

# Redox and metal profiling in ischemic stroke and endothelial cells adapted to physiological oxygen levels

G.E. Mann<sup>1</sup>

<sup>1</sup>King's British Heart Foundation Centre of Excellence, School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, 150 Stamford Street, London SE1 9NH, London, United Kingdom

In vivo, vascular and other cell types are exposed to physiological oxygen levels ranging from 2 – 13 kPa O<sub>2</sub> while cells cultured in vitro in standard CO<sub>2</sub> gas incubators are routinely exposed to hyperoxic oxygen levels (18 kPa O<sub>2</sub>). Although recent evidence highlights the importance of studying cellular redox signaling under physiological O<sub>2</sub> levels, few studies have examined the effects of short- or long-term adaptation of cells to different pericellular O<sub>2</sub> levels (see Keeley & Mann, *Physiol. Reviews* 2019; 99:161-234). As molecular mechanisms regulating NRF2 mediated redox signaling have primarily been studied in cells exposed to hyperoxia, we characterised NRF2 gene targets in endothelial cells following 5 d adaptation to hyperoxia (18 kPa), physiological normoxia (5 kPa) or hypoxia (1 kPa) in an O<sub>2</sub> regulated Scitiver workstation. Gene profiling established that activation of NRF2 and induction of GSH related genes were insensitive to alterations in O<sub>2</sub> whereas upregulation of HO-1 and NQO1 in response to electrophiles or NO was diminished under 5 kPa O<sub>2</sub> due to an upregulation of the NRF2 repressor Bach 1 (Chapple et al. *Free Radic Biol Med* 2016; 92: 152-62). We further established that a PP2A mediated feedback mechanism regulates Ca<sup>2+</sup> dependent endothelial NO synthesis (Keeley et al. *FASEB J* 2017; 31: 5172-5183) and NO bioavailability (Sevimli et al. *Redox Biol.* 2022; 53: 102319) under 5 kPa O<sub>2</sub> and that enhanced SERCA activity under 5 kPa O<sub>2</sub> protects endothelial cells against calcium overload (Keeley et al. *FASEB J* 2018; 32: 2531-2538). We recently employed ICP-MS and LA-ICP-MS to measure changes in total metal content in human coronary artery endothelial cells cultured long-term under 18, 5 kPa or 1 kPa O<sub>2</sub> and then subjected to ischemia reoxygenation injury. In a recent Expert Recommendation (Sies et al. *Nature Rev Mol Cell Biol* 2022; 23: 499-515), we emphasize the importance of maintaining physiologically relevant O<sub>2</sub> levels in cell culture to mimic redox reactions associated with specific cell types in vivo.

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