

**P042** Activation of nrf2 reverses oxidative stress in endothelial cells induced by hyperglycaemia *in vitro*. Critical role of transketolase

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Hyperglycaemia in diabetes induces oxidative stress and endothelial dysfunction associated with the development of vascular disease. We used human microvascular endothelial HMEC-1 cells in hyperglycaemic culture to model this effect. Incubation of HMEC-1 cells with high concentrations of D-glucose led to increased formation of reactive oxygen species (ROS). Similar concentrations of L-glucose had no effect. Increased ROS formation was prevented by p-trifluoromethoxycarbonyl cyanide phenylhydrazone, rotenone and myxothiazole, suggesting that dysfunction of mitochondria were a primary source of the increased ROS and electron flux through complex I and III contributed to this effect. Activation of NF-E2-related factor-2 (nrf2) by sulforaphane (SFN) prevented increased ROS, whereas ablation of nrf2 with antisense oligodeoxynucleotides (ODN) increased ROS formation. SFN induced nuclear translocation of nrf2 in normoglycaemia and hyperglycaemia and also increased nrf2 expression in hyperglycaemia. Nrf2 regulates the transcription of a battery of protective and metabolic enzymes including the pentosephosphate pathway enzyme transketolase (TK). Increased expression of TK was found to be pivotal in the protective effect of SFN as knockdown of TK expression with antisense ODN abolished the prevention of ROS formation by SFN. Therefore, physiological and pharmacological activation of nrf2 has a critical role in the prevention of oxidative stress linked to hyperglycemia.