

## Chronic nitric oxide deprivation induces an adaptive antioxidant status in human endothelial cells

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The endothelium explicates its physiological functions by producing active molecules, among which nitric oxide (NO) is particularly important. By diffusing into neighboring smooth muscle cells, NO induces vasorelaxation, thereby controlling blood pressure levels. Endothelial NO also has antiaggregant activity that protects the cardiovascular system from thrombosis and acute events. It is well known that endothelial dysfunction (ED), *i.e.* an impaired function of the endothelium coupled with a reduced release of NO, is a risk factor for atherosclerosis together with a list of conditions such as hypertension, hypercholesterolemia, smoking, diabetes, and the aging process itself. These conditions are also associated with a significant increase in Reactive Oxygen Species (ROS) in the vascular wall, that may contribute to the establishment of ED and to the development of its late effect on cardiovascular system.

Previous data from our laboratory<sup>1</sup> showed an increased cell motility due to the accumulation and transcriptional activation of the Hypoxia Inducible Factor-1 $\alpha$  (HIF-1 $\alpha$ ) in an *in vitro* model of ED represented by Human Umbilical Vein Endothelial Cells (HUVECs) chronically deprived of NO by a 48-h treatment with 5 mM N<sup>G</sup>-Nitro-L-Arginine Methyl Ester (L-NAME). In addition to the enhanced cell migration, chronic L-NAME treatment also induced a significant decrease in mitochondrial energy production, and a reduced endothelial nitric oxide synthase (eNOS) protein expression.

Here, in the attempt to unravel the pathway(s) linking NO deficiency to HIF-1 $\alpha$  accumulation and activity, we focus our attention on ROS since their formation has been involved in HIF-1 $\alpha$  stabilization in normoxia. We found that acute treatment with L-NAME induced in HUVECs an early and transient burst in ROS formation that was fully prevented in the presence of the antioxidant N-acetylcysteine (NAC). HIF-1 $\alpha$  accumulation was reduced by 45% in the presence of NAC indicating that the early ROS generation was partially involved in its stabilization. On the contrary, NAC did not affect the increase in cell migration induced by L-NAME as well as the reduction in eNOS protein expression that we consistently observed in ECs chronically deprived of NO. Regarding the loss in mitochondrial energy production induced in ECs by long term L-NAME exposure, it did not require neither ROS generation nor HIF-1 $\alpha$  activity, and was not due to autophagy.

At variance with acute treatment, chronic L-NAME exposure gave rise to an antioxidant environment characterized by a reduction in cellular ROS content accompanied by an increase in superoxide dismutase-2 (SOD-2) expression and activity. Importantly, this protective response was accompanied by the nuclear accumulation of the transcription factor NF-E2-related factor-2 (Nrf2) that was fully prevented in the presence of NAC.

In conclusion, our results suggest that the main effects observed in long term NO deprived HUVECs are independent of ROS generation, and must therefore depend on other still unknown pathways triggered by NO loss. On the contrary, ROS formation appears to be totally responsible for Nrf2 accumulation. Our observation that an endothelial deficit of NO, by mimicking the *in vivo* early phase of ED, induces in ECs an adaptive cellular response in the attempt to counteract the simultaneously action of other risk factor(s) such as oxidative stress, might contribute to a better knowledge of the endothelium behavior in the absence of NO, and finally to an improved comprehension of the molecular mechanisms involved in the onset of cardiovascular pathologies.

<sup>1</sup>Cattaneo et al. (2011). PLoS One.;6(12):e29680