Arginase inhibition restores in vivo coronary microvascular function in type 2 diabetic rats

Julia Grönros,1 Christian Jung,1 Jon O. Lundberg,2 Ruha Cerrato,1 Claes-Göran Östenson,3 and John Pernow4

1Department of Medicine, Division of Cardiology, 2Department of Physiology and Pharmacology, Division of Pharmacology, and 3Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden

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Grönros J, Jung C, Lundberg JO, Cerrato R, Östenson C, Pernow J. Arginase inhibition restores in vivo coronary microvascular function in type 2 diabetic rats. Am J Physiol Heart Circ Physiol 300: H1174–H1181, 2011. First published February 4, 2011; doi:10.1152/ajpheart.00560.2010.—Nitric oxide (NO) is crucial for maintaining normal endothelial function and vascular integrity. Increased arginase activity in diabetes might compete with NO synthase (NOS) for their common substrate arginine, resulting in diminished production of NO. The aim of this study was to evaluate coronary microvascular function in type 2 diabetic Goto-Kakizaki (GK) rats using in vivo coronary flow velocity reserve (CFVR) and the effect of arginine inhibition to restore vascular function. Different groups of GK and Wistar rats were given vehicle, the arginine inhibitor Nω-hydroxy-nor-1-arginine (nor-NOHA), L-arginine, and the NOS inhibitor Nω-monomethyl-L-arginine (L-NMMA). GK rats had impaired CFVR compared with Wistar rats (1.31 ± 0.09 vs. 1.87 ± 0.05, P < 0.001). CFVR was restored by nor-NOHA treatment compared with vehicle in GK rats (1.71 ± 0.13 vs. 1.23 ± 0.12, P < 0.05) but remained unchanged in Wistar rats (1.88 ± 0.10 vs. 1.79 ± 0.16). The beneficial effect of nor-NOHA in GK rats was abolished after NOS inhibition. CFVR was not affected by arginine compared with vehicle. Arginase II expression was increased in the aorta and myocardium from GK rats compared with Wistar rats. Citrulline-to-ornithine and citrulline-to-arginine ratios measured in plasma increased significantly more in GK rats than in Wistar rats after nor-NOHA treatment, suggesting a shift of arginine utilization from arginase to NOS. In conclusion, coronary artery microvascular function is impaired in the type 2 diabetic GK rat. Treatment with nor-NOHA restores the microvascular function by a mechanism related to increased utilization of arginine by NOS and increased NO availability.

color Doppler echocardiography; coronary flow velocity reserve; Goto-Kakizaki rat

TYPE 2 DIABETES is associated with macrovascular and microvascular dysfunction, which accelerates the development of end-organ damage. The causes of vascular dysfunction are multifactorial and include hyperglycemia, hyperinsulinemia, oxidative stress, and dyslipidemia (10). Several observations suggest that endothelial dysfunction, characterized by reduced bioavailability of nitric oxide (NO), plays an important role in the pathogenesis of vascular complications in diabetes (6). NO is produced by NO synthase (NOS), which converts arginine to citrulline and NO. Arginine is also a substrate for arginase, which converts arginine to ornithine and urea. Arginase may therefore interfere with the NO-producing pathway by competing for arginine with NOS (7, 31). We (15) have recently demonstrated that arginase inhibition increases NO availability and protects from myocardial ischemia-reperfusion injury in rats via an NO-dependent mechanism. In addition, there are observations suggesting that arginase is upregulated in diabetes. Arginase activity is increased in patients with type 2 diabetes (16). Furthermore, arginase activity has been demonstrated to be increased in the aorta of streptozotocin-induced diabetic rats (26), and it has been suggested that upregulation of arginase contributes to reduced bioavailability of NO in vitro.

Patients with diabetes have impaired coronary microvascular function that is also evident in the absence of obstructive coronary atherosclerosis (20). It is of importance that impaired coronary microvascular function is associated with an unfavorable prognosis (19). Coronary microvascular function can be evaluated in vivo by the determination of coronary flow velocity reserve (CFVR), which is a noninvasive ultrasound-based technique using color Doppler guidance. CFVR has been shown to correlate with coronary microvascular dysfunction (22) and coronary artery atherosclerosis in both clinical (12, 13, 29) and experimental (28, 32) studies. The present study was based on the hypothesis that the coronary microvascular dysfunction in type 2 diabetes is due to increased arginase activity resulting in reduced bioavailability of NO. Consequently, the aim was to explore whether an arginase inhibitor reverses coronary microvascular dysfunction via a mechanism resulting in increased bioavailability of NO in a rat model of type 2 diabetes in vivo.

METHODS

Animal Preparations

The present study was approved by the Ethics Committee for Laboratory Animal Experiments of Göteborg University and Karolinska Institute.

Twelve-week-old male GK rats (Stockholm GK colony) and Wistar rats (Charles River Laboratories) were used in the study. GK rats develop moderate hyperglycemia early in life and have impaired insulin secretion and reduced body weight but no ketoacidos (23, 24). These rats do not develop atherosclerosis but have been described to have endothelial dysfunction in the mesenteric artery and aorta when investigated ex vivo (1, 3). All animals were housed at constant temperature (21°C) with 12:12-h light-dark cycles and had free access to tap water and chow diet (Lactamin). During the experiments, rats were anesthetized with isoflurane gas (Abbott Scandinavia, Solna, Sweden) using a breathing mask. Rats were placed on a ventilated and heated bench, and rectal temperature was kept at 37.5–38°C using a thermo-regulating lamp. The used dose of isoflurane was titrated and 2.2–2.5% isoflurane.

Address for reprint requests and other correspondence: J. Grönros, Dept. of Medicine, Div. of Cardiology, N3:06, Karolinska Institute, Karolinska Univ. Hospital, Stockholm 171 76, Sweden (e-mail: julia.gronros@ki.se).
cream. A catheter (0.7 x 19 mm, Becton Dickinson Infusion Therapy, Helsingborg, Sweden) was inserted into the tail vein for basal blood sampling and drug administrations.

**Measurement of CFVR and Cardiac Parameters**

CFVR and cardiac function were measured as previously described in detail in mice (32). Briefly, the heart was visualized in a short-axis view with M-mode recordings for measurements of systolic function and a long-axis view to measure the length of the left ventricle using a 15-MHz linear transducer (15L8) connected to an echocardiographic system (Acuson Sequoia C512, Siemens Medical Solutions, Mountain View, CA). The proximal part of the left coronary artery was visualized in a parasternal long-axis view with color Doppler guidance with a gate size of 1 mm. For measurements of coronary hyperemic flow velocity, adenosine was infused via the tail vein at doses of 60, 100, and 140 mg·kg⁻¹·min⁻¹ for 2–3 min/dose. Each dose was separated by 1 min. The mean velocity was calculated from the delineated diastolic phase of the flow profile at baseline and the hyperemia obtained during the 140 mg·kg⁻¹·min⁻¹ dose using a software analysis program (Image Arena 2.9.1, Tomtec Imaging Systems, Unterschleidheim, Germany). CFVR is expressed as the ratio between the hyperemic blood flow velocity and baseline blood flow velocity. The intra- and interobserver coefficients of variation for CFVR measurements using this method are 9.2% and 10.4%, respectively (33). Cardiac measurements were made in accordance with guidelines from the American Society of Echocardiography (27). Stroke volume and end-diastolic volume were calculated using the cubic formula, and ejection fraction, fraction shortening, and cardiac output were calculated from previously validated formulas (18). Left ventricular mass was calculated according to the area-length formula (4). Wall thickness was calculated as follows: (intraventricular septum wall + left ventricular posterior wall)/2 during diastole in M-mode images. All data were analyzed offline by a person blinded to the strain and treatment of the animals.

**Measurements of Blood Pressure**

For the investigation of hemodynamics, blood pressure and heart rate were measured before and after study drug administration in a separate group of animals (see below). After isoflurane anesthesia, the left carotid artery was cannulated with a polyethylene-50 catheter connected to a pressure transducer for measurements of arterial pressure (equipment was made in house at Astra Zeneca, Möln达尔, Sweden). ECG leads were connected to the limbs for heart rate registrations.

**Experimental Protocols**

The main study consisted of five different protocols (n = 9–10 rats/group).

**Protocol 1.** Protocol 1 was used for the determination of CFVR in Wistar and GK rats without pharmacological interventions.

**Protocol 2.** Protocol 2 was used for the determination of CFVR in Wistar and GK rats given vehicle or the NOS inhibitor Nω-nitro-L-arginine (L-NMMA; 10 mg/kg). CFVR was determined 20 min after the administration of vehicle or NOS blockade in parallel groups.

**Protocol 3.** Protocol 3 was used for the determination of CFVR in Wistar and GK rats treated with the arginase inhibitor Nω-hydroxy-nor-L-arginine (nor-NOHA; 100 mg/kg) or L-arginine (100 mg/kg) and matched vehicle groups. CFVR was determined 15 min after the administration of the drug in parallel groups.

**Protocol 4.** Protocol 4 was used for the determination of CFVR in GK rats treated with nor-NOHA after the administration of L-NMMA or vehicle. L-NMMA/vehicle was given 5 min before nor-NOHA. CFVR was determined 15 min after the administration of nor-NOHA.

**Protocol 5.** Protocol 5 was used for the determination of blood pressure and heart rate at time points corresponding to CFVR in protocols 1–4 after the administration of adenosine, nor-NOHA, and L-NMMA in Wistar and GK rats.

**Drugs and Administration**

Drugs used in the functional experiments were adenosine (ITEM Development, Stockholm, Sweden), nor-NOHA (Bachem, Bubendorf, Switzerland), L-NMMA (Alexis Biochemicals, Lausen, Switzerland), and L-arginine (Sigma-Aldrich Sweden, Stockholm, Sweden). All drugs were dissolved and diluted in 0.9% saline and given as bolus injections.

**Measurement of Plasma Glucose and Insulin**

Basal blood samples were drawn from the tail vein of nonfasting anesthetized animals. The blood was placed in plastic tubes containing EDTA and centrifuged at 4,000 g for 10 min at 4°C, and the plasma was separated and stored at −80°C. Plasma glucose concentrations were analyzed with a blood glucose meter (YSI 2300, VWR, West Chester, PA). Plasma insulin concentrations were determined by radioimmunooassay (Pharmacia Insulin RIA kit, Pharmacia-Upjohn Diagnostics, Uppsala, Sweden) with rat insulin (Novo Nordisk, Bagsvaerd, Denmark) as a standard.

**Measurement of Plasma Nitrite and Amino Acids**

Nitrite was measured using a highly sensitive chemiluminescence method, as previously described in detail (8). The detection of arginine, citrulline, and ornithine was an application of the method of Lindroth and Mopper (17). Samples were diluted 10 times with distilled water, and an aliquot was mixed with an equal amount (10 μl) of an orthophtaldialdehyde-mercaptoethanol reagent by a CMA/200 autoinjector (CMA Microdialysis). After a 60-s reaction at 8°C, 15 μl were injected on a HPLC column (60 x 4 mm Nucleosil 100 C18, 5 μm, Knauer). Elution was achieved with a Na-acetate buffer (0.03 M), methanol [2.5% (vol/vol)], and tetrahydrofuran [2% (vol/ vol)]. A gradient of methanol (0–60%) was established between 4 and 28 min. The column was then regenerated with the initial buffer for 3 min. Detection was performed with a fluorescence detector (CMA/280), with excitation and emission bands of ~350 and 495 nm, respectively. Quantification was done with an integration program (EZ Chrom Chromatography Data system, Scientific Software) comparing peak heights. Samples were analyzed at Pronexus Analytical (Stockholm, Sweden).

**Immunoblot Analysis**

The thoracic aorta and left ventricle of the heart from GK and Wistar rats were collected and frozen at −80°C for subsequent evaluation of the expression of arginase I and II. Frozen samples were homogenized in ice-cold lysis buffer containing 20 mM Tris (pH 7.8), 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1% Triton X-100, 10% (wt/vol) glycerol, 10 mM Naf, 1 mM EDTA, 5 mM Na-pyrophosphate, 0.5 mM Na₃VO₄, 1 mg/ml leupeptin, 0.2 mM PMSF, 1 mg/ml aprotonin, and 1 mM benzamidine. Homogenates were centrifuged at 5,000 g for 20 min at +4°C, and the concentration of protein in the supernatant in each aliquot was determined using a bicinchoninic acid protein assay kit (Thermo Scientific, Pierce Biotechnology, Rockford, IL). Protein extracts (50 μg/lane) were loaded onto a 10% SDS gel and separated by electrophoresis. Extracts from the two groups were loaded on one gel, and the amount of protein was accordingly compared pairwise. Proteins were transferred to nitrocellulose membranes (Hybond-C pure, Amersham Biosciences UK, Little Chalfont, UK), and Ponceau staining was used to confirm the efficiency of transfer and to visualize protein loading. Membranes were incubated overnight at +4°C with antibodies against arginase I (HPA003595, Atlas Antibodies, AlbaNova University Center, Stockholm, Sweden).
and arginase II (HPA000663, Atlas Antibodies) at a concentration of 1:2,000 followed by anti-mouse (BD Biosciences Pharmingen) and anti-goat secondary antibodies (Santa Cruz Biotechnology), respectively. Proteins were visualized by enhanced chemiluminescence with an ECL advance Western blotting detection kit (Amersham Biosciences) and quantified using densitometry and Quantity One 4.5.1 software (Bio-Rad Laboratories, Hercules, CA). Finally, membranes were stripped and immunoblotted for β-actin at a concentration of 1:1,000 (Rockland Immunochemicals, Gilbertsville, PA) as a loading control.

Immunohistochemistry

From additional animals, the heart and aorta were dissected and fixed in formaldehyde before being embedded in paraffin. Serial cross-sections of 5 μm were made using a microtome (HM350, Dreamscience Lab) and placed on Superfrost Ultra plus slides (Thermo Fisher Scientific). Immunohistochemistry was performed in an automated immunostainer (Intellipath, Biocare Medical) using a MACH 3 rabbit HRP Polymer Detection kit from the same manufacturer and run according to their protocol. Primary antibodies for arginase I (HPA024006, Atlas Antibodies) were used in the dilution of 1:1,500 and arginase II (HPA000663, Atlas Antibodies) in the dilution of 1:300.

Statistical Analysis

Data are presented as means ± SE. Student’s t-test was used for comparisons between two groups and one-way ANOVA followed by Bonferroni’s multiple-comparison tests (Prism 4 software, GraphPad, San Diego, CA). *P < 0.05, **P < 0.01, ***P < 0.001. Student’s t-test was used for comparisons between two groups and one-way ANOVA followed by Bonferroni’s multiple-comparison tests (Prism 4 software, GraphPad, San Diego, CA). P values of <0.05 were considered statistically significant.

RESULTS

Impaired Coronary Artery Function in the GK Rat

CFVR was significantly impaired in GK rats compared with Wistar rats included in protocol 1 (1.31 ± 0.09 vs. 1.87 ± 0.05; Fig. 1A), which indicates coronary microvascular artery dysfunction. CFVR was significantly lower after NOS inhibition by l-NMMA compared with vehicle in Wistar rats (Fig. 2A). On the other hand, CFVR was not affected by l-NMMA in GK rats (1.53 ± 0.08 in l-NMMA-treated GK rats and 1.40 ± 0.06 in vehicle-treated GK rats; Fig. 1B).

Arginase Inhibition Improves CFVR in the GK Rat

As observed in protocol 1, CFVR was significantly impaired in GK rats compared with Wistar controls included in protocol 3. The arginase inhibitor nor-NOHA significantly improved CFVR compared with vehicle in GK rats (Fig. 2A). On the other hand, nor-NOHA had no effect on CFVR in Wistar rats. The increase in CFVR in GK rats by nor-NOHA was due to an increase in the hyperemic velocity, whereas the basal flow velocity was similar in all four groups (Fig. 2B).

NOS Inhibition Abolishes the Effect of Nor-NOHA in the GK Rat

To evaluate the mechanism underlying the restored CFVR in GK rats after nor-NOHA treatment, GK rats were given the NOS inhibitor l-NMMA with and without nor-NOHA in protocol 4. GK rats treated with nor-NOHA significantly improved in CFVR compared with vehicle-treated rats, as observed in protocol 3 (1.88 ± 0.08 vs. 1.40 ± 0.06, P < 0.001). l-NMMA did not affect CFVR per se but abolished the positive effect of nor-NOHA treatment on CFVR (P < 0.05; Fig. 3).

Effect of l-Arginine on CFVR

Treatment with l-arginine did not increase CFVR in GK rats compared with vehicle [1.42 ± 0.09 vs. 1.35 ± 0.10, respectively, P = not significant (NS)].

Plasma Glucose and Insulin

Plasma glucose levels were 7.2 ± 0.3 mmol/l in Wistar rats and 12.0 ± 1.1 mmol/l in GK rats (n = 9, P < 0.001). Insulin levels were 35.8 ± 1.7 μU/ml in Wistar rats and 44.8 ± 5.7 μU/ml in GK rats (n = 9; P = NS).

Plasma Nitrite and Amino Acids

Plasma nitrite levels were higher in nor-NOHA-treated than in vehicle-treated Wistar and GK rats, although the difference reached significance in GK rats only (Fig. 4). Plasma citrulline increased and ornithine decreased in both GK and Wistar rats after nor-NOHA administration, whereas arginine levels remained unchanged. As a result of this, the ratio of citrulline to ornithine increased markedly. Interestingly, the citrulline-to-ornithine ratio increased significantly more in GK rats than in Wistar rats treated with nor-NOHA (Fig. 5A). Furthermore, the citrulline-to-arginine ratio increased only in GK rats after nor-NOHA treatment and was higher in GK
rats than in Wistar rats treated with nor-NOHA (Fig. 5B). The ornithine-to-arginine ratio was markedly reduced in both GK and Wistar rats treated with nor-NOHA compared with vehicle (Fig. 5C).

**Immunoblot Analysis**

Arginase II expression was higher in both the aorta and left ventricle of GK rats compared with Wistar rats (Fig. 6, A and B). There was no significant difference in arginase I expression in the aorta (P = 0.05; Fig. 6C) or left ventricle (P = NS; Fig. 6D).

**Immunohistochemistry**

Arginase II was clearly localized in the endothelial cell layer and weak expression was also detected in smooth muscle cells of both the aorta and myocardium of GK and Wistar rats (Fig. 7). No expression of arginase I could be detected using immunohistochemistry.

**Cardiac and Hemodynamic Parameters**

Basal cardiac parameters measured with echocardiography are shown in Table 1. GK rats had lower body weight and smaller hearts. Left ventricular length and mass relative to body weight were higher in GK rats. However, wall thickness was similar in the two strains. No significant differences were found in functional parameters such as ejection fraction or stroke volume. Nor-NOHA and/or L-NMMA did not affect cardiac parameters in Wistar or GK rats (data not shown).

The hemodynamic effects of adenosine and nor-NOHA, alone and together with L-NMMA, were determined in separate experiments. Baseline heart rate, mean arterial blood pressure, and rate-pressure product did not differ between GK and Wistar rats. Adenosine infusion lowered heart rate (−32 ± 10 beats/min in Wistar rats and −79 ± 23 beats/min in GK rats at the 140 μg·kg⁻¹·min⁻¹ dose, P = NS between groups), mean arterial pressure (−17 ± 5 mmHg in Wistar rats and −25 ± 9 mmHg in GK rats, P = NS between groups), and rate-pressure product (−8,995 ± 2,429 mmHg·beats·min⁻¹ in Wistar rats and −19,800 ± 4,958 mmHg·beats·min⁻¹ in GK rats, P = NS between groups). Administration of nor-NOHA did not significantly affect heart rate (−7 ± 6 beats/min in Wistar rats and −28 ± 18 beats/min in GK rats), mean arterial pressure (−6 ± 2 mmHg in Wistar rats and −13 ± 7 mmHg in GK rats), or rate-pressure product (−2,963 ± 1,103 mmHg·beats·min⁻¹ in Wistar rats and −7,056 ± 3,804 mmHg·beats·min⁻¹ in GK rats) in either group. Administration of L-NMMA after nor-NOHA resulted in a significant increase in mean arterial pressure (16 ± 3 mmHg in Wistar rats and 33 ± 7 mmHg in GK rats, P = 0.05 between groups) during the first 6 min after administration, whereas heart rate was not significantly affected (1 ± 5 beats/min in Wistar rats and 14 ± 9 beats/min in GK rats). The increase in rate-pressure product induced by L-NMMA did not differ significantly between the groups (6,155 ± 1,435 mmHg·beats·min⁻¹ in Wistar rats and 12,260 ± 2,498 mmHg·beats·min⁻¹ in GK rats).

**DISCUSSION**

In the present study, we demonstrate in vivo coronary artery microvascular dysfunction in type 2 diabetic GK rats. These rats had increased expression of arginase II, and their microvascular function was normalized after arginase inhibition. The functional effect of arginase inhibition was accompanied by an
increase in citrulline-to-ornithine and citrulline-to-arginine ratios, indicating increased activity of NOS in relation to arginase. Furthermore, the positive effect of nor-NOHA on CFVR was abolished by the NOS inhibitor l-NMMA. Collectively, these observations suggest that blockade of arginase shifts the utilization of arginine from arginase to NOS, resulting in increased NO bioavailability and normalized coronary microvascular function in the GK type 2 diabetic rat.

We detected a decrease in CFVR measured with color Doppler-guided ultrasound in GK rats compared with healthy controls, which suggests an impairment in coronary artery microvascular function. Moreover, NOS inhibition reduced CFVR in Wistar rats but had no effect on the already impaired CFVR in GK rats. These observations indicate that GK rats have coronary artery microvascular dysfunction that is dependent on reduced NO bioavailability. Adenosine-induced coronary hyperemia used to determine CFVR has previously been shown to be attenuated after the inhibition of NOS, suggesting that it is mediated, at least partially, via NO-dependent pathways (2). Data from a clinical study (21) have demonstrated that patients with type 2 diabetes have reduced CFVR. Interestingly, a low value of coronary flow reserve has also been shown to be of prognostic value for future events in patients with diabetes (5). This suggests that the presently used rat model of type 2 diabetes is representative regarding myocardial microvascular dysfunction. It is therefore of importance to establish the mechanism behind coronary microvascular dysfunction and to identify targets for pharmacological interventions to restore vascular function in type 2 diabetes.

The present study shows that the arginase inhibitor acutely improved and normalized CFVR in GK rats. The positive effect of arginase inhibition was abolished by blockade of NOS. This finding clearly suggests that the improved CFVR after arginase blockade seen in the GK rats is due to increased NO bioavailability. The observations also indicate that arginine is shunted toward the NOS pathway instead of the arginase pathway, resulting in increased NO availability and restored coronary artery function in the GK rat after arginase blockade. In support of our functional effects, plasma nitrite levels were increased after nor-NOHA treatment, which indicates increased NO production. To further evaluate the relative activities of arginase and NOS, the levels of amino acid produced from arginine by these enzymes were analyzed. Since arginine and NOS metabolise arginine to ornithine and citrulline, respectively, the ratio between those gives information about the relative enzyme activities (31). Both citrulline-to-ornithine and citrulline-to-arginine ratios increased significantly more in GK rats than in Wistar rats treated with nor-NOHA. These results clearly indicate that the utilization of arginine is shifted from arginase toward NOS after arginase blockade and that this shift was more pronounced in GK rats. This conclusion is in line with functional data supporting the notion that the improvement in coronary artery function after arginase blockade is NO dependent.

The administration of l-arginine did not affect CFVR in GK rats. The finding that arginase blockade but not l-arginine improved CFVR may suggest that the increase in arginase activity rather than lack of arginine per se is of importance for microvascular dysfunction in GK rats. Moreover, previous studies (14, 25) have demonstrated that arginine administered systemically may be effectively metabolised by liver arginase and thereby lose its pharmacological effect. The effect of circulating arginine may also be dependent on a transport protein to reach its intracellular target. It has also been demonstrated that type 1 diabetic rats have both an increase in arginase activity and formation of ROS in coronary arteries. Romero et al. (25) concluded that arginase contributes to NOS uncoupling, resulting in an increased production of oxygen radicals. Since it is known that GK rats have an increased production of superoxide (1), it is possible that reduced production of ROS may have contributed to the beneficial effects of arginase inhibition on microvascular function in the present study.

Expression analyses of arginase revealed that arginase II was significantly enhanced in the aorta and myocardial tissue of GK rats compared with Wistar controls. Immunohistochemical analysis demonstrated the expression of arginase II in endothelial cells of the aorta and coronary arteries. No difference in the expression of arginase I was found between GK and Wistar rats, however. Although the relative importance of the two

Fig. 5. Ratios of plasma amino acid levels in Wistar and GK rats given vehicle or the arginase inhibitor nor-NOHA. A: citrulline-to-ornithine ratio (Cit/Orn). B: citrulline-to-arginine ratio (Cit/Arg). C: ornithine-to-arginine ratio (Orn/Arg). Data are means ± SE; n = 7 rats/group. **P < 0.01; ***P < 0.001.
isoforms for the regulation of coronary vascular function in type 2 diabetes remains to be clarified using isoform-specific inhibitors, the observation that the expression of arginase II was increased may suggest the involvement of this isoform in the functional effects of arginase inhibition observed in the present study.

In the complementary study, hemodynamic parameters were measured after drug administration. Adenosine reduced blood pressure and heart rate in both strains with no significant differences between the groups. Nor-NOHA did not affect hemodynamics in either strain. This suggests that changes in blood pressure or heart rate do not explain the differences in CFVR between GK and Wistar rats or the beneficial effect of nor-NOHA. In terms of cardiac parameters, left ventricular length and mass in relation to body weight were larger in GK rats than in Wistar rats. On the other hand, ejection fraction and fractional shortening were similar in both groups, indicating that CFVR is independent of cardiac systolic function. Inhibition of arginase or NOS did not affect any of the cardiac parameters. These observations suggest that the impaired and treatable CFVR in GK rats is dependent on microvascular dysfunction rather than differences in cardiac function.

The CFVR capacity of the coronary arteries is of prognostic value for future events not only in patients with diabetes (5, 21) and coronary stenosis (13, 29) but also in patients with less severe conditions, such as chest pain (30). Studies (28, 32) in mice and rats using CFVR have shown that coronary artery function is reduced in atherosclerosis, probably depending on both microvascular dysfunction and stenosis related to the disease. In addition, CFVR can be improved by statin treatment in atherosclerotic mice (9) or voluntary exercise in hypertensive rats (11). This highlights the importance of measuring in vivo coronary artery function in preclinical and clinical studies. Importantly, a comparative study performed in mice by Wikström et al. (33) also revealed that the flow velocity measure-
ments used in CFVR correlate well with its volumetric counterpart coronary flow reserve (33), which indicates that flow velocity and actual flow are comparable in this type of setting.

The present study has a few limitations that should be noted. First, the results of the study are limited to the GK model of type 2 diabetic rats. It is not known how other type 2 diabetic rat strains or patients with type 2 diabetes would respond to nor-NOHA treatment. Importantly, myocardial microvascular dysfunction was detected an in vivo setting using CFVR in the GK rat in a similar manner as in patients with type 2 diabetes, indicating that the GK rat is representative in this aspect. Second, arginase activity and NO production cannot be determined selectively in the myocardial microvasculature due to technical reasons. However, arginase II was demonstrated to be present in the coronary artery and aortic endothelium, and Western blot analysis revealed higher expression in the aorta of GK rats. Furthermore, the changed amino acid ratios clearly indicate that the pharmacological effect of nor-NOHA was more pronounced in GK rats than in Wistar rats, suggesting an increased sensitivity to arginase blockade in GK rats. It should also be noted that systemic nitrite levels are a fairly rough marker of overall NOS activity rather than a marker of NO generation in the coronary vasculature. Third, the hemodynamic effects of the drug intervention were determined in separate experiments and not in parallel with the determination of CFVR. This was chosen to keep the CFVR experiments as noninvasive as possible without arterial catheters. Therefore, we cannot relate any systemic hemodynamic change to the change in CFVR in the individual animal. Importantly, however, nor-NOHA, which markedly increased CFVR in GK rats, did not affect systemic hemodynamics.

In conclusion, the present study demonstrates that the GK type 2 diabetic rat has impaired coronary artery microvascular function and an increase in arginase expression. Short-term treatment with an arginase inhibitor normalizes the coronary artery function via a mechanism that is coupled to a shift of arginine utilization from arginase to NOS, resulting in increased bioavailability of NO. Arginase might be an important pharmaceutical target for endothelial microvascular dysfunction in type 2 diabetes.

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Table 1. Cardiac parameters measured with echocardiography

<table>
<thead>
<tr>
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<th>Wistar Rats</th>
<th>Goto-Kakizaki Rats</th>
<th>P Value</th>
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<tr>
<td>Body weight, g</td>
<td>395 ± 6</td>
<td>273 ± 7</td>
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<tr>
<td>LV length, cm</td>
<td>1.37 ± 0.04</td>
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<td>LV mass, mg</td>
<td>567 ± 15</td>
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<td>LV mass/body weight</td>
<td>1.44 ± 0.04</td>
<td>1.80 ± 0.07</td>
<td>&lt;0.001</td>
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<tr>
<td>End-diastolic volume, ml</td>
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<td>0.44 ± 0.04</td>
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<td>Ejection fraction, %</td>
<td>76 ± 1</td>
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<td>Fractional shortening, %</td>
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<td>39 ± 1</td>
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<td>Stroke volume, ml</td>
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<td>Cardiac output, ml/min</td>
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<td>Wall thickness, cm</td>
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<td>Heart rate, beats/min</td>
<td>399 ± 8</td>
<td>388 ± 16</td>
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Data are means ± SE. LV, left ventricular; NS, not significant.
REFERENCES


