低氧/厌氧产品案例——低氧与糖尿病研究

文章题目：Repression of hypoxia-inducible factor-1 contributes to increased mitochondrial reactive oxygen species production in diabetes

低氧诱导因子-1 的抑制可增加糖尿病患者线粒体活性氧的产生

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工作站使用情况：Ruskinn INVIVO2

使用气体浓度：低氧（1% O_2）

摘要：线粒体活性氧(ROS)的过度产生是糖尿病并发症发生的中心机制。最近，缺氧已被证实在糖尿病中发挥额外的致病作用。在这项研究中，我们假设 ROS 过量产生是继发于高血糖抑制缺氧诱导因子-1 (HIF-1)导致的缺氧反应受损。研究人员分析了暴露于低氧环境下的健康人和 1 型糖尿病患者血液中的 ROS 水平。在糖尿病小鼠模型中，研究了肾 mIMCD-3 细胞和肾脏中 HIF-1、葡萄糖水平、ROS 产生及其功能后果的关系。研究发现在糖尿病患者中，低氧暴露会增加循环 ROS，但在非糖尿病患者中则不会；高糖通过 HIF 脯氨酸羟化酶(PHD)依赖机制抑制了糖尿病动物的缺氧细胞和肾脏中的 HIF-1 的表达；HIF-1 信号通路受损，通过丙酮酸脱氢酶激酶 1 (PDK1)介导的线粒体呼吸增加，促进线粒体 ROS 的过量产生；在糖尿病患者中，HIF-1 功能的恢复降低了持续高血糖情况下 ROS 的过度产生，并对细胞凋亡和肾损伤具有保护作用。表明 HIF-1 的抑制在糖尿病线粒体 ROS 过剩产生中起着核心作用，并且是糖尿病并发症的潜在治疗靶点。

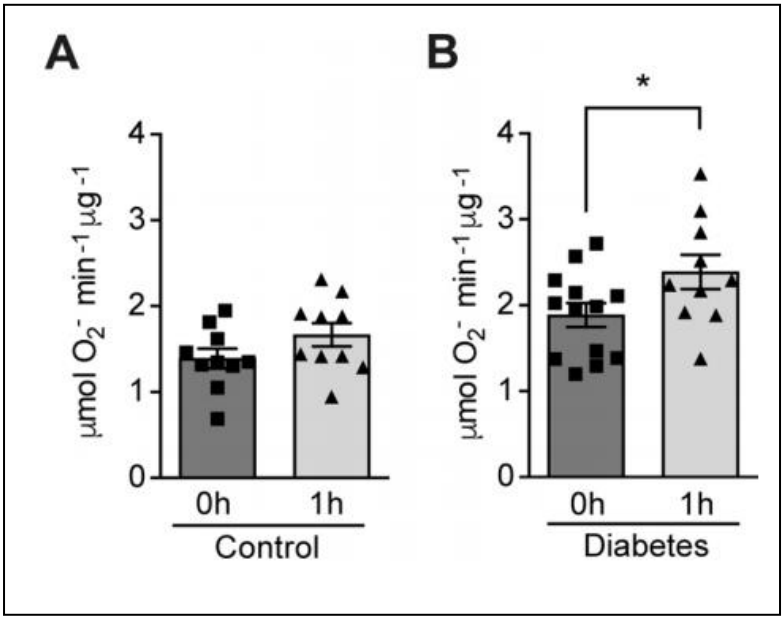

Figure 1. Hypoxia increases circulating ROS in patients with diabetes but not in control subjects without diabetes. Healthy controls (A) and subjects with type 1 diabetes (B) were exposed to intermittent hypoxia for 1 hr. Peripheral blood was taken before (0h) and after (1h) hypoxia exposure. ROS levels were analyzed using Electron Paramagnetic Resonance (EPR) Spectroscopy with CPH spin probes (n = 10–13). Data are represented as mean ± SEM. *, $p < 0.05$ analysed using unpaired two-sided Student's t-test. This figure has one figure supplement

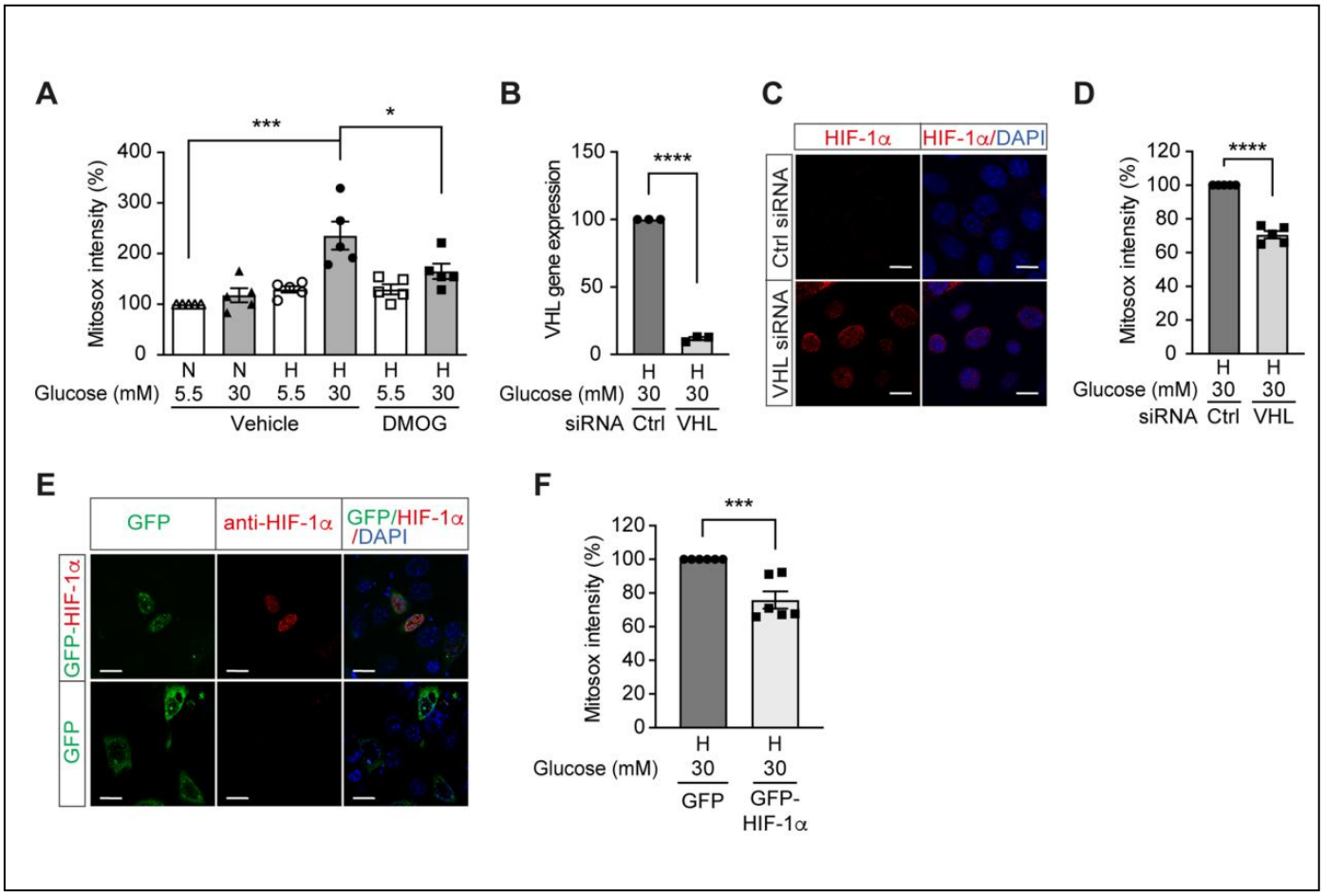

Figure 3. High glucose levels induce mitochondrial ROS overproduction in hypoxia, which can be rescued by promoting HIF-1 function. (A) Mitochondrial ROS levels were measured as mitosox intensity in mIMCD-3 cells cultured in normal (5.5 mM) or high (30 mM) glucose media in normoxia (N) or hypoxia (H) for 24 hr in the presence of DMOG or vehicle (n = 5). (B–D) mIMCD-3 cells were transfected with von Hippel–Lindau tumour suppressor (VHL) or control (Ctrl) siRNA, and exposed to hypoxia (H) and 30 mM glucose for 24 hr. VHL gene expression (B, n = 3), endogenous HIF-1α expression (red) and DAPI staining (blue) (C) and mitochondrial ROS levels (D, n = 5) were assessed using quantitative RT-PCR, fluorescent immunocytochemistry and flow cytometry, respectively. (E and F) mIMCD-3 cells were transfected with plasmids encoding GFP or GFP-HIF-1α,and exposed to hypoxia and 30 mM glucose for 24 hr. (E) Expression of GFP and GFP-HIF-1α (green) were detected using confocal microscopy. The nuclear HIF-1α expression was confirmed by immucytochemistry using anti-HIF-1α antibody (red). Nuclei were stained blue with DAPI. (F) Mitochondrial ROS levels are shown (n = 6). The mitosox intensity of cells cultured under control conditions were considered as 100%.

参与者间歇性低氧(13% O_2) 1 小时，研究发现糖尿病患者缺氧时外周血 ROS 水平升高。然而，在血糖正常的对照组中，缺氧并没有改变 ROS 水平（图 1AB）；

我们进一步研究了 HIF-1 对糖尿病患者线粒体 ROS 产生的影响。肾髓质上皮细胞 mIMCD-3 细胞低氧（1% O_2）培养 24 h；研究发现暴露于高糖水平和缺氧的细胞线粒体 ROS 水平升高，HIF-1 激活剂 DMOG 通过激活 HIF，减少缺氧时高糖水平引起的线粒体 ROS 过度产生(图 3A)；在暴露于缺氧和高糖水平的 mIMCD3 细胞中，沉默 VHL 基因(图 3B)后，核 HIF-1α表达增加(图 3C)，导致线粒体 ROS 下降(图 3D)；在低氧和高血糖条件下，过表达 HIF-1α的 mIMCD3 细胞线粒体 ROS 减少(图 3E-F)；表明缺氧和高血糖联合暴露的细胞中 ROS 的过度产生依赖于 HIF-1 功能的损害，当 HIF-1 活性保持时，ROS 可以被减弱。