Coronary Heart Disease

Arginase Inhibition Improves Endothelial Function in Patients With Coronary Artery Disease and Type 2 Diabetes Mellitus

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Background—Endothelial dysfunction plays an important role in the early development of atherosclerosis and vascular complications in type 2 diabetes mellitus. Increased expression and activity of arginase, metabolizing the nitric oxide substrate L-arginine, may result in reduced production of nitric oxide and thereby endothelial dysfunction. We hypothesized that inhibition of arginase activity improves endothelial function in patients with coronary artery disease (CAD) and type 2 diabetes mellitus.

Methods and Results—Three groups of subjects were included: 16 patients with CAD, 16 patients with CAD and type 2 diabetes mellitus (CAD+Diabetes), and 16 age-matched healthy control subjects. Forearm endothelium-dependent and endothelium-independent vasodilatation were assessed with venous occlusion plethysmography before and during intra-arterial infusion of the arginase inhibitor \(N^\text{G}\)-hydroxy-nor-L-arginine (nor-NOHA; 0.1 mg/min). Nor-NOHA was also coinfused with the nitric oxide synthase inhibitor \(N^\text{G}\)-monomethyl L-arginine. The expression of arginase was determined in the internal mammary artery of patients undergoing bypass surgery. Nor-NOHA markedly increased endothelium-dependent vasodilatation (up to 2-fold) in patients with CAD+Diabetes and CAD (\(P<0.001\)) but not in the control group. \(N^\text{G}\)-monomethyl L-arginine completely inhibited the increase in endothelium-dependent vasodilatation induced by nor-NOHA. Endothelium-independent vasodilatation was slightly improved by nor-NOHA in the CAD+Diabetes group. Arginase I was expressed in vascular smooth muscle cells and endothelial cells, and arginase II was expressed in endothelial cells of patients with and without diabetes mellitus.

Conclusions—Arginase inhibition markedly improves endothelial function in patients with CAD and type 2 diabetes mellitus suggesting that increased arginase activity is a key factor in the development of endothelial dysfunction. (Circulation. 2012; 126:2943-2950.)

Key Words: nitric oxide ■ coronary disease ■ diabetes mellitus type 2 ■ vasodilation ■ endothelial dysfunction

Diabetes mellitus is associated with a considerable risk for cardiovascular complications such as peripheral artery disease, coronary artery disease (CAD), and myocardial infarction.1 Available data indicate that endothelial dysfunction plays an important role in the development of vascular complications in diabetes mellitus2 and that these complications are a major cause of morbidity and mortality in these patients.3 Furthermore, the presence of endothelial dysfunction is an independent risk factor for future cardiovascular events in patients with CAD.4

Clinical Perspective on p 2950

Endothelial dysfunction is characterized by reduced bioavailability of the vasodilator and anti-inflammatory molecule nitric oxide (NO). NO is produced by the endothelial isoform of nitric oxide synthase (eNOS) from the amino acid L-arginine. The mechanism behind impaired endothelial function and reduced bioavailability of NO in patients with type 2 diabetes mellitus and cardiovascular complications is multifactorial and incompletely understood. Factors of importance

Received August 30, 2012; accepted November 2, 2012.

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The online-only Data Supplement is available with this article at http://circ.ahajournals.orglookup/suppl/doi:10.1161/CIRCULATIONAHA.112.140335/-/DC1.

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Circulation is available at http://circ.ahajournals.org

DOI: 10.1161/CIRCULATIONAHA.112.140335
include reduced availability of L-arginine, reduced activity of eNOS, uncoupling of eNOS resulting in superoxide production, and increased inactivation of NO due to accumulation of superoxide radicals. Recently, arginase has emerged as an important regulator of NO bioavailability by competing with eNOS for their common substrate L-arginine. Upregulation of arginase, which metabolizes L-arginine to L-ornithine and urea, may result in reduced NO production by shunting L-arginine from the eNOS pathway to the arginase pathway. Although arginase plays its dominant role in the hepatic urea cycle, it is upregulated in the vasculature of experimental models of atherosclerosis, myocardial ischemia, hypertension, and ageing. Increased arginase expression and activity has also been described in animal models of diabetes and in diabetic erectile tissue in humans, as well. This mechanism may be of importance for reduced NO availability and endothelial dysfunction associated with diabetes mellitus. This is supported by a recent study showing that arginase I is upregulated in coronary arterioles of patients with type 2 diabetes mellitus, contributing to a reduced NO production in vitro. However, whether arginase activity contributes to endothelial dysfunction in patients with CAD and diabetes mellitus in vivo is unknown. Thus, the main objective of the current study was to test the hypothesis that arginase contributes to the occurrence of endothelial dysfunction and that arginase blockade improves endothelial function in patients with CAD and type 2 diabetes mellitus.

Methods

Subjects

The study was conducted on 48 male subjects belonging to 3 different groups: (1) 16 patients with CAD, (2) 16 patients with both CAD and type 2 diabetes mellitus (CAD+Diabetes) and (3) 16 healthy control subjects. Patients were classified as having type 2 diabetes mellitus if fasting blood glucose exceeded 7.0 mmol/L on at least 2 occasions or blood glucose was >11.0 mmol/L 2 hours after an oral glucose load (75 g). CAD was classified as previous myocardial infarction or significant CAD determined from a coronary angiogram. The control subjects were matched for age, were free of medication, had no medical history of any cardiovascular disease, performed a bicycle exercise test that did not reveal any signs of myocardial ischemia, and had fasting blood glucose <6.0 mmol/L. Participants were informed of the nature, purpose, and possible risk involved in the study before giving informed consent. The investigation was conducted in accordance with the Declaration of Helsinki and was approved by the regional ethics committee.

Blood Flow Measurements

Investigations were performed with the subjects in the supine position in a quiet laboratory with controlled temperature. Subjects arrived at the laboratory at 8 a.m. They were allowed to have a light breakfast on the day of experiment, but they were instructed not to use caffeine or nicotine-containing products. A percutaneous catheter was inserted under local anesthesia in the proximal direction into the brachial artery of the nondominant arm for infusions. Forearm blood flow (FFB) was measured simultaneously in both arms by venous occlusion plethysmography, using a mercury-in-silastic strain gauge applied around the widest part of the forearm. A cuff placed around the upper arm was inflated to 50 mm Hg for 10 seconds to obstruct the venous outflow for recording of FBF. The circulation of the hands was excluded by inflation of a wrist cuff to 30 mm Hg above systolic blood pressure.

Study Protocol

Baseline FBF was determined during a continuous intra-arterial infusion of saline (0.9%) at a rate of 1 mL/min. Baseline endothelium-dependent (EDV) and endothelium-independent vasodilation was determined by intra-arterial infusions of serotonin (21, 70, and 210 ng/min) and sodium nitroprusside (SNP; 1, 3, and 10 μg/min), respectively. Each dose was given for 2 minutes at a rate of 2.5 mL/min with a 2-minute washout period between each dose. Serotonin has previously been demonstrated to evoke EDV and NO-dependent vasodilation in the human forearm of healthy subjects, patients with hypercholesterolemia, and those with type 2 diabetes mellitus. Thereafter an intra-arterial infusion of the arginase inhibitor Nω-hydroxy-nor-L-arginine (nor-NOHA) was started and maintained for 120 minutes at a dose of 0.1 mg/min (1 mL/min). This dose was calculated to result in a local plasma concentration of 30 μmol/L based on an estimated resting plasma flow of 20 mL/min. Nor-NOHA is an analog of an intermediate in the conversion from L-arginine to NO that is a potent inhibitor of arginase with Ki values for arginase I and II of 500 and 50 nmol/L, respectively. At the presently used concentration, nor-NOHA is highly specific and does not inhibit eNOS activity. EDV and endothelium-independent vasodilation was reassessed at 60 and 120 minutes of nor-NOHA infusion.

Five of the patients with CAD and type 2 diabetes mellitus also participated in a separate protocol including 100 minutes intra-arterial infusion of nor-NOHA (0.1 mg/min; 1 mL/min), followed by coinfusion with the NOS inhibitor Nω-monomethyl L-arginine (L-NMMA) (2 mg/min; 1 mL/min) for 20 minutes. Endothelium-dependent and -independent vasodilation was assessed at baseline and at the end of the protocol. The further characterize the involvement of NO in the vasodilator response to serotonin in patients with type 2 diabetes mellitus and CAD, a separate group of 10 patients were investigated. These patients were 69 ± 3 years of age, had a body mass index of 28 ± 2 kg/m², had a previous myocardial infarction (n = 7), had undergone coronary artery bypass grafting surgery (n = 6) or percutaneous coronary intervention (n = 3) and had type 2 diabetes mellitus according to the criteria above. Serotonin was infused as described above before and following a 20 minutes intra-arterial infusion of the NOS inhibitor L-NMMA (2 mg/min). The vasodilator response to serotonin was markedly inhibited in the presence of L-NMMA (online-only Data Supplement Figure I).

Blood Sampling

Screening blood sampling included fasting blood glucose, HbA1c, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides.

Substances

Nor-NOHA (Bachem, Bubendorf, Switzerland) and serotonin (Sigma-Aldrich, Schnelldorf, Germany) were dissolved in double-distilled water, sterile filtered through a Millipore filter, tested for bacterial toxins and sterility, and stored frozen at −80°C. All substances, including SNP (Abbot, Chicago) and L-NMMA (Cilag, Laufenlingen, Switzerland) were diluted to the proper concentrations in 0.9% NaCl on the day of the experiment.

Immunohistochemistry

Samples of internal mammary artery specimens were harvested from 6 patients (3 with and 3 without type 2 diabetes mellitus) undergoing coronary artery bypass grafting surgery owing to stable CAD. The distal end of the artery was cut and snap frozen on dry ice. The samples were sectioned (10 μm), fixed by using acetone, and stained by using alkaline phosphatase MACH 3 technology (Biocare Medical). The sections were incubated with primary antibodies against arginase I (1:50 dilution, Atlas Antibodies, Stockholm, Sweden), arginase II (1:50 dilution, Atlas Antibodies), α-actin (1:600, Dako, Glostrup, Denmark), and von Willebrand factor (1:4000, Dako) for 1
hour in room temperature and counterstained with hematoxylin. The specificity of the primary antibodies was tested by preadsorption of the antibodies with arginase proteins and the peptides used to produce the antibodies (10:1) for 2 hours. Preadsorption with arginase I antigen prevented the staining induced by the arginase I antibody but did not affect the staining induced by the arginase II antibody. Conversely, preadsorption with the arginase II antigen prevented the staining induced by the arginase II but did not affect the staining induced by the arginase I antibody.

Calculations and Statistics
Data are presented as means±SEM. FBF was calculated as the mean of 4 to 8 inflow recordings during 2 minutes. During the vasodilator response to serotonin and SNP, the 4 highest flow recordings at the end of the infusion were used for calculations. Because no infusions affected blood pressure or contralateral FBF, all hemodynamic effects mediated by serotonin and SNP are expressed as absolute blood flow changes from baseline blood flow during infusion of saline. Differences in FBF change in response to different doses of serotonin and SNP between saline and in the presence of arginase inhibition were assessed by 2-way analysis of variance. Changes in baseline flows were calculated both as absolute blood flow changes in the infusion arm and the percentage of changes in the ratio between flows in the infused and noninfused arms. The latter was calculated as blood flow in the infused arm divided by flow in the noninfused control arm at the start of the experiment (set as 100%). Any change in this ratio during the experiment was calculated as the percentage of change of the baseline ratio. Changes in baseline flow were assessed by 1-way repeated-measures analysis of variance followed by the Dunnett multiple comparison test. Differences in basal flow between the groups were assessed by 2-way analysis of variance with the Bonferroni multiple comparison test. A value of P<0.05 was considered significant.

Results

Study Subjects
Basal characteristics of study subjects are presented in Tables 1 and 2. Blood pressure did not differ between the groups at baseline and was not changed during the study protocol. Subjects within all 3 groups were overweight, but the control subjects had a lower waist-hip ratio. Fasting glucose and HbA1c levels were significantly higher in the diabetic group. Control subjects had significantly higher total cholesterol and low-density lipoprotein cholesterol levels. Ongoing medication and degree of coronary disease for patients included in the CAD and CAD+Diabetes groups are presented in Table 2. None of the subjects in the control group were on any medication.

Baseline FBF
Baseline FBF before the start of nor-NOHA infusion did not differ significantly between the groups (Table 3). Changes in FBF during the infusion of nor-NOHA, presented as absolute blood flow in the infusion arm and the percentage of change in blood flow from baseline with a correction for blood flow changes in control arm, are summarized in Table 3. There was no significant change in basal FBF in any of the groups during the course of the experiment (Table 3).

Endothelium-dependent and -independent Vasodilatation
Basal (ie, during infusion of saline) serotonin-induced increase in FBF was significantly lower in both the CAD and the CAD+Diabetes groups than in the control group (online-only Data Supplement Figure II). Furthermore, basal EDV was lower in the CAD+Diabetes group than in the CAD group (P<0.01). In addition, the vasodilator response to SNP at baseline was lower in the CAD+Diabetes group (P<0.01) and the CAD group (P<0.05) than in the control group. Infusion of nor-NOHA induced a significant increase in

Table 1. Basal Study Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n=16)</th>
<th>CAD Group (n=16)</th>
<th>CAD+Diabetes Group (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>61±1</td>
<td>62±2</td>
<td>64±2</td>
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<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>125±2</td>
<td>133±5</td>
<td>136±4</td>
</tr>
<tr>
<td>Diastolic</td>
<td>78±2</td>
<td>78±2</td>
<td>75±3</td>
</tr>
<tr>
<td>MAP</td>
<td>94±2</td>
<td>96±3</td>
<td>96±3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26±1</td>
<td>30±3</td>
<td>29±1</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.94±0.01</td>
<td>0.97±0.01</td>
<td>0.99±0.01**</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.2±0.1</td>
<td>5.4±0.2</td>
<td>8.1±0.6***</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>38±1</td>
<td>38±1</td>
<td>52±2***,<strong>,</strong>,<strong>,</strong></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.1±0.2</td>
<td>1.2±0.2</td>
<td>2.1±0.4*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.6±0.2</td>
<td>4.1±0.2**</td>
<td>3.8±0.2**</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>3.7±0.2</td>
<td>2.4±0.1***</td>
<td>2.0±0.2**</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.4±0.1</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>79±6</td>
<td>82±4</td>
<td>92±13</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>1.1±0.2</td>
<td>1.5±0.4</td>
<td>2.0±0.4</td>
</tr>
</tbody>
</table>

Data are mean±SEM. Significant differences by 1-way analysis of variance with Bonferroni multiple comparison test are shown: *P<0.05, **P<0.01, ***P<0.001 versus the control group and ###P<0.001 vs the CAD group. BMI indicates body-mass index; CAD, coronary artery disease; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; HbA1c, glycosylated hemoglobin; LDL, low-density lipoprotein; and MAP, mean arterial pressure.

Table 2. Study Subject Characteristics: Medication and Degree of Coronary Stenosis

<table>
<thead>
<tr>
<th></th>
<th>CAD Group (n=16)</th>
<th>CAD+Diabetes Group (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE-inhibitors or ARB</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>β-blockers</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Antiplatelet drugs</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Lipid-lowering drugs</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Diuretics</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Nitrates</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Antiarrhythmic drugs</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Insulin</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Biguanides/sulfonylureas</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Degree of coronary disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-vessel</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>2-vessel</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>3-vessel</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

Data are number of patients. ACE indicates angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; and CAD, coronary artery disease.
serotonin-induced vasodilatation in the CAD+Diabetes group. The increase was apparent after 60 minutes of nor-NOHA infusion (data not shown). After 2 hours infusion of nor-NOHA, a 2-fold increase in the vasodilator response to serotonin was observed ($P<0.0001$; Figure 1A). Nor-NOHA also enhanced serotonin-induced vasodilatation in the CAD group at 2 hours ($P<0.001$; Figure 1C). The increase in serotonin-induced vasodilatation in response to nor-NOHA was significantly greater in the CAD+Diabetes group than in the CAD group at 2 hours ($P<0.05$; Figure 2). In addition, the vasodilator response to SNP was slightly, but significantly enhanced by nor-NOHA in the CAD+Diabetes group ($P<0.001$; Figure 1B), but not in the CAD group (Figure 1D). Nor-NOHA did not affect the vasodilator response to serotonin or SNP in the control group (Figure 1E and 1F).

### Table 3. Forearm Blood Flow During Infusion of Saline and 2 Hours of nor-NOHA Infusion

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>CAD Group</th>
<th>CAD+Diabetes Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBF (mL/min/1000 mL)</td>
<td>% Baseline</td>
<td>FBF (mL/min/1000 mL)</td>
<td>% Baseline</td>
</tr>
<tr>
<td>Baseline (NaCl)</td>
<td>30.6±2.1 100±9</td>
<td>31.1±2.2 100±5</td>
<td>38.6±3.4 100±8</td>
</tr>
<tr>
<td>Nor-NOHA 60 min</td>
<td>26.7±2.0 106±9</td>
<td>30.7±2.4 100±6</td>
<td>37.1±2.9 92±5</td>
</tr>
<tr>
<td>Nor-NOHA 120 min</td>
<td>25.8±2.2 87±6</td>
<td>28.1±2.3 86±8</td>
<td>33.2±3.1 87±5</td>
</tr>
</tbody>
</table>

Forearm blood flow (FBF) in the infusion arm is expressed in absolute flow in the experimental arm and in percentage of baseline flow after correction for blood flow in the control arm. Data are mean±SEM. No significant changes from baseline were observed in any group (1-way repeated measures analysis of variance followed by the Dunnett multiple comparison test). CAD indicates coronary artery disease; Nor-NOHA, $N^\omega$-hydroxy-nor-L-arginine.

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**Figure 1.** Change in endothelium-dependent vasodilatation (EDV; A, C, and E) and in endothelium-independent vasodilatation (EIDV; B, D, and F) during NaCl and after 2 hours of nor-NOHA infusion in patients with coronary artery disease (CAD) and type 2 diabetes mellitus (CAD+Diabetes group; A and B; n=16), patients with CAD only (C and D; n=16) and control subjects (Control group; E and F; n=16). Significant differences with 2-way ANOVA with repeated measures are shown. Nor-NOHA indicates $N^\omega$-hydroxy-nor-L-arginine; ANOVA, analysis of variance; and SNP, sodium nitroprusside.
L-nor-L-arginine; ANOVA, analysis of variance; and L-NMMA, L-NMMA, L-N-monomethyl arginine.

Expression of Arginase

The expression of arginase in arteries from patients with CAD was visualized by immunohistochemical detection in mammary arteries of patients with stable CAD. Arginase I was clearly expressed in vascular smooth muscle cells of the intima and in endothelial cells (Figure 4). Although less pronounced, arginase II was also expressed in endothelial cells (Figure 4). A similar expression pattern was observed in arteries obtained from patients with and without diabetes mellitus.

Discussion

The main finding of the present study is that EDV is markedly improved following local administration of the arginase inhibitor nor-NOHA in patients with CAD with and without type 2 diabetes mellitus and that this effect depends on the production of NO. The beneficial effect was particularly pronounced in patients with type 2 diabetes mellitus. These observations suggest that upregulation of arginase activity is a key mechanism behind endothelial dysfunction among these patients and that inhibition of arginase results in improved endothelial function.

Recent experimental data suggest that arginase expression and activity is of importance for the development of vascular dysfunction in various cardiovascular disorders and in diabetes mellitus. Arginase activity is increased and contributes to endothelial dysfunction in the aortas of atherosclerotic mice.6 It has been demonstrated that arginase I is of importance for vascular dysfunction in a rat model of type 1 diabetes11,23 and that the arginase II isoform contributes to renal injury in mice with type 1 diabetes.24 In the type 2 diabetic Goto-Kakizaki rat model, arginase II was upregulated in aorta and myocardium.10 Arginase inhibition restored microvascular function by a mechanism related to increased use of arginine by NOS and increased NO availability.10 Recently, it was demonstrated that selective upregulation of endothelial cell arginase II induces endothelial dysfunction and promotes atherosclerosis.25 These observations clearly suggest an important role of arginase in the regulation of NO bioavailability and endothelial function in experimental models of atherosclerosis and diabetes mellitus. In clinical studies of type 2 diabetic subjects, an increase in plasma arginase activity, and accompanying impairment in NOS activity has been reported.26 Furthermore, increased expression of arginase II was detected in human diabetic corpus cavernosum that may contribute to the diabetes-induced erectile dysfunction.12 Of further importance is the observation that arginase expression is upregulated in coronary arterioles of patients with type 2 diabetes mellitus, contributing to a reduced NO production in vitro.13 However, no previous study has evaluated the functional importance of arginase activity for endothelial function in patients with atherosclerosis and type 2 diabetes mellitus in vivo.

In the current study we demonstrate that patients with CAD with and without diabetes have impaired EDV in comparison with control subjects. Inhibition of arginase with nor-NOHA resulted in increased EDV in both patients groups. Interestingly, patients with both CAD and diabetes mellitus had a significantly greater improvement in EDV following arginase blockade than patients with CAD without diabetes mellitus. These novel observations suggest that arginase activity is of importance for endothelial dysfunction in patients with CAD with and without diabetes mellitus and that the functional
importance is even greater in the presence of diabetes mellitus. The improvement in the vasodilator response to serotonin induced by nor-NOHA was blocked by the NOS inhibitor L-NMMA. Collectively, these observations suggest that the enhanced vasodilator response to serotonin following arginase inhibition is mediated by increased bioavailability of NO.

The observation that the improvement in endothelial function was more pronounced in patients with CAD and diabetes mellitus than in those without diabetes mellitus is of interest. Previous data suggest that arginase inhibition attenuates production of reactive oxygen species in diabetes which is an important mechanism in diabetes-induced endothelial dysfunction. Increased oxidative stress may result in reduced levels of NO because of a direct reaction between NO and reactive oxygen species. Oxidative stress is also associated with uncoupling of eNOS resulting in impaired production of NO and further production of superoxide. These mechanisms may underlie endothelial dysfunction in diabetes mellitus, and are counteracted by arginase blockade via increased availability of L-arginine and increased NO production.

Our data also demonstrate that arginase inhibition leads to enhanced endothelium-independent vasodilatation induced by SNP in patients with CAD and diabetes mellitus, which indicates improvement in other vascular functions besides endothelial function via a mechanism related to arginase activity in this group of patients. This finding is in agreement with our previous study, in which we demonstrated that intra-arterial infusion of endothelin-1 antagonists improved endothelium-independent vasodilatation in patients with insulin resistance, but not in healthy controls. An alternative explanation is that SNP via increased blood flow and shear stress partly induces EDV that is modulated by arginase inhibition.

Our results are in line with a recent in vitro study by Beleznai and coworkers, who demonstrated that arginase blockade enhanced acetylcholine-induced relaxation of isolated coronary arterioles from patients with diabetes mellitus, but did not affect diminished responses in coronary arterioles from patients without diabetes mellitus. Besides obvious differences between data obtained in vitro and in vivo, the lack of effect in the group of patients without diabetes mellitus observed by Beleznai et al may be because of concomitant CAD. Our results are also in accordance with the observation that arginase blockade augments reflex cutaneous vasodilatation in patients with essential hypertension, suggesting that arginase also is of importance for regulation of vascular function in hypertension.

Immunohistochemical analysis of arginase expression revealed clear expression of arginase I in intimal vascular smooth muscle cells and in endothelial cells of mammary arteries of patients undergoing coronary artery bypass grafting. Although less abundant, arginase II was also expressed in endothelial cells. A similar expression pattern was observed in patients with and without diabetes mellitus. This observation suggests that both arginase I and II may be responsible for regulation of endothelial function in arteries of patients with CAD. However, the expression of arginase in the forearm arteries is not known and regional differences in arginase expression may exist between the forearm arteries and the mammary artery. Because it was not possible to obtain arterial biopsies from the forearm, it cannot with certainty be determined which arginase isoform accounts for reduced NO bioavailability in the functional experiments.

There are certain limitations with this study. As expected, there were differences in baseline characteristics between the patient groups and the control that may affect endothelial function. The control group had higher total and low-density lipoprotein cholesterol levels that most likely are due to the fact that the majority of the patients were on lipid-lowering drugs. However, despite the higher cholesterol level, the control group had significantly better basal endothelial function. Other differences between the groups such as higher...
waist-hip ratio, fasting glucose, and HbA1c are expected in a diabetic patient group. Ongoing medication in addition to lipid-lowering drugs such as angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, and antidiabetic drugs in the patient groups may affect baseline endothelial function and be a confounder in a study of the present design. However, baseline EDV was markedly lower in CAD and CAD+Diabetes groups than in the control group despite adequate medication. Any improvement in baseline EDV by these drugs would, if anything, mask any difference in response to arginase inhibition between the groups. Furthermore, it cannot be excluded that ongoing medication interferes with arginase activity in the patient groups, because little is known regarding the interactions with arginase. However, available data indicate that statins\(^{31}\) and insulin\(^{26}\) reduce arginase activity, suggesting that these classes of drugs do not explain the response to arginase inhibition in the present study. During the protocol, a nonsignificant trend toward a decrease in basal blood flow was observed in all groups. This could be explained by the fact that subjects participating in the protocol were resting in a supine position for ~3 hours. Importantly, these changes were of similar magnitude in all groups and are therefore unlikely to have contributed to the differences in response to arginase inhibition between the groups. Furthermore, changes in basal blood flow are not affecting measurements of EDV, because EDV was expressed as a delta change from basal blood flow.

In conclusion, the present study demonstrates that arginase inhibition acutely improves endothelial function in patients with CAD and, in particular, among patients with concomitant type 2 diabetes mellitus. This suggests that upregulation of arginase activity is a key mechanism behind endothelial dysfunction. Arginase may be a promising therapeutic target for the treatment of vascular dysfunction among these patients.

### Sources of Funding
This study was supported by the European Foundation for the Study of Diabetes, Swedish Research Council Medicine (10857), the Swedish Heart and Lung Foundation, the Stockholm County Council (ALF), Karolinska Institutet/Stockholm County Council Strategic Cardiovascular Programme, King Gustav V and Queen Victoria Foundation, and Novo Nordisk Foundation.

### Disclosures
None.

### References

**CLINICAL PERSPECTIVE**

Vascular complications associated with type 2 diabetes mellitus are a widespread clinical problem. The mechanism behind these complications is far from understood, and specific treatment is lacking. Endothelial dysfunction with reduced bioavailability of nitric oxide has been suggested to be of importance for development of atherosclerosis and diabetic vascular complications. Emerging evidence suggests that increased activity and the expression of arginase via the metabolism of L-arginine, which is a substrate for both arginase and nitric oxide synthase, may result in reduced levels of arginine available for nitric oxide production and hence endothelial dysfunction. In the current study, we demonstrate that patients with coronary artery disease with and without diabetes mellitus have impaired endothelial function in comparison with age-matched control subjects. Furthermore, we show that inhibition of arginase activity in the forearm of patients with diabetes mellitus and coronary artery disease markedly improves endothelial function. The improvement in endothelial function is more pronounced in patients with coronary artery disease and diabetes mellitus than in those with coronary artery disease alone. The beneficial effect of arginase inhibition is completely dependent on the increased bioavailability of nitric oxide. These novel observations suggest that arginase activity is a key factor for endothelial dysfunction in patients with coronary artery disease, in particular, among those with diabetes mellitus, by regulating the bioavailability of nitric oxide. Our findings suggest that arginase inhibition could be an effective pharmacological intervention to improve the vascular function in these patients.
Arginase Inhibition Improves Endothelial Function in Patients With Coronary Artery Disease and Type 2 Diabetes Mellitus
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Circulation. 2012;126:2943-2950; originally published online November 26, 2012;
doi: 10.1161/CIRCULATIONAHA.112.140335
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/126/25/2943

Data Supplement (unedited) at:
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Supplemental figure 1. Change in endothelium-dependent vasodilatation induced by serotonin under basal conditions (saline) and following blockade of NOS with L-NMMA in patients with coronary artery disease and type 2 diabetes (n=10). Significant difference with 2-way ANOVA with repeated measures is shown.
Supplemental figure 2. Basal endothelium-dependent vasodilatation (EDV) in patients with coronary artery disease and type 2 diabetes (CAD+Diabetes group; n=16), patients with CAD only (n=16) and control subjects (Control group; n=16). Significant differences with 2-way ANOVA and Bonferroni's multiple comparison test are shown.