Arginase II is emerging as an important player in endothelial dysfunction with implications in vascular disease associated with atherosclerosis, diabetes mellitus, and hypertension. Arginase, of which there are 2 isoforms, is a metalloenzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. Arginase I is a cytosolic enzyme found primarily in the liver, whereas arginase II is a mitochondrial enzyme with a more widespread distribution, including the vasculature. Arginase II is expressed in endothelial and vascular smooth muscle cells and has been identified in many vascular beds including the aorta, coronary, carotid, and pulmonary arteries. The exact function of vascular mitochondrial arginase II still remains elusive, although there is growing evidence suggesting that arginase inhibits nitric oxide (NO) synthesis by competing with NO synthase (NOS) for L-arginine, which is the exclusive substrate for NOS. Inhibition of arginase stimulates NO production in endothelial cells, whereas overexpression of arginase II decreases intracellular L-arginine content and reduces NO production. In addition, reduced NO substrate promotes endothelial NOS (eNOS) uncoupling. As such, increased activation of arginase II has the potential to impair endothelial function by reducing NO bioavailability and increasing oxidative stress. Arginase-mediated endothelial dysfunction has been demonstrated in hypertension, atherosclerosis, ischemia-reperfusion injury, erectile dysfunction, and diabetes mellitus and has also been shown in aging blood vessels.

Arginase II is constitutively expressed in endothelial cells and is activated by a wide range of agents such as thrombin, EGF (epidermal growth factor), lipopolysaccharides, TNFα (tumor necrosis factor alpha), and H₂O₂. Factors that suppress arginase expression and activity include genistein, cocoa flavanols, and simvastatin. Arginase expression and activity are increased in various pathological conditions, including atherosclerosis, where oxidized low-density lipoprotein (oxLDL) seems to be especially important. Extensive work has delineated the pathway, whereby oxLDL regulates arginase II in endothelial cells, and it is now clear that oxidized low density lipoprotein receptor-1 (LOX-1) and Rho kinase (ROCK) are important. This is evidenced by the fact that inhibition of LOX-1, RhoA (Ras homolog gene family, member A), and ROCK, using various pharmacological and mouse model strategies, attenuates endothelial cell arginase II activity. Signaling pathways and downstream targets of oxLDL arginase II have also been well defined, and it is now clear that arginase II activation through LOX-1 causes eNOS uncoupling and reduced NO generation. However, there is a gap in our understanding of what links oxLDL-LOX-1 to arginase II-regulated NOS.

In the current issue, Pandey et al provide insights into the missing link. Using HUVECs (human umbilical vein endothelial cells) and mouse aortic intima and several experimental strategies to interrogate up- and downstream arginase regulators, the authors show that oxLDL stimulates arginase II by inducing translocation from the mitochondria to the cytosol through processes that are dependent on mitochondrial processing peptidases (MPPs). MPPs cleave off N-terminal cleavable presequences and play a role in the mitochondrial protein import/export machinery. Using mass spectrometry and Edman degradation analysis, arginase II was found to have a putative MPP cleavage site in its N terminus, which probably influences mitochondrial to cytoplasmic translocation. Cytoplasmic arginase II promoted eNOS uncoupling, decreased NO production, and increased reactive oxygen species (ROS) generation leading to endothelial dysfunction. The importance of MPP in this process was confirmed in studies where knockdown of MPP prevented oxLDL-induced arginase translocation, eNOS uncoupling, and impaired vascular function. To demonstrate the pathophysiological significance of this paradigm, studies in atherosclerotic apolipoprotein E–deficient mice crossed with arginase 2 knockout mice, demonstrated reduced redox-sensitive atheromatous plaque burden, decreased oxidative stress, and increased NO.

What is emerging is that ROS are both upstream and downstream of arginase II. By stimulating eNOS uncoupling, arginase II promotes an increase in superoxide and ROS generation and oxidative stress, which can activate redox-sensitive ROCK to increase MPP-induced translocation and activation of arginase II. ROS also play a critical role in regulating oxidation of lipoproteins, resulting in oxLDL formation, which is critically involved in atherosclerosis and arginase II activity, as demonstrated before. This circuitous feed-forward system, where ROS regulates oxLDL, which stimulates arginase II through ROCK and MPP, leading to eNOS uncoupling and increased ROS formation may cause amplification of injurious arginase II–dependent processes underlying vascular dysfunction in atherogenesis.

Despite the elegant studies under discussion, there are some aspects that warrant further consideration. First, although
the study under discussion focuses on endothelial arginase II, where NOS is the primary downstream target, it should be appreciated that in vascular smooth muscle cells, rather than modulating NO generation, arginase II seems to function mainly to increase synthesis of proline and polyamines, which influence collagen production and cell growth, respectively. In the vascular media, this impacts structural remodeling, vascular stiffness, and intimal hyperplasia, processes associated with vascular injury. Whether the oxLDL-LOX-1-ROCK-MPP pathway, and the importance of mitochondrial to cytoplasm translocation of arginase II as demonstrated in endothelial cells, also affects vascular smooth muscle cell arginase II and vascular injury is unclear. Second, in addition to arginase II, arginase I is expressed in endothelial and vascular smooth muscle cells and could contribute to overall arginase activity, especially in the cytoplasm, where arginase I localizes. This could explain, at least in part, why in the Pandey study arginase activity in the cytosol and mitochondrial fractions were comparable, despite the majority of arginase II being confined to the mitochondria. Moreover, the significance of arginase isoform expression in different species needs to be considered because whereas human endothelial cells seem to express both isoforms, rodent endothelial cells may only express one isoform type. Considering that the study under discussion examined both human aortic endothelial cells and murine aortic tissue, it is possible that different isoforms may differentially influence downstream signaling pathways and cell function. Finally, although the study addresses reverse translocation of arginase II relative to oxLDL-LOX-1-ROCK activation and atherothrombosis, it would be interesting to know whether this is oxLDL specific or whether it is a more generalized phenomenon. This is particularly important considering that there are many vasoactive agents that are redox sensitive (like oxLDL) and that stimulate ROCK. If it is oxLDL specific, mechanisms underlying such specificity await identification.

Despite its limitations, the study of Pandey et al is important because it advances the field by identifying an important mechanism through the mitochondria, linking proatherogenic factors with arginase II-mediated NO/SNO systems. This is an exciting time in the field of arginase biology because interruption of arginase signaling may provide an attractive strategy for the management of endothelial dysfunction and vascular injury associated with atherosclerosis, diabetes mellitus, and hypertension. There is currently much interest in the development of arginase inhibitors as novel therapeutics for vascular disease.

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Disclosures
None.

References


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