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

Running title: Shemyakin et al.; Arginase inhibition improves endothelial function

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Abstract:

**Background**—Endothelial dysfunction plays an important role in the early development of atherosclerosis and vascular complications in type 2 diabetes. Increased expression and activity of arginase, metabolizing the nitric oxide (NO) substrate L-arginine, may result in reduced production of NO 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.

**Methods and Results**—Three groups of subjects were included: 16 patients with CAD, 16 patients with CAD and type 2 diabetes (CAD+Diabetes) and 16 age-matched healthy control subjects. Forearm endothelium-dependent (EDV) and endothelium-independent (EIDV) vasodilatation were assessed with venous occlusion plethysmography before and during i.a. infusion of the arginase inhibitor N^α^-hydroxy-nor-L-arginine (nor-NOHA; 0.1 mg/min). Nor-NOHA was also co-infused with the NO synthase inhibitor (L-NMMA). The expression of arginase was determined in internal mammary artery of patients undergoing bypass surgery. Nor-NOHA markedly increased EDV (up to 2-fold) in patients with CAD+Diabetes and CAD (P < 0.001) but not in the control group. L-NMMA completely inhibited the increase in EDV induced by nor-NOHA. EIDV 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.

**Conclusions**—Arginase inhibition markedly improves endothelial function in patients with CAD and type 2 diabetes suggesting that increased arginase activity is a key factor in the development of endothelial dysfunction.

**Key words:** coronary disease; endothelial dysfunction; nitric oxide; type 2 diabetes mellitus; vasodilation
Diabetes mellitus is associated with a considerable risk for cardiovascular complications such as peripheral artery disease, coronary artery disease (CAD) and myocardial infarction.\textsuperscript{1} Available data indicate that endothelial dysfunction plays an important role in the development of vascular complications in diabetes,\textsuperscript{2} and that these complications are a major cause of morbidity and mortality in these patients.\textsuperscript{3} Furthermore, presence of endothelial dysfunction is an independent risk factor for future cardiovascular events in patients with coronary artery disease.\textsuperscript{4}

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 and cardiovascular complications is multifactorial and incompletely understood. Factors of importance 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.\textsuperscript{5} 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,\textsuperscript{6} myocardial ischemia,\textsuperscript{7} hypertension\textsuperscript{8} and ageing.\textsuperscript{9} Increased arginase expression and activity has also been described in animal models of type 2 diabetes\textsuperscript{10,11} as well as in diabetic erectile tissue in humans.\textsuperscript{12} This mechanism may be of importance for reduced NO availability and endothelial dysfunction associated with diabetes. This is supported by a recent study showing that arginase I is upregulated in coronary arterioles.
of patients with type 2 diabetes, contributing to a reduced NO production in vitro.\textsuperscript{13} However, whether arginase activity contributes to endothelial dysfunction in patients with CAD and diabetes 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.

\textbf{Methods}

\textbf{Subjects}

The study was conducted on 48 male subjects belonging to three different groups: (1) 16 patients with CAD, (2) 16 patients with both CAD and type 2 diabetes (CAD+Diabetes) and (3) 16 healthy control subjects. Patients were classified as having type 2 diabetes if fasting blood glucose exceeded 7.0 mmol/L on at least two occasions or blood glucose was higher than 11.0 mmol/L 2 h after an oral glucose loading (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 less than 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 carried out in accordance with the Declaration of Helsinki and was approved by the regional ethics committee.

\textbf{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 am. They were allowed to have a
light breakfast on the day of experiment, but 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 non-dominant arm for infusions. Forearm blood flow (FBF) 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 mmHg for 10 sec to obstruct the venous outflow for recording of FBF. The circulation of the hands was excluded by inflation of a wrist cuff to 30 mmHg above systolic blood pressure.

**Study protocol**

Baseline FBF was determined during a continuous i.a. infusion of saline (0.9%) at a rate of 1 mL/min. Baseline endothelium-dependent (EDV) and endothelium-independent vasodilatation 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 min at a rate of 2.5 mL/min with a 2 min washout period between each dose. Serotonin has previously been demonstrated to evoke EDV and NO-dependent vasodilatation in the human forearm of healthy subjects, patients with hypercholesterolemia and type 2 diabetes. Thereafter an i.a. infusion of the arginase inhibitor N^6^-hydroxy-nor-L-arginine (nor-NOHA) was started and maintained for 120 min at a dose of 0.1 mg/min (1 mL/min). This dose was calculated to result in a local plasma concentration of 30 μM based on an estimated resting plasma flow of 20 mL/min. Nor-NOHA is an analogue 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 nM, respectively. At the presently used concentration nor-NOHA is highly specific and does not inhibit eNOS activity. EDV and endothelium-independent vasodilatation was reassessed at 60 and 120 min.
of nor-NOHA infusion.

Five of the patients with CAD and type 2 diabetes also participated in a separate protocol including 100 min i.a. infusion of nor-NOHA (0.1 mg/min; 1 mL/min), followed by co-infusion with the NOS inhibitor L-NMMA (2 mg/min; 1 mL/min) for 20 min. Endothelium-dependent and -independent vasodilatation 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 and CAD, a separate group of 10 patients were investigated. These patients were 69 ± 3 years old, had a BMI of $28 \pm 2$ kg/m$^2$, had a previous myocardial infarction (n=7), had undergone CABG (n=6) and/or percutaneous coronary intervention (n=3) and had type 2 diabetes according to the criteria above. Serotonin was infused as described above before and following a 20 min i.a. infusion of the NOS inhibitor L-NMMA (2 mg/min). The vasodilator response to serotonin was markedly inhibited in the presence of L-NMMA (Supplemental Fig. 1).

**Blood sampling**

Screening blood sampling included fasting blood glucose, HbA1c, total cholesterol, LDL, HDL cholesterol, and triglycerides.

**Substances**

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

Samples of internal mammary artery specimens were harvested from six patients (three with and three without type 2 diabetes mellitus) undergoing coronary artery bypass grafting surgery due to stable CAD. The distal end of the artery was cut and snap frozen on dry ice. The samples were sectioned (10 μm), fixed using acetone and stained 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 one 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 h. 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 I antibody but did not affect the staining induced by the arginase II antibody.

Calculations and statistics

Data are presented as means ± SEM. FBF was calculated as the mean of 4 to 8 inflow recordings during 2 min. During the vasodilator response to serotonin and SNP the 4 highest flow recordings at the end of the infusion were used for calculations. Since 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 ANOVA. Changes in baseline
flows were calculated both as absolute blood flow changes in the infusion arm and per cent changes in the ratio between flows in the infused and non-infused arms. The latter was calculated as blood flow in the infused arm divided by flow in the non-infused control arm at the start of the experiment (set as 100%). Any change in this ratio during the experiment was calculated as per cent change of the baseline ratio. Changes in baseline flow was assessed by 1-way repeated measures ANOVA followed by Dunnett’s multiple comparison test. Differences in basal flow between the groups were assessed by 2-way ANOVA with Bonferroni’s 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 three 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 LDL 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 percent 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 (i.e. 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 (Supplemental Fig. 2). Furthermore, basal EDV was lower in the CAD+Diabetes group than in the CAD group ($P<0.01$). Also 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 serotonin-induced vasodilatation in the CAD+Diabetes group. The increase was apparent after 60 min of nor-NOHA infusion (data not shown). After 2 h infusion of nor-NOHA, a 2-fold increase in the vasodilator response to serotonin was observed ($P<0.0001$; Fig. 1 A). Nor-NOHA also enhanced serotonin-induced vasodilatation in the CAD group at 2 h ($P<0.001$; Fig. 1 C). 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 h ($P<0.05$; Fig. 2). In addition, the vasodilator response to SNP was slightly, but significantly enhanced by nor-NOHA in the CAD+Diabetes group ($P<0.001$; Fig. 1 B), but not in the CAD group (Fig. 1 D). Nor-NOHA did not affect the vasodilator response to serotonin or SNP in the control group (Fig. 1 E and F).

The NO-dependency of the increase in serotonin-induced vasodilatation mediated by nor-NOHA was tested by co-infusion of nor-NOHA and the eNOS inhibitor L-NMMA in a subgroup (n=5) of the patients with CAD+Diabetes. The improvement in serotonin-induced vasodilatation by nor-NOHA was completely blocked by L-NMMA (Fig. 3).

**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 (Fig. 4). Although less pronounced, arginase II was also expressed in endothelial cells (Fig. 4). A similar expression pattern was observed in arteries obtained from patients with and without diabetes.

**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 and that this effect is dependent on production of NO. The beneficial effect was particularly pronounced in patients with type 2 diabetes. 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. Arginase activity is increased and contributes to endothelial dysfunction in aorta of atherosclerotic mice. It has been demonstrated that arginase I is of importance for vascular dysfunction in a rat model of type 1 diabetes and that the arginase II isoform contributes to renal injury in mice with type 1 diabetes. In the type 2 diabetic Goto-Kakizaki rat model arginase II was upregulated in aorta and myocardium. Arginase inhibition restored microvascular function by a mechanism related to increased utilization of arginine by NOS and increased NO availability. Recently, it was demonstrated that selective upregulation of
endothelial cell arginase II induces endothelial dysfunction and promotes atherosclerosis.\textsuperscript{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. In clinical studies of type 2 diabetic subjects, an increase in plasma arginase activity and accompanying impairment in NOS activity has been reported.\textsuperscript{26} Furthermore, increased expression of arginase II was detected in human diabetic corpus cavernosum that may contribute to the diabetes-induced erectile dysfunction.\textsuperscript{12} Of further importance is the observation that arginase I is upregulated in coronary arterioles of patients with type 2 diabetes, contributing to a reduced NO production \textit{in vitro}.\textsuperscript{13} However, no previous study has evaluated the functional importance of arginase activity for endothelial function in patients with atherosclerosis and type 2 diabetes \textit{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 had a significantly greater improvement in EDV following arginase blockade than patients with CAD without diabetes. These novel observations suggest that arginase activity is of importance for endothelial dysfunction in patients with CAD with and without diabetes and that the functional importance is even greater in the presence of diabetes. 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 than in those without diabetes is of interest. Previous data
suggest that arginase inhibition attenuates production of reactive oxygen species in diabetes\textsuperscript{11} which is an important mechanism in diabetes-induced endothelial dysfunction. Increased oxidative stress may result in reduced levels of NO due to 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\textsuperscript{27, 28} and are counteracted by arginase blockade via increased availability of L-arginine and increased NO production\textsuperscript{11}.

Our data also demonstrate that arginase inhibition leads to enhanced endothelium-independent vasodilatation induced by SNP in patients with CAD and diabetes, 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 i.a. infusion of endothelin-1 antagonists improved endothelium-independent vasodilatation\textsuperscript{29} 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\textsuperscript{13}, who demonstrated that arginase blockade enhanced acetylcholine-induced relaxation of isolated coronary arterioles from patients with diabetes, but did not affect diminished responses in coronary arterioles from patients without diabetes. Besides obvious differences between data obtained in vitro and in vivo, the lack of effect in the group of patients without diabetes observed by Beleznai et al. may be due to the fact that less than 50\% of patients in that group had concomitant CAD. Our results are also in accordance with the observation that arginase blockade augmented reflex cutaneous vasodilatation in patients with essential hypertension suggesting that
arginase also is of importance for regulation of vascular function in hypertension.\textsuperscript{30}

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. Similar expression pattern was observed in patients with and without diabetes. 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. Since it was not possible to obtain arterial biopsies from the forearm, it cannot with certainty be determined which arginase isoform that 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 LDL cholesterol levels that most likely is 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 ACE inhibitors, angiotensin receptor antagonists and anti-diabetic 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 since 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 non-significant trend towards 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 supine position for about 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 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. 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.

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**Conflict of Interest Disclosures:** None.
References:


**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><strong>Age (y)</strong></td>
<td>61 ± 1</td>
<td>62 ± 2</td>
<td>64 ± 2</td>
</tr>
<tr>
<td><strong>Blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<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><strong>BMI (kg/m^2)</strong></td>
<td>26 ± 1</td>
<td>30 ± 3</td>
<td>29 ± 1</td>
</tr>
<tr>
<td><strong>Waist-hip ratio</strong></td>
<td>0.94 ± 0.01</td>
<td>0.97 ± 0.01</td>
<td>0.99 ± 0.01**</td>
</tr>
<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
<td>5.2 ± 0.1</td>
<td>5.4 ± 0.2</td>
<td>8.1 ± 0.6***###</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>38 ± 1</td>
<td>38 ± 1</td>
<td>52 ± 2***###</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/L)</strong></td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>2.1 ± 0.4*</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>5.6 ± 0.2</td>
<td>4.1 ± 0.2***</td>
<td>3.8 ± 0.2***</td>
</tr>
<tr>
<td><strong>LDL (mmol/L)</strong></td>
<td>3.7 ± 0.2</td>
<td>2.4 ± 0.1***</td>
<td>2.0 ± 0.2***</td>
</tr>
<tr>
<td><strong>HDL (mmol/L)</strong></td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1*</td>
</tr>
<tr>
<td><strong>Creatinine (μmol/L)</strong></td>
<td>79 ± 6</td>
<td>82 ± 4</td>
<td>92 ± 13</td>
</tr>
<tr>
<td><strong>hsCRP (mg/L)</strong></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. BMI, body-mass index; CAD, coronary artery disease; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; HbA1c, glycosylated hemoglobin; LDL, low-density lipoprotein; MAP, mean arterial pressure. Significant differences by 1-way ANOVA with Bonferroni’s multiple comparison test are shown:*P<0.05, **P<0.01, ***P<0.001 vs. the control group and ##P<0.01, ###P<0.001 vs. the CAD group.
**Table 2.** Study subject characteristics: medication and degree of coronary stenosis

<table>
<thead>
<tr>
<th>Medication (no.)</th>
<th>CAD group (n=16)</th>
<th>CAD+Diabetes group (n=16)</th>
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<tbody>
<tr>
<td>ACE-inhibitors or ARB</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>beta-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>
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<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 (no.)</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, Angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; CAD, coronary artery disease.

**Table 3.** Forearm blood flow during infusion of saline and 2 h of nor-NOHA infusion.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>CAD group</th>
<th>CAD+Diabetes group</th>
</tr>
</thead>
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<tr>
<td></td>
<td>FBF (mL/min/1000mL)</td>
<td>Percent of baseline</td>
<td>FBF (mL/min/1000mL)</td>
</tr>
<tr>
<td>Baseline (NaCl)</td>
<td>30.6 ± 2.1</td>
<td>100 ± 9</td>
<td>31.1 ± 2.2</td>
</tr>
<tr>
<td>Nor-NOHA 60'</td>
<td>26.7 ± 2.0</td>
<td>106 ± 9</td>
<td>30.7 ± 2.4</td>
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<tr>
<td>Nor-NOHA 120'</td>
<td>25.8 ± 2.2</td>
<td>87 ± 6</td>
<td>28.1 ± 2.3</td>
</tr>
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</table>

Forearm blood flow (FBF) in the infusion arm expressed in absolute flow in the experimental arm and in per cent 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 ANOVA followed by Dunnett’s multiple comparison test).
Figure Legends:

**Figure 1.** Change in endothelium-dependent vasodilatation (EDV; panels A, C and E) and in endothelium-independent vasodilatation (EIDV; panels B, D and F) during NaCl and following 2 h nor-NOHA infusion in patients with coronary artery disease and type 2 diabetes (CAD+Diabetes group; panels A and B; n=16), patients with CAD only (panels C and D; n=16) and control subjects (Control group; panels E and F; n=16). Significant differences with 2-way ANOVA with repeated measures are shown.

**Figure 2.** Difference in the change in endothelium-dependent vasodilatation (EDV) in response to 2 h infusion of nor-NOHA between the group of patients with coronary artery disease and type 2 diabetes (CAD+Diabetes group; n=16) and the group of patients with CAD only (n=16). Significant difference with 2-way ANOVA with repeated measures is shown.

**Figure 3.** Difference in the change in endothelium-dependent vasodilatation (EDV) from baseline to 2 h infusion of nor-NOHA alone and co-infusion of nor-NOHA and L-NMMA in patients with coronary artery disease and type 2 diabetes (n=5). Significant difference with 2-way ANOVA with repeated measures is shown.

**Figure 4.** Representative images of the localization of arginase I and II expression in the mammary artery of a patient without diabetes. Black arrows indicate staining in endothelial cells and vascular smooth muscle cells. Vascular smooth muscle cells and endothelial cells are visualized with staining of α-actin and von Willebrand factor, respectively.
Increase in EDV

- CAD + Diabetes group
- CAD group

Serotonin (ng/min)

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$P < 0.05$

$\text{mL/min/1000 mL}$
Difference in EDV change

- **Nor-NOHA**
- **Nor-NOHA + L-NMMA**

Serotonin (ng/min)

mL/min/1000 mL

$P < 0.0001$
Arginase I

Arginase II

α-actin

von Willebrand factor
Sprint Fidelis Lead Fractures in Patients with Cardiac Resynchronization Therapy Devices: Insight from the Resynchronization/Defibrillation for Ambulatory Heart Failure (RAFT) Study

Ratika Parkash, Bernard Thibault, Larry Sterns, John Sapp, Andrew Krahn, Mario Talajic, Marilynn Luce, Elizabeth Yetisir, Patricia Theoret-Patrick, George Wells and Anthony Tang

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