Mitochondrial arginase II constrains endothelial NOS-3 activity

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Mitochondrial arginase II constrains endothelial NOS-3 activity. Am J Physiol Heart Circ Physiol 293: H3317–H3324, 2007. First published September 7, 2007; doi:10.1152/ajpheart.00700.2007.—Emerging evidence supports the idea that arginase, expressed in the vascular endothelial cells of humans and other species, modulates endothelial nitric oxide (NO) synthase-3 (NOS-3) activity by regulating intracellular L-arginine bioavailability. Arginase II is thought to be expressed in the mitochondria of a variety of nonendothelial cells, whereas arginase I is known to be confined to the cytosol of hepatic and other cells. The isoforms that regulate NOS-3 and their subcellular distribution, however, remain incompletely characterized. We therefore tested the hypothesis that arginase II is confined to the mitochondria and that mitochondrial arginase II reciprocally regulates vascular endothelial NO production. Western blot analysis, immunocytochemistry with MitoTracker, and immunoelectron microscopy confirmed that arginase II is confined predominantly but not exclusively to the mitochondria. Arginase activity was significantly decreased, whereas NO production was significantly increased in the aorta and isolated endothelial cells from arginase II knockout (ArgII/−/−) mice compared with wild-type (WT) mice. The vasorelaxation response to acetylcholine (ACh) was markedly enhanced and the vasoconstrictor response to phenylephrine (PE) attenuated in ArgII/−/− in pressurized mouse carotid arteries. Furthermore, inhibition of NOS-3 by Nω-nitro-L-arginine methyl ester (L-NAME) impaired ACh response and restored the PE response to that observed in WT vessels. Vascular stiffness, as assessed by pulse wave velocity (PWV), was significantly decreased in ArgII/−/− compared with WT mice. On the other hand, 14 days of oral L-NAME treatment significantly increased PWV in both WT and ArgII/−/− mice, such that they were not significantly different from one another. These data suggest that arginase II is predominantly confined to the mitochondria and that this mitochondrial arginase II regulates NO production, vascular endothelial function, and vascular stiffness by modulating NOS-3 activity.

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Arginase shares L-arginine as a substrate with NOS-3 and hydrolyzes L-arginine to ornithine and urea as part of the urea cycle. It is increasingly recognized that arginase modulates NO activity by regulating intracellular L-arginine bioavailability (2, 26). Thus the balance between arginase and NOS-3 activities, in part, regulates vascular endothelial NO production. Arginase activation/upregulation results in arginase/NOS imbalance and decreased NO production and has been demonstrated to contribute to endothelial dysfunction in a number of disease/pathophysiological processes, such as aging (2), diabetes (3, 6), hypertension (7, 12, 28), and atherosclerosis (18).

The two isoforms of mammalian arginase, arginase I and II, encoded by different genes (25), are expressed in different tissues. Furthermore, there appears to be significant species heterogeneity in isoform expression. Arginase I, located in the cytoplasm, is expressed most abundantly in the liver and is a critical enzyme in the urea cycle. Arginase II, on the other hand, is thought to be a mitochondrial enzyme and is expressed primarily in extrahepatic tissues, such as kidney (11), brain, small intestine, mammary gland, and macrophages. Accumulating evidence suggests that arginase II is the major isoenzyme in the endothelial cells (ECs) of humans and other species. The role of arginase II in ECs, however, remains incompletely understood, although there is now emerging evidence that it might be an important regulator of NO production (17, 18).

In cardiac myocytes, we recently demonstrated that arginase II, confined to the mitochondria, plays an important role in regulating neuronal NOS (NOS-1)-dependent myocardial contractile function (21). Considering that mitochondrial arginase II in myocytes regulates distinct and spatially confined pools of L-arginine and thus modulates NOS-1, we wished to determine whether arginase II is also confined to the mitochondria in vascular ECs and whether mitochondrial arginase II regulates vascular endothelial NO production and thereby endothelial function.

METHODS

Animals. Ten-week-old male wild-type (WT; C57BL/6J) mice from Jackson Laboratories were used as a control and fed a normal diet for 6 wk. Arginase II knockout (ArgII/−/−) mice were a gift from Dr. O’Brien, Baylor College of Medicine, and were bred and housed in our Institution. Male 10-wk-old ArgII/−/− mice were also fed a normal diet for 6 wk. All procedures and protocols were approved by the Institutional Animal Care and Use Committee of The Johns Hopkins University School of Medicine.

Aorta preparation. Heparin was administered 1 h before death. The animals were anesthetized with ketamine-acepromazine intraperitoneally.

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ally, and the thoracic aorta, from distal aortic arch to the diaphragmatic level, was dissected, removed, and immersed in Krebs solution containing (in mM) 118 NaCl, 4.7 KCl, 1.2 KH$_2$PO$_4$, 2.5 CaCl$_2$, 1.2 MgSO$_4$, 25 NaHCO$_3$, and 11.1 glucose. The vessels were carefully cleaned of connective tissue and cut into 2- to 3-mm rings. Aortic rings were immediately frozen in liquid nitrogen and stored at −80°C until assayed.

**Isolation of mouse ECs.** ECs were isolated from WT and ArgII$^{-/-}$ mice vessel rings, as previously described (13). In brief, the dissected aorta from heparinized mice was immersed in heparin-containing 20% FBS-DMEM and washed with serum-free DMEM. After the aorta was filled with collagenase type II (2 mg/ml in serum-free DMEM, Sigma) and incubated for 45 min at 37°C, ECs were removed from the aorta by flushing with 5 ml of 20% FBS-DMEM. After centrifugation of this solution to harvest the ECs, they were cultured in a collagen type I-coated dish. To remove smooth muscle cells, the cells were washed with warmed PBS, and complete medium G containing 20% FBS, 100 µg/ml penicillin-G, 100 µg/ml streptomycin, 2 ml 1-glutamine, 1× nonessential amino acids, 1× sodium pyruvate, 25 mM HEPES (pH 7.0–7.6), 100 µg/ml EC growth supplements, and DMEM was added. All experiments were performed on second-passage number mouse aortic ECs.

**Arginase activity.** Arginase activity was determined by quantitating urea production using the spectrophotometric method with α-isonitrosopropiophenone as described previously (21). Briefly, cell lysates were incubated with 75 µl manganese chloride solution (50 mM Tris·Cl, pH 7.5) at 60°C for 10 min and further reacted with 50 µl substrate l-arginine (0.5 mol/l, pH 9.7) at 37°C for 1 h. After the reaction was stopped by adding 400 µl of the acid solution mixture, α-isonitrosopropiophenone (25 µl, 9% in absolute ethanol) was added. The optical density of the mixture heated at 100°C for 45 min was then measured spectrophotometrically at 550 nm.

**Nitrare and nitrite or nitrogen oxide measurement.** NO production from tissue lysates was evaluated by measuring nitrite levels using a NO assay kit (CaliBiochem) by Griess reaction as previously described (21).

**Estimation of NO generation with fluorescence probes, 4,5-dimino-fluorescein.** ECs isolated from aorta of WT and ArgII$^{-/-}$ mice were labeled with a fluorescent probe to NO [4,5-diamino-fluorescein (DAF), 5 µmol/l, 30 min] at room temperature and protected from light. After being washed with PBS, cells were then fixed with paraformaldehyde (3%) for 20 min. Images were acquired using a Nikon TE-200 epifluorescence microscope (with a DAF) and analyzed with Dose Response Software (AD Instruments). Vessel rings were preconstricted with phenylephrine (1 µmol/l), and their vasorelaxant dose response to ACh (1 nM to 10 µM) were recorded. After washout and return to resting tension, vessels were again preconstricted with PE and their response to sodium nitroprusside (SNP) was determined (1 nM to 10 µM).

**In vitro vascular reactivity in carotid arteries.** Carotid arteries were dissected free from connective tissue in Krebs solution under a microscope and placed in a vessel chamber. Both ends of the carotid arteries were cannulated and sutured to two glass micropipettes. The chamber was filled with oxygenated Krebs solution (95% O$_2$–5% CO$_2$, pH 7.4, 37°C). The vessel was carefully cleaned and cut into 1.5-mm rings and suspended for isometric tension recording in organ chambers, as previously described (27). Protocols were performed on rings beginning at their optimum resting tone, previously determined to be 500 mg for mouse aorta. This resting tone was reached by stretching rings in 100-nm increments separated by 10-min intervals. Data were collected using a MacLab system and analyzed using Dose Response Software (AD Instruments). Vessel rings were preconstricted with phenylephrine (1 µM), and their vasorelaxant dose response to ACh (1 nM to 10 µM) were recorded. After washout and return to resting tension, vessels were again preconstricted with PE and their response to sodium nitroprusside (SNP) was determined (1 nM to 10 µM).

**Pulse wave velocity.** Vascular stiffness was determined pre- and posttreatment by measuring pulse wave velocity (PWV) using an ECG-triggered 10-MHz Doppler probe (Indus Instruments) at thoracic and abdominal aorta locations. The animals were anesthetized and maintained with ~1% to 1.5% isoflurane. Animals were positioned supine with legs and arms taped to ECG electrodes incorporated into a temperature-controlled printed circuit board (THM100, Indus Instruments, Houston, TX). Rectal temperature was monitored with a probe (Physitemp, Clifton, NJ) and maintained at 37°C throughout the procedure. Both thoracic and abdominal aortic flows were acquired at a depth of ~2 to 4 and ~5 to 6 mm, respectively, with a 2-mm diameter, 10-MHz Doppler probe (Indus Instruments). These sites of...
measurement were marked upon image acquisition, and the separation
distance between them was measured. PWV (in m/s) was calculated as
the quotient of separation distance and the time difference between
pulse arrivals, as measured from R-peaks of the ECG. Data analysis of
Doppler and ECG signals was performed off-line using DSPW soft-
ware from Indus Instruments.

Statistics. All data are represented as means ± SE. Statistical
significance was determined by t-test or two-way ANOVA with
Bonferroni posttests (Graphad Prism 4 software). A values of P <
0.05 was considered significant.

RESULTS

Arginase II is present predominantly in the mitochondria. Mitochondria were isolated from the cytoplasm using a previ-
ously described technique (21). To determine the subcellular
localization of arginase II, Western blot analysis was per-
formed with a mitochondrial-specific [voltage-dependent anion
channel (VDAC)] and a cytoplasm-specific protein (β-tubulin) in
addition to arginase II and NOS-3. As demonstrated in
Fig. 1A, there is adequate isolation/purification of mitochondria
since the mitochondrial protein, VDAC, is found exclusively in
the mitochondrial fraction, whereas β-tubulin is found only in
the cytoplasmic fraction. Importantly, arginase II is found
predominantly, though not exclusively, in the mitochondrial
fraction. To further determine the subcellular localization of arginase II, we costained ECs from WT and ArgII−/− mice
with the mitochondrial-specific dye MitoTracker (red) and an
arginase II-specific dye (green). As illustrated in Fig. 1B,
arginase II is expressed in ECs from WT but not ArgII−/−
mice. Furthermore, arginase II appears to be located in the
mitochondria, as is demonstrated in the merged image showing
both arginase II and MitoTracker. To further confirm the
compartment-specific site of arginase II, we used immunoel-
tron microscopy. As demonstrated in Fig. 1C, in the isolated
human ECs, the preponderance of immunogold staining is
confined to the mitochondria.

Mitochondrial arginase II reciprocally regulates NO. We have previously demonstrated that an increase in EC arginase
activity is responsible for a reciprocal decrease in NO activity in
ECs in aging rats (26) and in atherosclerosis (18). Further-
more, we demonstrated that arginase II reciprocally regulates
myocyte NOS-1-dependent myocardial contractility at baseline
(21). We wished to determine, under normal physiological
conditions, whether mitochondrial arginase II constrains NOS-
3-dependent NO production. As demonstrated in Fig. 2A,
there is a significant reduction in arginase activity in aorta from
ArgII−/− compared with WT mice. This is associated with a
significant increase in NO production in aorta from ArgII−/−
compared with WT mice (Fig. 2B). Furthermore, there is a
significant increase in the fluorescence staining in cells loaded
with the NO-sensitive fluorescent dye, DAF, in isolated ECs from
ArgII−/− compared with WT mice (Fig. 2C). We next

...more, we were unable to demonstrate the expression of arginase I in aorta of WT or ArgII−/− mice, suggesting that
no compensatory effect in isoform expression is induced in
the ArgII−/− mice (mouse liver was used as a positive control).
Interestingly, there is a significant reduction in NOS-3 expres-
sion in ArgII−/− mice compared with control, despite an
increase in NO production. This is consistent with the idea
that it is the availability of substrate, cofactors, and dimerization
that is more important than protein abundance with regard to
NO production by the NOS-3 enzyme (8, 14, 24).

Arginase II deficiency is associated with improved endothel-
ial function. We tested the hypothesis that increased NO
activity in ArgII−/− mice results in 1) enhanced vascular
endothelial function and 2) decreased vascular stiffness. To
examine vascular endothelial function, two vascular bioassays
were used. Mouse thoracic aorta and carotid arteries from
ArgII−/− and WT mice were dissected free of their connective
tissue. Mouse aortic rings were placed in an organ chamber
attached to a force transducer, whereas mouse carotids were
 cannulated using glass pipette, and mounted in a perfusion bath
(Living System). The aorta was stretched to a passive tension
of 500 mg, whereas the carotids were pressurized to 50 mmHg
and the diameter of the vessels determined using video-dimen-
sion analysis. As demonstrated in Fig. 3A, ACh that resulted
mediated a dose-dependent relaxation in the aorta. Interest-
ingly, there was a significantly enhanced response in the aorta
from ArgII−/− compared with WT mice. This was reflected as
an increase in the maximal elastance (62.7 ± 4.6% vs. 89.3 ±
11.6%, n = 6, P < 0.01). There was, however, no difference
in the response to SNP in the aorta (Fig. 3B), suggesting an
enhancement of endothelial-dependent function. With regard
to the carotid vascular bioassay, there was no significant
difference in the diameter (WT vs. ArgII−/−, 459.6 ± 6.4 vs.
460.0 ± 12.4 μm) of the vessels before PE preconstriction
between the ArgII−/− and WT mice. The vessels were precon-
stricted with PE (1 μM), and the responses to the endothelial-
dependent vasodilator ACh and the endothelial-independent
NO donor SNP were determined. As demonstrated in Fig. 3C,
ACh resulted in a dose-dependent vasodilatation response to
ACh in both ArgII−/− and WT mouse carotid arteries. How-
ever, there was a significant augmentation in the ACh response
in vessels from ArgII−/− mice (log EC50, WT vs. ArgII−/−,
−7.6 ± 0.1 vs. −8.1 ± 0.1, n = 4, P < 0.05). The responses
of the NO donor SNP were identified in the two groups (Fig.
3B). Since NO-dependent vasodilatation is one of the potential
mechanisms responsible for ACh-mediated vasodilatation, we
examined ACh responses in the presence of the NOS inhibitor
l-NAME. As demonstrated in Fig. 3C, NOS inhibition mark-
edly attenuated the response to ACh. Furthermore, there was
no significant difference in the non-NO-dependent vasodilator
responses to ACh between WT and ArgII−/− mice. As with the
aorta, there was no significant difference in the responses of the
ArgII−/− and WT mouse carotids to SNP (Fig. 3D). This
suggests that endothelial NO-dependent increases in vasodila-
tor function are a function to ArgII−/− deficiency. We next
examined the vasoconstrictor responses to PE. As demon-
strated in Fig. 3E, there is an attenuation of the vasoconstrictor
response to PE in ArgII−/− mice (maximal elastance, WT vs.
ArgII−/−, 16.8 ± 0.5 vs. 19.9 ± 1.1, n = 4, P < 0.01),
indicating an attenuated pressor response in ArgII−/− mice. To
determine whether this attenuated pressor response was medi-
Fig. 1. Subcellular localization of arginase II. A: in Western blot, voltage-dependent anion channel (VDAC) and β-tubulin are used as mitochondrial and cytoplasmic marker proteins, respectively. Arginase II is found in mitochondrial fraction (M), but little is present in the cytoplasmic fraction (C) in mouse (MAECs) and human (HAECs) aortic endothelial cells (n = 3 experiments). B: endothelial cells from wild-type (WT) and arginase II knockout (ArgII−/−) mice were stained with a mitochondrial specific dye MitoTracker (red) and costained with arginase II (green). Arginase II appears to colocalize with the mitochondria in endothelial cells from WT mice but not expressed in ArgII−/− mice (n = 3 experiments). C: some gold particles are seen in the cytoplasm in immunoelectron microscopy, but gold particles are predominantly confined to the mitochondria (arrows) of the isolated HAECs. Comb, combined; NOS, nitric oxide (NO) synthase.
Fig. 2. Effect of arginase II deficiency on arginase activity and NO production. A: arginase activity was significantly reduced in aorta from ArgII−/− compared with WT mice (*P < 0.01; n = 8 experiments from 4 animals). B: ArgII−/− mice had a significant increase in NO production compared with WT mice (*P < 0.01; n = 8 experiments from 4 animals). C: phenotype of isolated cells was confirmed by using endothelial cell-specific protein NOS-3 (red). NO production in isolated endothelial cells from ArgII−/− and WT mice was measured by NO-sensitive dye, 4,5-diaminofluorescein (DAF; green). DAF fluorescence was significantly increased in ArgII−/− compared with WT mice (n = 3 experiments). D: Western blot and cumulative quantitative data (E) demonstrating the absence of arginase II in ArgII−/− mice (K, kidney, positive control). Lack of expression of arginase I in either WT or ArgII−/− mice (L, liver, positive control). Downregulation of NOS-3 in ArgII−/− mice (*P < 0.05, #P < 0.01, n = 4 experiments). AU, arbitrary units.
Fig. 3. Effect of arginase II deficiency and the NOS-3 inhibitor \textit{N}-nitro-L-arginine methyl ester (L-NAME) on vascular endothelial function in mouse aorta and carotid. A: endothelium-dependent relaxation responses to acetylcholine (ACh) in aortic rings were markedly enhanced in ArgII\textsuperscript{-/-} compared with WT mice (*$P < 0.01$, WT vs. ArgII\textsuperscript{-/-}, $n = 6$ experiments). B: responses to sodium nitroprusside (SNP) were no different. C: similarly endothelium-dependent responses to ACh were enhanced in carotid arteries of ArgII\textsuperscript{-/-} mice (*$P < 0.01$, WT vs. ArgII\textsuperscript{-/-}, $n = 4$ experiments). Although ACh responses after preincubation with \textit{l}-NAME were significantly impaired in the carotid arteries of both WT and ArgII\textsuperscript{-/-} mice, the responses were no different between the groups. D: vasodilatory responses to the endothelial-independent vasodilator SNP were identical in ArgII\textsuperscript{-/-} and WT mice ($n = 4$ experiments). E: contractile response to phenylephrine (PE) was significantly attenuated in carotid rings from ArgII\textsuperscript{-/-} compared with WT mice. After preincubation with \textit{l}-NAME, the pressor response to PE of ArgII\textsuperscript{-/-} was restored and identical to that of WT mice (*$P < 0.01$ vs. WT, $\#P = $ not significant vs. WT + \textit{l}-NAME, $n = 4$ experiments).

Arginase II deficiency is associated with a decrease in vascular stiffness. We next wished to determine whether the in vitro measures of enhanced endothelial function translated into an improvement in baseline arterial compliance in ArgII\textsuperscript{-/-} mice. There was, however, no significant difference between ArgII\textsuperscript{-/-} and WT mice but a greater increase in ArgII\textsuperscript{-/-} mice. There was, however, no significant difference between ArgII\textsuperscript{-/-} mice and their WT controls after 14 days of NOS inhibition (WT vs. ArgII\textsuperscript{-/-}, $4.3 \pm 0.2$ vs. $4.2 \pm 0.1$, $P = $ not significant). This supports the idea that ArgII\textsuperscript{-/-} mice have increased vascular compliance as a result of increased basal NO production.

Thus, in summary, arginase II is confined predominantly to the mitochondria. Furthermore, ArgII\textsuperscript{-/-} mice have increased basal NO production, enhanced endothelial function, and increased vascular compliance.

DISCUSSION

We have demonstrated for the first time that arginase II, the predominant isofrom in ECs, is confined mainly to the mitochondria. Moreover, arginase II regulates endothelial NO production since ECs from ArgII\textsuperscript{-/-} mice have increased endothelial NO production, enhanced NO-mediated endothelial-dependent vasorelaxation, and increased arterial compliance.

There is accumulating evidence in human ECs that arginase II is the predominantly expressed isofrom (17, 18). Our previous data (18) demonstrated that arginase II is the predominant
have characterized three pools of L-arginine, one of which is not changeable with extracellular L-arginine. Moreover, these observations are consistent with those of Simon et al. (19), who demonstrated copurification of NOS-3 with mitochondria in human umbilical vein endothelial cells, suggests a potential mechanism underlying reciprocal NOS-3 regulation by mitochondrial arginase II. Moreover, this group also demonstrated that NOS-3 was docked to the outer mitochondrial membrane by a novel anchoring mechanism that was sensitive to protease cleavage. The data support the idea that NOS-3 may indeed be colocalized with arginase II in a functional domain that could share a common L-arginine pool. This is completely consistent with our findings regarding the subcellular distribution of NOS-3 and arginase II.

Our findings could have important implications for our understanding of pathophysiological disease processes. A recent review of mitochondria and endothelial function by Davidson and Duchen (5) highlights the role of endothelial mitochondria in the pathogenesis of atherosclerosis and diabetic vascular disease. For example, endothelial mitochondria-mediated increases in reactive oxygen species (ROS) production in response to oxidized LDL exacerbate endothelial dysfunction (29). Furthermore, high EC turnover in atherosclerosis and the associated necessary activation of apoptotic pathways regulated by mitochondria also implicate these organelles in this process (4). Our group has recently demonstrated that oxidized LDL activates arginase by a mechanism that involves a release of its constraint by the microtubular structure (17).

It is now well recognized that large-artery vascular stiffness, as measured by PWV and the augmentation index, is an important characteristic of vascular health, and an increase in vascular stiffness represents an independent risk factor for adverse cardiovascular events. We have demonstrated that PWV is a sensitive indicator of endothelial dysfunction in that even small changes in endothelial function with resultant effects on the underlying vascular smooth muscle cause significant changes in PWV. In isolated vascular endothelial function studies, we demonstrated enhanced NO-dependent vasorelaxant responses in ArgII−/− mice. Interestingly, this translated to a significant basal decrease in PWV. Furthermore, acute treatment with the NOS inhibitor L-NAME (not long enough to induce profound vascular remodeling; see Ref. 12a) increased vascular stiffness in both WT and ArgII−/− mice, such that vascular stiffness was similar in both groups. The data support two important concepts: 1) that the basal decrease in PWV in ArgII−/− mice is most likely a function of an increase in basal NO, and 2) that endothelial NO is a significant modulator of vascular stiffness and that the latter may be a sensitive in vivo indicator of vascular endothelial function/dysfunction.

In summary, we have demonstrated that endothelial arginase II is confined predominantly to the mitochondria where it regulates NO production and vascular stiffness. Its spatial confinement has important implications for diseases, for example, atherosclerosis and diabetes, in which endothelial dysfunction is in part modulated by mitochondria.

Previous studies resulted in the conventional understanding of NOS-3 regulation, which suggested that NOS-3 was restricted primarily to the caveolae and confined to a signaling domain with surface receptors and the cationic amino acids transport (16). This is at odds with the concept that arginase II, a mitochondrially located enzyme, could reciprocally regulate NOS-3. It is now recognized, however, that dynamic trafficking of NOS-3 (in lipid rafts from the surface to the inside of the cell) may be critical in its regulation. Furthermore, it appears that there are a number of different NOS-3 pools that might be active and respond to different physiological and pharmacological stimuli (9, 20, 22). Recent work by Gao et al. (10), demonstrating copurification of NOS-3 with mitochondria in isoform present in human and mouse ECs. In nonendothelial tissue, it is well established that arginase II is confined predominantly but not exclusively to the mitochondria (kidney, etc.) (15). However, this subcellular distribution has never previously been demonstrated in ECs. Our group has also previously determined that the arginase II isoform confined to the mitochondria in cardiac myocytes is capable of regulating NOS-1. This is due to the close juxtaposition of the mitochondria and the sarcoplasmic reticulum-containing NOS-1 and likely reflects dual enzymatic competition for their shared substrate L-arginine in specific subcellular compartments/pools. Using both immunoprecipitation and Western blotting techniques in subcellular fractions and immunoelectron microscopy, we have now demonstrated this phenomenon in ECs. Topal et al. (23) demonstrated that depletion of freely exchangeable L-arginine pools using extracellular L-lysine did not modulate the influence of arginase on EC NO release. This suggests the presence of different L-arginine pools, at least one of which is accessible to NOS and arginase, but is not exchangeable with extracellular L-arginine. Moreover, these observations are consistent with those of Simon et al. (19), who have characterized three pools of L-arginine, one of which is exchangeable with extracellular L-arginine and two which are not. One of these latter appears to be that which is accessible to arginase.

Fig. 4. Vascular stiffness using pulse wave velocity (PWV). PWV was measured before and after treatment with the NOS inhibitor L-NAME for 14 days in both WT and ArgII−/− mice. The baseline PWV was significantly decreased in ArgII−/− compared with WT mice. After L-NAME treatment, PWV was significantly increased in both WT and ArgII−/− mice (*P < 0.01, **P < 0.001, n = 6 experiments).
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GRANTS

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