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Systemic changes in arginase and arginine metabolism in a model of atherosclerosis: a comparison of apoE-/- and C57 mice

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Increased arginase activity is implicated in cardiovascular pathogenesis. Therefore we studied systemic changes in arginase and arginine metabolism in a model of atherosclerosis. ApoE-/- and C57 mice were placed on 45 or 60% fat diets for 2 months. Results showed increased circulating arginase activity with no difference between 45% (14-fold) and 60% (16-fold) fat diet in the apoE-/-. There was a mild increase in the C57 with 45% fat (2.4-fold) and a large increase due to 60% (19-fold). In the plasma, circulating arginine was reduced in the apoE-/- and showed a decreasing trend in the C57. The plasma arginine/ornithine ratio was significantly decreased in all groups that showed profound increased plasma arginase activity. Markers of tissue (LDH) and liver (ALT) injury were markedly elevated in mice with increased arginase activity. Interestingly, blood cell gene expression in the apoE-/- fed 60% fat showed an 11-fold increase in arginase II (AII) and no increase in arginase I (AI). Tissue gene expression showed increased AI (20-5.5-fold) and AII (14- 19-fold) in aorta and heart respectively in 60% fat apoE-/- (45% to be completed) with no increase in C57. In conclusion, increased circulating arginase activity and reduced arginine are likely the result of liver and/or tissue injury. Increased arginase gene expression in the blood and tissue (apoE-/- only) are likely associated with the development of atherosclerosis.

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Arginase contributes to arteriolar endothelial dysfunction following hemorrhage

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Vascular tissues express arginase that metabolizes L-arginine (L-Arg) to L-ornithine and urea. Arginase competes for L-Arg with nitric oxide synthase (NOS) and contributes to endothelial dysfunction in hypertension and diabetes. Blood loss promotes endothelial dysfunction which contributes to compromised tissue perfusion. This study tests the hypothesis that increased arginase activity contributes to endothelial dysfunction following hemorrhage. Male Sprague-Dawley rats (300-350g) were subjected to a 45% blood loss over 5min. Blood pressure spontaneously returned to 90% of baseline within 1hr. Plasma arginase activity was increased 36hrs post-hemorrhage by 22%. Isolated first-order gracilis muscle arterioles were superfused with Krebs buffer and exposed to constant midpoint, but altered endpoint pressures to establish graded levels of luminal flow (0-50µl/min). In post-hemorrhage arterioles, flow-induced dilation was abolished (Δ_{max} shock: 0±0 vs unbled: 18±1µm). Acute *in vitro* treatment with an inhibitor of arginase, 100µM N^{ω}-hydroxy-nor-L-arginine (Δ_{max} 22±2µm) or the NOS substrate, 1mM L-Arg restored flow-induced dilation to unbled control levels (Δ_{max} 17±1µm). In unbled controls, flow-induced dilation was abolished by the NOS inhibitor, 1mM L-NAME. These results suggest that arginase activity is increased following massive hemorrhage and contributes to endothelial dysfunction in resistance vessels by inhibiting endothelial NOS.

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967.7

Methionine cycle kinetics and arginine supplementation in endothelial dysfunction of ESRD.

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To investigate the effect of arginine supplementation on metabolic pathways involved in endothelial dysfunction of end stage renal disease (ESRD), we conducted a study on 11 ESRD patients age 49±16; wt 93±26 kg receiving an adequate protein and energy intake for 1week, followed by a primed, continuous, 9h infusion (4h fast;5h fed) of L-[1-^{13}C] methionine, L-[$^{2}H_3$] methyl methionine, and determination of flow mediated vasodilatation (FMD). After a break period, they received daily arginine supplementation of 21g, for 4weeks, and the studies were repeated. Diets were isonitrogenous. The rates of methionine transmethylation (TM), Transulfuration (TS) and Remethylation RM are shown in Table 1. There was an effect of the metabolic period, fast vs. fed on remethylation rates for the arginine supplemented diet, and on the TS rates for the control and arginine supplemented periods. However, there was no effect of arginine supplementation on the rates of TM, TS, RM or on FMD. Arginine supplementation does not affect methionine cycle metabolism. NIH DK62363, Ajinomoto 3ARP Program.

Table.1 Methionine cycle kinetics during control diet and arginine supplementation.

	Control Diet[1]	Arg suppl Diet
TM µmol.kg^{-1}.h^{-1}.		
Fast	10.5±4.5	9.8±3.0
Fed	16.1±5.8	13.9±5.2
RM µmol.kg^{-1}.h^{-1}.		
Fast	7.45±4.4	7.28±2.4*
Fed	11.6±5.1	10.2±3.2
TS µmol.kg^{-1}.h^{-1}.		
Fast	3.1±0.5*	3.29±0.9*
Fed	5.0±1.3	5.22±1.5
FMD (%)		
	5.8±3.8	5.22±1.5

[1]All values are mean±SD. *p<0.05 fast vs. fed.

967.8

Enzymes of L-arginine metabolism and their regional induction in the heart following myocardial infarction

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L-Arginase 1 (ARG1) is the single most highly induced transcript in the mouse left ventricle after acute myocardial infarction (AMI). Within ischemic/infarcted tissue (IF), the ARG1 transcript is induced 121-fold by 24 h post-AMI. In addition to induction of the ARG1 transcript, arginase specific activity is elevated 34-fold at 48 h increasing to 42-fold by 1-week. The L-arginase 2 (ARG2) transcript is also induced along with transcripts encoding enzymes of polyamine biosynthesis and a protein inhibitor of nitric oxide synthase (NOS) activity. Thus, nitric oxide production may be regulated, in part, by inhibition of NOS and depletion of the NOS substrate, L-arginine, by L-arginase. In addition, L-arginine may undergo conversion to polyamines as part of the stress response and/or to proline (collagen biosynthesis). To test these hypotheses, we produced antibodies against ARG1, ornithine decarboxylase, antizyme inhibitor and protein-inhibitor of NOS. As judged by western blot and immunohistochemical analyses, ARG1 and the antizyme inhibitor polypeptides show induction in the IF. We have further evaluated the regional and cell-type specificity of their induction by AMI within the mouse left ventricle. *Support: NIH grants P20 RR15640 and R15 HL71239*

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