Diabetes-induced vascular dysfunction involves arginase I


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Submitted 2 August 2011; accepted in final form 21 October 2011

Romero MJ, Iddings JA, Platt DH, Ali MI, Cederbaum SD, Stepp DW, Caldwell RB, Caldwell RW. Diabetes-induced vascular dysfunction involves arginase I. Am J Physiol Heart Circ Physiol 302: H159–H166, 2012. First published November 4, 2011; doi:10.1152/ajpheart.00774.2011.—Arginase can cause vascular dysfunction by competing with nitric oxide synthase for l-arginine and by increasing cell proliferation and collagen formation, which promote vascular fibrosis/stiffening. We have shown that increased arginase expression/activity contribute to vascular endothelial cell (EC) dysfunction. Here, we examined the roles of the two arginase isoforms, arginase I and II (AI and AII, respectively), in this process. Experiments were performed using streptozotocin-induced diabetic mice: wild-type (WT) mice and knockout mice lacking the AII isoform alone (AII−/−) or in combination with partial deletion of AI (AII−/− AI−/−). EC-dependent vasorelaxation of aortic rings and arterial fibrosis and stiffness were assessed in relation to arginase activity and expression. Diabetes reduced mean EC-dependent vasorelaxation markedly in diabetic WT and AII−/−AII−/− aortas (53% and 44% vs. controls, respectively) compared with a 27% decrease in AII−/−AII−/− vessels. Coronary fibrosis was also increased in diabetic WT and AII−/−AII−/− mice (1.9- and 1.7-fold vs. controls, respectively) but was not altered in AII−/−AII−/− diabetic mice. Carotid stiffness was increased by 142% in WT diabetic mice compared with 51% in AII−/−AII−/− mice and 19% in AII−/−AII−/− mice. In diabetic WT and AII−/−AII−/− mice, aortic arginase activity and AI expression were significantly increased compared with control mice, but neither parameter was altered in AII−/−AII−/− mice. In summary, AII−/−AII−/− mice exhibit better EC-dependent vasodilation and less vascular stiffness and coronary fibrosis compared with diabetic WT and AII−/−AII−/− mice. These data indicate a major involvement of AI in diabetes-induced vascular dysfunction.

IN VASCULAR ENDOTHELIAL CELLS (ECs), nitric oxide (NO) synthase (NOS) uses l-arginine to produce NO, which reduces blood flow and reduces inflammation (21, 22). Reduced availability of l-arginine to NOS and the resultant reduction of NO production have been implicated in the vascular dysfunction associated with diabetes and other cardiovascular disease states. Acute l-arginine supplementation can prevent or reverse EC dysfunction and restore EC-dependent vasodilation in diabetes (1, 31). Reduced availability of l-arginine for NOS can occur via increased activity or expression of arginase, an enzyme that competes with NOS for l-arginine, producing ornithine and urea.

Two isoforms of arginase exist in mammals, arginase I and II (AI and AII, respectively) (8, 23). AI, which is located in the cytoplasm, is expressed most abundantly in liver, whereas AII is a mitochondrial enzyme expressed primarily in the kidney. Both AI and AII have been found in EC populations (11, 34). During diabetes, impaired vascular function is closely associated with oxidative stress and inflammation (12, 28), both of which have been associated with elevated arginase activity and expression (4, 18).

Enhanced arginase activity appears to be involved in conditions characterized by vascular endothelial dysfunction (VED), such as diabetes, pulmonary hypertension, ischemia-reperfusion, and aging (5, 19, 29, 33, 42). Additionally, high chronic l-arginine intake can induce arginase expression/activity, thereby inducing vascular dysfunction (20, 21, 30, 35). Competition between arginase and NOS for their common substrate, arginine, suggests a cause-and-effect relationship in which increased arginase activity/expression decreases arginine bioavailability for NOS. We (33) previously tested this concept in diabetic rats and high-glucose (HG)-treated bovine coronary ECs (BCECs). We showed that diabetes-induced impairment of coronary vasorelaxation to ACh was correlated with increases in ROS, arginase activity, and AI expression in the aorta and liver. Treatment of diabetic coronary arteries with arginase inhibitors reversed the impaired vasodilation to ACh. Treatment of BCECs with HG (25 mM) also increased arginase activity and superoxide levels and diminished NO production in ECs. Additionally, transfection of BCECs with AI small interfering RNA prevented the elevation of arginase activity in HG-treated cells and normalized NO production, suggesting an involvement of AI in the reduced NO production. Our results indicate that increased arginase activity in diabetes contributes to VED by decreasing l-arginine availability to NOS.

In addition to the arginase-induced decreases in NO formation, elevated ornithine production, resulting from increased arginase function, gives rise to an enhanced synthesis of polyamines and proline (16, 47). Increases in polyamines can enhance cell proliferation, whereas proline supports collagen production. These processes can contribute to vascular fibrosis and stiffness, which are characteristics of diabetes-induced vascular pathology (24, 36).

Based on this evidence, we hypothesized that an upregulation of arginase expression and activity is involved in diabetes-induced vascular dysfunction, increased superoxide production, coronary fibrosis, and vascular stiffness. This was tested in streptozotocin (STZ)-diabetic wild-type (WT) mice and arginase knockout mice with either partial deletion of AI and complete deletion of AII (AII−/− AII−/−) or complete deletion of AII alone (AII−/− AII−/−).
METHODS

Animals and Induction of Diabetes

Experiments were performed using C57BL/6J WT mice, A1+/+; All+/− mice, and All+/−/All−− mice. A1 homozygous knockout mice do not survive beyond 2 wk due to hyperammonemia. The C57BL/6J A1+/−/All−− mice developed by Cederbaum et al. (15) and O’Brien et al. (27) were provided by Dr. Stephen Cederbaum and have been backcrossed for at least 10 generations. At 10 wk old, mice were rendered diabetic with STZ (65 mg/kg, ip, every other day for up to 4 injections). Mice were considered diabetic once blood glucose values of >350 mg/dl were achieved. Our study protocol was approved by the Institutional Animal Care and Use Committee of the Georgia Health Sciences University.

Tissue Harvest

After 8 wk of diabetes, mice were anesthetized with ketamine (100 mg/kg, ip) and xylazine (10 mg/kg, ip), and blood samples were obtained via cardiac puncture using heparinized syringes. Plasma was isolated by centrifugation. Tissues were harvested for immediate use (aorta), fixed in formalin (heart), or snap frozen in liquid nitrogen (heart and aorta) and stored at −80°C for later assay. Carotid arteries were initially placed in Krebs buffer at 0°C and then preserved/vitrified stepwise cold exposure to increasing concentrations of propanediol [10–40% (wt/wt) for 30 min each with constant 15% (wt/wt) trehalose] in low ionic strength HEPS buffer at 0°C and stored at −80°C until use (32). At the time of use, stored carotid arteries were allowed to warm to 0°C and exposed to the reverse order of propanediol concentrations used for preservation (30 min each) ending in Krebs buffer.

Arginase Activity

Frozen mouse aortas were pulverized and homogenized on ice in lysis buffer [1:4 (wt/vol), 50 mM Tris-HCl, 0.1 mM EDTA, and 0.1 mM EGTA, pH 7.5] containing protease inhibitors. Homogenates were centrifuged for 10 min at 14,000 g, and the resulting supernatants were used for assay. Arginase activity was measured using a colorimetric determination of urea production from L-arginine, as previously described (13). Briefly, 25 μl of 10 mM MnCl2 were added to 25 μl of tissue homogenate, and samples were heated at 56°C for 10 min to activate arginase; 50 μl of 0.5 M l-arginine were then added to each tube, and samples incubated at 37°C for 1 h to hydrolyze l-arginine. Then, 400 μl of an acid solution mixture (10% H2SO4, 30% H3PO4, and 70% H2O) was added to stop the reaction. Afterward, 25 μl of 9% α-isonitrosopropiophenone were then added to each sample, and samples were heated at 100°C for 45 min. Samples were then placed in the dark at room temperature for 10 min, after which 200 μl of samples were loaded into 96-well plates and absorbance was read at 540 nm in a microplate reader (BioTek Instruments). Results were standardized according to sample protein concentration, as determined by a protein assay (Bio-Rad). Values were corrected for basal urea levels obtained in the absence of L-arginine (controls). Results were standardized according to sample protein concentration. The properties of carotid arteries were examined. After stepwise removal from vitrification solutions, carotid artery segments were cannulated at both ends, and mechanical characteristics were determined in Ca2+-free Krebs solution composed of (in mM) 118.3 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, and 25 NaHCO3 using a pressure myograph. Vessel wall thickness (WTh; in μm) was calculated as follows: WTh = (OD − ID)2/2, where OD and ID are outer and inner carotid artery diameters (in μm), respectively, as measured by video microscopy. Transmural luminal pressure was converted from mmHg to N/m², where 1 mmHg = 1.334 × 102 N/m², for the calculation of circumferential stress (σ). σ, or the amount of hydrostatic force applied over a cross-sectional area of the luminal surface, was calculated as follows: σ = P IL × ID/2WTh, where P IL is transmural luminal pressure. Circumferential strain (ε), or the ratio of the observed change in the luminal diameter compared with the initial luminal diameter, was calculated as follows: ε = ID − ID20/ID20, where ID20 is the internal carotid artery diameter at the lowest luminal pressure (20 mmHg). Each measurement was taken as a hydrostatic sample at increments of 10 mmHg, ranging from 40 to 120 mmHg. Each stress-strain relationship was defined by the following equation: σ = α × εβ, where α is the intercept, β is the “slope” of the exponential fit, and χ is wall strain. The β-coefficient was used as a relative measure of vascular stiffness, or vascular compliance. Each β-coefficient is the average plot of individual stress-strain data obtained from one carotid artery/animal.

Coronary Fibrosis

Hearts were embedded in paraffin blocks after fixation in 10% formalin. Paraffin-embedded sections (5 μm thick) were deparaffinized with xylene and rehydrated by immersion in a graded series of ethanol washes. Sections were stained by Masson’s trichrome (Accustain Kit, Sigma-Aldrich) according to the manufacturer’s protocol. Collagen deposition around the coronary vessels was detected by blue staining. The area of collagen staining relative to the vessel surface area was quantified using ImageJ (NIH). Perivascular fibrosis data are expressed as the collagen-to-vessel surface area ratio.

Hydroxyproline Levels

Hydroxyproline levels in cardiac tissue homogenates were assayed with a commercial ELISA kit for mouse hydroxyproline according to the manufacturer’s instructions (Cederlane Labs). Sample levels were measured in a microplate reader at 450 nm, and the concentration of hydroxyproline in cardiac mouse samples was determined by com-
paring the optical density of the samples to standard curves. Values were standardized according to sample protein concentrations.

Lipid Peroxidation Levels

Lipid peroxidation was assessed in mouse plasma samples using a Lipid Hydroperoxide Assay Kit (Cayman Labs) according to the manufacturer’s instructions. In brief, after chloroform extraction of lipid hydroperoxides from plasma, hydroperoxide levels were measured using redox reactions with ferrous ions and detected at 500 nm in a microplate reader.

Statistical Analysis

Data are given as means ± SE. Statistical analysis was performed by one-way ANOVA with a Tukey post test. In some experiments, statistical differences were determined by a Student’s t-test. Results were considered significant when P values were <0.05.

RESULTS

Arginase Activity and Expression

Arginase activity in the aorta of diabetic WT mice was increased twofold compared with levels in nondiabetic control mice (Fig. 1A). In diabetic AI<sup>+/+</sup> AII<sup>−/−</sup> mice, aortic arginase activity was elevated by 1.61-fold versus nondiabetic controls. In contrast, arginase activity was not altered in AI<sup>+/+</sup> AII<sup>−/−</sup> mice compared with their nondiabetic controls. There were no significant differences among the three control groups.

AI and AII expressions were also assessed (Fig. 1B). AI expression was elevated in diabetic WT mice (6.1-fold) compared with nondiabetic WT mice. AI expression was also elevated in diabetic AI<sup>+/+</sup> AII<sup>−/−</sup> mice (2.2-fold) versus nondiabetic group levels. There were no differences between the diabetic and nondiabetic AI<sup>+/+</sup> AII<sup>−/−</sup> groups. Expression of AI in nondiabetic AI<sup>+/+</sup> AII<sup>−/−</sup> and AI<sup>−/−</sup> AII<sup>−/−</sup> mice was modestly greater than that in nondiabetic WT mice. The basal elevation of AI was likely a compensation for the complete lack of AII in these mice. As expected, AII expression was not evident in aortas from either arginase knockout group (data not shown) and not different between control and diabetic WT groups.

Vascular Function

Vasorelaxation. Endothelium-dependent and -independent vasodilator function was examined in the aorta of mice. In WT mice (Fig. 2A), the endothelium-dependent vasodilator
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ACh produced a maximum relaxation (E\text{max}) of 61.8 ± 3.7% in the control group and 32.1 ± 2.9% in the diabetic group. In AI\textsuperscript{+/−}AII\textsuperscript{−/−} mice (Fig. 2A), the E\text{max} to ACh was 66.2 ± 3.3% in the control group, which was not different than that in WT controls, and the E\text{max} in the diabetic group was 42.2 ± 4.1%, which was similar to that of WT diabetics. In AI\textsuperscript{+/−}AII\textsuperscript{−/−} mice (Fig. 2B), the E\text{max} to ACh in controls was 75.9 ± 3.1%, a value significantly greater than WT controls. The E\text{max} for diabetic mice of this genotype was 54.3 ± 4.3%, a value much greater than that of the diabetic WT group.

We also examined mean vasorelaxation responses of the aorta to ACh over the range of 10\textsuperscript{−9}−10\textsuperscript{−4} M in diabetic mice of each genotype compared with their respective control/ nondiabetic responses (Fig. 2, A and B). When calculated as percentage of their respective control responses over the range of ACh concentrations, the WT group value was 46.8 ± 3.6% and the AI\textsuperscript{+/−}AII\textsuperscript{−/−} value was 56.3 ± 4.1%. However, the AI\textsuperscript{+/−}AII\textsuperscript{−/−} value was significantly better at 72.5 ± 4.8% of control responses (P < 0.05 vs. other genotypes). Thus, the vasorelaxant responses of AI\textsuperscript{+/−}AII\textsuperscript{−/−} mice to ACh were better protected from the effects of diabetes.

Vasorelaxation responses to the endothelium-independent NO donor SNP were not affected by diabetes in any of the genotypes (Fig. 2C).

Vasoconstriction. Contractile responses to PE (10\textsuperscript{−6} M) were approximately twofold greater in aortas of diabetic versus nondiabetic WT mice (Fig. 3). Among nondiabetic mice, aortic contractile responses to PE were 1.35- to 1.4-fold higher in WT mice versus both of the knockout strains, which did not differ in responses. Contractile responses of aortas from diabetic AI\textsuperscript{+/−}AII\textsuperscript{−/−} mice were 1.52-fold greater than those from their nondiabetic controls. However, contractile responses of AI\textsuperscript{+/−}AII\textsuperscript{−/−} aortas were not different between the diabetic and nondiabetic groups.

Carotid Artery Stiffness

Passive mechanical properties of carotid arteries from diabetic and age-matched nondiabetic mice of each genotype were determined after 8 wk of diabetes. Stiffness was determined as the change in circumferential wall strain per unit change in circumferential wall stress as hydrostatic pressure was incrementally increased. The greater the slope of this relationship (β-coefficient), the greater the stiffness and the lesser the compliance. In WT mice, arteries from diabetic mice exhibited a 142% greater β-coefficient value than nondiabetic mice (Fig. 4A). For the AI\textsuperscript{+/−}AII\textsuperscript{−/−} groups, diabetic mice exhibited a 51% greater β-coefficient value than nondiabetic controls, which had a greater β-coefficient value than WT controls (7.4 vs. 5.4; Fig. 4B). In AI\textsuperscript{+/−}AII\textsuperscript{−/−} mice, diabetic mice had only a 19% greater β-coefficient that their control counterparts, which exhibited a β-coefficient similar to AI\textsuperscript{+/−}AII\textsuperscript{−/−} con-
controls (Fig. 4C). Thus, diabetes enhances the stiffness of carotid arteries of WT mice to a greater extent than Al-/-Al-/- or Al+/-Al-/- mice. The latter genotype exhibited the smallest increase in carotid artery stiffness in response to diabetes among the three genotypes.

Coronary Perivascular Fibrosis

Fibrosis, as defined by enhanced levels of tissue collagen, is important in vascular remodeling and negatively affects vascular function. As shown in Fig. 5A, diabetic WT mice exhibited enhanced coronary perivascular fibrosis, as evident by the increased blue staining of collagen around vessels compared with control WT mice. Collagen staining was also elevated in Al+/-Al-/- diabetic mice to a level similar to that observed in diabetic WT mice. However, collagen staining was not altered in diabetic Al+/-Al-/- mice versus their respective controls. Additionally, the ratio of coronary perivasual fibrosis to total vessel surface area increased markedly for diabetic WT mice (1.9-fold) and Al+/-Al-/- mice (1.7-fold), whereas only marginal changes were observed in Al+/-Al-/- knock-out mice (Fig. 5B). These data indicate that arginase activation is associated with increased collagen production and that Al is the predominant isoform contributing to the proline pathway for collagen synthesis.

Hydroxyproline Levels

Levels of hydroxyproline are indicative of tissue collagen content. In diabetic WT and Al+/-Al-/- mice, myocardial levels of hydroxyproline were elevated similarly (by 1.66- and 1.71-fold) compared with their respective controls (Fig. 6). There were no differences in cardiac hydroxyproline levels between diabetic and nondiabetic Al+/-Al-/- mice. This profile of hydroxyproline levels among the groups was the same as that observed for coronary fibrosis.

Plasma Oxidant Levels

Levels of lipid hydroperoxide in plasma provide a measure of the systemic production of oxidative species. Diabetes elevated plasma lipid hydroperoxide above nondiabetic levels to 42.3 ± 2.5 μM in WT mice versus 26.4 ± 1.6 μM in controls (P ≤ 0.05), but it did not alter levels in Al+/-Al-/- mice (33.7 ± 2.8 vs. 27.9 ± 1.8 μM in nondiabetic controls).

Diabetes elevated vascular (aortic) arginase activity and expression in our WT mice, similar to our observations in a previous study of diabetic rats (33). Arginase activity and Al expression were also elevated in diabetic Al+/-Al-/- mice but not in Al+/-Al-/- mice, indicating that enhanced Al expression accounts for elevated enzyme activity. However, it is important to note that Al may play a role in the diabetes-induced increase in Al in our model. Diabetic Al+/-Al-/- mice exhibited less elevation of arginase activity and Al expression than WT mice. This suggests that Al contributes to the diabetes-induced enhancement of Al expression and activity in diabetic WT mice. The interplay between the two arginase isoforms leading to Al transcriptional regulation in diabetes requires further investigation. We did observe that nondiabetic Al knockout mice exhibited higher Al expression than nondiabetic WT mice but that arginase activity in the two strains was not different. The increased Al expression under control conditions may be a compensatory response to the lack of Al to maintain basal activity.

**Discussion**

**Arginase Activity/Expression**

Diabetes elevated vascular (aortic) arginase activity and expression in our WT mice, similar to our observations in a previous study of diabetic rats (33). Arginase activity and Al expression were also elevated in diabetic Al+/-Al-/- mice but not in Al+/-Al-/- mice, indicating that enhanced Al expression accounts for elevated enzyme activity. However, it is important to note that Al may play a role in the diabetes-induced increase in Al in our model. Diabetic Al+/-Al-/- mice exhibited less elevation of arginase activity and Al expression than WT mice. This suggests that Al contributes to the diabetes-induced enhancement of Al expression and activity in diabetic WT mice. The interplay between the two arginase isoforms leading to Al transcriptional regulation in diabetes requires further investigation. We did observe that nondiabetic Al knockout mice exhibited higher Al expression than nondiabetic WT mice but that arginase activity in the two strains was not different. The increased Al expression under control conditions may be a compensatory response to the lack of Al to maintain basal activity.
Vascular Function

Our present findings of elevated arginase activity and expression associated with vascular dysfunction in diabetic WT mice are in agreement with our previous study (33) showing that STZ-induced diabetes in rats causes impaired coronary EC-dependent vasodilation and elevated vascular arginase activity and expression. Our present data further demonstrate the prominent role of AI in VED resulting from diabetes. Diabetic mice that lacked one copy of the AI gene and both copies of AII (AII<sup>−/−</sup>/AII<sup>−/−</sup>) showed substantially greater endothelium-dependent vasorelaxation than was seen in either WT mice or mice deficient in AII alone (AII<sup>−/+</sup>/AII<sup>−/−</sup>). Endothelium-independent vasodilation to the NO donor SNP was not affected by diabetes or by the absence of either the AI or AII genes. In contrast, and as expected, the contractile responses of aortas to PE were enhanced by diabetes (9). Impaired endothelial function is also responsible for this enhancement, as reduced NO production in response to PE allows greater contraction. This profile of PE-induced contraction in diabetic mice of the three genotypes mirrored the profile of impaired vasorelaxant responses to ACh observed in diabetes. WT aortas exhibited the greatest enhancement of PE-induced contraction and the most impairment of ACh-induced vasorelaxation during diabetes.

These data strongly indicate that AI is the isoform primarily responsible for VED in these diabetic mice. Others (34) have reported that mice lacking AII genes are largely protected from atherosclerosis-induced VED, vascular stiffness, and enhanced endothelial ROS production. Additionally, Goto-Kakizaki diabetic rats have been shown to exhibit impaired coronary function, which was restored by an arginase inhibitor, and elevated aortic AII, but not AI, expression (16). However, a study (35) of myocardial ischemia-reperfusion and coronary endothelial dysfunction in mice showed only the involvement of AI. We (25) have also shown that STZ-induced diabetes-induced impairment of coronary vasorelaxation to ACh in rats was correlated with increases in ROS, arginase activity, and AI expression in the aorta. Additionally, a recent study (2) of diabetic human coronary arterioles demonstrated the involvement of upregulated AI in reduced NO production and vasodilation. Together, these results suggest that the involvement of arginase isoforms in vascular dysfunction varies depending on the model and disease condition.

Vascular Fibrosis and Stiffness

Coronary arterial perivascular fibrosis is a serious complication of diabetes (24) that contributes to vascular dysfunction (19). Vascular remodeling with pathological fibrosis may be caused by increased collagen production and/or impaired collagen turnover due to deficient matrix metalloproteinase activation (31, 36). Elevated vascular stiffness is a known cardiovascular risk factor (3). We found that 8-wk diabetic WT mice have markedly increased perivascular collagen deposition around coronary vessels compared with control WT mice. We also found no differences in coronary fibrosis between diabetic WT and diabetic AII<sup>−/−</sup>/AII<sup>−/−</sup> mice. However, less perivascular collagen deposition was observed in AII<sup>−/+</sup>/AII<sup>−/−</sup> mice, indicating that AI is the predominant isoform contributing to collagen synthesis. This fibrotic process appears to be caused by an elevation of arginase activity, which increases the production of ornithine and the subsequent production of pyrroline-5-carboxylate and proline, a precursor of collagen. Preventing the elevation of arginase activity or expression prevents the vascular fibrotic response and preserves vascular compliance (1, 7).

Increased vascular stiffness contributes to the elevation of systolic and mean arterial blood pressure as well as the incidence of vascular rupture and thrombosis (36). Our finding of greater stiffness (reduced compliance) of isolated carotid arteries from diabetic WT mice versus their nondiabetic WT controls is in line with our findings of greater coronary vascular fibrosis in this genotype. Compared with WT mice, we did observe a smaller elevation of diabetes-induced stiffness in AII<sup>−/+</sup>/AII<sup>−/−</sup> mice versus their controls, which suggests that AI activity contributes to stiffness in diabetes. Stiffness of carotid arteries from diabetic AII<sup>−/−</sup>/AII<sup>−/−</sup> mice was only modestly elevated compared with their controls, which correlates with a low degree of coronary fibrosis in these mice. There was a progressive reduction in vascular stiffness values (B-coefficients; Fig. 4, A–C) when diabetic WT, AII<sup>−/+</sup>/AII<sup>−/−</sup>, and AII<sup>−/+</sup>/AII<sup>−/−</sup> tissues were compared versus their controls, indicating that both isoforms are involved in diabetes-induced vascular stiffness. However, this reduced stiffening to diabetes in all AII<sup>−/−</sup> vessels partially resulted from a moderate elevation of basal stiffness in the arteries of control, nondiabetic AII<sup>−/−</sup> versus control WT mice. We do not know the basis of the enhanced basal vascular stiffness in mice lacking AII. However, it may relate to the elevated systemic blood pressure and sympathetic nervous function observed in AII knockout mice (17).

The present results indicate that arginase plays a critical role in collagen synthesis and fibrosis by providing the substrate for the collagen precursor proline. This conclusion is supported by our finding of elevated hydroxyproline levels in diabetic hearts of WT and AII<sup>−/+</sup>/AII<sup>−/−</sup> mice compared with diabetic AII<sup>−/−</sup>/AII<sup>−/−</sup> or control WT mice. These findings correlate with the Masson’s trichrome staining results for collagen. However, we cannot exclude the possibility of impaired collagen degradation and turnover in diabetes. Another mechanism may account for the effect of elevated arginase activity on coronary fibrosis, since elevated arginase can diminish NO biosynthesis and NO-dependent processes. Several studies (5, 20, 33) have reported that long-term inhibition of NOS induces perivascular fibrosis and arterial stiffness in experimental animal models and humans. This effect is associated with elevated plasminogen activator inhibitor 1 production and the subsequent inhibition of matrix metalloproteinases, which leads to decreased matrix degradation and increased collagen deposition (25). It is probable that the overall improvement in vascular function of diabetic AII<sup>−/−</sup>/AII<sup>−/−</sup> mice, due to enhanced levels of NO production, is a central event in preventing coronary perivascular fibrosis (collagen deposition) and the impairment of vascular compliance.

It is important to note that ANG II, a well-recognized mediator of diabetic vascular complications including coronary fibrosis, is a known activator of arginase (26, 30). We have found that ANG II can exert its actions on arginase through the activation of a p38 MAPK pathway. This pathway has been reported to be involved in the fibrotic actions of ANG II in heart tissue (10, 29). Elevation of arginase activity and protein expression in response to ANG II and HG also have been
shown to involve the activation of the small GTPase RhoA and its effector Rho kinase (25, 26).

**ROS**

Diabetes and elevated levels of arginase activity and expression are associated with increased levels of ROS (15). Our study showed that whole body levels of ROS are elevated in diabetic WT mice, as indicated by measurements of plasma lipid hydroperoxide levels. In our study, we showed that preventing the elevation of AI activity, by partial gene deletion of AI in AI^{+/−}/AI^{−/−} mice, prevents diabetes-induced increases of plasma lipid hydroperoxide levels. These data indicate an important role for AI in the enhanced oxidative milieu during diabetes.

ROS generation from mitochondria as well as from cytosolic NADPH oxidase have been shown to contribute to oxidative organ damage in diabetes (6, 14). However, we believe that endothelial NOS uncoupling due to enhanced arginase activity and reduced \( \tau \)-arginine is also an important source of superoxide formation in the vasculature. Determining the role of arginase on signaling pathways associated with NADPH oxidase-derived and mitochondrial ROS requires further study.

**Summary**

Our data indicate that elevated arginase function has an important role in the vascular complications of diabetes. Diabetic AI^{+/−}/AI^{−/−} mice exhibited less arginase activity/expression, less impairment of endothelium-dependent vasodilation, less tissue oxidation, and less coronary fibrosis and vascular stiffness than diabetic WT and AI^{+/−}/AI^{−/−} mice. Thus, AI appears to be the primary arginase isoform involved in diabetes-induced vascular dysfunction.

**ACKNOWLEDGMENTS**

The authors appreciate the excellent assistance of Dr. Lin Yao and Anil Bhatta (Georgia Health Sciences University).

**GRANTS**

This work was supported by National Institutes of Health Grants R01-HL-70215 and R01-EY-11766 (to R. W. Caldwell and R. B. Caldwell).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


