Hypoxia. 2. Hypoxia regulates cellular metabolism

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Wheaton WW, Chandel NS. Hypoxia. 2. Hypoxia regulates cellular metabolism. Am J Physiol Cell Physiol 300: C385–C393, 2011. First published December 1, 2010; doi:10.1152/ajpcell.00485.2010.—Adaptation to lowering oxygen levels (hypoxia) requires coordinated downregulation of metabolic demand and supply to prevent a mismatch in ATP utilization and production that might culminate in a bioenergetic collapse. Hypoxia diminishes ATP utilization by downregulating protein translation and the activity of the Na-K-ATPase. Hypoxia diminishes ATP production in part by lowering the activity of the electron transport chain through activation of the transcription factor hypoxia-inducible factor-1. The decrease in electron transport limits the overproduction of reactive oxygen species during hypoxia and slows the rate of oxygen depletion to prevent anoxia. In this review, we discuss these mechanisms that diminish metabolic supply and demand for adaptation to hypoxia.

sodium-potassium-ATPase; electron transport chain; oxygen levels

Themes

METAZOANS have developed mechanisms for adaptation to lower oxygen levels (hypoxia) (97). One of these mechanisms is metabolic suppression as measured by a decrease in the mitochondrial oxygen consumption of cells (i.e., the respiratory rate) occurring at oxygen levels (1–3% O2) well above the threshold where oxygen becomes limiting (<0.3% O2) to cytochrome c oxidase (COX) (complex IV), a phenomenon referred to as oxygen conformance (53). Complex IV is the main enzyme in the electron transport chain (ETC) that utilizes oxygen and couples it to the generation of ATP (oxidative phosphorylation). Based on the ability of deep sea diving mammals to survive long periods of hypoxia, Peter Hochachka (52) elegantly surmised that animals tolerant of hypoxia have the ability to undergo oxygen conformance as an adaptive mechanism. Furthermore, studies of intact myocardium indicated that regional decreases in oxygen delivery can elicit local decreases in oxygen demand as reflected by a diminished contractile function (2, 3, 75, 88). However, most studies in isolated cells failed to observe oxygen conformance under hypoxic conditions (6, 8, 109), even though metabolic suppression was observed during anoxia (0% O2) (5, 7). Because this metabolic suppression occurred at oxygen levels where COX is inhibited, the oxygen conformance phenomenon remained theoretical. In the 1990s, multiple studies observed that isolated cells are able to diminish the respiratory rate at oxygen levels in the range of 1–3% O2 by decreasing the cellular ATP-utilizing processes (metabolic demand) (22, 23, 31, 96). Previous studies failed to observe a decrease in the respiratory rate during hypoxia because isolated cells were exposed to hypoxia for seconds (8, 109). However, when cells are exposed to chronic hypoxia (minutes to hours), they display a reversible suppression of oxygen consumption without any detectable cell injury (22, 23, 31, 96). In recent years, it has been observed that hypoxic suppression of the respiratory rate also involves the activation of the transcription factor hypoxia-inducible factor (HIF-1), which regulates ATP generation (metabolic supply) (98). In this review we focus on the mechanisms underlying the coordinated regulation of metabolic demand and supply during hypoxia resulting in metabolic adaptation to maintain cellular homeostasis.

What Controls the Respiratory Rate of Cells?

To understand how hypoxia causes a decrease in the respiratory rate, we briefly review the factors that are responsible for the control of respiration. The respiratory rate is the rate of oxygen consumption by mitochondria in living cells. Although mitochondria consume the majority of cellular oxygen, there are other cellular processes that also consume oxygen. It is important to separate nonmitochondrial oxygen consumption from mitochondrial oxygen consumption in living cells by measuring oxygen consumption in the presence of mitochondrial inhibitors, such as the complex I and III inhibitors rotenone and antimycin, respectively. Mitochondrial respiration proceeds when reducing equivalents (NADH and FADH2) are generated by the tricarboxylic acid (TCA) cycle (93). The electrons generated from NADH and FADH2 undergo oxidation to NAD+ and FAD+ by complex I and II of the ETC located in the inner mitochondrial membrane. Subsequently, these electrons are sequentially transferred to complex III, cytochrome c, and complex IV, which transfers the electrons to molecular oxygen. The movement of electrons through the ETC is coupled to proton translocation from the mitochondrial matrix across the inner membrane to the intermembrane space, creating an electrochemical gradient of protons consisting of a pH gradient and a membrane potential. These protons can either run down their gradient through the F1Fo-ATP synthase (complex V) or the protons can leak back across the inner membrane to the mitochondrial matrix (14). Complex V couples the transport of protons to the generation of ATP from ADP and phosphate (Pi). ATP generated in the matrix is exported to the cytosol in exchange for ADP by the adenine nucleotide translocase (ANT) located in the inner membrane. ATP in the cytosol is utilized by variety of processes such as the Na-K-ATPase, which regenerates ADP pools. ADP is then transported back to the mitochondria, so the cycle can continue. Thus mitochondrial oxygen consumption is a combination of coupled respiration and uncoupled respiration. Coupled respiration is the rate of oxygen consumption by complex IV coupled to the generation of ATP synthesis by complex V. By contrast, uncoupled respiration is the rate of oxygen consumption by complex IV that is not coupled to the generation of ATP due to the proton leak. Most cells display high levels of coupled respiration. The notable exception are
brown fat cells that exhibit uncoupled respiration due to an abundance of uncoupling proteins, which increase proton leak by allowing protons to flow back into mitochondria without driving the generation of ATP (40).

Initial work in the 1950s by Chance and Williams (29) proposed that the respiratory rate in cells is controlled by cellular ATP utilization. In this model, the increase in cellular ATP utilization decreases cytosolic ATP levels and increases cytosolic ADP and P_i levels. The rise in cytosolic ADP levels leads to a rise in mitochondrial ADP via the increased activity of the adenine nucleotide carrier. The increased mitochondrial ADP concentration stimulates the ATP synthase to augment the rate of ATP synthesis, which results in a decrease in the mitochondrial membrane potential, thus stimulating the respiratory chain to consume oxygen. However, in the ensuing decades it has become clear that other factors also control the respiratory rate, such as the availability of reducing equivalents provided by the TCA cycle, electron flux through the ETC, the availability of ADP provided by cellular ATPases, the adenine nucleotide translocase, and the magnitude of the proton leak (64). How to quantitatively measure the relative control exerted by these processes on the respiratory rate remained elusive until the pioneering work of Kacser and Burns (66) in the early 1970s describing metabolic control analysis. Their work describing metabolic control analysis helped them determine control coefficients of a particular protein over metabolic flux through a pathway. The control coefficient is the percent change in the respiratory rate divided by the percent change in the protein or complex causing the change in the respiratory rate. For example, if a 10% change in the ANT results in a 10% change in the respiratory rate, then the control coefficient of the proton leak would be 1. However, if the 10% change resulted in 1% change in the respiratory rate, then the control coefficient would be 0.1. In the late 1980s, Brand and colleagues began to utilize metabolic control analysis on isolated rat hepatocytes exposed to ambient air and determined that 15–30% of respiration is controlled by the NADH supply (these include pyruvate supply to the mitochondria, the TCA cycle, and any other NADH-supplying reaction); 20% is controlled by the proton leak; and 0–15% is controlled by the ETC (16, 17). The remaining 50% is controlled by ATP synthesis, transport, and utilization of which in most cells the dominant factor is the rate of ATP utilization by cellular processes. Chandel et al. (31) performed metabolic control analysis in isolated rat hepatocytes and determined that there was no major difference between normoxia and hypoxia with respect to factors that controlled the respiratory rate-specifically NADH supply, ETC activity, and ATP utilization.

**Hypoxia Diminishes NADH Supply to the ETC Through HIF-1**

Under normal oxygen conditions, pyruvate derived from glycolysis is transported into the mitochondria and converted into acetyl-CoA by the pyruvate dehydrogenase (PDH) complex (112). Acetyl-CoA combines with oxaloacetate to form citrate as the initial step in the TCA cycle. The reducing equivalents NADH and FADH2 generated by the TCA cycle drive ETC to generate ATP for energy and ROS for signaling, while the TCA cycle intermediates are utilized for biosynthetic processes such as lipid synthesis (107). During acute exposure to hypoxia (seconds to minutes), cells do not display changes in carbon flux into the TCA cycle. However, as cells are exposed to prolonged hypoxia (hours), the activation of the transcription factor HIF-1 diminishes the carbon flux into the TCA cycle (71, 82, 89). HIF-1 is a heterodimer of two basic helix loop-helix/PER-SIM-ARNT(PAS) proteins: HIF-1α and the aryl hydrocarbon nuclear trans-locator (ARNT or HIF-1β) (104). Both HIF-1α and HIF-1β protein subunits are expressed ubiquitously, but the stability of each protein is differentially regulated by oxygen levels (104). HIF-1α protein is rapidly degraded under normal oxygen conditions, whereas HIF-1β protein levels are constitutively stable. During normoxia, HIF-1α protein undergoes hydroxylation at two proline residues 402 and 564 within the oxygen-dependent degradation domain of HIF-1α (61, 63, 83). This hydroxylation reaction is catalyzed by prolyl hydroxylases (PHDs) that require Fe (II), oxygen, and 2-oxoglutarate (21, 41). Hydroxylated proline serves as a binding site for the von Hippel-Lindau protein (pVHL), the substrate recognition component of the VBC-CUL-2 E3 ubiquitin ligase complex (55). Once bound, pVHL tags HIF-1α with ubiquitin thereby targeting it for proteasomal degradation (84). Hypoxia prevents hydroxylation of HIF-1α protein resulting in the stabilization of the protein. Hypoxia diminishes hydroxylation collectively by I) limiting the oxygen as a substrate (67) and 2) through an increase in the release of superoxide from mitochondrial complex III (10) (Fig. 1). The mechanisms linking mitochondrially generated superoxide with the stabilization of the HIF are an area of active investigation. According to one hypothesis, complex III-generated superoxide is converted to hydrogen peroxide in the cytosol where it oxidizes the PHDs cofactor Fe (II) to Fe (III) making the PHDs catalytically inactive (45).

The two major targets of HIF-1 that decrease pyruvate conversion to acetyl-CoA are lactate dehydrogenase A

![Fig. 1. Hypoxia activates hypoxia inducible factor-1 (HIF-1). HIF1α subunit is hydroxylated by pyruvate dehydrogenase 2 (PHD2) at distinct proline residues thereby targeting the protein for von Hippel-Lindau protein (pVHL)-mediated proteasomal degradation. Hypoxia concomitantly diminishes PHD2 activity and induces the production of mitochondrial reactive oxygen species (ROS) at complex III resulting in an inhibition of hydroxylation of HIF1α subunit. Once HIF1α subunit is stabilized, it binds with HIF-β and p300 coactivators to hypoxic response elements (HREs) in the promoters and enhancers of target genes that modulate metabolism.](http://ajpcell.physiology.org/)
(LDH-A) and pyruvate dehydrogenase kinase 1 (PDK1) (43, 71, 89). PDK1 phosphorylates and inactivates the catalytic subunit of PDH (54). An increase in PDK1 protein levels decreases PDH activity thereby preventing the conversion of pyruvate to acetyl-coA while also driving pyruvate conversion to lactate. LDH-A converts pyruvate to lactate by utilizing the NADH generated from glycolysis (103). This is an efficient manner to oxidize cytosolic NADH to NAD^+ (110), which allow for an increase in the glycolytic rate because the regeneration of NAD^+ is a rate-limiting step for glycolysis. Normally, reducing equivalents from cytosolic NADH are shuttled into the mitochondria via the malate aspartate shuttle where NADH is regenerated to NAD^+ and subsequently shuttled back to the cytosol. The ectopic expression of LDH-A alone is sufficient to increase lactate production (102). The coordinated upregulation of PDK1 and LDH-A diverts pyruvate from fueling the mitochondria to generation of lactate (Fig. 2). The decreased acetyl-coA levels in the mitochondria diminish TCA cycle activity, resulting in reduced generation of mitochondrial NADH and FADH2 levels and reduced electron flux through the ETC. Based on this model, we hypothesize that cells that are efficient in conducting fatty acid oxidation to generate mitochondrial acetyl-coA would be refractory to PDK1/LDH-A dependent decrease in TCA cycle activity. We predict that only cells that utilize pyruvate as the major carbon source to fuel the TCA cycle would be sensitive to inhibition of the TCA cycle by the PDK1/LDH-A axis.

Hypoxia Diminishes Electron Transport

Multiple studies throughout the 1970s and 1980s examined the oxygen dependence of the ETC (108, 109). These studies observed that exposure of cells to acute hypoxia (minutes to seconds) did not attenuate the flux of electrons through the ETC nor increase NADH levels in mitochondria. However, in the mid-1990s we reported that isolated mitochondria decreased coupled respiration and that isolated COX decreased its maximal velocity ($V_{\text{max}}$) when exposed to chronic hypoxia (2 h) (30, 32). Thus there is an intrinsic oxygen dependence of COX during prolonged hypoxia. Another important regulator of COX activity is nitric oxide (NO) (36). Low concentrations (nM range) of NO reversibly inhibit isolated COX by competing with oxygen (15, 35, 80). Under aerobic conditions, oxygen levels are high enough to prevent NO from inhibiting COX activity (36). However, as oxygen levels fall, the low levels of NO are sufficient to inhibit COX activity. Low levels of NO under normoxia do not injure cells. However, the same low levels of NO are sufficient to inhibit respiration and initiate cell death under hypoxia (1.5% O2) (76). In the absence of NO, hypoxia alone does not have any deleterious effects on cells. It is likely that COX activity is compromised in inflammatory conditions where NO levels are high with concomitant tissue hypoxia. Furthermore, the NO-generating enzyme inducible NO synthase (iNOS) is a target of HIF-1 (65, 85). We propose that hypoxia diminishes COX activity by decreasing the $V_{\text{max}}$ of COX activity and by increasing NO levels to inhibit COX activity. Although this mechanism diminishes COX activity during hypoxia, the activity cannot be diminished to the point where respiration fails to meet the basal metabolic demands of cells. Therefore, cells ensure optimal COX activity during hypoxia by activating HIF-1 to induce subunit switch from COX4–1 subunit to COX4–2 (44). COX has 13 subunits, of which the three catalytic subunits COX I-III are encoded by mitochondrial DNA. The remaining regulatory 10 subunits including COX4 subunits are encoded by nuclear DNA. HIF-1 induces both the expression of the COX4–2 subunit and the mitochondrial protease LON, which targets COX4–1 subunit degradation to complete the switching of the COX4 subunits during hypoxia. Recently, another mechanism to downregulate the ETC is the finding that micro-RNA 210 (mir-210) blocks the expression of the iron-sulfur cluster assembly proteins ISCU1/2, which are required for the functions of complex I, COX subunit 10, aconitase, and subunit D of succinate dehydrogenase (28, 33, 42, 91). Using a miRNA microarray, Kulshreshtha et al. (74) first discovered that miR-210 is regulated by hypoxia, and recently it was proposed to be the major micro-RNA upregulated during hypoxia. HIF-1, but not HIF-2,
is responsible for the induction of mir-210 during hypoxia (57). The ectopic expression of mir-210 is sufficient to decrease mitochondrial respiration and upregulate glycolysis (33). Thus there are multiple mechanisms by which HIF-1 can coordinately diminish electron flux through the ETC (Fig. 3).

**Hypoxia Diminishes Cellular ATP Utilization**

A major ATP consumer is the Na-K-ATPase, which can account for 20–70% of the oxygen expenditure of mammalian cells (86). Na-K-ATPase is a plasma membrane protein that transports Na\(^+\) and K\(^+\) across the plasma membrane to maintain ionic gradients (69). The Na-K-ATPase is a heterodimer composed of \(\alpha\) and \(\beta\)-subunits. The \(\alpha\)-subunit is a transmembrane protein that cleaves high-energy phosphate bonds and exchanges intracellular Na\(^+\) for extracellular K\(^+\) by coupling the exchange to the hydrolysis of ATP. The smaller \(\beta\)-subunit is a glycosylated transmembrane protein that controls the heterodimer assembly and insertion into the plasma membrane. Multiple investigations have reported that hypoxia reversibly suppresses Na-K-ATPase activity (27, 38, 81, 90, 110). Initially, the Na-K-ATPase catalytic activity was proposed to be regulated through changes in substrate affinity. However, recent reports have demonstrated that the Na-K-ATPase activity is regulated by phosphorylation, which results in either endocytosis or exocytosis of this molecule from the plasma membrane. Exposure to hypoxia for as little as 15 min decreases Na-K-ATPase activity due to endocytosis of the \(\alpha\)-subunit from the plasma membrane (37). AMP-activated protein kinase (AMPK) directly phosphorylates PKC-\(\xi\) at Thr410 to promote Na-K-ATPase endocytosis during hypoxia (46) (Fig. 4). Whereas hypoxia does not affect the total Na-K-ATPase protein abundance in cell lysates during this short exposure (<1 h), chronic exposure causes hypoxia-induced endocytosis of the Na-K-ATPase triggering pVHL-mediated degradation of plasma membrane Na-K-ATPase in a HIF independent manner (113).

The other major consumer of ATP is mRNA translation to protein. Multiple studies have demonstrated that hypoxia inhibits mRNA translation (4, 11–13, 58, 59, 72, 73, 77). The initiation of mRNA translation is regulated by the active eukaryotic initiation factors eIF4F and eIF2 (51). The suppression of mammalian target of rapamycin (mTOR) complex 1 (mTORC1) and pancreatic eIF2\(\alpha\) kinase (PERK) are the key regulators of diminished global translation observed during hypoxia (78). mTORC1 consists of the catalytic subunit mTOR and several regulatory proteins: raptor, PRS40, DEPTOR, and mLST8/G\(_{\text{p64}}\) (99). mTORC1 controls translation by phosphorylating the p70 ribosomal S6 kinase (S6K1) and 4E-BP1, the eukaryotic initiation factor 4E (eIF4E) binding protein 1. mTORC1 is activated by the small Ras-like GTPase Rheb, which is inhibited by TSC2. During growth factor stimulation, AKT phosphorylates and inhibits TSC2 thereby allowing Rheb to activate mTORC1. Hypoxia causes rapid (within 15 min) and reversible hypophosphorylation of mTORC1 effectors 4E-BP1 and S6K (4, 58). The rapid inhibition of mTOR is HIF
independent and occurs through the activation of AMPK (4, 77). AMPK phosphorylates TSC2 and raptor resulting in suppression of mTORC1 (100). The loss of TSC2 effectively suppresses mTOR inhibition during hypoxia (20). The sustained inhibition of mTORC1 over hours involves the HIP-dependent transcription of REDD1, which releases TSC2 from its growth factor-stimulated association with 14–3–3 proteins to allow TSC2 inhibition of mTORC1 (19). AMPK can also phosphorylate eEF2 kinase (eEF2K) resulting in eEF2 phosphorylation and inhibition of translation elongation (18, 56). Another negative regulator of mTORC1 during hypoxia is the promyelocytic leukemia protein, which promotes sequestration of mTOR to the nucleus during hypoxia (11). Finally, mRNA translation during hypoxia is decreased by activation of PERK (12, 13, 72, 73). PERK activation results in eIF2α phosphorylation, which inhibits mRNA translation initiation (95). PERK activation during hypoxia requires mitochondrial ROS (79) (Fig. 4). Collectively, these studies highlight that multiple mechanisms contribute to the decrease in global protein synthesis during hypoxia.

It is becoming increasingly clear that hypoxic activation of AMPK diminishes processes that consume ATP such as Na-K-ATPase and global protein synthesis allowing metabolic adaptation to decreasing oxygen levels. AMPK is a heterotrimeric serine/threonine consisting of a catalytic α subunit and two regulatory β- and γ-subunits (68). AMPK is ubiquitously expressed and is activated upon nutrient-limiting conditions. AMPK has evolved to serve as a metabolic checkpoint in cells by halting cell growth and suppressing ATP-consuming processes (48). AMPK is activated either by an increase in the AMP-to-ATP ratio through the protein kinase LKB1 or an increase in calcium through the Ca2+/calmodulin-dependent kinase (CaMKK) (49, 50, 101, 111). Multiple studies have demonstrated that acute hypoxia (minutes to several hours) does not alter AMP-to-ATP ratio but does robustly activate AMPK (39, 77). This is not surprising since oxygen becomes limiting to the generation of ATP as cells approach anoxic conditions. Since COX is not limited under hypoxic conditions, there is no reason for cells to experience a decrease in the ability to generate ATP during acute hypoxia (32). As it is well established that cells under hypoxia elicit an increase in calcium (105), the more likely scenario is that CaMKK is the upstream kinase required for hypoxic activation of AMPK. However, the experimental evidence for CaMKK-dependent hypoxic activation of AMPK is still lacking. It is also possible that LKB1 activates AMPK independent of changes in the AMP-to-ATP ratio during hypoxia. Interestingly, the increase in calcium during hypoxia has been suggested to be under the control of mitochondrial complex III-generated ROS (106). We recently demonstrated that rapid activation of AMPK during hypoxia is dependent on mitochondrial complex III ROS (39). This is consistent with the observations that the suppression of ATP-consuming processes is dependent on mitochondrial ROS generation. Therefore, metabolic demand during hypoxia is likely initiated by mitochondrial ROS activation of AMPK (Fig. 4).

Physiological Consequences of Metabolic Suppression

An advantage of cells reducing their respiratory rate as oxygen delivery diminishes would be to delay the onset of local tissue anoxia. Furthermore, an attenuated respiratory rate might render cells less susceptible to anoxia-induced cell injury. Enhanced resistance to anoxic injury might develop if the adapted cells could selectively inhibit facultative metabolic activities related to organ function, thereby preserving limited energy production for obligatory functions necessary for cell survival (52). Metabolic suppression is observed in intact hearts where regional decreases in myocardial oxygen delivery results in decreased contractile activity and oxygen consumption, a phenomenon termed hibernating myocardium (2, 3, 75, 88). In the early 1990s, Bristow and colleagues (2, 3) observed diminished contraction in swine myocardium due to a reduction in epicardial flow. Downey and Lee (75) also found evidence of metabolic suppression in isoprenaline-stimulated canine myocardium during partial ischemia. Importantly, no detectable cellular injury was observed, and contractile function was recovered upon restoration of blood flow to the ischemic regions. Thus hibernating myocardium represents a state where a reduction in ATP demand in response to a decrease in regional oxygen supply serves to protect the myocardium from developing ischemic injury when the blood flow is severely reduced.

The other major advantage of diminishing respiratory rate during hypoxia is to limit the production of ROS (98). Within minutes, hypoxia increases ROS production from complex III to stabilize the HIF-1α protein (10). ROS at low levels initiate cellular signaling events but at high levels can initiate damage and cell death (47). Interestingly, cells maintained for several days under hypoxia (chronic hypoxia) decrease the levels of ROS back to normoxic levels (71). However, HIF-1 knockout cells continue to display increased ROS during hypoxia resulting in cell death, and the addition of ROS scavengers decrease hypoxia-induced cell death in HIF-1 knockout cells indicating that HIF-1 dampening of ROS levels is an adaptive mechanism (71). Furthermore, expression of PDK1 in HIF-1-deficient cells reduces ROS levels and apoptosis during hypoxia (71). COX4–2 subunit switching to COX4–1 subunit during hypoxia also affects ROS production, as hypoxic cells in which COX4–2 is diminished but still express COX4–1 display increased ROS levels and apoptosis (44). The expression of

**Fig. 5.** Hypoxia depresses the respiratory rate for metabolic adaptation. The downregulation of ATP demand and supply diminishes the respiratory rate, which prevents the overproduction of ROS and depletion of oxygen under hypoxic conditions.
mir210 also decreases ROS generation during hypoxia by decreasing ETC activity (28, 33, 42). Collectively, these in vitro data indicate that reduced flux of carbons through the TCA cycle and subsequent electron flux through the ETC during hypoxia is an adaptive response to diminish overproduction of ROS (Fig. 5).

Presently, the full in vivo significance of HIF-1-mediated decrease in ROS production is not fully understood. To date, the best evidence suggesting that HIF-1-mediated depression of the ETC and ROS generation is relevant in vivo comes from studies on ischemia-reperfusion injury. Tissues exposed to ischemia followed by restoration of blood supply undergo oxidative stress-induced damage called reperfusion injury. Multiple studies have demonstrated that reducing flux through the ETC during ischemia and reperfusion phases prevents reperfusion injury (24). If reducing ETC flux prevents injury, then one would predict that activation of HIF-1 repression of the TCA cycle and the ETC would prevent reperfusion injury. To test this possibility Semenza and colleagues (62) have utilized heterozygous HIF-1α (HIF1α+/−) mice because homozgyous HIF-1α mice are embryonic lethal. They observed that exposure of wild-type mice to intermittent hypoxia resulted in protection of isolated hearts against ischemia-reperfusion injury 24 h later (26) and that this cardiac protection was lost in HIF1α+/− mice (25). Subsequently, multiple studies have demonstrated that activation of HIF-1 pharmacologically using PHD inhibitors or genetically through small interfering RNA (siRNA) against PHDs or via constitutive expression of HIF-1α also prevents ischemia-reperfusion injury in the heart (9, 70, 87). PHD2 hypomorph mice are also protected against ischemia-reperfusion injury in the heart (60). Recent studies using PHD1 null mice further corroborate these studies. Aragones et al. (1) observed that ablation of PHD1 results in increased in expression of PDK1 and PDK4 leading to repression of mitochondrial metabolism. PHD1 null mice were found to be remarkably protected against ischemia-reperfusion injury in the liver. Short-term inhibition of PHD1 through siRNA also prevents liver ischemia-reperfusion injury (94). The loss of PHD1 production correlated with a decrease in oxidative stress due to depression of mitochondrial metabolism. However, it is not yet clear whether these HIF-1-induced cytoprotective effects observed against ischemia-reperfusion injury are primarily due to diminished mitochondrial metabolism and ROS generation as HIF-1 can also activate a variety of other protective mechanisms such as the production of the hormone erythropoietin (EPO), which is sufficient to prevent ischemia-reperfusion injury (25). It will be interesting to test whether HIF-1-mediated protection against ischemia-reperfusion injury acts through the inhibition of mitochondrial metabolism and ROS generation through induction of mir-210 or PDK1.

In conclusion, there is an ongoing lively debate in the bioenergetic field over what controls the rate of cellular respiration during hypoxia. Historically, investigators attributed the majority of metabolic control to the activity of cellular ATPases, which inhibit respiration by decreasing the availability of ADP. Recent work from several groups of investigators has challenged this model, suggesting that reducing the supply of carbon to the TCA cycle and reducing electron flux through the ETC can also inhibit respiration. This debate is roughly analogous to the ongoing debate over what controls the United States economy; one camp argues for a strategy that increases demand through public sector spending while the other argues for a strategy that increases the money supply through lowered interest and tax rates. Currently, available data suggests that the cell employs strategies regulating both supply and demand simultaneously to reduce metabolism during hypoxia to maintain homeostasis. The downregulation of ATP utilization by bioenergetic processes, including protein synthesis and Na-K-ATPase activity, is coordinated with a reduction in carbon flux through the TCA cycle and electron flux through the ETC by activating HIF-1-mediated induction of PDK1 and mir-210 as well as subunit switching of COX subunit 4. This coordinated downregulation of demand and supply prevents a mismatch in ATP production and utilization that might culminate in bioenergetic collapse while limiting the production of ROS and slowing the rate of oxygen depletion under ischemic conditions (Fig. 5).

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