The Basic Science of Metabolism in Pulmonary Arterial Hypertension

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Pulmonary hypertension (PH) encompasses a group of progressive and incurable cardiopulmonary disorders characterized by pulmonary vascular remodeling and increased mean pulmonary artery pressure leading to right heart failure. Emerging data suggest a common etiology for the reported diverse molecular and physiological abnormalities observed in the pulmonary vasculature: the metabolic theory of PH. This theory proposes that aberrations in metabolism and mitochondrial function are a major underlying cause for the cellular and organ level PH phenotype. Additionally, the metabolic theory of PH provides a rationale for the observed metabolic defects in other organs and systems outside of the pulmonary vasculature, including the right ventricle (RV), immune system, and skeletal muscle. However, whether these metabolic changes are driving disease and the timing and extent of these aberrations are still unknown. This review highlights: 1) key examples of metabolic alterations in the pulmonary vasculature, RV, inflammatory cells, and skeletal muscle; 2) examples of promising therapeutic interventions directly modifying metabolism; and 3) key remaining questions about the role of metabolic remodeling in PH.

Pulmonary hypertension (PH) is a chronic, progressive, and incurable disease of the pulmonary vasculature and right heart that is characterized by abnormal pulmonary artery (PA) vasoconstriction and remodeling. These processes lead to elevated PA pressure and result in increased right ventricular (RV) afterload and failure.[1,2] Based on the current World Health Organization (WHO) clinical classification, PH is grouped into 5 distinct types based on clinical presentation, disease association, and histopathology.[3] An estimated 70 million patients in the United States with common cardiopulmonary diseases such as chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, sleep-disordered breathing, and left heart disease have PH or are at major risk for developing PH.[4-9] Interest has centered on WHO Group 1 PH (known as pulmonary arterial hypertension [PAH]), a less common but highly aggressive and fatal type of PH (survival is just 60% at 3 years[2,10]) that can arise spontaneously, hereditarily, or as a complication of drug and toxin use, congenital heart disease, HIV infection, autoimmune disease, or liver cirrhosis.[3] In fact, most of our current understanding of PH pathology and therapeutic interventions comes from the study of PAH. However, despite advances in the treatment of PAH, several challenges exist in diagnosing and treating this disease, contributing to its high mortality rate: patients often do not develop symptoms until late in disease progression, current diagnostic approaches primarily rely on invasive hemodynamic assessment, and histopathological assessment of the degree of pulmonary vascular remodeling in a living patient is not feasible.[2] Furthermore, currently available therapies primarily target vasodilatory responses and are unable to reverse or prevent disease progression. Because of these hurdles, diagnoses are often made at the end stages of PAH progression when current therapeutic interventions have limited impact on survival.[11] Finally, while PAH is a more aggressive form of PH, there are no approved drugs to treat other and more common forms of PH (including those due to left heart disease or chronic lung diseases). Therefore, there is a critical need for approved therapies to affect pulmonary vascular remodeling in addition to vasomotor tone and be effective in other PH patient populations.

PAH development is thought to be initiated by an injury or insult to the pulmonary vasculature resulting in a wave of PA endothelial cell (PAEC) apoptosis.[1] The surviving PAECs are dysfunctional, proproliferative, and antiapoptotic.[12] Subsequently, these remaining abnormal PAECs produce dysregulated autocrine and paracrine signals and recruit inflammatory cells, processes that induce the hyperproliferation of PA smooth muscle cells (PASMCs) and other cells of the PA wall, resulting in abnormal vasoconstriction, vascular remodeling, and eventual obliteration

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of pulmonary vessels.[13] Although PAH has long been thought to be a disease of the pulmonary vasculature, increasing evidence shows that other extrapulmonary tissues and cells aberrantly behave in PAH and can adversely affect the pulmonary vasculature, thus contributing to the progression of disease.[14] For instance, the RV of patients with PAH undergoes molecular and physiological changes as it adapts to the changes in the pulmonary vasculature. Importantly, RV function is the major determinant of survival in PAH and a significant percentage of patients demonstrate a significant decrease in RV ejection fraction despite having a positive hemodynamic response to therapy.[15,16] This suggests there may be intrinsic and RV-specific mechanisms independent of PA remodeling, which determine the progression of RV failure in PAH. Furthermore, patients with PAH who do not have a coexisting autoimmune disorder have evidence of increased activated immune cells that contribute to a persistent inflammatory state and promote fibrosis.[14] Finally, the skeletal muscle from nonobese or diabetic patients with PAH exhibits evidence of metabolic abnormalities as seen in diabetes mellitus and metabolic syndrome, such as increased glucose and insulin levels and increased lipid deposition.[14]

While our knowledge of the molecular determinants of PAH has become increasingly complex, until recently there has not been a concerted effort to establish a unifying pathway or master regulator responsible for driving the complex and multivariate pathogenic processes in PAH. One recent unifying pathway with growing supportive evidence is the metabolic theory, which proposes that altered metabolism is a major driving force behind PAH pathogenesis.[14,17] This theory posits that the diverse molecular signatures of PAH are related to and a consequence of metabolic changes in the pulmonary vasculature and extrapulmonary tissues. In the current review, we focus on the key findings from the lungs, RV, inflammatory cells, and skeletal muscle of disease models and patients that have been pivotal in our understanding of the role of altered metabolism in PAH (Figure 1). We highlight promising therapeutic interventions targeting metabolism in PAH and conclude by identifying remaining questions surrounding the metabolic theory of PAH.

ALTERED METABOLISM IN PAH: FROM MOUSE TO MAN

Cellular metabolism encompasses the biochemical pathways and basic components through which the cell generates new molecules, breaks down and removes waste, generates energy, and promotes survival. Many of these reactions occur in the mitochondria, which are responsible for integrating environmental and molecular cues (ie, hypoxia, endoplasmic reticulum stress, inflammation) with the available cellular fuel supply inputs of oxygen, glucose, lipids, and amino acids to optimize the cellular responses of energy production, cell survival, growth, and/or death.[18] In many cell types, oxidative phosphorylation is the principle energy-generating pathway, although it should be noted that PAECs and activated inflammatory cells (in particular, T cells) primarily rely on glycolysis for normal energy production.[17,19-21] Glycolysis is the oxygen-independent metabolic pathway that converts glucose into pyruvate to generate energy (adenosine triphosphate [ATP]) and reduced nicotinamide adenine dinucleotide (NADH).[14,22] This pathway occurs in the cytoplasm of the cell, and pyruvate generated from glycolysis can then be shuttled to the mitochondria where it is converted to acetyl-CoA by pyruvate dehydrogenase (PDH), linking glycolysis to the citric acid cycle and subsequent energy production via oxidative phosphorylation.[22,23] It should be noted that in addition to glucose, acetyl-CoA may be derived from fats and proteins through other pathways as energy sources become more or less bioavailable.[24] Acetyl-CoA next enters the citric acid/Krebs cycle where it can generate energy precursors NADH and succinate, which are then oxidized (via the oxidative phosphorylation pathway) and enter the electron transport chain, finally resulting in the generation of ATP.[23] Glucose oxidation refers to the processes through which a glucose molecule is converted to pyruvate via glycolysis, then converted to acetyl-CoA and enters the citric acid cycle and is oxidized via the oxidative phosphorylation pathway, finally resulting in the generation of energy/ATP.[17,22]

During acute stress such as hypoxia, the cell can rapidly alter its metabolic pathways to adjust and adapt as needed to the stressor to ensure cell survival.[25] For example, PASMCs and fibroblasts grown under hypoxic conditions undergo a metabolic shift from oxidative phosphorylation to glycolysis to sustain ATP production.[26] Typically, once the stressor is removed from the cell (ie, the cells return to normoxia and/or the hypoxia regulator hypoxia inducible factor [HIF] is degraded), the metabolic activity of the cell returns to the more efficient oxidative phosphorylation pathway.[24] If stress is sustained and cell survival is no longer energy efficient or feasible, the cell will undergo apoptosis (or other types of cell death).[27] However, in PAH, a growing body of evidence suggests that the metabolic pathways of the cell are unable to return to homeostatic conditions.[24,28] This metabolic shift is similar to what has been described in cancer, where cancer cells shift from oxidative phosphorylation to glycolysis and lactic acid fermentation even in the absence of hypoxia to produce ATP (termed *aerobic glycolysis* or the Warburg effect).[29] While energetically less efficient, the Warburg effect facilitates cell survival and supports neoplastic growth.[17] Cellular metabolism is an intricate and interconnected network of cycles and pathways that will not be presented in detail in this review. For a more in-depth examination of the role of mitochondrial remodeling, metabolic signaling, and how these pathways are altered in PAH, we refer the reader to several excellent reviews.[14,17,21,28,30-32]

Altered Metabolism in the Pulmonary Vasculature in Experimental Models of PH

Metabolic changes in PASMCs: As previously mentioned, under homeostatic conditions PASMCs rely on oxidative phosphorylation via glucose oxidation for energy production

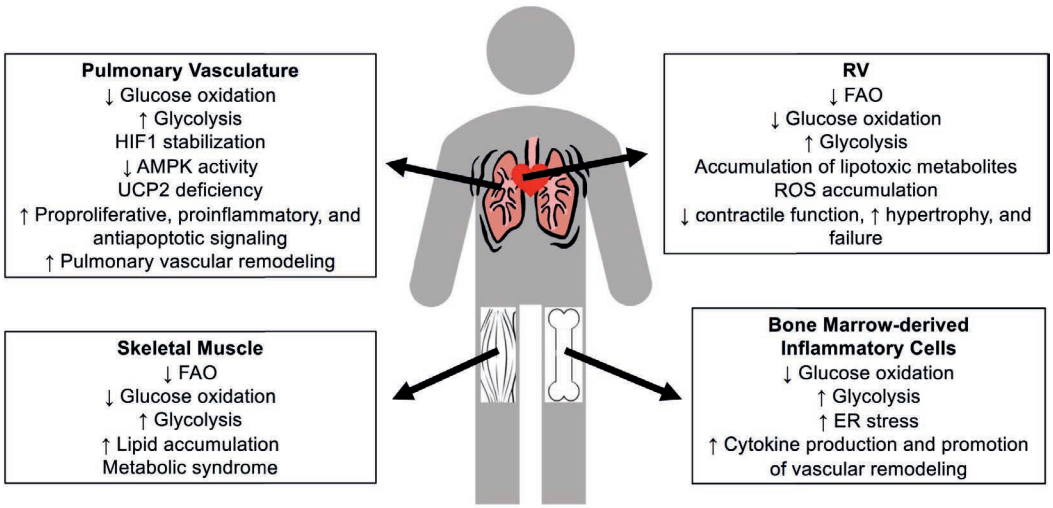

Figure 1: Metabolic Remodeling Occurs in Multiple Tissues in PAH. Current evidence has identified aberrant mitochondrial and metabolic changes in multiple organs/tissues of patients with PAH characterized by an increased shift to aerobic glycolysis and a suppression of oxidative phosphorylation (ie, glucose oxidation or fatty acid oxidation [FAO]) to meet cellular and tissue energy demands). Major metabolic changes are identified (boxed text) in each tissue. The exact contribution of each of these molecular metabolic changes to PAH pathogenesis is unknown and remains to be determined. Functionally, these changes promote a proproliferative, proinflammatory, and antiapoptotic cell phenotype and promote pulmonary vascular remodeling and right ventricular (RV) failure. Abbreviations: AMPK: 5′ adenosine monophosphate-activated protein kinase; ER: endoplasmic reticulum; FAO: fatty acid oxidation; HIF-1: hypoxia-inducible factor 1; ROS: reactive oxygen species; UCP2: uncoupling protein 2.

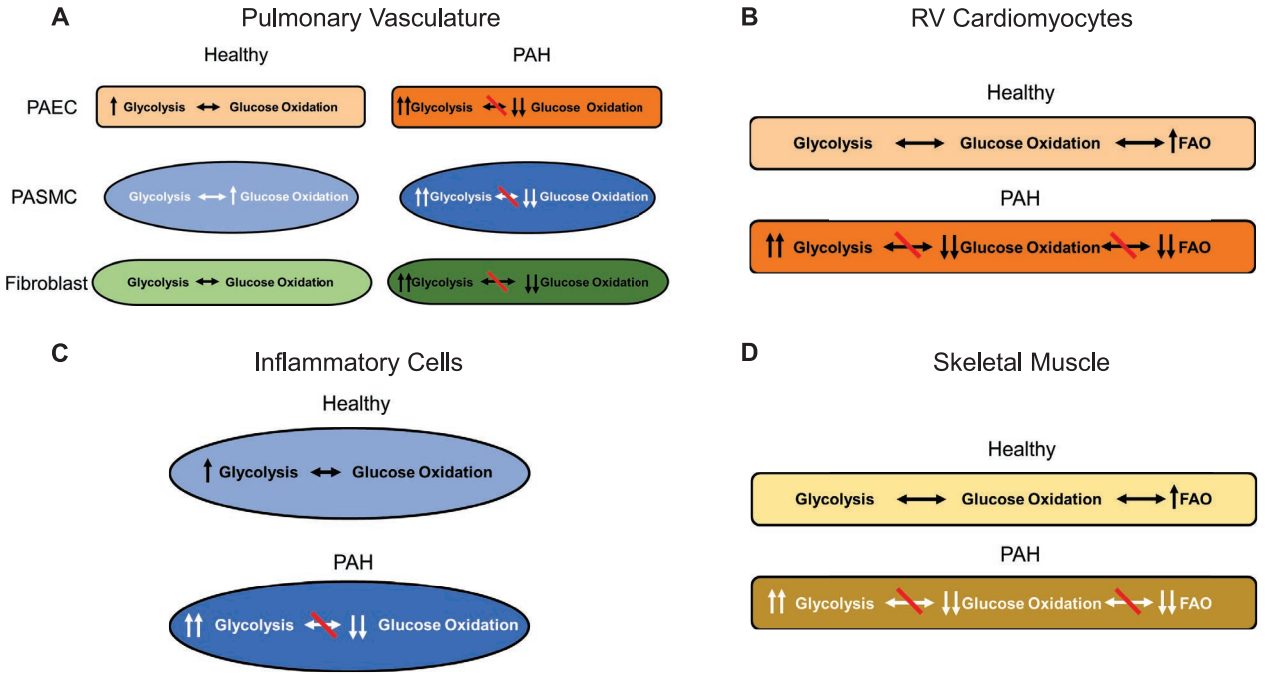

Figure 2: Simplified Schematic of the Proposed Cellular Metabolic Shift From Oxidative Phosphorylation to Glycolysis in PAH. (A) Pulmonary artery endothelial cells (PAECs), pulmonary artery smooth muscle cells (PASMCs), and fibroblasts are the major cell types of the pulmonary vasculature. Current evidence suggests these cells undergo a metabolic shift from a balance between glycolysis and oxidative phosphorylation (ie, glucose oxidation) to suppression of oxidative phosphorylation and enhancement of aerobic glycolysis to meet the energy needs of the cell in PAH. How the metabolic remodeling in one cell type affects the others, and whether the driver and molecular pathway leading to metabolic remodeling in all pulmonary vascular cell types is the same is currently unknown and is the subject of multiple investigations. (B) In the nondiseased right ventricle (RV), fatty acid oxidation (FAO) is the dominant source of energy production. Glucose oxidation and FAO exist in a mutually competitive cycle known as the Randle cycle. In PAH, RV injury stimulates glycolytic gene expression and leads to the repression of FAO. However, as the RV decompensates, glucose oxidation is also reduced leading to increased glycolysis. It is not known if the endothelial cells and fibroblasts in the RV undergo similar metabolic changes in PAH; however, drugs targeting the Randle cycle are currently being investigated. (C) Activated inflammatory cells are already highly glycolytic. However, there is evidence in PAH that aerobic glycolysis is further enhanced in inflammatory cells and glucose oxidation is suppressed. These include resident and recruited macrophages, dendritic cells, T cells, B cells, and mast cells. (D) Skeletal muscles in PAH patients have evidence of metabolic syndrome and lipid deposition, as well as suppression of mitochondrial function, glucose and FAO oxidation, and upregulation of glycolysis. Arrows indicate the preferred metabolic pathway of each cell type; red slash indicates suppression/inhibition of metabolic cycle interaction or shifting between glycolysis and the Krebs cycle to generate energy.

(Figure 2A). Conversely, mitochondria in PAH PASMCs exhibit suppressed glucose oxidation and increased aerobic glycolysis.[14,17,33] Importantly, the metabolic shift from glucose oxidation to glycolysis, promoted by HIF-1 stabilization even under normoxic conditions, leads to nuclear factor of activated T-cells (NFAT) signaling, tyrosine kinase activity, inflammation, and altered mitochondrial fission, fusion, biogenesis, or enzyme activity, all converging to inhibit the glucose oxidation master regulator PDH.[17,34,35] This inhibition prevents pyruvate from entering the Krebs cycle and suppresses the electron transport chain, leading to a decrease in diffusible redox signaling, reactive oxygen species (ROS), and active metabolite α-ketoglutarate (αKG). In turn, this can promote the continued stabilization and activation of transcription regulators NFAT or HIF-1 even in the absence of hypoxia, and subsequently drive pulmonary vascular remodeling. αKG plays a central role, as it is required for the activity of HIF-1 inhibitor prolyl hydroxylase.[36] It can also promote epigenetic changes by modifying histone methylation.[17] Other downstream effects stemming from the suppression of glucose oxidation include inhibition of voltage-gated potassium (Kv) channels and intracellular calcium accumulation.[17,31] A resulting feed-forward loop then sustains the hyperproliferative, antiapoptotic PAH cellular phenotype. Interestingly, pharmacologic activation of PDH using dichloroacetate (DCA) treatment (a pyruvate dehydrogenase kinase [PDK] inhibitor that redirects glucose metabolism away from lactate production and toward mitochondrial oxidative metabolism) prevents and reverses established PH in monocrotaline and Fawn-hooded rat models.[34,37] Further demonstrating this pathway's importance to the PAH cellular phenotype, knockdown of PDK or knockout of malonyl-CoA decarboxylase (an enzyme mediating fatty acid oxidation, that when silenced promotes glucose oxidation through the Randle cycle) prevent and reserve established PH and normalize PAH PASMCs through upregulation of Kv channels, degradation of HIF-1 and NFAT, decreased intracellular calcium, decreased proliferation, and increased apoptosis.[33,37,38] Taken together, the data demonstrating that targeting and stimulating PDH activity reverses established PAH identify this as an attractive metabolic therapeutic target. Indeed, clinical studies have yielded promising results (reviewed in the following Clinical Trials section).

Another interesting and targetable metabolic regulator in PAH is uncoupling protein 2 (UCP2), a mitochondria-specific calcium transporter that increases mitochondrial calcium levels.[39] Many mitochondrial enzymes (including PDH) are dependent on calcium for their activation; therefore, UCP2 deficiency inhibits their function.[39] Indeed, UCP2-deficient mice develop spontaneous PH, providing a direct link between mitochondria dysfunction and PH in mice. PASMCs from UCP2-deficient mice also show evidence of the hyperproliferative PH cellular phenotype with NFAT and HIF-1 stabilization and activation, as well as decreased glucose oxidation.[39]

Metabolic changes in PAECs: While most studies have focused on the role of metabolic changes in PASMCs in PAH, increasing evidence suggests a substantial change in PAH PAEC metabolic signaling as well (Figure 2A). Vascular ECs have a lower number of mitochondria and a higher rate of glycolysis compared to PASMCs, and this is proposed to confer several advantages, namely decreased generation of ROS, preserved amount of oxygen for transfer to perivascular cells, and use of lactate as a proangiogenic signaling molecule.[40,41] Despite the increased rate of glycolysis compared to PASMCs, PAH PAECs demonstrate a further increase in glycolysis, as well as decreased mitochondrial dehydrogenase activity, mitochondrial number, and oxygen consumption versus control PAECs. Furthermore, cellular ATP does not decrease in PAH PAECs exposed to hypoxia, whereas hypoxic control PAECs exhibit a 35% decrease.[42] Subsequent studies identified HIF-1 as a driver of the metabolic switch from mitochondrial glucose oxidation to glycolysis in PAH PAECs.[43]

More recently, studies using microarray and metabolic intermediate analyses in human pulmonary microvascular ECs (PMVECs) overexpressing PAH-associated mutations in the bone morphogenetic protein receptor 2 (BMPR2) showed that PMVECs with BMPR2 mutations demonstrate increased glycolysis and reduced glucose oxidation compared to controls, but also upregulation of the pentose phosphate shunt and pathways associated with glutamine and aspartate metabolism.[44]

In addition to repressed glucose oxidation and upregulated pentose phosphate shunt pathways, PAH PAECs also exhibit aberrant nitric oxide (NO) production.[45] This reduction in NO is thought to be due to downregulation of AMP-activated protein kinase (AMPK), observed in PAECs from chronically hypoxic mice and patients with PAH. While AMPK activity has been associated with antiapoptotic effects in PAECs, it also exerts proapoptotic effects in PASMCs, suggesting AMPK may be a key mediator in both processes postulated to be necessary for PAH pathogenesis. In fact, endothelial-specific deletion of AMPK in hypoxic mice exacerbates the PH phenotype and promotes PASMC proliferation.[45] In PASMCs, in response to the inhibition of oxidative phosphorylation and subsequent reduced ROS generation, AMPK activity is associated with inhibition of Kv channels.[46] Interestingly, metformin, an antidiabetic drug and known stimulator of AMPK, protects against PH in chronic-hypoxia and MCT-induced PH models.[45,47-49] Taken together, these recent studies suggest that AMPK may serve as an attractive drug target. Currently, a phase 1 clinical trial is underway to examine the effects of metformin on pulmonary vascular function in patients with PAH (NCT01884051, discussed in the Clinical Trials section).[31] As in PASMCs, the metabolic changes in PAECs not only affect cellular bioenergetics and growth but also influence multiple signaling responses. This includes increased sensitivity to apoptosis, calcium release affecting contraction, proliferation, and migration, as well as alterations in ROS signaling.[50]

Metabolic changes in fibroblasts: Recent data suggest that in addition to PASMCs and PAECs, PAH fibroblasts also have metabolic abnormalities that directly promote a proinflammatory and proproliferative environment.[21,26] For example, fibroblasts from calves with severe hypoxia-induced PH and humans with PAH undergo increased aerobic glycolysis even under normoxic conditions.[51] This is accompanied by increased NADH levels and levels of the NADH/NAD+ ratio sensor C-terminal binding protein 1 (CtBP1). Pharmacologic inhibition of NADH in hypoxic mice corrects the glycolytic shift of fibroblasts, attenuates proinflammatory signaling and PASMC and fibroblast proliferation, and decreases pulmonary vascular remodeling.[51]

Evidence of Altered Metabolism in the RV

While metabolic abnormalities in the pulmonary vasculature are increasingly recognized as regulators of PAH etiology, more recently the role of metabolism in the RV is emerging as a critical disease modifier (Figure 2B).

Under basal conditions, the RV derives 60% to 90% of its energy through fatty acid oxidation and the remaining 10% to 40% through glucose oxidation and glycolysis.[30] The mutually competitive balance between these metabolic pathways, known as the Randle cycle, reciprocally regulates the activation and inhibition of each (ie, as fatty acid oxidation increases in the RV, glucose oxidation decreases and vice versa).[32] Recently, evidence from animal models and human RV tissue demonstrated increased lipid accumulation and decreased fatty acid oxidation in PAH. As a result of lipid accumulation in the RV, lipotoxic metabolites and ROS accumulate and initiate apoptosis in the surrounding tissue.[52,53]

In addition to lipid accumulation, the PAH-RV exhibits abnormalities in glucose oxidation. As the RV hypertrophies and compensates, HIF-1 is stabilized; this is associated with preserved glucose oxidation.[17,32] However, as the RV decompensates, HIF-1 is inhibited, glucose oxidation and fatty acid oxidation are suppressed, and glycolysis is stimulated, resulting in continued RV hypertrophy and reduced RV contractile function.[54] However, DCA treatment in monocrotaline-PH and PA banded rats restores cardiac output and RV function, inhibits PDK activity, stimulates glucose oxidation and partially rescues Kv1.5 function.[54] Finally, in a PA banding model, ranolazine, a fatty acid oxidation inhibitor, increases glucose oxidation, decreases RV hypertrophy and improves RV function.[55] However, despite these interesting data, the long-term effect of stimulating glucose oxidation at the expense of fatty acid oxidation (via the Randle cycle) on RV function is not yet clear.[52]

Altered Metabolism in Inflammatory Cells

A possible underlying mechanism for the observed metabolic remodeling in multiple organs in PAH patients is that these changes are mediated through circulating factors, and immune cells in particular. Resident and recruited macrophages, dendritic cells, T cells, B cells, and mast cells have all been identified as perpetuators of the inflammatory response in PAH lungs.[14] Most immune cells are highly glycolytic upon activation and this activation is associated with suppressed mitochondrial function (Figure 2C).[17] However, there is evidence indicating that circulating immune cells from patients with PAH are also metabolically altered. For instance, circulating peripheral blood mononuclear cells (PBMCs) from patients with scleroderma-associated PAH demonstrate markers of increased endoplasmic reticulum stress (ER stress) and unfolded protein response (UPR) pathways.[56] Prolonged upregulation of ER stress and UPR pathways typically leads to profound mitochondrial abnormalities.[57] Activation of these pathways is also associated with increased cytokine production, which would contribute to and promote pulmonary vascular remodeling.[17] However, the extent to which immune cells are metabolically altered and the contributions of metabolically abnormal circulating immune cells from patients with PAH to other organs of the body are not currently known.

One of the most compelling pieces of data demonstrating altered metabolism of immune cells comes from the study of macrophages, cells critical for proper initiation and resolution of inflammatory responses.[20] Macrophages accumulate in remodeled vessels in PAH.[21] In fact, in rodent models of PH, macrophage accumulation may be a prerequisite for vessel remodeling, as ablation of monocytes attenuates macrophage accumulation.[21] These macrophages express surface markers GLUT1 (glucose transporter 1) and CtBP1, suggestive of not only an alternative macrophage activation phenotype but also of metabolic reprogramming.[26] PH fibroblast-activated macrophages also highly express arginase 1, VEGF-A, and IL-1beta, all of which require HIF-1 and STAT3 signaling, 2 major targets and stimulators of metabolic reprogramming.[21]

Finally, although it has not yet been definitively demonstrated in PAH, it is possible the mitochondria can directly trigger inflammation: 1) through induction of the NLRP3 inflammasome and/or 2) by repression of glucose oxidation and oxidative phosphorylation in immune cells, promoting the subsequent shift to glycolysis that is required for immune cell activation, thus promoting a persistent inflammatory state.[17,21] Interestingly, the highly glycolytic state required for the activation of many immune cells may make them responsive to interventions targeting metabolism.

Altered Metabolism in Skeletal Muscle

Even in the absence of diabetes and obesity, the presence of metabolic syndrome has become increasingly recognized in patients with PAH, suggesting that there may be an underlying overall metabolic dysfunction in these patients.[14] In fact, PAH patients with insulin resistance demonstrate worse clinical outcomes than patients without insulin resistance.[58] The skeletal muscle is particularly sensitive to insulin resistance and metabolic syndrome, and indeed, the skeletal muscle of PAH patients exhibits signs of such abnormalities as well as mitochondrial dysfunction (Figure 2D).[59] Proteomic analysis of PAH skeletal muscles revealed decreased expression of PDH and increased expression of glycolytic

enzymes (eg, lactate dehydrogenase), as well as abnormal mitochondrial morphology suggestive of a glycolytic metabolism shift.[59,60] This finding is significant because it suggests that the impaired mitochondrial and metabolic functions observed in the cardiopulmonary system are also present in extrapulmonary tissues.

Another study demonstrated that mitochondria isolated from the gastrocnemius muscle of MCT rats are characterized by ATP synthase activity, decreased expression of transcription factor A, mitochondrial (TFAM, a DNA-binding protein that activates transcription of mitochondrial DNA), and accumulation of oxidatively modified proteins, suggesting that mitochondria from these animals are unable to efficiently clear damaged or misfolded proteins, thus contributing to muscle atrophy in PAH.[59]

Finally, while most studies focused on metabolic disorders in PAH, a recent study demonstrated that in a novel model of PH from heart failure with preserved ejection fraction (PH-HFpEF), chronic nitrite or metformin treatment reduce hyperglycemia and attenuate PH endpoints via a sirtuin 3 (SIRT3)-AMPK-GLUT4 dependent signaling pathway in the skeletal muscle, suggesting that glucose uptake and mitochondrial function in the skeletal muscle are linked to systemic glucose levels and pulmonary vascular function.[61]

CLINICAL TRIALS TARGETING METABOLIC ABNORMALITIES IN PATIENTS WITH PAH

With the emergence of the metabolic theory of PAH, there has been an ongoing effort to therapeutically target metabolic dysfunction. While several currently available or emerging therapies may only target the PA or the RV in isolation (or even have opposing effects in these 2 compartments), metabolic modulators have the potential to target the entire cardiopulmonary axis. In addition, metabolic strategies have the potential to be used in a more personalized and patient-specific treatment approach. While several approaches are promising, we highlight 3 strategies with strong preclinical support and clinical potential. We refer the reader to several excellent reviews discussing in greater detail the ongoing clinical trials targeting metabolic pathways in PAH.[28,31]

Dichloroacetate

As described in the previous section, preclinical data demonstrate that DCA blocks the glycolytic shift in energy metabolism by inhibiting PDK. A 4-month phase 1, open-label, 2-center study in patients with PAH on background therapy found that DCA reduced mean PA pressure and pulmonary vascular resistance and increased functional capacity.[62] However, these responses were highly variable. Demonstrating the importance of personalized approaches to medicine, Michelakis et al found that a lack of response was associated with loss-of-function genetic variants in SIRT3 and UCP2. Interestingly, deletion of these proteins is associated with development of PH via PDK-independent mitochondrial dysfunction.[62] This clinical trial not only was the first to target the mitochondria directly in PAH, but also identified a genetically susceptible PAH patient population that would not benefit from DCA treatment.

Metformin

Also discussed above, metformin is an antidiabetic drug with promising therapeutic potential for treating PAH, especially given the increasing evidence of insulin resistance and metabolic syndrome in PAH.[58,63,64] Metformin is protective against experimental models of PH through the activation of AMPK, although it should be mentioned that there are multiple proposed mechanisms of action for metformin including inhibition of mitochondrial glycerophosphate dehydrogenase, inhibition of the mitochondrial respiratory chain (complex I), and inhibition of glucagon-induced cyclic adenosine monophosphate (cAMP) and decreased protein kinase A activity.[65,66] It remains to be seen if these mechanisms will be applicable in PAH. Interestingly, metformin also has therapeutic potential for treating Group 2 PH, specifically HFpEF.[61] Finally, a phase 1 clinical trial is currently enrolling to evaluate the effects of metformin on insulin resistance, oxidant stress, and RV lipid accumulation in PAH (NCT01884051).

Ranolazine

Another approach to regulate metabolism and promote RV function in PAH is to enhance the Randle cycle by targeting the fatty acid oxidation cycle. Ranolazine does this by inhibiting beta oxidation and enhancing glucose oxidation.[31] Ranolazine is currently being used in a number of clinical trials for PAH.[31] A phase 3 prospective, open-label clinical trial found that 11 patients with PAH demonstrated improvement in functional class and RV size and a decrease in RV strain. However, this study did not report any significant improvement in hemodynamics.[67] Taken together, these data suggest that targeting the metabolic abnormalities in the cardiopulmonary system (and beyond) is a promising therapeutic approach and as our knowledge of metabolic abnormalities in PAH increases, new targetable pathways will emerge.

CONCLUSION

The emergence of the metabolic theory of PAH as the underlying and unifying driver of PAH has led to the development of new therapeutic targets and an increase in our understanding of the underlying mechanisms regulated by mitochondria and metabolism in the pulmonary vasculature and beyond. However, this theory has yet to completely explain the complex and at times disparate effects of metabolic remodeling in PAH. Several questions remain (Table 1), and a better understanding of the molecular and cell-specific energetic needs will be necessary to develop the most effective and personalized approaches to treating PAH. While there is promise that metabolic therapies could be effective in non-PAH PH as well, it remains to be seen if these other groups of PH demonstrate similar metabolic abnormalities as PAH and whether current approaches targeting the mitochondria will be effective in these PH syndromes as well. Despite these remaining questions, the metabolic theory of PAH has provided an attractive explanation for our current understanding of the

Table 1. Altered Metabolism in PAH: Current Gaps in Knowledge

Is altered cellular metabolism the driver of early vascular injury and cell death in PAH, or merely a response to early vascular insult and apoptosis?
Is altered metabolism a symptom of a cellular stress response gone awry or a driver of PAH pathogenesis?
Is mitochondrial suppression a symptom or cause of PAH?
What causes metabolic dysfunction in PAH? Are patients genetically predisposed?
What is the timing of mitochondrial remodeling and metabolic dysfunction in PAH? Are the sequences of events the same for all patients?
Is altered metabolism present in non-PAH PH? Are the abnormalities the same as what has been observed in PAH?
Does altered RNA metabolism affect PAH?
Are there sex differences in metabolic abnormalities and mitochondrial dysfunction?
Are there sex differences in metabolic adaptive and maladaptive responses?
Do other cell types, such as endothelial cells and fibroblasts, have the same metabolic alterations in the right ventricle and skeletal muscle as cardiomyocytes?
Do right ventricular endothelial cells share the same mitochondrial/metabolic alterations as the pulmonary artery endothelial cells?
If multiple organs have metabolic dysfunction in PAH, why is the main site of disease manifestation in the pulmonary vasculature?
What lessons from clinical trials using metabolic-targeting drugs in cancer can we apply for PAH?

disparate and often discordant pathways making up and contributing to PAH pathogenesis. Finally, novel therapeutic avenues targeting metabolism offer a means to significantly improve patient outcomes.

References

1. Rabinovitch M. Molecular pathogenesis of pulmonary arterial hypertension. *J Clin Invest*. 2012;122(12):4306-4313.
2. Tuder RM, Archer SL, Dorfmüller P, et al. Relevant issues in the pathology and pathobiology of pulmonary hypertension. *J Am Coll Cardiol*. 2013;62(25 Suppl):D4-D12.
3. Simonneau G, Gatzoulis MA, Adatia I, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol*. 2013;62(25 Suppl):D34-41.
4. Chaouat A, Weitzenblum E, Krieger J, Oswald M, Kessler R. Pulmonary hemodynamics in the obstructive sleep apnea syndrome. Results in 220 consecutive patients. *Chest*. 1996;109(2):380-386.
5. Hyduk A, Croft JB, Ayala C, et al. Pulmonary hypertension surveillance–United States, 1980-2002. *MMWR Surveill Summ*. 2005;54(5):1-28.
6. Lloyd-Jones D, Adams RJ, Brown TM, et al. Executive summary: heart disease and stroke statistics–2010 update: a report from the American Heart Association. *Biochim Biophys Acta*. 2015;1852(12):2722-2731.
7. Mohammed SF, Hussain I, AbouEzzeddine OF, et al. Right ventricular function in heart failure with preserved ejection fraction: a community-based study. *Circulation*. 2014;130(25):2310-2320.
8. Morrison DA, Adcock K, Collins CM, et al. Right ventricular dysfunction and the exercise limitation of chronic obstructive pulmonary disease. *J Am Coll Cardiol*. 1987;9(6):1219-1229.
9. Redfield MM, Jacobsen SJ, Burnett JC Jr, et al. Burden of systolic and diastolic ventricular dysfunction in the community: appreciating the scope of the heart failure epidemic. *JAMA*. 2003;289(2):194-202.
10. Humbert M, Sitbon O, Yaïci A, et al. Survival in incident and prevalent cohorts of patients with pulmonary arterial hypertension. *Eur Respir J*. 2010;36(3):549-555.
11. Lajoie AC, Bonnet S, Provencher S. Clinical trial research in focus: improving drug development and trial design in pulmonary arterial hypertension. *Lancet Respir Med*. 2017;5(7):544-546.
12. Aldred MA, Comhair SA, Varella-Garcia M. Somatic chromosome abnormalities in the lungs of patients with pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2010;182(9):1153-1160.
13. Morrell NW, Adnot S, Archer SL, et al. Cellular and molecular basis of pulmonary arterial hypertension. *J Am Coll Cardiol*. 2009;54(1 Suppl):S20-S31.
14. Paulin R, Michelakis ED. The metabolic theory of pulmonary arterial hypertension. *Circ Res*. 2014;115(1):148-164.
15. Vonk-Noordegraaf A1, Haddad F2, Chin KM, et al. Right heart adaptation to pulmonary arterial hypertension: physiology and pathobiology. *J Am Coll Cardiol*. 2013;62(25 Suppl):D22-D33.
16. van de Veerdonk MC, Kind T, Marcus JT, et al. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. *J Am Coll Cardiol*. 2011;58(24):2511-2519.
17. Sutendra G, Michelakis ED. The metabolic basis of pulmonary arterial hypertension. *Cell Metab*. 2014;19(4):558-573.
18. Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis*. 2013;4:e532.
19. Marcu R, Zheng Y, Hawkins BJ. Mitochondria and angiogenesis. *Adv Exp Med Biol*. 2017;982:371-406.
20. Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. *Circ Res*. 2014;115(1):165-175.
21. D'Alessandro A, El Kasmi KC, Plecitá-Hlavatá L, et al. Hallmarks of pulmonary hypertension: mesenchymal and inflammatory cell metabolic reprogramming. *Antioxid Redox Signal*. 2018;28(3):230-250.
22. Fernie AR, Carrari F, Sweetlove LJ. Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. *Curr Opin Plant Biol*. 2004;7(3):254-261.
23. Pelley JW. Citric acid cycle, electron transport chain, and oxidative phosphorylation. In: Pelley JW, ed. *Elsevier's Integrated Review Biochemistry*. 2nd ed. Philadelphia, PA: W.B. Saunders; 2012:57-65.
24. Ryan JJ, Archer SL. Emerging concepts in the molecular basis of pulmonary arterial hypertension: part I: metabolic plasticity and mitochondrial dynamics in the pulmonary circulation and right ventricle in pulmonary arterial hypertension. *Circulation*. 2015;131(19):1691-1702.
25. Mizock BA. Alterations in carbohydrate metabolism during stress: a review of the literature. *Am J Med*. 1995;98(1):75-84.
26. Stenmark KR, Tuder RM, Kasmi KCE. Metabolic reprogramming and inflammation act in concert to control vascular remodeling in hypoxic pulmonary hypertension. *J Appl Physiol* (1985). 2015;119(10):1164-1172.
27. Plas DR, Thompson CB. Cell metabolism in the regulation of programmed cell death. *Trends Endocrinol Metab*. 2002;13(2):75-78.
28. Chan SY, Rubin LJ. Metabolic dysfunction in pulmonary hypertension: from basic science to clinical practice. *Eur Respir Rev*. 2017;26(146).
29. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009;324(5930):1029-1033.
30. Talati M, Hemnes A. Fatty acid metabolism in pulmonary arterial hypertension: role in right ventricular dysfunction and hypertrophy. *Pulm Circ*. 2015;5(2):269-278.
31. Harvey LD, Chan SY. Emerging metabolic therapies in pulmonary arterial hypertension. *J Clin Med*. 2017;6(4).

32. Ryan JJ, Archer SL. The right ventricle in pulmonary arterial hypertension: disorders of metabolism, angiogenesis and adrenergic signaling in right ventricular failure. *Circ Res*. 2014;115(1):176-1788.
33. Sutendra G, Bonnet S, Rochefort G, et al. Fatty acid oxidation and malonyl-CoA decarboxylase in the vascular remodeling of pulmonary hypertension. *Sci Transl Med*. 2010;2(44):44ra58.
34. Bonnet S, Michelakis ED, Porter CJ, et al. An abnormal mitochondrial-hypoxia inducible factor-1alpha-Kv channel pathway disrupts oxygen sensing and triggers pulmonary arterial hypertension in fawn hooded rats: similarities to human pulmonary arterial hypertension. *Circulation*. 2006;113(22):2630-2641.
35. Tuder RM, Chacon M, Alger L, et al. Expression of angiogenesis-related molecules in plexiform lesions in severe pulmonary hypertension: evidence for a process of disordered angiogenesis. *J Pathol*. 2001;195(3):367-374.
36. MacKenzie ED, Selak MA, Tennant DA, et al. Cell-permeating alpha-ketoglutarate derivatives alleviate pseudohypoxia in succinate dehydrogenase-deficient cells. *Mol Cell Biol*. 2007;27(9):3282-3289.
37. McMurtry MS, Bonnet S, Wu X, et al. Dichloroacetate prevents and reverses pulmonary hypertension by inducing pulmonary artery smooth muscle cell apoptosis. *Circ Res*. 2004;95(8):830-840.
38. Sutendra G, Kinnaird A, Dromparis P, et al. A nuclear pyruvate dehydrogenase complex is important for the generation of acetyl-CoA and histone acetylation. *Cell*. 2014;158(1):84-97.
39. Dromparis P, Paulin R, Sutendra G, et al. Uncoupling protein 2 deficiency mimics the effects of hypoxia and endoplasmic reticulum stress on mitochondria and triggers pseudohypoxic pulmonary vascular remodeling and pulmonary hypertension. *Circ Res*. 2013;113(2):126-136.
40. Draoui N, de Zeeuw P, Carmeliet P. Angiogenesis revisited from a metabolic perspective: role and therapeutic implications of endothelial cell metabolism. *Open Biol*. 2017;7(12).
41. Eelen G, de Zeeuw P, Simons M, Carmeliet P. Endothelial cell metabolism in normal and diseased vasculature. *Circ Res*. 2015;116(7):1231-1244.
42. Xu W, Koeck T, Lara AR, et al. Alterations of cellular bioenergetics in pulmonary artery endothelial cells. *Proc Natl Acad Sci USA*. 2007;104(4):1342-1347.
43. Fijalkowska I, Xu W, Comhair SA, et al. Hypoxia inducible-factor1alpha regulates the metabolic shift of pulmonary hypertensive endothelial cells. *Am J Pathol*. 2010;176(3):1130-1138.
44. Fessel JP, Hamid R, Wittmann BM, et al. Metabolomic analysis of bone morphogenetic protein receptor type 2 mutations in human pulmonary endothelium reveals widespread metabolic reprogramming. *Pulm Circ*. 2012;2(2):201-213.
45. Omura J, Satoh K, Kikuchi N, et al. Protective roles of endothelial AMP-activated protein kinase against hypoxia-induced pulmonary hypertension in mice. *Circ Res*. 2016;119(2):197-209.
46. Moral-Sanz J, Mahmoud AD, Ross FA, et al. AMP-activated protein kinase inhibits Kv 1.5 channel currents of pulmonary arterial myocytes in response to hypoxia and inhibition of mitochondrial oxidative phosphorylation. *J Physiol*. 2016;594(17):4901-4915.
47. Agard C, Rolli-Derkinderen M, Dumas-de-La-Roque E, et al. Protective role of the antidiabetic drug metformin against chronic experimental pulmonary hypertension. *Br J Pharmacol*. 2009;158(5):1285-1294.
48. Zhou G, Myers R, Li Y, et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*. 2001;108(8):1167-1174.
49. Goncharov DA, Goncharova EA, Tofovic SP, et al. Metformin therapy for pulmonary hypertension associated with HFpEF versus PAH. *Am J Respir Crit Care Med*. 2018;198(5):681-684.
50. Frump A, Prewitt A, de Caestecker M. BMPR2 mutations and endothelial dysfunction in pulmonary arterial hypertension (2017 Grover Conference Series). *Pulm Circ*. 2018;8(2):2045894018765840.
51. Li M, Riddle S, Zhang H, et al. Metabolic reprogramming regulates the proliferative and inflammatory phenotype of adventitial fibroblasts in pulmonary hypertension through the transcriptional co-repressor C-terminal binding protein-1. *Circulation*. 2016;134(15):1105-1121.
52. Brittain EL, Talati M, Fessel J, et al. Fatty acid metabolic defects and right ventricular lipotoxicity in human pulmonary arterial hypertension. *Circulation*. 2016;133(20):1936-1944.
53. Talati MH, Brittain EL, Fessel JP, et al. Mechanisms of lipid accumulation in the bone morphogenetic protein receptor type 2 mutant right ventricle. *Am J Respir Crit Care Med*. 2016;194(6):719-728.
54. Piao L, Fang YH, Cadete VJ, et al. The inhibition of pyruvate dehydrogenase kinase improves impaired cardiac function and electrical remodeling in two models of right ventricular hypertrophy: resuscitating the hibernating right ventricle. *J Mol Med (Berl)*. 2010;88(1):47-46.
55. Fang YH, Piao L, Hong Z, et al. Therapeutic inhibition of fatty acid oxidation in right ventricular hypertrophy: exploiting Randle's cycle. *J Mol Med (Berl)*. 2012;90(1):31-43.
56. Lenna S, Farina AG, Martyanov V, et al. Increased expression of endoplasmic reticulum stress and unfolded protein response genes in peripheral blood mononuclear cells from patients with limited cutaneous systemic sclerosis and pulmonary arterial hypertension. *Arthritis Rheum*. 2013;65(5):1357-1366.
57. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol*. 2012;13(2):89-102.
58. Zamanian RT, Hansmann G, Snook S, et al. Insulin resistance in pulmonary arterial hypertension. *Eur Respir J*. 2009;33(2):318-324.
59. Moreira-Gonçalves D, Padrão AI, Ferreira R, et al. Signaling pathways underlying skeletal muscle wasting in experimental pulmonary arterial hypertension. *Biochim Biophys Acta*. 2015;1852(12):2722-2731.
60. Malenfant S, Potus F, Fournier F, et al. Skeletal muscle proteomic signature and metabolic impairment in pulmonary hypertension. *J Mol Med (Berl)*. 2015;93(5):573-584.
61. Lai YC, Tabima DM, Dube JJ, et al. SIRT3-AMP-activated protein kinase activation by nitrite and metformin improves hyperglycemia and normalizes pulmonary hypertension associated with heart failure with preserved ejection fraction. *Circulation*. 2016;133(8):717-731.
62. Michelakis ED, Gurtu V, Webster L, et al. Inhibition of pyruvate dehydrogenase kinase improves pulmonary arterial hypertension in genetically susceptible patients. *Sci Transl Med*. 2017;9(413).
63. Hansmann G, Wagner RA, Schellong S, et al. Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator–activated receptor-gamma activation. *Circulation*. 2007;115(10):1275-1284.
64. West J, Niswender KD, Johnson JA, et al. A potential role for insulin resistance in experimental pulmonary hypertension. *Eur Respir J*. 2013;41(4):861-871.
65. Rena G, Pearson ER, Sakamoto K. Molecular mechanism of action of metformin: old or new insights? *Diabetologia*. 2013;56(9):1898-1890.
66. Madiraju AK, Erion DM, Rahimi Y, et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature*. 2014;510(7506):542-546.
67. Khan SS, Cuttica MJ, Beussink-Nelson L, et al. Effects of ranolazine on exercise capacity, right ventricular indices, and hemodynamic characteristics in pulmonary arterial hypertension: a pilot study. *Pulm Circ*. 2015;5(3):547-556.