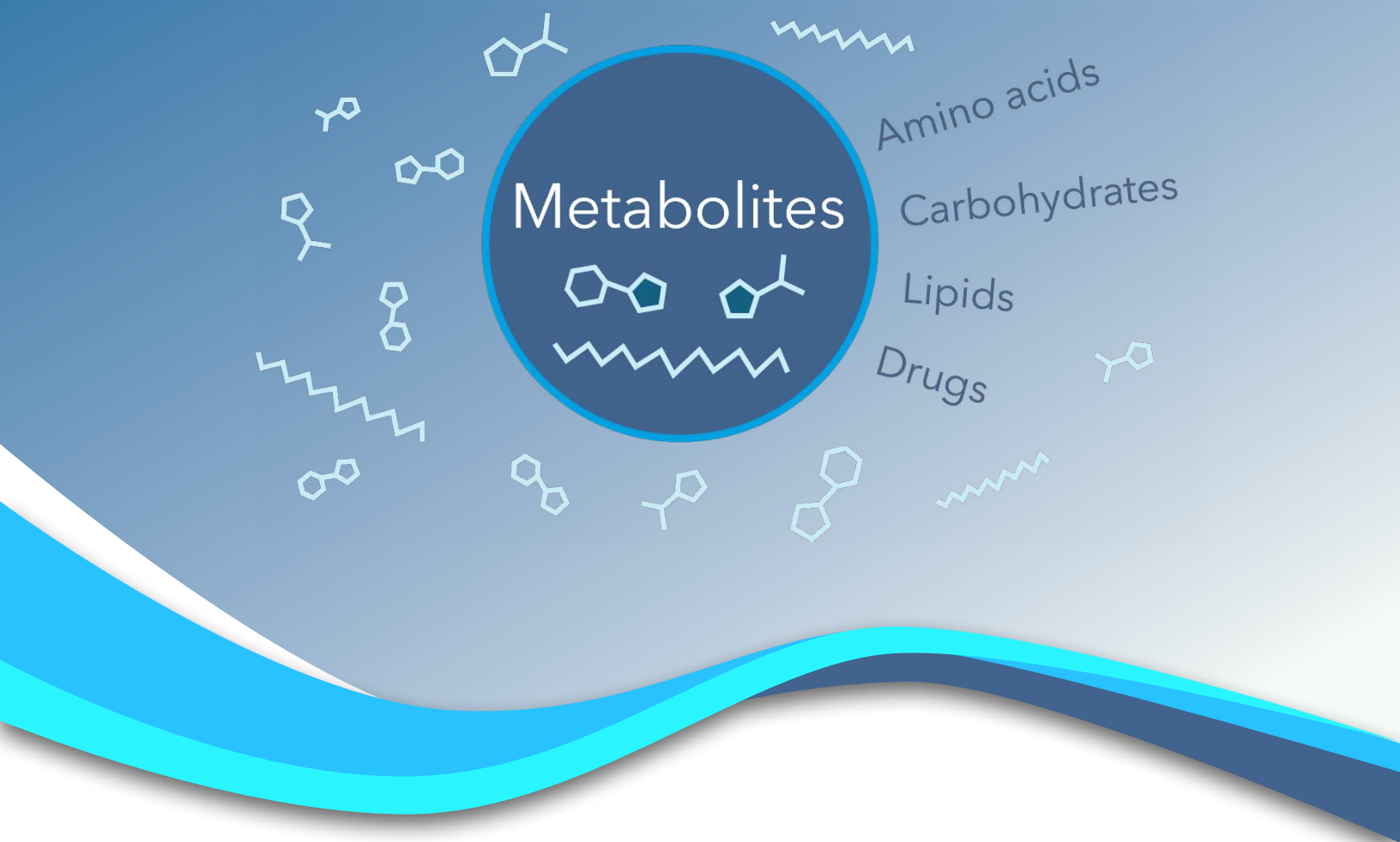




Technical Note

Effects of Hypoxia and Physioxia on the Metabolome



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Introduction

The cellular metabolome, which refers to the complete set of small-molecule metabolites within a biological system, is highly sensitive to changes in oxygen availability. Hypoxia (low oxygen conditions less than 1%) and physioxia (physiological oxygen levels, typically lower than atmospheric 21% oxygen) significantly alter metabolic pathways, influencing energy production, redox balance, biosynthetic processes, and signaling mechanisms. Understanding these metabolic shifts is crucial for fields such as cancer biology, regenerative medicine, and physiology, where oxygen tension plays a pivotal role.

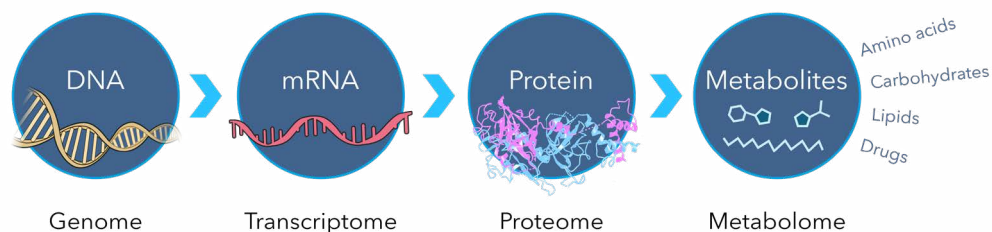


Figure 1. The genome, transcriptome, proteome, and metabolome represent different layers of biological information in an organism. The genome is the complete set of an organism's DNA, containing all the genetic instructions necessary for its structure, function, and development. The transcriptome refers to the collection of RNA molecules transcribed from the genome, reflecting which genes are actively being expressed at any given time. The proteome encompasses the full range of proteins produced by a cell or organism, providing insights into cellular function and regulation. The metabolome is the comprehensive set of small molecules, or metabolites, such as sugars, lipids, and amino acids, that result from biochemical processes within the organism and reflect its metabolic state. Together, these "omes" form an interconnected system that helps scientists understand the dynamic processes underlying life and disease, enabling therapeutic discoveries.

Oxygen Levels and Cellular Metabolism

Cells *in vivo* often exist in physioxia conditions rather than the standard 21% atmospheric oxygen used in traditional cell culture. For example, tissue oxygenation ranges from ~1–8% O₂ in most organs. Hypoxia, typically defined as <1% O₂, induces a metabolic shift that can be adaptive or pathological, depending on the context. Oxygen-dependent enzymes, particularly those in mitochondrial respiration and redox reactions, exhibit altered activity under these conditions.

Metabolic Adaptations to Hypoxia

1. Glycolytic Shift and Energy Metabolism

Under hypoxic conditions, cells reduce their reliance on oxidative phosphorylation (OXPHOS) and increase anaerobic glycolysis. This is primarily driven by the stabilization of hypoxia-inducible factors (HIFs), which upregulate glycolytic enzymes (e.g., hexokinase, phosphofructokinase, and lactate dehydrogenase) and glucose transporters (GLUT1, GLUT3). Consequently, lactate production increases, leading to extracellular acidification and metabolic reprogramming often observed in cancer cells (Warburg effect).

2. Tricarboxylic Acid (TCA) Cycle Rewiring

Hypoxia leads to a suppression of the TCA cycle due to limited oxygen availability for oxidative metabolism. Intermediates such as succinate and fumarate accumulate, which can further stabilize HIFs by inhibiting prolyl hydroxylases (PHDs). Additionally, cells may divert glutamine metabolism toward reductive carboxylation to maintain biosynthetic processes, supplying citrate for lipid biosynthesis.

3. Lipid Metabolism Adjustments

Lipid metabolism is significantly altered under hypoxia. Cells reduce β -oxidation of fatty acids due to impaired mitochondrial respiration and instead increase lipid storage as triglycerides. Hypoxia also promotes *de novo* lipogenesis via activation of sterol regulatory element-binding proteins (SREBPs), contributing to membrane biosynthesis and cellular adaptation to low oxygen levels.

4. Amino Acid Metabolism Alterations

Amino acids play critical roles in hypoxic adaptation, particularly glutamine, serine, and aspartate. Glutamine metabolism supports TCA cycle anaplerosis, nucleotide biosynthesis, and redox balance through glutathione production. Hypoxia can also impair aspartate synthesis due to reduced electron transport chain (ETC) activity, limiting purine and pyrimidine synthesis, thereby affecting cell proliferation.

5. Redox Homeostasis and Reactive Oxygen Species (ROS)

Hypoxia affects mitochondrial ROS production in a biphasic manner: acute hypoxia may transiently increase ROS, which serves as a signaling molecule for HIF stabilization, while chronic hypoxia often results in reduced ROS due to ETC downregulation. Cells enhance antioxidant defenses by upregulating enzymes such as superoxide dismutase (SOD) and glutathione peroxidase, preventing oxidative damage.

Physioxia: A More Accurate Cellular Model

Physioxia represents the actual oxygen conditions experienced by cells in tissues, making it a more relevant model than normoxia (21% O₂) for *in vitro* studies. Cells cultured under physioxic conditions exhibit metabolic profiles distinct from both normoxic and hypoxic states:

- **Reduced Glycolysis:** Compared to hypoxia, cells under physioxia rely more on mitochondrial respiration while maintaining an efficient glycolytic flux.
- **Optimized Mitochondrial Function:** Mitochondria under physioxia operate at a more physiological rate, reducing artificial oxidative stress observed in hyperoxic (21% O₂) conditions.
- **Balanced Lipid and Amino Acid Metabolism:** Unlike hypoxia, physioxic conditions maintain a metabolic balance that supports normal proliferation and differentiation in stem cells and tissue-specific cells.

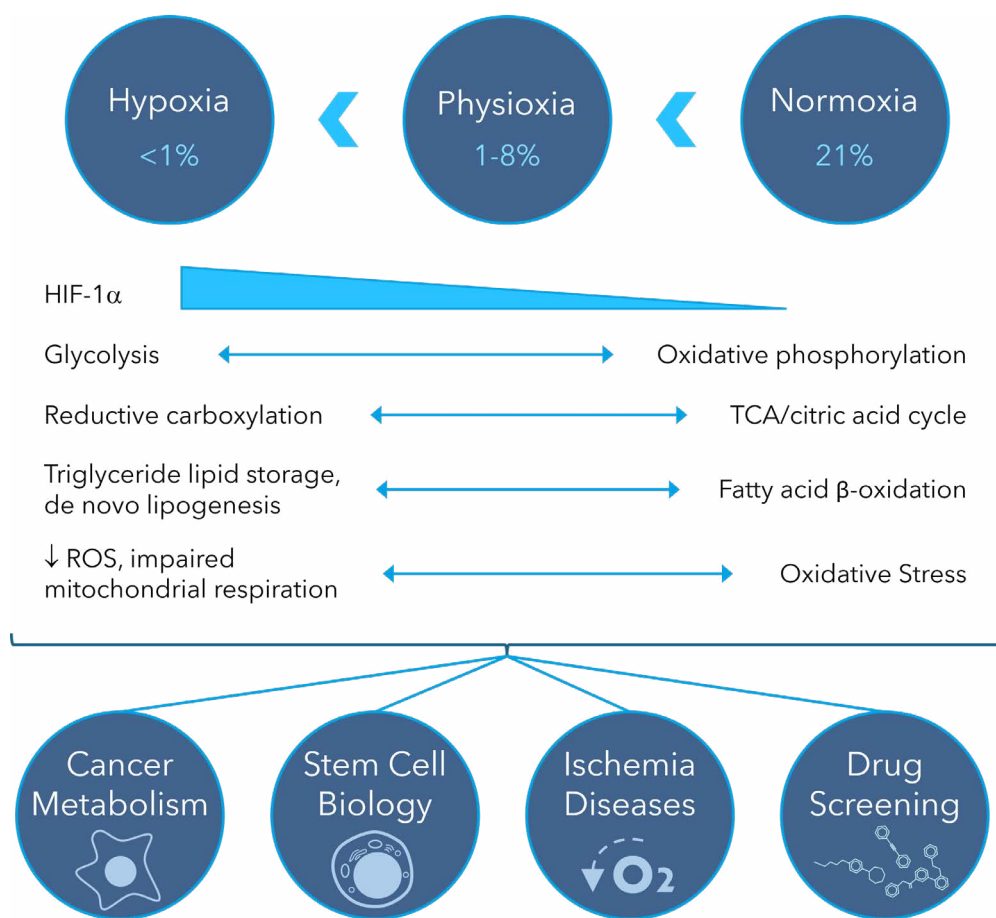


Fig. 2. The “Goldilocks” of Physioxia. In between the two extremes of normoxia (21% oxygen) and hypoxia (<1% oxygen) lies the region of physiological oxygen where cells thrive, balanced in between the two distinct metabolic outcomes. Understanding the divergent behavior of cells and cell models under each condition has significant impact on how researchers can interpret disease and develop therapeutics.

Implications for Disease and Therapeutics

- 1. Cancer Metabolism:** Tumors often experience heterogeneous oxygen levels, leading to metabolic plasticity that enables survival and resistance to therapy. Hypoxia-induced glycolytic dependency provides a therapeutic target through metabolic inhibitors such as lactate dehydrogenase A (LDHA) inhibitors.
- 2. Stem Cell Biology:** Physioxia maintains stemness and differentiation potential better than normoxia, making it crucial for regenerative medicine applications.

3. **Ischemia and Hypoxia-related Diseases:** Understanding metabolic shifts in ischemic conditions can aid in designing treatments for stroke, myocardial infarction, and other hypoxia-driven pathologies, as well as reperfusion therapies.
4. **Impact on Drug Screening and Discovery:** Oxygen levels significantly influence drug metabolism and efficacy. Incorporating physioxia and hypoxic conditions into drug screening platforms enhances the predictive value of preclinical models, leading to more accurate assessments of drug responses, particularly for anticancer and ischemia-targeted therapies.

Conclusion

Hypoxia and physioxia, relative to normoxia, exert profound effects on cellular metabolism, influencing pathways related to energy production, biosynthesis, and redox balance. Recognizing these metabolic alterations is essential for improving experimental models, developing targeted therapies, and advancing biomedical research.

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