

Forum Original Research Communication

Supplement

Folic acid promotes recycling of tetrahydrobiopterin and protects against hypoxia-induced pulmonary hypertension by recoupling endothelial nitric oxide synthase

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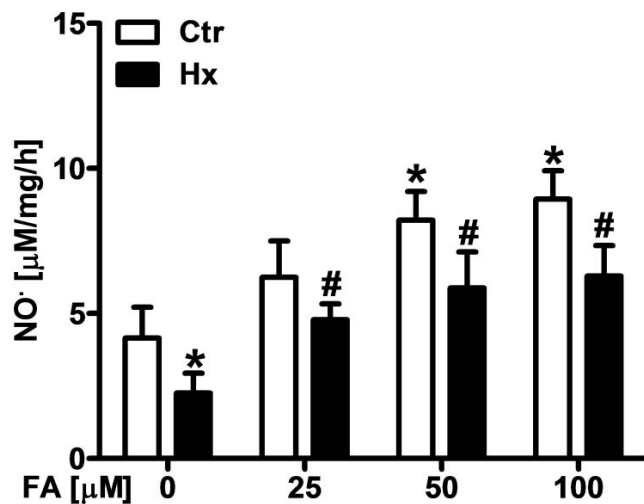
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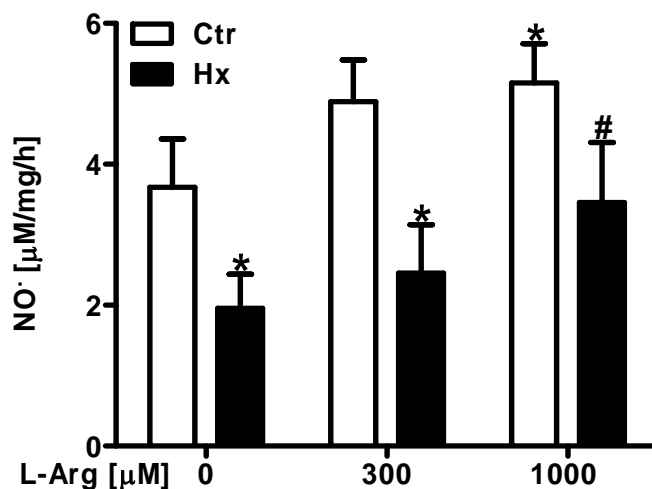
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Supplementary figures



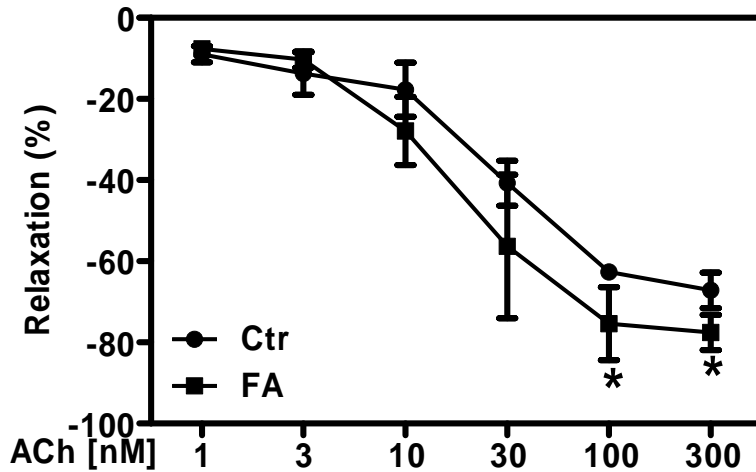
Supplement Fig.1: Folic acid dose dependently increases NO bioavailability

Human pulmonary artery endothelial cells (HPAEC) were exposed to normoxia (Ctr) or hypoxia (1% oxygen, Hx) for 24 h in the presence of folic acid (FA, 0-100 μ M). NO levels were measured by electron paramagnetic resonance (EPR) using iron(2+) diethyldithiocarbamic acid [Fe(2+)(DETC)] complex as a NO radical-specific spin probe under hypoxic conditions (n=3; * p <0.05, (1- β) \geq 0.841 vs. CtrCtr; # p <0.05, (1- β) \geq 0.99 vs. HxCtr).



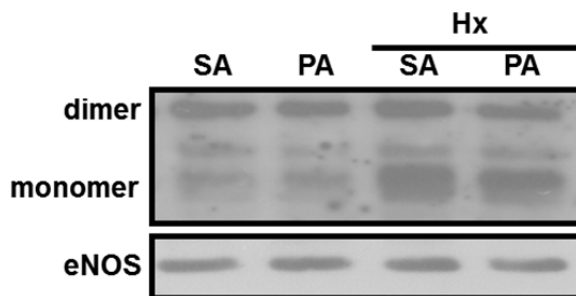
Supplement Fig.2: High dose arginine supplementation improves NO bioavailability

Human pulmonary artery endothelial cells (HPAEC) were exposed to normoxia (Ctr) or hypoxia (1% oxygen, Hx) for 24 h in the presence of L-arginine (L-Arg, 0, 300 and 1000 μ M). NO levels were measured by electron paramagnetic resonance (EPR) using iron(2+) diethyldithiocarbamic acid [Fe(2+)(DETC)] complex as a NO radical-specific spin probe under hypoxic conditions (n=3; * p <0.05, (1- β) \geq 0.804 vs. CtrCtr; # p <0.05, (1- β) \geq 0.801 vs. HxCtr).



Supplement Fig.3: Folic acid improves pulmonary artery vasodilation

Explanted pulmonary artery (PA) segments were placed in a myograph chamber, precontracted with serotonin (Ser, 1 μ M), and increasing concentrations of the vasodilator acetylcholine (ACh, 1-300 nM) were added in the presence or absence of folic acid (FA, 50 μ M). Vessel relaxation was determined by wire myography (n=3; * p <0.05, (1- β) \geq 0.8 vs. respective Ctr).



Supplement Fig.4: Hypoxia increases eNOS monomer formation

Systemic (SA) and pulmonary arteries (PA) were explanted and exposed to hypoxia (1% oxygen, Hx) for 24 h. Total proteins were isolated in non-denaturing conditions and low temperature Western blotting was performed for the visualization of endothelial nitric oxide synthase (eNOS) monomers and dimers (upper panel). Standard Western blot with denaturated protein samples was performed for total eNOS (lower panel).