of the endothelium reduces bioavailability of the anti-atherosclerotic signalling radical nitric oxide (NO). We explored the effects of increasing insulin signalling in the endothelium, using novel transgenic mice, over-expressing Type A human Insulin Receptor (HIRECO) in the endothelium, driven by the Tie-2 promoter-enhancer.

**Methods** Semi-quantitative RT-PCR was carried out on various tissues and isolated endothelial cells from lungs to confirm significant levels of human insulin receptor mRNA while the protein expression was confirmed by western blotting on aortic sections or endothelial cells. Lucigenin-enhanced chemiluminescence was exploited to measure superoxide anion levels while; vasomotor functions were assessed in thoracic aortic rings mounted in organ baths.

**Results** No significant changes in morphological features, metabolic phenotypes or blood pressure regulation were observed between the HIRECO and wild type (WT) littermates. However, plasma insulin levels were similar following an overnight fast, but were decreased in the HIRECO after glucose challenge. HIRECO mice demonstrated significant endothelial dysfunction measured by a blunted response to acetylcholine (Emax, WT vs HIRECO: 84±5% vs 62±5% respectively, n=5, p<0.05). Endothelium-independent response to sodium nitroprusside remained unchanged. The impaired aortic response to acetylcholine was normalised by the specific NADPH oxidase inhibitor peptide, gp91ds-tat, (Emax: 93±5%; n=6, p<0.05), as well as the superoxide dismutase mimetic, Mn(III)tetraakis (1-merihyl-4-pyrydil) porphyrin pentachloride. Isolated aortic rings of HIRECO exhibited a hypercontractile response to phenylephrine compared to wild type mice (log EC50, WT vs HIRECO: 6.96±0.03 vs 7.24±0.08, n=6, p<0.01). Indeed, HIRECO mice elicited a 1.65-fold increase in the level of superoxide anion production compared to WT. Basal NO bioactivity was decreased in HIRECO compared to WT littermates (Emax upon exposure to eNOS inhibitor, L-NAMe in phenylephrine-constricted aorta, WT vs HIRECO: 144±27.9% vs 52±33%, n=5, p<0.05). However, basal eNOS phosphorylation levels in isolated endothelial cells of HIRECO mice was enhanced 1.56-fold compared to WT littermates.

**Conclusions/Implications** These data demonstrate enhanced oxidative stress in a novel murine model of increased insulin signalling in the endothelium, leading to reduced bioavailability of nitric oxide and atherosclerosis. These data also demonstrate for the first time, that increased insulin sensitivity in the endothelium, increases the generation of superoxide anion and reduces NO bioavailability.

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