Cholesterol lowering is more important than pleiotropic effects of statins for endothelial function in patients with dysglycaemia and coronary artery disease

Magnus Settergren*, Felix Böhm, Lars Rydén, and John Pernow

Department of Cardiology, Karolinska University Hospital, Stockholm SE-171 76, Sweden

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Aims
The importance of pleiotropic effects of statins on endothelial function and inflammatory markers was investigated in patients with dysglycaemia and coronary artery disease (CAD).

Methods and results
Thirty-nine patients were randomized to simvastatin 80 mg daily (S80; n = 20) or ezetimibe 10 mg and simvastatin 10 mg daily (E10/S10; n = 19) for 6 weeks, aiming at similar cholesterol reduction. Endothelial function, evaluated by brachial artery flow-mediated vasodilatation (FMD) and the effect of endothelin receptor blockade, serum lipid, and inflammatory markers were evaluated at baseline and follow-up. At follow-up, low-density lipoprotein cholesterol decreased from 3.1 (2.8–3.4) (median and quartiles) to 1.5 mmol/L (1.4–1.7) and from 3.0 (2.5–3.4) to 1.3 mmol/L (1.1–1.8), in the S80 and E10/S10 groups, respectively. In the entire study group, FMD increased from 4.3% (3.4–6.1) at baseline to 5.5% (3.4–6.6) at follow-up, while C-reactive protein decreased from 3.1 (1.7–7.6) to 2.3 mg/L (0.9–6.5). The changes in FMD and C-reactive protein from baseline to follow-up were not significantly different between patients on S80 and E10/S10 groups. Endothelin blockade enhanced endothelium-dependent vasodilatation both at baseline and follow-up.

Conclusion
Lipid lowering is more important than pleiotropic effects of statins for improvement in endothelial function and inflammatory markers in patients with dysglycaemia and CAD.

Keywords
Statins • Pleiotropic effects • Ezetimibe • Endothelial function • Type 2 diabetes

Introduction
Cholesterol-lowering therapy with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) decreases mortality and morbidity in patients with cardiovascular disease, not the least among those with type 2 diabetes mellitus.1,2 There is a close correlation between the reduction in low-density lipoprotein (LDL) cholesterol and benefits seen after the institution of statins supporting the notion that LDL-lowering is the primary mechanism underlying the effects of statins.3,4 In addition, statins exert anti-inflammatory and anti-oxidative effects that may be unrelated to cholesterol-lowering. Post hoc analysis of large intervention trials as well as experimental studies suggest that a part of the beneficial effects of statins is independent of their lipid-lowering capacity, the so-called pleiotropic effects.5–7 Such effects may include improved endothelial function, characterized by increased availability of nitric oxide (NO) and decreased levels of endothelin-1 (ET-1).8,9 It is, however, difficult to distinguish the beneficial effects related to cholesterol-lowering from those owing to possible pleiotropic effects, when the degree of cholesterol-lowering is not comparable.

A new possibility to separate pleiotropic from lipid-lowering effects of statins is offered by the introduction of ezetimibe, a pharmacological agent that lowers blood cholesterol by inhibition

*Corresponding author. Tel: +46 8 51770807, Fax: +46 8 311044, Email: magnus.settergren@karolinska.se

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of intestinal lipid absorption without influencing the mevalonate pathway.10,11 The combination of ezetimibe and a low-dose statin will result in a comparable degree of cholesterol-lowering as that achieved by high-dose statin treatment.11 The present study tested the hypothesis that cholesterol reduction achieved with a high dose of simvastatin, because of pleiotropic effects, would be more efficient than a similar cholesterol reduction accomplished by the combination of ezetimibe and a low-dose simvastatin. This was studied by evaluation of the effects on endothelial function and inflammatory markers in patients with dysglycaemia and coronary artery disease (CAD).

**Methods**

**Subjects**

A total of 43 patients with type 2 diabetes or impaired glucose tolerance (IGT) and stable CAD were recruited. Patients were classified as having type 2 diabetes mellitus or IGT according to the WHO criteria.13 Stable CAD was defined as present by means of a coronary angiogram or a history of previous myocardial infarction (MI). The exclusion criteria were as follows: treatment with statins or other lipid-lowering agents during the preceding 12 weeks, change in dosage of any vasodilator drugs during the preceding 6 weeks, age >80 years; any concomitant disease that was considered to have limited the possibility for the patient to complete the study protocol, MI or a coronary intervention during the preceding 2 months, planned coronary intervention within the nearest 3 months, known allergic reaction to acetylsalicylic acid, disturbed hepatic function according to a standard laboratory assessment, warfarin treatment or an international normalized ratio >2.0, untreated hypertension, participation in an ongoing study. All patients gave their written informed consent. The study protocol, conducted according to the Declaration of Helsinki, was approved by the ethics committee of the Karolinska University Hospital.

**Study design**

The study was a randomized double-blind controlled clinical trial. The study protocol included an evaluation of endothelial function during flow-mediated vasodilatation (FMD) on day 1 and a forearm venous occlusion plethysmography the following day. After completion of these investigations, the patients were using the double dummy technique, randomized to one of the two following treatment groups: simvastatin 80 mg and ezetimibe/simvastatin placebo (S80) or ezetimibe 10 mg/simvastatin 10 mg and simvastatin placebo (E10/S10). All study personnel and participants were blinded to treatment assignment for the duration of the study. All drugs were given once daily in the evening. After 6 weeks of treatment, the patients were re-examined as above.

All tests were initiated in the morning after 12 h of fasting. Following blood sampling, the patients were served a standard breakfast consisting of a cheese sandwich and lingonberry juice. The patients were not allowed any caffeine-containing drinks or tobacco consumption on the day of the study. All drugs except aspirin, clopidogrel, and glucose-lowering medications were withheld on the morning of the test. Treatment compliance was checked through pill count. A computer-generated randomization list was delivered by the producer of the drug/placebo (Merck Sharp Dome, Stockholm, Sweden). The subjects were randomized in blocks of four. The randomization code was kept in sealed envelopes and was not opened until completion of data collection and analyses.

**Flow-mediated vasodilatation**

The patients were investigated in a quiet, dimly lit room in the supine position. Non-invasive examination of the brachial artery of the non-dominant arm13 was performed by means of an 8 MHz linear-array transducer connected to an Acuson Sequoia® (Acuson Corporation, Mountain View, CA, USA). Baseline images were saved every third second during 1 min and a mean value was calculated from these values. Subsequently, a blood pressure cuff positioned below the elbow was inflated to 260 mmHg for 5 min. The artery was continuously imaged for 3 min during the hyperaemia following release of the cuff pressure to determine endothelium-dependent vasodilatation (EDV). A mean value was calculated from three recordings at maximum dilatation. Endothelium-independent vasodilatation (EIDV) was determined following sublingual administration of nitroglycerine (0.4 mg). All images were analysed using proprietary software (Brachial analyzer®; Medical Imaging Applications, Iowa City, IA, USA) by a technician, blinded to treatment allocation. The maximum lumen diameter, found through beat-to-beat analysis, was measured using an automated contour detection system. Lumen diameter was defined as the distance between the intima of the far and near vessel walls. Dilatation was calculated as maximal lumen diameter after ischaemia or nitroglycerine minus lumen diameter at baseline divided by lumen diameter at baseline. Four examinations in the S80 group and one in the E10/S10 group could not be analysed because of difficulties in obtaining clear identification of the intima. The coefficient of variation for FMD determination on two study occasions was 18%.

**Forearm plethysmography**

This investigation was performed in order to evaluate the effect of ET receptor blockade on basal vascular tone and EDV. Following local anaesthesia, a percutaneous catheter was inserted into the brachial artery of the non-dominant arm. Forearm blood flow (FBF) was measured simultaneously in both arms using a mercury-in-silastic strain gauge applied around the widest part of the forearm.14 Upper arm cuffs were inflated to 50 mmHg for 10 s in order to obstruct the venous flow during the recording of FBF. Wrist cuffs were inflated to 30 mmHg above systolic blood pressure to exclude the hand from circulation during the determination of FBF. During the recording, 0.9% saline (1 mL/min) was continuously infused in the brachial artery.

Basal FBF was recorded during an additional infusion of saline, 30 min after the arterial cannulation. Thereafter, acetylcholine (3, 10, and 30 μg/min) was infused into the brachial artery to assess EDV. This was followed by an infusion of the NO donor sodium nitroprusside (SNP; 1 and 3 μg/min) for determination of EIDV. Each dose was given during 2 min at a rate of 2.5 mL/min. The ET-1-induced vasoconstrictor tone was then assessed by co-infusion of the ET₄-receptor antagonist BQ123 and the ET₄-receptor antagonist BQ788. The antagonists were infused for 80 min at a rate of 10 nmol/min and FBF was determined every 10 min. After 60 min of infusion, EDV and EIDV were re-assessed. The acute vasodilator effect of acetylcholine and nitroprusside are expressed in absolute change in FBF. The prolonged effect of ET receptor blockade on baseline FBF is expressed as per cent change of the ratio between the experimental and control arms according to previous recommendations.15 Average values of the FBF were obtained from four to eight inflow recordings during 2 min. One examination in the S80 group could not be analysed because of unstable inflow curves. The NO-dependent property of the vasodilatation induced by acetylcholine has been validated in this model previously.16 The coefficient of variation between two consecutive determinations of EDV in one subject is 5.1%.
Biochemical analysis
Fasting plasma glucose, haemoglobin A1c (HbA1c), total serum cholesterol, LDL, high-density lipoprotein (HDL), triglycerides, apolipoprotein A1 and B (ApoA1 and ApoB) were assessed at baseline and at the end of the study according to local laboratory routines. Plasma ET immunoreactivity was analysed by radioimmunoassay using commercially available antiserum (rabbit anti-ET-1 6901, Peninsula, Merseyside, UK) following ethanol extraction.17 High-sensitive C-reactive protein was measured using the Behring Nephelometer Analyzer II with particle-enhanced immunonephelometric assay.18 Intercellular adhesion molecule-1 (ICAM-1) and interleukin-6 (IL-6) were analysed on the Evidence® biochip array analyzer (Randox Laboratories, Ltd, Crumlin, UK).19

Drugs
BQ123 (Clinalfa, Laufelfingen, Switzerland), BQ788 (Neosystem SA, Strasbourg, France), and acetylcholine (CIBA Vision, Roskilde, Denmark) were dissolved in sterile 0.9% sodium chloride (NaCl) and stored at −80°C. On the day of the experiment all substances including SNP (Abbott, Chicago, IL, USA) were diluted to the proper concentrations in sterile 0.9% NaCl. Simvastatin, ezetimibe, and placebo were supplied by Merck Sharp and Dome.

Statistical analysis
All results are expressed as median and quartiles. A two-sided P-value <0.05 was considered significant. Group comparison with respect to FMD, ET-1-mediated vascular effects, clinical characteristics, and laboratory results were made by the Mann–Whitney rank sum test. The difference regarding the effect of the two modes of treatment on ET-1-mediated vascular effects was analysed by comparing the changes in EDV induced by ET-receptor blockade from baseline to follow-up between the two groups. The change in EDV was calculated as the difference in the mean of the responses to the three dosages of acetylcholine. The primary endpoint of the study was the absolute change in FMD. The secondary endpoints were change in ET-1-mediated vascular effects determined by venous occlusion plethysmography and change in inflammatory markers. All analyses were according to the protocol. The number of patients in each group needed to detect a difference in FMD of 2% with a power of 80% and a two-tailed t-test at the 5% level is approximately 22, assuming a normal shift model with a standard deviation of 2.4 for difference in FMD. All statistical analysis were performed using GraphPad Prism version 4 (GraphPad Software, San Diego, CA, USA) and Minitab version 14 (Minitab Inc., PA, USA) for power calculation.

Results
Subjects
Four patients failed to fulfil the protocol and were therefore excluded from the study; two because of unwillingness to complete the study (both in the simvastatin 80 mg group), one because of stroke and one because of rash while on study medication (both in the ezetimibe 10 mg/simvastatin 10 mg group). Thirty-nine patients completed the study (Figure 1). As shown in Table 1, the two groups were well balanced regarding baseline characteristics. There were 17 patients with diabetes and three with IGT in the S80 group while all patients in the E10/S10 group had diabetes.

Lipids and inflammatory markers
Baseline lipids were well balanced between the groups (Table 2). Total and LDL cholesterol and triglycerides decreased substantially and by similar magnitude in both groups, while HDL cholesterol did not change significantly. ApoB decreased to numerically similar levels but statistically more in the S80 group, whereas ApoA1 remained unchanged (Table 2).

C-reactive protein, IL-6, and ICAM-1 did not differ significantly between the two groups and there was no significant difference between the two groups following treatment. Plasma ET-1 was not significantly affected in any of the groups (Table 2).

Flow-mediated vasodilatation
Because of image quality, FMD could not be analysed in two patients in the S80 group and three patients in the E10/S10 giving a total number of 34 patients who were evaluated. Baseline brachial artery diameter was similar in the two groups (3.8 mm (3.2–4.3) and 3.7 mm (3.6–4.2), respectively). Following 6 weeks of treatment, FMD increased in the entire study group from 4.3% (3.4–6.1) to 5.5% (3.4–6.6; P = 0.005). FMD increased from 4.3% (2.7–6.0) to 5.8% (3.4–8.4) in the E10/S10 group, and from 4.3% (1.7–6.2) to 5.2% (3.0–6.0). There was no statistical difference in the increase of FMD between the two groups (Figure 2) (P = 0.39, 95%CI −0.7 to 1.9). EIDV, induced by nitroglycerine, did not change during treatment in either group.
Table 1 Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>E10/S10 (n = 19)</th>
<th>S80 (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>74 (66–77)</td>
<td>70 (62–74)</td>
</tr>
<tr>
<td>Male/female, n (%)</td>
<td>11 (58)/8 (42)</td>
<td>15 (75)/5 (25)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28 (26–29)</td>
<td>28 (25–31)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>4 (21)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>6.2 (4.3–7.1)</td>
<td>5.5 (5.0–7.2)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>150 (140–160)</td>
<td>150 (120–160)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>60 (50–70)</td>
<td>60 (50–75)</td>
</tr>
<tr>
<td>Type 2 diabetes/impaired glucose tolerance, n (%)</td>
<td>19 (100)/0 (0)</td>
<td>17 (85)/3 (15)</td>
</tr>
<tr>
<td>Insulin treatment, n (%)</td>
<td>6 (31)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Oral hypoglycaemic, n (%)</td>
<td>8 (42)</td>
<td>9 (45)</td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
<td>16 (84)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Clopidogrel, n (%)</td>
<td>2 (10)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Beta-blockers, n (%)</td>
<td>15 (79)</td>
<td>18 (90)</td>
</tr>
<tr>
<td>Calcium channel-blockers, n (%)</td>
<td>6 (31)</td>
<td>8 (40)</td>
</tr>
<tr>
<td>ACE-inhibitors, n (%)</td>
<td>10 (52)</td>
<td>9 (45)</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Values are presented as median and quartiles. There were no significant differences between the groups.

Table 2 Lipids, inflammatory markers, and endothelin-1 at baseline and follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>E10/S10 Baseline</th>
<th>Follow-up</th>
<th>S80 Baseline</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.8 (4.7–5.2)</td>
<td>3.1 (2.9–3.6)</td>
<td>4.8 (4.2–5.6)</td>
<td>3.0 (2.5–3.4)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.1 (2.8–3.4)</td>
<td>1.5 (1.4–1.7)</td>
<td>3.0 (2.5–3.7)</td>
<td>1.3 (1.1–1.8)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.0 (0.9–1.2)</td>
<td>1.0 (0.9–1.2)</td>
<td>0.9 (0.8–1.2)</td>
<td>0.9 (0.8–1.3)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.4 (1.0–2.2)</td>
<td>1.0 (0.7–1.5)</td>
<td>1.8 (1.2–2.6)</td>
<td>1.1 (0.8–1.4)</td>
</tr>
<tr>
<td>ApoA1 (g/L)</td>
<td>1.4 (1.2–1.6)</td>
<td>1.4 (1.2–1.5)</td>
<td>1.4 (1.2–1.6)</td>
<td>1.4 (1.1–1.7)</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.1 (1.0–1.6)</td>
<td>0.6 (0.5–0.8)</td>
<td>1.1 (1.0–1.3)</td>
<td>0.6 (0.5–0.6)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>3.1 (1.6–8.4)</td>
<td>2.8 (1.0–6.9)</td>
<td>2.7 (1.7–4.6)</td>
<td>2.3 (0.9–4.2)</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>5.1 (2.4–11.5)</td>
<td>4.6 (2.0–6.0)</td>
<td>2.9 (1.8–8.8)</td>
<td>3.1 (1.7–6.9)</td>
</tr>
<tr>
<td>ICAM-1 (ng/mL)</td>
<td>338 (299–417)</td>
<td>344 (276–390)</td>
<td>378 (326–448)</td>
<td>378 (316–417)</td>
</tr>
<tr>
<td>Endothelin-1 (pg/mL)</td>
<td>6.0 (5.2–6.7)</td>
<td>5.8 (5.1–6.2)</td>
<td>5.2 (4.7–6.5)</td>
<td>5.5 (5.0–6.8)</td>
</tr>
</tbody>
</table>

Values are median and quartiles. LDL, low-density lipoprotein; HDL, high-density lipoprotein; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; IL-6, interleukin-6; ICAM-1, intercellular adhesion molecule-1.

[12.1% (7.1–14.3) and 10.8% (8.2–14.8) at baseline and 12.9% (7.9–17.3) and 11.6% (6.9–14.5) at follow-up, respectively].

**Forearm plethysmography**

Flow recordings from one patient in the S80 group could not be analysed because of unstable recordings. Thus, 38 patients were available for the final analysis of the plethysmographic recordings. Infusion of BQ123 and BQ788 increased FBF by 20% (−5 to 45) (P < 0.001) in the entire study group at baseline. This response remained at 24% during follow-up. ET-receptor blockade caused a significant increase in EDV at baseline and this effect remained during follow-up (Figure 3). EDV was not altered by ET-receptor blockade either during baseline or during follow-up (not shown). The increase in FBF in response to BQ123 and BQ788 were similar in the two groups at baseline [27% (13–46) and 15% (−8 to 35), respectively] and at follow-up [22% (8–49) and 24% (−1 to 42), respectively]. Furthermore, the two treatment strategies did not differ regarding their effect on the improvement in EDV induced by ET-receptor blockade (P = 0.66) (Figure 3).

**Discussion**

The main finding of this study is that aggressive lipid-lowering using the two treatment strategies, simvastatin 80 mg and simvastatin...
10 mg in combination with ezetimibe 10 mg, did not differ with regard to their effect on endothelial function. This suggests, in contrast to our hypothesis, that lipid-lowering per se rather than the pleiotropic effect of statins is important for the improvement of endothelial function.

The present study compares the effect of two different pharmacological approaches, causing a similar and pronounced reduction in LDL cholesterol, on endothelial function in patients with dysglycaemia and CAD. The study population was chosen as patients with dysglycaemia are at high risk for cardiovascular morbidity and mortality and benefit particularly from aggressive cholesterol reduction with statins.20 The prevalence of type 2 diabetes and IGT is high among patients with CAD,21 and endothelial dysfunction is strongly related to future cardiovascular events in such patients.22 In the present study, improvement in endothelial function with lipid-lowering treatment was observed although baseline cholesterol was just above the threshold for treatment according to international guidelines.23 At the time of follow-up, the cholesterol levels were substantially reduced to levels lower than those previously obtained with aggressive lipid-lowering in clinical studies.24,25 This indicates that an aggressive lipid-lowering is beneficial for vascular function and inflammation also in patients with

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**Figure 2** Absolute changes in flow-mediated endothelium-dependent vasodilatation in the E10/S10 and S80 group from baseline to follow-up. Data are depicted as medians and quartiles; \( n = 18 \) in the E10/S10 group and \( n = 16 \) in the S80 group.

**Figure 3** Effect of intra-arterial infusion of acetylcholine on forearm blood flow (FBF) before and during co-administration of the \( \text{ET}_a \) receptor antagonist BQ123 and the \( \text{ET}_b \) receptor antagonists BQ788 at baseline and at follow-up in the E10/S10 and S80 group. Data are presented as median and quartiles; \( n = 19 \) in all groups. The two groups did not differ with regard to their effect on the improvement in endothelium-dependent vasodilatation induced by endothelin receptor blockade (\( P = 0.66 \)).
seemingly low cholesterol, and that reduction in LDL cholesterol per se, rather than pleiotropic effects of statins, is important for the improvement in endothelial function. This conclusion is supported by the finding that reduction in LDL cholesterol by acute LDL apheresis\(^{26}\) and diet\(^{27}\) also improves endothelial function. Furthermore, equal lowering of LDL cholesterol by a low-dose statin plus ezetimibe or a high-dose statin provided equivalent reduction of C-reactive protein.\(^{28}\) Bulut et al.\(^{29}\) recently showed that switching from atorvastatin given as a daily dose of 40 mg to 10 mg together with ezetimibe 10 mg, induced a significant additional reduction in cholesterol and improved endothelial function in patients with the metabolic syndrome, supporting the notion that lipid-lowering is more important than the potential pleiotropic effects of statins. This assumption is also supported by the outcome of two meta-analyses of clinical trials on lipid-lowering therapy underlining that it is the reduction in LDL cholesterol that reasonably explains the reduction of clinical events and the C-reactive protein levels.\(^{30,31}\) However, the present study is, to the best of our knowledge, the first study comparing high dose simvastatin with the combination of ezetimibe and low dose statin resulting in identical reduction in LDL cholesterol on endothelial function.

The present data may appear to contrast those reported in two recent studies, one on patients with heart failure\(^ {32}\) and another in patients with CAD.\(^{33}\) Both reports suggest that the pleiotropic effects of statins might be of clinical benefit. Thus, Landmesser et al. comparing the effect of simvastatin 10 mg with that of ezetimibe 10 mg as monotherapy on endothelial function in patients with heart failure, demonstrated improved EDV with simvastatin but not with ezetimibe. One important difference between that and the present investigation is that Landmeser et al.\(^{32}\) excluded patients with diabetes. Another difference is the use of ezetimibe as monotherapy, which only resulted in a 15% reduction in LDL in comparison with a 50% reduction when using the clinically approved combination of ezetimibe with simvastatin in the present study. Fichtlscherer et al.\(^{33}\) studied the effect of ezetimibe and simvastatin as monotherapy and in various combinations. The interpretation of their data is hampered by the fact that the groups differed significantly in LDL cholesterol levels at baseline and follow-up. This underlines the importance of well-matched groups regarding baseline cholesterol and treatment effects when addressing the relative impact of LDL reduction and pleiotropic effects of lipid-lowering agents. With well-matched cholesterol levels, as in the present study, the combination of ezetimibe and simvastatin did indeed cause a significant improvement in endothelial function.

ET-1 is a potent vasoconstrictor and pro-inflammatory peptide that is elevated in type 2 diabetes and in patients with high LDL cholesterol.\(^{34–36}\) Previous studies have shown that ET-1 induces impaired EDV,\(^{16}\) and that ET-receptor antagonists improve endothelial function in patients with atherosclerosis.\(^{34,16}\) In the present study, improvements were seen in EDV during ET-receptor blockade at baseline. This extends previous observations to a diabetic population. Previous in vitro studies indicate that lipid-lowering treatment suppresses the expression of ET-1 in endothelial cells,\(^{27}\) thereby attenuating the negative effect of ET-1 on endothelial function. Therefore, the effect of ET-receptor blockade was evaluated before and after lipid-lowering treatment. Both the unchanged vasodilator effect and the effect on EDV following cholesterol-lowering treatment suggest that the vascular effects induced by ET-1 was unaffected by 6 weeks of aggressive cholesterol-lowering therapy. This finding indicates that ET-receptor blockade exerts beneficial effects on endothelial function on top of aggressive lipid-lowering therapy.

The lack of effect of lipid-lowering therapy on acetylcholine-induced vasodilator response in forearm resistance vessels is in line with previous statin studies in patients with type 2 diabetes.\(^ {38–40}\) A possible explanation for the differences in outcome when using FMD and venous occlusion plethysmography may be that FMD detects endothelial function in conduit arteries, whereas venous occlusion plethysmography reflects resistance vessel function. Accordingly, these two methods are not significantly related to each other.\(^{41}\) Importantly, EDV obtained with both methods are associated with cardiovascular events in patients with CAD, and correlate with endothelial function in coronary arteries\(^ {22}\) as well as Framingham risk score.\(^ {41}\)

**Limitations**

In order to achieve comparable cholesterol-lowering in the two study groups it is necessary to combine ezetimibe with a low-dose of simvastatin instead of using ezetimibe as monotherapy. This may be a limitation as the dose–response relation for the potential pleiotropic effect of simvastatin remains uncertain. On the other hand, the present combination therapy corresponds to what is approved for clinical practice. If pleiotropic mechanisms are important, it is surprising that the high-dose simvastatin did not improve FMD. It is, however, not possible to exclude that the high-dose of simvastatin would result in a significant improvement in FMD in a larger study group. On the other hand, there are indications that statins exert biphasic dose-related effects, which has been reported regarding statin-mediated effects on endothelial progenitor cells and angiogenesis.\(^ {42,43}\) It cannot be excluded that 6 weeks of treatment is too short to affect the vascular effects of ET-1. Besides, being a vasoconstrictor, ET-1 is a potent mitogen and such effects may take longer to appear than vasoconstrictor effects.

**Clinical implications**

There are numerous studies supporting the notion that ‘the lower, the better’ with regard to cholesterol levels.\(^ {3,25}\) To achieve aggressive reduction in LDL cholesterol, higher doses of statins are required which may be problematic, as side effects of statins tend to be dose-related.\(^ {44}\) To combine a lower dose of a statin with ezetimibe in order to achieve the same degree of lipid-lowering might therefore be an attractive alternative considering the beneficial effects on endothelial function and pro-inflammatory parameters demonstrated in the present study.

**Conclusion**

Aggressive cholesterol-lowering improved endothelial function and reduced inflammatory markers in patients with dysglycaemia...
and CAD. The effect on endothelial function and inflammatory markers did not differ between the two treatment strategies, simvastatin 80 mg and simvastatin 10 mg in combination with ezetimibe 10 mg, which suggests that cholesterol-lowering rather than a statin-mediated pleiotropic effect is important. This makes the long-term prognostic effect of the combination of ezetimibe and statin of considerable interest to explore in patients with CAD and dysglycaemia.

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Conflict of interest: none declared.

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References


