating unit (Peritemp 4005, Perimed, Sweden). The occlusion of the brachial artery was then performed for

3 min by inflating the tourniquet of a sphygmomanometer to 20 mm Hg above the systolic pressure. After a fast decompression, the flow changes were recorded to evaluate the postocclusive reactive hyperemia (PORH). The thermal hyperemia test was performed following a 5-min stabilization period. Skin temperature was increased to 44 °C in the heating chamber and maintained for the next 5 min. During this period, the course of

thermal hyperemia (TH) was recorded. Based on the measurements, the following parameters were calculated for both postocclusive and thermal tests: 1) the peak flow after stimulation (in perfusion units – PU) as the difference between maximum and baseline perfusion (PORH<sub>max</sub>, TH<sub>max</sub>). 2) the mean velocity of the perfusion increase (in PU.s<sup>-1</sup>, PORH<sub>max</sub>/t, TH<sub>max</sub>/t).

**Table 1.** Baseline demographic and laboratory characteristics of the study groups.

	Controls	HLP <sub>0</sub>	HLP <sub>CAD</sub>	DM1 <sub>0</sub>	DM1 <sub>RNP</sub>	DM2 <sub>0</sub>	DM2 <sub>RNP</sub>
<i>Number</i>	26	27	8	20	24	18	18
<i>Sex (male/female)</i>	16 / 10	8 / 19	8 / 0	10 / 10	12 / 12	13 / 5	10 / 8
<i>Age [years]</i>	38±16	52±12	55±17	32±9	44±9	56±8	58±9
<i>BMI[kg/m<sup>2</sup>]</i>	23.5±2.4	26.7±4.2	25.4±3.9	23.1±2.4	24.2±2.3	29.0±4.7	32.0±4.0
<i>SBP [mmHg]</i>	121±15	129±13	131±21	120±12	126±14	141±11	152±15
<i>DBP [mmHg]</i>	78±11	82±8	85±18	78±8	81±7	86±8	86±10
<i>TC [mmol/l]</i>	5.0±1.1	8.5±1.5	5.7±0.6	NA	NA	5.5±1.0	6.5±0.9
<i>LDL-C [mmol/l]</i>	3.0±0.6	6.1±1.3	4±0.5	NA	NA	3.3±0.9	4.0±1.2
<i>HDL-C [mmol/l]</i>	1.6±0.3	1.6±0.4	1±0.2	NA	NA	1.2±0.3	1.0±0.2
<i>TG [mmol/l]</i>	1.24±0.7	1.75±0.8	1.5±0.5	NA	NA	2.2±1.0	3.5±2.1
<i>Glucose [mmol/l]</i>	4.5±0.2	5.2±0.5	1±0.2	8.8±4.3	8.9±3.0	9.5±2.8	11.9±4.2
<i>HbA<sub>1c</sub> [%]</i>	4.7±0.3	NA	NA	7.6±1.1	7.9±1.3	7.4±1.6	8.7±1.2

*BMI* – body mass index, *SBP* – systolic blood pressure, *DBP* – diastolic blood pressure, *TC* – total cholesterol, *LDL-C* – LDL-cholesterol, *HDL-C* – HDL-cholesterol, *TG* – triglycerides, *HbA<sub>1c</sub>* – glycated hemoglobin *HbA<sub>1c</sub>*, *NA* – not available.

**Table 2.** Comparison of the microvascular reactivity between the study groups.

	Controls	HLP <sub>0</sub>	HLP <sub>CAD</sub>	DM1 <sub>0</sub>	DM1 <sub>RNP</sub>	DM2 <sub>0</sub>	DM2 <sub>RNP</sub>
<i>PORH<sub>max</sub> [PU]</i>	51±23	64±32	40±23	37±16 <sup>x,b</sup>	27±13 <sup>x,b</sup>	36±18 <sup>x</sup>	30±16 <sup>x</sup>
<i>PORH<sub>max</sub>/t [PU/s]</i>	4.1±2.4	5.2±2.6	3.6±2.4	4.7±2.5 <sup>b</sup>	2.5±1.3 <sup>x,b</sup>	4.1±2.1	3.4±1.8
<i>TH<sub>max</sub> [PU]</i>	115±46	173±89 <sup>x,a</sup>	86±46 <sup>a</sup>	99±41 <sup>b</sup>	70±24 <sup>x,b</sup>	83±36 <sup>x</sup>	73±31 <sup>x</sup>
<i>TH<sub>max</sub>/t [PU/s]</i>	1.7±0.7	2.4±1.3 <sup>x,a</sup>	0.9±0.4 <sup>x,a</sup>	1.6±0.7 <sup>b</sup>	1.2±0.6 <sup>x,b</sup>	1.1±0.4 <sup>x</sup>	1.0±0.4 <sup>x</sup>

Peak blood flow and the velocity of perfusion increase were assessed during the postocclusive (*PORH<sub>max</sub>*, *PORH<sub>max</sub>/t*) and thermal (*TH<sub>max</sub>*, *TH<sub>max</sub>/t*) tests; <sup>x</sup> *p* < 0.05 versus the control group, <sup>a</sup> *p* < 0.05 HLP<sub>0</sub> versus the HLP<sub>CAD</sub> group, <sup>b</sup> *p* < 0.05 DM1<sub>0</sub> versus the DM1<sub>RNP</sub> group.

#### Laboratory measurements and statistics

The basic biochemical tests were performed by automated analyzer methods. All results are expressed as means ± SD. Differences between groups were tested by the Mann-Whitney U test; values before and after the

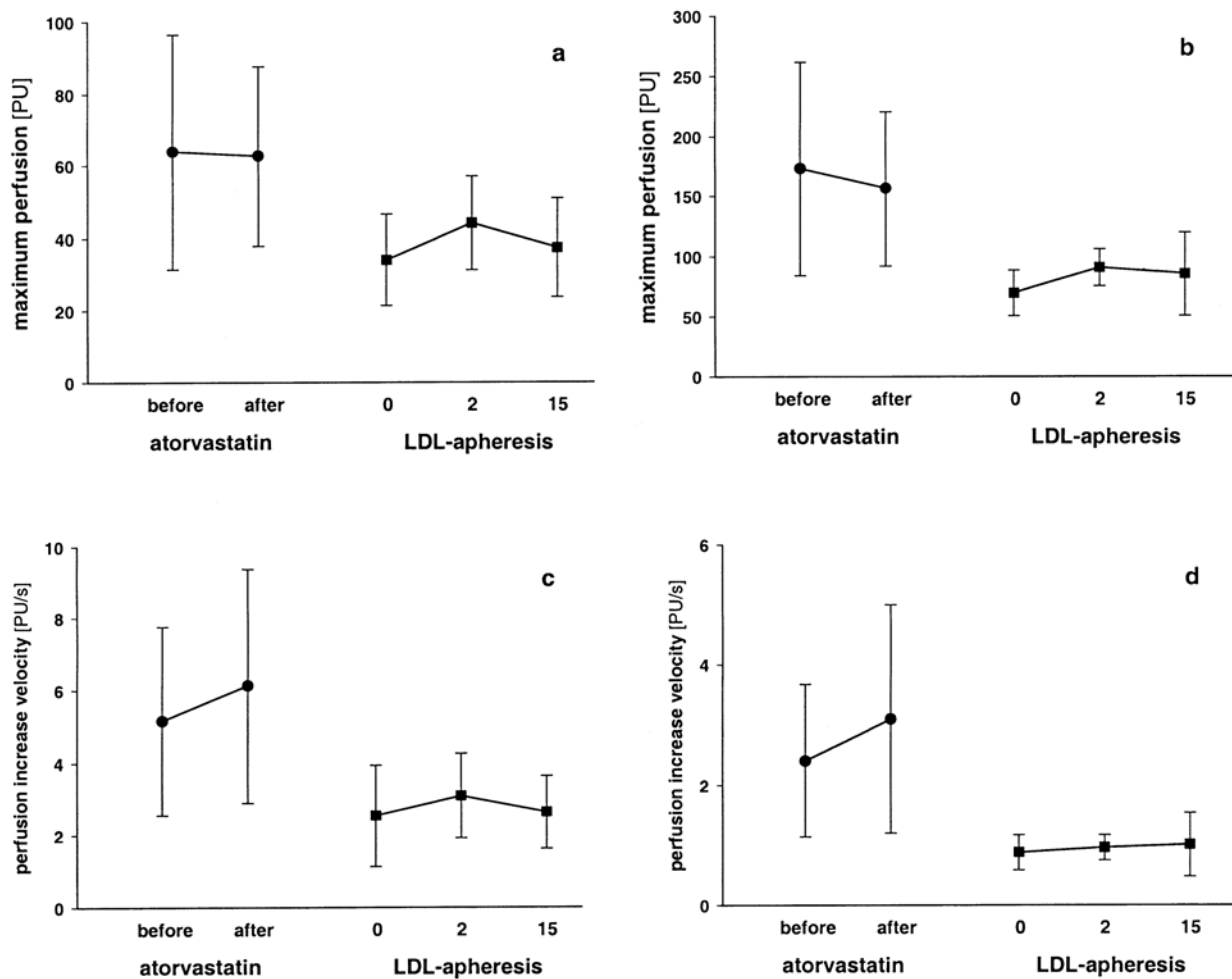
treatment were compared using Wilcoxon matched pairs test; all probabilities are two-sided. The Spearman correlation was used to test for the relation between variables.

## Results

### Comparison of microvascular reactivity between the groups studied

Results of the laser-Doppler measurements are shown in Table 2. In the HLP<sub>0</sub> patients, the MVR was not impaired in comparison with the control group despite severe hypercholesterolemia. Conversely, there was a

trend towards higher values of MVR (significant for the TH parameters). In the HLP<sub>CAD</sub> patients, MVR was decreased compared to the controls (significantly in case of the TH parameters). MVR in the HLP<sub>CAD</sub> patients was also consistently lower than that in HLP<sub>0</sub> patients, the differences being significant for the TH tests and marginally significant for the PORH tests.



**Fig. 1.** The effect of lipid lowering treatment on the skin microvascular reactivity during postocclusive ( $PORH_{max}$  – a,  $PORH_{max}/t$  – c) and thermal ( $TH_{max}$  – b,  $TH_{max}/t$  – d) tests. The HLP patients without CAD were examined before and after the 10 weeks of atorvastatin treatment (circles). The CAD patients were examined at the baseline and on the days after the first and the third apheresis (day 0, 2 and 15, squares).

In the HLP<sub>0</sub> patients, no significant associations were found between parameters of microvascular function and blood lipid or glucose levels, patient age or BMI. In the HLP<sub>CAD</sub> group, similar analyses were not performed because of the limited number of patients.

In diabetic patients without retinopathy, there was no impairment of MVR in the DM1<sub>0</sub> group and a moderate impairment in the DM2<sub>0</sub> group compared to the healthy subjects. In the patients with retinopathy (DM1<sub>RNP</sub> and DM2<sub>RNP</sub> groups), MVR was consistently

and significantly lower than in the control group. When diabetic patients with and without retinopathy were compared (DM1<sub>0</sub> vs. DM1<sub>RNP</sub> and DM2<sub>0</sub> vs. DM2<sub>RNP</sub> groups), the MVR decrease was more profound in patients with retinopathy; the difference, however, was significant in type I diabetic patients only.

An inverse relationship was observed in type I diabetic patients between PORH<sub>max</sub>, TH<sub>max</sub> and TH<sub>max</sub>/t (but not PORH<sub>max</sub>/t) values and fasting blood glucose levels ( $r = -0.49, -0.39$  and  $-0.42, p = 0.001-0.007$ ) or glycated hemoglobin levels ( $-0.45, -0.41$  and  $-0.43, p = 0.002-0.005$ ). No similar relationship was detected in the subjects with type II diabetes. No association was found between the MVR and age, BMI or duration of the disease in any diabetic group.

#### *Effect of lipid lowering in patients with hypercholesterolemia*

Effects of atorvastatin and LDL-apheresis treatments on the MVR in HLP<sub>0</sub> and HLP<sub>CAD</sub> groups are summarized in Fig. 1. In the HLP<sub>0</sub> patients, atorvastatin treatment resulted in a decrease of total cholesterol by 31±12 % (from 8.59±1.60 to 5.86±1.12 mmol/l), LDL-cholesterol by 40±14 % (from 6.20±1.39 to 3.67±0.94 mmol/l) and triglycerides by 14±31 % (from 1.67±0.63 to 1.33±0.38 mmol/l); HDL-cholesterol remained unchanged (1.63 mmol/l). Despite this profound lipid lowering, no significant changes in the MVR were observed; there was only a non-significant trend towards improvement in the increase of perfusion velocity (PORH<sub>max</sub>/t, TH<sub>max</sub>/t).

In the HLP<sub>CAD</sub> group, LDL-apheresis resulted in a decrease of total cholesterol by 39±12 % after the first apheresis and by 40±15 % after the third apheresis (5.70±0.64 to 3.44±0.33 and to 3.40±0.44 mmol/l, respectively). The corresponding changes in LDL-cholesterol were 50±12 and 52±17 % (from 3.95±0.54 to 1.96±0.43 and to 1.95±0.46 mmol/l, respectively). There was some increase in all parameters of the MVR immediately after both apheresis treatments (7-30 %), but this remained insignificant, obviously because of the small number of patients in this pilot study.

## **Discussion**

The principal finding of our study is that microvascular reactivity (as assessed by laser-Doppler flowmetry) is not impaired in hypercholesterolemic patients without a manifest vascular disease, and that

lipid lowering with atorvastatin has little effect on microvascular functions in these patients.

So far, only a few studies have addressed similar topics with different results. Khan *et al.* (1999) studied patients with hyperlipidemia and peripheral artery obstructive disease. Fluvastatin failed to improve the NO-dependent MVR (acetylcholine iontophoresis), and some improvement in the NO-independent reactivity (sodium-nitroprusside iontophoresis) was only observed after a prolonged treatment. In another study of hypercholesterolemic patients (Haak *et al.* 2001), fluvastatin led to early and markedly improved postischemic MVR (as assessed by capillaroscopy), but only marginally improved thermal MVR (laser-Doppler) after prolonged treatment. Asberg *et al.* (2001) studied the effect of atorvastatin in cyclosporin-treated renal-transplant patients with moderate hypercholesterolemia. Mild improvement (+17 %,  $p=0.042$ ) in the MVR was observed after acetylcholine iontophoresis but no change after ischemic stimulation (PORH). Substantial differences in the design of our and the above-mentioned studies preclude direct comparisons, but in general, changes in MVR induced by lipid lowering range from mild or moderate improvement to no change at all. For some parameters, trends towards minor improvement are evident which fail to reach significance due to the wide variability of the measurements.

Comparisons of hyperlipidemic patients with healthy controls were only done in our study and in that of Khan *et al.* (1999). In our study, hypercholesterolemic patients without vascular disease had quite normal (or even better than normal) MVR. In the study by Khan *et al.* (1999), patients with hyperlipidemia and peripheral artery obstructive disease had substantial impairment of MVR compared to the healthy controls. Similarly, in our patients with hyperlipidemia and CAD, MVR was decreased compared to the controls and to the HLP<sub>0</sub> patients. There was also no correlation between microvascular function and blood lipid levels in our study, a finding similar to those of Khan *et al.* (1999) and Haak *et al.* (2001). These results together suggest that hyperlipidemia has a minor direct effect on the microvascular function. Probably, MVR is not a sensitive marker of early vascular dysfunction induced by hyperlipidemia, but rather reflects functional changes associated with an advanced form of the vascular disease.

The lack of consistent association between hyperlipidemia and microvascular function, as well as the small effect of lipid lowering on MVR, contrast with the well documented effect of hypercholesterolemia on the

endothelial function in resistance vessels and with the improvement induced by treatment (Tamai *et al.* 1997, Dupuis *et al.* 1999). Both tests are supposed to reflect endothelium-dependent vasomotor functions. However, microvascular bed is different from muscular arteries, and various parts of the vascular bed may be differentially involved in pathological processes; the effects of various treatments may also be different.

In type I and type II diabetic patients, microvascular changes were more pronounced in subjects with retinopathy than in those without retinopathy (in type I patients without retinopathy, there were hardly any changes at all). The relationship between diabetes, retinopathy and microvascular dysfunction therefore runs in parallel with the relationship between hypercholesterolemia, atherosclerosis and microvascular reactivity: it is associated rather with clinically manifest

vascular involvement than with a predisposing condition (i.e. diabetes).

In conclusion, severe hypercholesterolemia in patients without manifest atherosclerosis was not associated with an impairment of microvascular reactivity. Atorvastatin treatment had no significant effect on the MVR, despite the profound changes in blood lipid levels. A decrease in the MVR, however, was evident in patients with hyperlipidemia and severe coronary artery disease. These results suggest that the microcirculation is not involved in the early vascular changes induced by HLP and that MVR rather reflects changes which appear later in the course of the atherosclerotic disease.

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