Endothelial cell Nrf2-KO attenuates endothelial function and skeletal muscle antioxidant capacity

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INTRODUCTION: Endothelial cells line the inner surface of blood vessels and play a major role in modulating blood flow and gas exchange. Endothelial dysfunction is thought to be a contributor to cardiovascular disease development, and it is well-accepted that excessive reactive oxygen species (harmful molecules) likely contribute to endothelial dysfunction. The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is considered the master regulator of cellular protection in response to elevated reactive oxygen species. Therefore, Nrf2 may be a potential therapeutic target to protect against endothelial dysfunction. However, the roles of endothelial cell-specific Nrf2 on endothelial function are not known. The purpose of this study was to investigate the impacts of endothelial cell-specific Nrf2 deletion on vascular function (endothelium-dependent and endothelium-independent vasodilation) and skeletal muscle antioxidant status. **METHODS:** Leg arteries were harvested from 6-mo old C57BL/6 mice (WT, n = 6) and endothelial cell-specific Nrf2-knockout mice (Tie2-Cre-Nrf2 floxed-KO, n = 6). Endothelium-dependent vasodilation was assessed in response to flow (30 $uL \cdot min^{-1}$) and acetylcholine (ACh, 10^{-7}-10^{-3} M) with and without N_{ω}-Nitro-L-arginine methyl ester (L-NAME), and endothelium-independent vasodilation was assessed with sodium nitroprusside (SNP, 10^{-9}-10^{-4} M) using videomicroscopy. Skeletal muscle antioxidant protein expression for glutathione peroxidase-1 (GPX-1) and catalase (CAT) was assessed by immunoblotting. **RESULTS:** Endothelium-dependent vasodilation was lower in Nrf2-KO compared to WT induced by flow (WT: 34.8±2.9%, Nrf2-KO: 20.7±3.7%, $P<0.01$) and ACh (10^{-3}M, WT: 68.3±8.2%, Nrf2-KO: 44.5±7.1%, $P<0.01$). L-NAME incubation attenuated endothelium-dependent vasodilation in WT mice in response to flow (12.8±4.5%, $P<0.01$) and ACh (10^{-3} M, 19.1±4.4%, $P<0.01$) but did not change in Nrf2-KO in response to flow (15.6±6.8%, $P=0.28$) or ACh (10^{-3} M, 37.7±7.0%, $P = 0.16$). Endothelium-independent vasodilation was not different (SNP 10^{-4} M, WT: 92.7±3.6%, Nrf2-KO: 81.9± 0.2%, $P=0.157$). In addition, GPX-1 was lower in Nrf2-KO mice (WT: 0.47±0.06, Nrf2-KO: 0.001±0.003, $P<0.01$), but CAT was not different (WT: 0.16±0.04, Nrf2-KO: 0.37±0.21, $P=0.08$). **CONCLUSIONS:** Endothelial cell Nrf2 may play a key role in endothelial-mediated vasodilatory function. The nitric oxide synthase inhibitor L-NAME attenuated endothelial-mediated vasodilation in WT but not in endothelial cell Nrf2-KO. Furthermore, endothelial cell Nrf2 may play a role in skeletal muscle antioxidant homeostasis, which suggests potential systemic implications of endothelial cell Nrf2 deletion. These results collectively suggest that the endothelial cell Nrf2 system is linked to endothelial dysfunction and changes in the skeletal muscle redox environment, likely through nitric oxide- and oxidative stress-related mechanisms.