The effect of CO-releasing molecules on the activation of the hypoxia response

Emma Klemola, Joona Tapio, Peppi Koivunen

Biocenter Oulu and Faculty of Biochemistry and Molecular Medicine, University of Oulu, Finland

Metabolic syndrome (MetS) is a cluster of risk factors that increase the risk for developing cardiovascular disease (CVD) and type 2 diabetes (T2D). The main cellular response to insufficient availability of oxygen (O_2), hypoxia, is regulated by the hypoxia-inducible factor (HIF). The stability of HIF is regulated by three O_2-dependent HIF prolyl 4-hydroxylases (HIF-P4Hs 1-3). HIF upregulates genes involved in key biological pathways including erythropoiesis and metabolism. Mice with a genetically activated HIF response due to partial depletion of HIF-P4H-2, are leaner and resist various metabolic conditions. Hemoglobin (Hb) is the main carrier of O_2 in the cardiovascular system, and its levels mediate tissue oxygenation. Lower Hb levels within normal variation are associated with an overall healthier metabolic profile, and these associations associate with higher expression of HIF target genes. Carbon monoxide (CO) has a 220-fold affinity to Hb compared to O_2. Binding of CO to Hb decreases levels of O_2Hb and tissue oxygenation. CO-releasing molecules (CORMs) release low amounts of CO *in vivo*. CORMs have been reported to improve various metabolic conditions in obese mice, via an uncoupling of mitochondrial respiration, leading to a metabolic switch towards glycolysis. In this study we used mice to evaluate whether long-term CORM treatment leads to activation of the HIF pathway via reduced levels of circulatory O_2Hb and tissue oxygenation. We hypothesized that the reported beneficial effects of CORMs on metabolism are mediated by HIF. In the study wild-type mice were fed a high-fat diet and administered either CORM-401 or vehicle 3 times a week for 6-8 weeks. A glucose tolerance test was conducted to evaluate glucose handling. Tissues from mice were collected and quantitative real-time PCR was performed to analyze mRNA expression levels of HIF target genes. Histological and immunohistochemical analyses were used to further evaluate tissue level effects of CORM-401.