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Metabolism in Pulmonary Hypertension

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Abstract

Pulmonary arterial hypertension (PAH) is characterized by impaired regulation of pulmonary hemodynamics and vascular growth. Alterations of metabolism and bioenergetics are increasingly recognized as universal hallmarks of PAH, as metabolic abnormalities are identified in lungs and hearts of patients, animal models of the disease, and cells derived from lungs of patients. Mitochondria are the primary organelle critically mediating the complex and integrative metabolic pathways in bioenergetics, biosynthetic pathways, and cell signaling. Here, we review the alterations in metabolic pathways that are linked to the pathologic vascular phenotype of PAH, including abnormalities in glycolysis and glucose oxidation, fatty acid oxidation, glutaminolysis, arginine metabolism, one-carbon metabolism, the reducing and oxidizing cell environment, and the tricarboxylic acid cycle, as well as the effects of PAH-associated nuclear and mitochondrial mutations on metabolism. Understanding of the metabolic mechanisms underlying PAH provides important knowledge for the design of new therapeutics for treatment of patients.

1. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive lethal disorder disproportionately afflicting women, characterized by impaired regulation of pulmonary hemodynamics and vascular growth, in which right ventricular failure leads to death. PAH is defined clinically by pulmonary artery pressures >20 mm Hg at rest (1, 2) and classified into five categories by the World Symposium on Pulmonary Hypertension (WSPH). This review focuses on category 1 disease, which includes idiopathic, heritable, and drug- and toxin-induced PAH, as well as PAH associated with connective tissue diseases and congenital heart diseases (2, 3) (**Table 1**). Pathologically, PAH is

Table 1 World Symposium on Pulmonary Hypertension (WSPH) grouping of pulmonary hypertension patients

WSPH 1/Pulmonary Arterial Hypertension (PAH)
1.1 Idiopathic
1.2 Heritable
1.2.1 *BMPR2*
1.2.2 *ALK1*, *ENG*, *SMAD9*, *CAV1*, *KCNK3*
1.2.3 Unknown
1.3 Drug- and toxin-induced
1.4 Associated with
1.4.1 Connective tissue disease
1.4.2 HIV infection
1.4.3 Portal hypertension
1.4.4 Congenital heart diseases
1.4.5 Schistosomiasis
1.5 PAH long-term responders to calcium channel blockers
1.6 PAH with overt features of venous/capillaries (PVOD/PCH) involvement
1.7 Persistent PH of the newborn syndrome
WSPH 2/Pulmonary Hypertension (PH) due to left heart disease
2.1 PH due to heart failure with preserved LVEF
2.2 PH due to heart failure with reduced LVEF
2.3 Valvular heart disease
2.4 Congenital/acquired cardiovascular conditions leading to postcapillary PH
WSPH 3/PH due to lung disease and/or hypoxia
3.1 Obstructive lung disease
3.2 Restrictive lung disease
3.3 Other lung disease with mixed restrictive/obstructive pattern
3.4 Hypoxia without lung disease
3.5 Developmental lung disorders
WSPH 4/PH due to pulmonary artery obstructions
4.1 Chronic thromboembolic PH
4.2 Other pulmonary artery obstructions
WSPH 5/PH with unclear and/or multifactorial mechanisms
5.1 Hematological disorders
5.2 Systemic and metabolic disorders
5.3 Others
5.4 Complex congenital heart disease

Abbreviations: *ALK1*, activin A receptor-like type 1; *BMPR2*, bone morphogenetic protein receptor type 2; *CAV1*, caveolin-1; *ENG*, endoglin; HIV, human immunodeficiency virus; *KCNK3*, potassium channel subfamily K member 3; LVEF, left ventricular ejection fraction; PAH, pulmonary arterial hypertension; PCH, pulmonary capillary hemangiomatosis; PVOD, pulmonary veno-occlusive disease; *SMAD9*, SMAD family member 9.

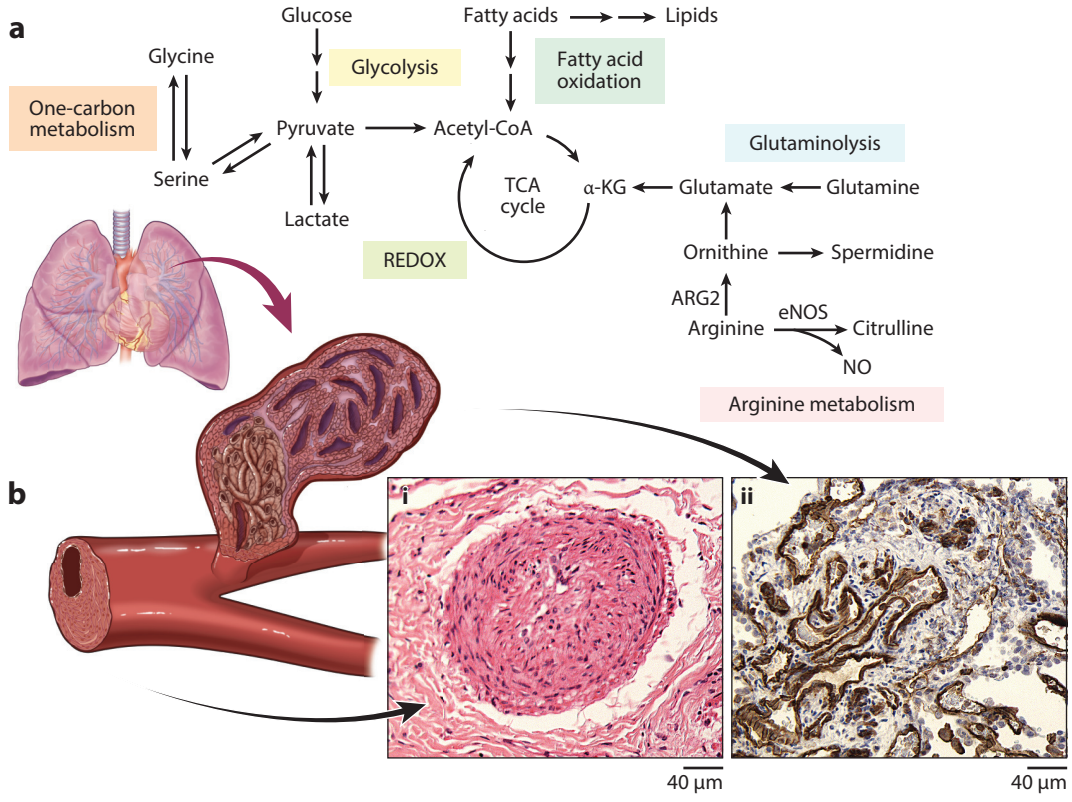

Figure 1

(*a*) An overview of metabolism in the lung and heart. Glucose is converted to pyruvate and lactate via glycolysis. Pyruvate can be further converted to acetyl coenzyme A (acetyl-CoA) for entry into the tricarboxylic acid (TCA) cycle. Fatty acids supply acetyl-CoA via β-oxidation. Serine and glycine required for one-carbon metabolism are biosynthetically linked and can be used to supply pyruvate. Glutamine is converted to glutamate and α-ketoglutarate (α-KG) to fuel the TCA cycle via glutaminolysis. Nitric oxide (NO) is produced in the lung by endothelial nitric oxide synthase (eNOS). Arginine is the substrate for both eNOS and arginase (ARG). Mitochondrial arginase 2 (ARG2) catabolizes arginine to ornithine, which can provide glutamate and α-KG. The reducing and oxidizing (REDOX) environment affects cell function and metabolism. (*b*) Model of abnormal pulmonary vasculature, including the plexiform lesion, and the hypertrophic right ventricle in pulmonary arterial hypertension (PAH) and histopathology of endothelial cell lesions in the PAH lung. (*b*, *i*) A concentric laminar intimal lesion obliterating the pulmonary blood vessel. (*b*, *ii*) CD31-positive staining in proliferative endothelial cells lining the vascular lumen of plexiform lesion. Histopathology figures are modified from Figure 4G–4H in Reference 9.

defined as panvasculopathy of the pulmonary arteries because abnormalities are found in all three types of pulmonary arteries—elastic, muscular, and nonmuscular—and all cell types of the artery, including endothelial and smooth muscle cells (4, 5). Plexiform lesions, which are seen in PAH lungs, are composed of proliferative endothelial cells and arise only in muscular arteries, or supernumerary muscular arteries, ~200 μm in diameter (4, 6) (**Figure 1**). Increased pulmonary vascular resistance (PVR) due to obstructive lung panvasculopathy leads to progressive right ventricular failure.

Endothelial and smooth muscle cell dysfunction in pulmonary arteries is mechanistically linked to the pathobiology of PAH (7–16). Previous studies identify downregulation of endothelium-derived vasodilators, e.g., prostacyclin and nitric oxide (NO), and upregulation of endothelium-derived vasoconstrictors, e.g., endothelin-1, in models of pulmonary hypertension (PH) and in

human PAH (7, 10, 17–20). Existing therapies target vasodilatory pathways and have improved survival; i.e., 5-year survival for newly diagnosed patients is 70% (21). It is increasingly recognized that alterations of metabolism and bioenergetics are universal hallmarks of PAH in patients and animal models of the disease, but the metabolic pathways have not yet been targeted by therapies (7–16, 22–31). Here, we review the metabolic pathways and mechanisms that are linked to the pathophysiology of PAH (**Figure 1**).

2. METABOLIC PATHWAYS

Mitochondria are remarkable organelles that integrate energy production, biosynthetic pathways, and signal transduction. The pathways localized in mitochondria are dependent on cytosolic reactions and/or molecules. Some mitochondrial pathways are also found in the cytosol. In this review, seven major metabolic pathways that are implicated in PAH are considered, including glucose and fatty acid oxidation, glutaminolysis, arginine metabolism, one-carbon metabolism, reducing and oxidizing (REDOX) reactions, the tricarboxylic acid (TCA) cycle, and the electron transport chain (ETC). The TCA cycle is (*a*) the central hub for all metabolic pathways in the mitochondria, (*b*) necessary for life-sustaining energy production, and (*c*) essential for cell biosynthetic functions in which TCA intermediates mediate signal transduction and regulate cell functions (32). Fueling the TCA cycle are acetyl coenzyme A (acetyl-CoA) derived from glucose via glycolysis and fatty acids via β-oxidation and α-ketoglutarate (α-KG) derived from the amino acids glutamate and arginine. Reactive oxygen species (ROS) are produced as a by-product of oxidative phosphorylation and are imperative in the REDOX homeostasis of the cell. One-carbon metabolism in the mitochondria delivers methyl groups for synthesis of essential components of cells and tissues to maintain integrity of human health and body repair (**Figure 2**). The following details of the pathways identify the magnitude of effects of the mitochondrion for human health and in the pathobiology of PAH.

2.1. Glycolysis and Glucose Oxidation

Glucose is converted to pyruvate via the glycolytic pathway (**Figures 1** and **2*a***). Pyruvate can subsequently be converted to lactate or further oxidized in the mitochondria. Glycolysis is an important source for energy in vascular endothelial cells but much less so in cardiomyocytes (33). In PAH, glucose metabolism is shifted away from complete mitochondrial oxidative phosphorylation and toward greater cytoplasmic glycolysis to pyruvate and ultimately lactate in primary pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASMCs) derived from PAH lungs in culture, in hearts and lungs of patients with PAH in vivo, and in animal models of PAH (7, 8, 10, 11, 15, 16, 26, 29, 31, 34–45) (**Table 2**; **Figures 1** and **2*a***).

Many studies have identified increased glycolysis in PAH PAECs and PASMCs (**Table 2**), including definitive studies using radioisotopes and stable isotopes (10, 16). Despite greater glucose metabolism to pyruvate, PAH PAECs have less mitochondrial respiration than PAECs from control lungs (10, 12) (**Table 3**). Oxygen consumption is decreased in PAH PAECs compared to control PAECs when measured using TCA cycle intermediates glutamate-malate or succinate as substrates (10). Furthermore, PAH PAECs have lower oxygen consumption than control cells for any glucose concentration provided, supporting the idea that the shift away from oxidative metabolism of glucose in PAH endothelial cells is independent of oxygen availability, i.e., the Warburg effect (12). Overall, there is abundant evidence that PAH PAECs have less oxidative metabolism of glucose (12).

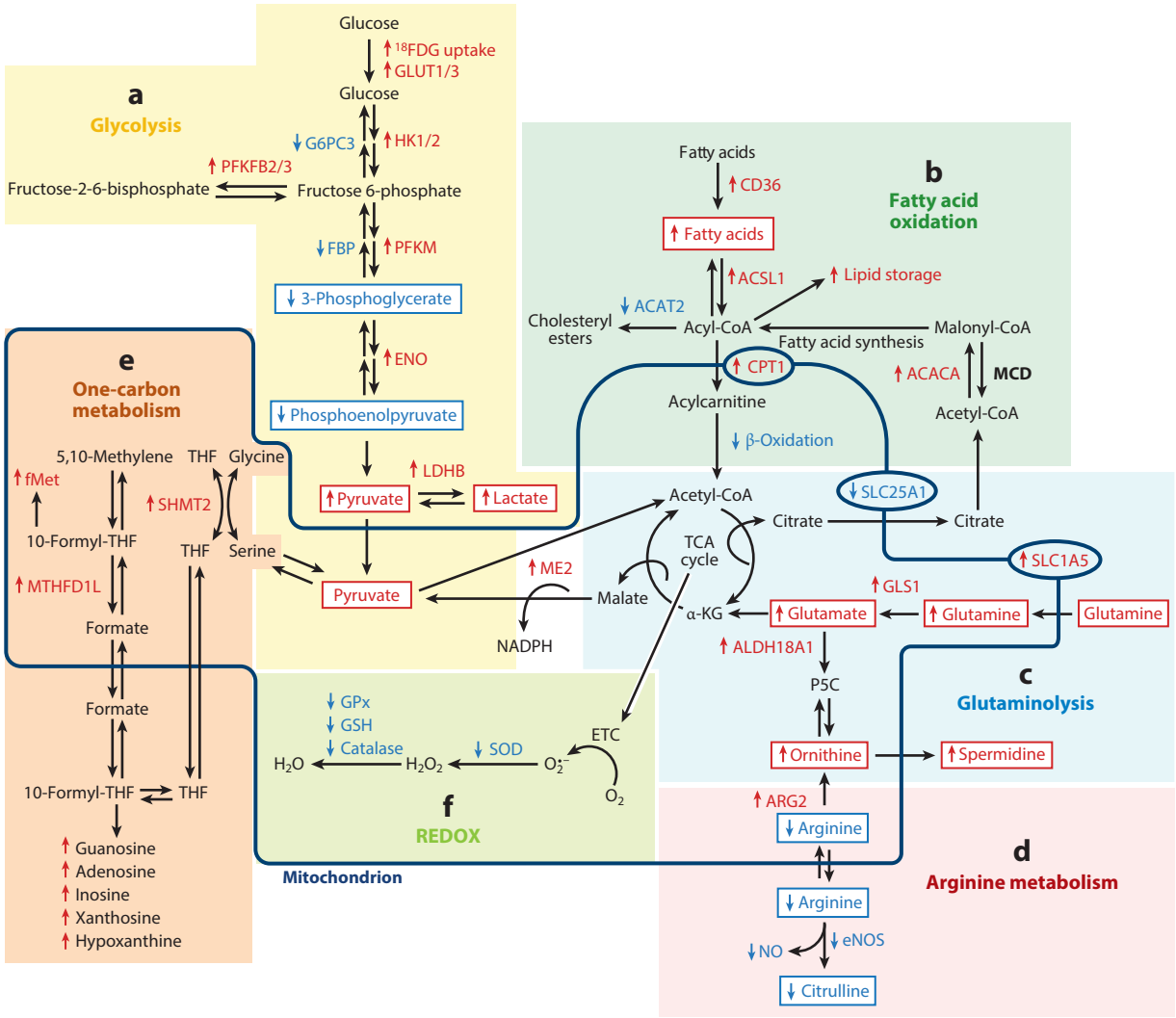

Figure 2

Alterations of metabolic pathways in PAH described in this review. (*a*) Glucose uptake and glycolysis, (*b*) fatty acid uptake and oxidation, (*c*) glutamine uptake and glutaminolysis, (*d*) arginine metabolism, (*e*) one-carbon metabolism, and (*f*) REDOX. The line delineates those molecules and reactions found in the mitochondria. Some proteins, e.g., SOD1 and eNOS, are located in both mitochondria and cytosol. Abbreviations: ACACA, acetyl-CoA carboxylase 1; ACAT2, acetyl-CoA acetyltransferase 2; Acetyl-CoA, acetyl coenzyme A; ACSL1, fatty acetyl-CoA L1; Acyl-CoA, acyl-coenzyme A; ALDH18A1, aldehyde dehydrogenase 18 family member A1; α-KG, α-ketoglutarate; ARG2, arginase 2; CPT1, carnitine palmitoyltransferase 1; ENO, enolase; eNOS, endothelial nitric oxide synthase; ETC, electron transport chain; FBP, fructose 1,6-bisphosphate; ^{18}FDG, ^{18}F-fluorodeoxyglucose; fMet, *N*-formylmethionine; G6PC3, glucose-6-phosphatase catalytic subunit 3; GLS1, glutaminase 1; GLUT, glucose transporter; GPx, glutathione peroxidases; GSH, glutathione; H_2O_2, hydrogen peroxide; HK, hexokinase; LDHB, lactate dehydrogenase B; MCD, malonyl-CoA decarboxylase; ME2, malic enzyme 2; MTHFD1L, monofunctional C1-tetrahydrofolate synthase; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; $O_2^{\bullet -}$, superoxide; P5C, Δ1-Pyrroline-5-carboxylate; PAH, pulmonary arterial hypertension; PFKFB, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase; PFKM, phosphofructokinase; REDOX, reducing and oxidizing; SHMT2, mitochondrial serine hydroxymethyltransferase 2; SLC1A5, solute carrier family 1 member 5; SLC25A1, solute carrier family 25 member 1; SOD, superoxide dismutase; TCA, tricarboxylic acid; THF, tetrahydrofolate. Red ↑ indicates increase, and blue ↓ indicates reduction.

Table 2 Increased glycolysis and hypoxia-inducible factor (HIF) expression in pulmonary arterial hypertension

Sources	Parameters	References
Human data		
Pulmonary artery endothelial cells (PAECs) in vitro	HIF presence in nuclear extract by Western blot analyses ↑5-^{3}H-glucose to $^{3}H_2O$ ↑^{13}C-fructose bisphosphate from ^{13}C-glucose (↑proximate glycolysis pathway intermediates) ↑Lactate by spectrophotometry ↑Hepatocyte growth factor (HGF) and stromal-derived factor 1 (SDF1) by ELISA	10, 11, 16, 26
Pulmonary artery smooth muscle cells (PASMCs) in vitro	↑^{13}C-pyruvate and ^{13}C-lactate from ^{13}C-glucose (↑glucose uptake and utilization) ↑Enolase 1 (ENO1) by Western blot analyses	16, 35
Lung	HIF presence by immunohistochemistry ↑^{18}F-fluorodeoxyglucose (FDG) uptake ↑6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) and lactate dehydrogenase (LDH)B by Western blot analyses ↑HGF and SDF1 by immunohistochemistry ↑PFKFB2 and LDHB by gene microarray analyses ↑Glucose to sorbitol, fructose, and fructose 6-phosphate ↓Fructose 1,6-bisphosphate (FBP), 3-phosphoglycerate (3-PGA), and phosphoenolpyruvate (PEP) by metabolomics ↓Glucose-6-phosphatase catalytic subunit 3 (G6PC3) by Western blot analyses, immunohistochemistry, and gene microarray analyses	10, 11, 15, 26, 31, 34, 36, 37
Small pulmonary arteries	HIF presence by immunofluorescence	14, 15
Right ventricle	HIF presence in nuclei of cardiomyocytes by immunohistochemistry ↑^{18}FDG uptake ↑Glycolytic gene, e.g., hexokinase 2 (HK2) and solute carrier family 2 member 3 (*SLC2A3*), expression by microarray analyses	29, 31, 38, 39, 47, 48
Animal models		
Shunt lambs[a]		
Lung	↑Lactate/pyruvate ratio by spectrophotometry	40, 45
Fawn hooded rats[b]		
PASMC	HIF presence by immunofluorescence	14, 15
Small rat pulmonary arteries	HIF presence by Western blot analyses	14, 15
Hypoxia/Sugen rats[c]		
Lung	↑ENO ↑PFKFB3 mRNA by qRT-PCR, protein by Western blot analyses, and activity by Fructose-2,6-bisphosphate assay	35, 37, 41
Right ventricle	↑HK1 and phosphofructokinase (PFKM) by gene microarray analyses	41
Monocrotaline (MCT) rats[d]		
Lung	↑Glucose transporter 1 (GLUT1) and LDHA mRNA by qRT-PCR and protein by Western blot	44
Right ventricle	↑^{18}FDG uptake ↑GLUT1 protein expression by Western blot analyses ↑GLUT1, HK2, and LDHA mRNA by qRT-PCR	42–44
Liver	↑Metabolic fluxes including glucose, lactate, and pyruvate in liver perfusion	55

[a]Shunt lambs, with pulmonary hypertension secondary to increased pulmonary blood flow with surgically created heart defect.
[b]Fawn hooded rats, a spontaneously pulmonary hypertensive strain with a hereditary bleeding tendency due to a genetic defect in platelet aggregation.
[c]Hypoxia/Sugen rats, with hypoxia- or sugen-induced pulmonary hypertension.
[d]MCT rats, a maladaptive model (heart failure model) combined with PAH and right ventricular hypertrophy induced by MCT.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; PAH, pulmonary arterial hypertension; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

Table 3 Decreased glucose oxidation and abnormal mitochondria in pulmonary arterial hypertension

Sources	Parameters	References
Human data		
Pulmonary artery endothelial cells (PAECs) in vitro	↓Oxygen consumption with a Seahorse XF24 analyzer ↓Oxygen consumption under state 3 and state 4 respiration using the classical method of circulator chambers and a 5300A biological oxygen monitor and Clark-type polarographic oxygen electrode ↓Complex IV activity by spectrophotometry and mitochondrial dehydrogenase activity by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay ↓mtDNA by Southern blot ↓Mitochondrial numbers by quantitation of mitochondria in electron microscopy images of cells	10, 12
Pulmonary artery smooth muscle cells (PASMCs) in vitro	↓Mitochondria by immunofluorescence	14, 15
Small pulmonary arteries	↓Complex I by immunofluorescence	14, 15
Animal models		
Shunt lambs		
Lung	↑Uncoupling protein 2 (UCP2) expression by Western blot analyses	40
Chickens with pulmonary hypertension syndrome[a]		
Lung	↓Oxygen consumption under state 3 and state 4 respiration using the classical method	50, 51
Breast and heart muscle	↓Oxygen consumption under state 3 respiration using the classical method ↓Complex I activity by spectrophotometry ↑Succinate metabolism	50, 51
Fawn hooded rats		
PASMCs	↓Complex I by immunofluorescence Small, dense, dysmorphic mitochondria (swollen cristae) by transmission electron microscopy	14, 15
Small rat pulmonary arteries	↓Complexes I, III, and IV by Western blot analyses	14, 15
Monocrotaline (MCT) rats		
Right ventricle	↓Oxygen consumption by high-resolution respirometry ↓^{14}C-glucose oxidation	42, 43
Endothelial nitric oxide synthase (eNOS)$^{-/-}$ mice[b]		
Lung	↓Oxygen consumption with Oxymax metabolic chamber	52
Brain, liver, heart tissue, and brown adipocytes or brown adipose tissue	↓Complex IV and cytochrome complex (Cyt c) by Western blot analyses ↓UCP1 and peroxisome proliferator–activated receptor gamma coactivator 1α, nuclear factor erythroid 2–related factor 1, and mitochondrial transcription factor A mRNA by qRT-PCR ↓mtDNA by Southern blot	52
Soleus muscles	↓Oxygen consumption by a Clark electrode in an oxygraphic cell	53
Brown adipocytes or brown adipose tissue	↓Number of mitochondria by MitoTracker fluorescence	52

[a]Chickens with pulmonary hypertension syndrome and increased right ventricle/total ventricle weight ratio; chickens developed pulmonary hypertension syndrome under cool temperatures combined with feed to support rapid growth rate.
[b]eNOS$^{-/-}$ mice, with eNOS knockout.
Abbreviations: eNOS, endothelial nitric oxide synthase; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

Unlike in the vascular endothelium, in healthy adult hearts mitochondrial fatty acid β-oxidation is the major source of energy production and oxygen consumption (46). In right ventricular hypertrophy, as occurs in PAH, energy production is increasingly dependent on glycolysis. Glucose uptake, as a measure of cardiac glucose use, can be quantitatively measured by ^{18}F-fluorodeoxyglucose (FDG) uptake in the heart using positron emission tomography (FDG-PET) in vivo. FDG-PET studies show much greater FDG uptake in the hearts and lungs of patients with PAH compared to healthy individuals (10, 29, 31, 36, 38). High levels of right ventricular FDG uptake are associated with a poor heart rate recovery after a 6-min walk, higher right ventricular systolic pressure (RVSP), higher PVR, and lower cardiac index, all of which are clinical indicators of more severe disease (47, 48). The relationship of FDG uptake to clinical phenotype of severe disease suggests that functional metabolic imaging may be useful in monitoring patients over time for response to therapy and/or progression of disease in anticipation of transplantation (29). In accord with the greater FDG uptake in lungs and hearts of PAH patients, increased levels of glycolysis-related enzymes have been reported in PAH lungs, i.e., 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB2) and lactate dehydrogenase B (LDHB) (34), and in biopsies of right ventricles from PAH patients, i.e., hexokinase 2 (HK2) and solute carrier family 2 member 3 (*SLC2A3*), which encodes glucose transporter 3 (GLUT3) (39, 49) (**Table 2**; **Figure 2*a***). Similar to findings in patients, there is reduced mitochondrial oxygen consumption in the lungs and hearts of PH animal models (42, 43, 50–53) (**Table 3**).

Among molecular mechanisms that likely promote the shift to glycolysis and away from oxidative glucose metabolism in PAH is the pathologic accumulation of hypoxia-inducible factor 1α (HIF1α). Transcription factor HIF1α plays a role in a variety of cellular functions, including proliferation, angiogenesis, survival, and metabolism (11, 14, 15, 29, 48, 49, 54). Upregulation of HIF1α is found in plexiform lesions, pulmonary arteries, and cardiomyocytes from PAH patients (11, 13–15, 29). Secretion of the HIF-regulated factors hepatocyte growth factor (HGF) and stromal-derived factor 1 (SDF1) is significantly increased in PAH PAECs compared to that of healthy control cells (26). Increased glycolysis, greater FDG uptake, and increased levels of glycolysis-related enzymes and activity in lungs and right ventricles in PH animal models have been mechanistically associated with HIF accumulation (14, 15, 35, 37, 40–45, 55) (**Table 2**).

2.2. Fatty Acid Oxidation

Fatty acid metabolism involves pathways such as cellular fatty acid uptake and storage, fatty acid transport into mitochondria, mitochondrial fatty acid β-oxidation, and fatty acid synthesis. Imbalanced fatty acid metabolism is reported in hearts and lungs of PAH patients (12, 16, 34, 39, 40, 44, 52, 56–64) (**Table 4**; **Figure 2*b***). The healthy adult heart relies on fatty acids as the main energy source to power contractile function, with approximately 60–90% of ATP generated from fatty acid oxidation and the remaining 10–40% from oxidation of glucose, lactate, amino acids, or ketone bodies (65, 66). Disruption in fatty acid metabolism contributes to cardiac contractile dysfunction and hypertrophy (39, 56, 57, 59, 60). Although glycolysis and glutaminolysis can help maintain normal function in hypertrophic cardiomyocytes during the initial stages of heart failure, these pathways are inadequate in progressive right ventricular hypertrophy, as occurs in PAH (39, 56). PAH patients show higher right ventricular lipid accumulation in the form of triglycerides, diacylglycerols, and ceramides that associate with right ventricular dysfunction and failure (39, 64). Excess cellular lipid uptake mediated by CD36, the main transporter responsible for fatty acid uptake into contracting cardiomyocytes, results in lipid accumulation in right ventricular tissue from individuals with heritable PAH with a bone morphogenetic protein receptor type 2 (*BMPR2*) mutation as well as mice genetically deficient in *Bmpr2* (39, 56, 61). CD36 mRNA and

Table 4 Abnormalities of fatty acid synthesis/oxidation in pulmonary arterial hypertension

Sources	Parameters	References
Human data		
Pulmonary artery endothelial cells (PAECs) in vitro	↓^{13}C-α-ketoglutarate (α-KG) from ^{13}C-long-chain fatty acids (LCFAs) ↓Acetyl-CoA acetyltransferase 2 (ACAT2) by global proteomics ↑Enoyl-CoA delta isomerase 1 by global proteomics	12, 16
Lung	↑Carnitine, dicarboxylic acid, and long- and medium-chain free fatty acid products, including caproate, caprylate, myristate, and palmitoleate by metabolomics ↑Adrenate (a prothrombotic lipid that can cause obstructions) by metabolomics ↑Expression of genes coding the enzymes fatty acetyl-CoA L1 (*ACSL1*) and acetyl-CoA carboxylase 1 (*ACACA*) by gene microarray	34
Right ventricle	↓Fatty acid uptake by ^{123}I-labeled 15-(*p*-iodophenyl)-3-(*R*,*S*)-methylpentadecanoic acid (BMIPP) single-photon emission computed tomography (SPECT) ↓Fatty acid oxidation by gene microarray analyses ↓Long-chain acylcarnitines by liquid chromatography/mass spectrometric analysis ↑Lipid content by oil red O staining ↑Ceramide by liquid chromatography/mass spectrometric analysis ↑Triglycerides by cardiac imaging with proton spectroscopy	39, 60, 62, 64
Plasma	↑Free fatty acids by enzymatic assay ↑Long-chain acylcarnitines by liquid chromatography/mass spectrometric analysis	64
Animal models		
Shunt lambs		
Lung	↑Acylcarnitines by HPLC	40
Monocrotaline (MCT) rats		
Pulmonary artery smooth muscle cells (PASMCs) in vitro	↑Carnitine palmitoyltransferase 1 (CPT1) by Western blot and immunofluorescence	63
Lung	↑CPT1 and CD36 mRNA by qRT-PCR and protein by Western blot	44, 63
Right ventricle	↑CPT1 and CD36 mRNA by qRT-PCR and protein by Western blot ↑Myocardial lipid peroxidation in right ventricle in late stage PH by thiobarbituric acid reactive substances (TBARS) assay	44, 58, 63
Severe pulmonary hypertension (SPH) rats[a]		
Right ventricle	↓14-(*R*,*S*)-[^{18}F]fluoro-6-thia-heptadecanoic acid (^{18}F-FTHA) (fatty acid analog) uptake	59
***Bmpr2*$^{-/-}$ mice**[b]		
Right ventricle	↓Fatty acid β-oxidation of palmitate by oxygen consumption measurement using Oroboros O_2k Oxygraph ↑Fatty acid uptake by ^{14}C-palmitate ↑CD36 fatty acid transporter molecule by immunofluorescence and Western blot analyses ↑Fatty acid metabolites by metabolomics ↑Lipid accumulation in the form of triglycerides, diacylglycerol, and ceramides by metabolomics ↑Lipid by oil red O staining	39, 56, 61, 64

(*Continued*)

Table 4 (*Continued*)

Sources	Parameters	References
***Cav1*$^{-/-}$ mice**[c]		
Heart	↓Triglycerides, fatty acids, and cholesterol measured enzymatically ↑Lipid uptake by measure of the uptake of ^{14}C-palmitate ↑^{14}C-palmitate oxidation ↑cAMP by cAMP (^{3}H) assay	57
eNOS$^{-/-}$ mice		
Brown adipocytes or brown adipose tissue	↑Lipid droplets in adipocytes by oil red O staining	52

[a]SPH rats, SPH induced by treatment with the VEGF receptor inhibitor SU5416 and under hypoxia.
[b]*Bmpr2*$^{-/-}$ mice, with *Bmpr2* knockout, do not develop PH spontaneously but are more susceptible to hypoxic or chemically induced PH.
[c]*Cav1*$^{-/-}$ mice, with caveolin-1 knockout with PH and left ventricular hypertrophy.
Abbreviations: *Bmpr2*, bone morphogenetic protein receptor type 2; cAMP, cyclic adenosine monophosphate; *Cav1*, caveolin-1; eNOS, endothelial nitric oxide synthase; HPLC, high-performance liquid chromatography; PH, pulmonary hypertension; qRT-PCR, quantitative reverse transcription polymerase chain reaction; VEGF, vascular endothelial growth factor.

protein expression is also increased in the lungs and hearts of the monocrotaline (MCT)-induced PH rat model (44). On the other hand, single-photon emission computed tomography (SPECT) using the fatty acid analog ^{123}I-β-iodophenyl pentadecanoic acid suggests impaired fatty acid uptake in the right ventricles of patients with very severe PAH (60, 62), perhaps suggesting that the failing heart has limited ability to uptake fatty acid. Once taken up, fatty acids are converted into acylcarnitine and transported into the mitochondria by carnitine palmitoyltransferase (CPT), the rate-limiting enzyme in the fatty acid oxidation pathway. Expression of CPT1 in the right heart is upregulated in the MCT-induced PH rat model (44, 63), but the transport of fatty acids into mitochondria is not accompanied by enhanced mitochondrial fatty acid oxidation. Several reports suggest disruption in mitochondrial fatty acid oxidation in *BMPR2*-mutant cardiomyocytes (39, 56, 64). Abnormalities of cardiac mitochondrial fatty acid oxidation in patients are supported by findings of increased levels of carnitine and acylcarnitine in plasma from PAH patients (44), which suggest incomplete mitochondrial fatty acid oxidation. Abnormalities of carnitine metabolism are also linked to mechanisms of PH in a lamb model (40, 45). In the MCT-induced PH rat model, right heart hypertrophy, physiological signs of heart failure, and myocardial lipid peroxidation are found in the late stages, 6 weeks after MCT administration (58). As in cell studies of glucose metabolic abnormalities, increased HIF1α accumulation in the right ventricle is associated with decreased fatty acid oxidation and cardiomyocyte lipid accumulation in PAH (67, 68).

Fatty acid abnormalities in the lung have also been described. Accumulation of carnitine and dicarboxylic acid in PAH lung, smooth muscle, and endothelial cells is associated with enhanced expression of genes involved in fatty acid oxidation, such as acetyl-CoA carboxylase 1 (*ACACA*), which is associated with proliferation in cancer cells (12, 34, 69–71). Using in vitro stable isotope metabolic flux analysis, PAH PAECs have less mitochondrial fatty acid oxidation, which results in less contribution of fatty acid–derived carbons to support the TCA cycle in PAH cells (16). Similar to findings in the PAH heart, CPT1 is also upregulated in lungs and pulmonary arteries of the MCT rat model (63). Upregulation of fatty acid oxidation by overexpressing CPT1 in PASMCs increases cellular ATP and promotes cellular proliferation, while CPT1 inhibition abrogates PASMC proliferation (63). In murine studies, abolishing fatty acid oxidation by genetic deletion of malonyl-CoA decarboxylase prevents development of PH in mice exposed to MCT or hypoxia, as compared to wild-type mice (72). Taken together, there are fatty acid metabolic

Table 5 Increased glutamate metabolism in pulmonary arterial hypertension

Sources	Parameters	References
Human data		
Pulmonary artery endothelial cells (PAECs) in vitro	↑^{13}C-succinate from ^{13}C-glutamine (↑glutamine-derived anaplerosis) ↑Aldehyde dehydrogenase 18 family member A1 (ALDH18A1) by global proteomics ↑Spermidine by metabolomics	12, 16
Lung	↑Glutamine uptake measured at right heart catheterization ↑Glutaminase 1 (GLS1) by immunofluorescence ↑ALDH18A1 by metabolomics and Western blot analyses	34, 76, 78
Right ventricle	↑Glutamine transporter solute carrier family 1 member 5 (SLC1A5) by immunofluorescence	43
Plasma	↑Glutamine ↑Glutamate by metabolomics	12, 76
Animal models		
Monocrotaline (MCT) rats		
Right ventricle	↑^{14}C-glutamine metabolism ↑SLC1A5 by Western blot analyses ↑SLC1A5 and mitochondrial malic enzyme 2 (ME2) by qRT-PCR	43
Plasma	↑Glutamine and malate	43
***Bmpr2*$^{-/-}$ mice**		
Pulmonary microvascular endothelial cells (PMVECs) in vitro	↑^{13}C-glutamine metabolism ↑Glutamine-supported ATP-linked mitochondrial respiration with a Seahorse XF96 analyzer	76

Abbreviations: *Bmpr2*, bone morphogenetic protein receptor type 2; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

disturbances in PAH that are not fully understood. Additional studies in cells, tissues, and animal models will help to define the relationship to PAH pathophysiology.

2.3. Glutaminolysis

Glutamine is an important source for a variety of biochemical functions, including as a carbon donor in the TCA cycle and nitrogen donor for purine synthesis and for protein and lipid synthesis, cell energy, and the biosynthesis of purine nucleosides. With a shift away from glucose oxidation in PAH, an alternative source of carbons for macromolecule synthesis may be provided via glutamine metabolism. Glutaminolysis starts with a two-step process of (*a*) deamination of glutamine to glutamate by glutaminase (GLS1 and 2), followed by (*b*) conversion of glutamate to α-KG by glutamate dehydrogenase (**Figure 2*c***). This process is critical for rapid growth of cancer cells and other proliferating cells (73–75). HIF1α promotes glutamine metabolism toward biosynthetic pathways (76).

Glutamine metabolism is increased in the PAH right ventricle. Glutamine uptake is mediated by the alanine-serine-cysteine-transporter ASCT2 (solute carrier family 1 member 5, SLC1A5) (77). SLC1A5 is upregulated in the hypertrophic right ventricle of PAH patients and in the right ventricle of the MCT rat model (43) (**Table 5**). The glutamine antagonist 6-Diazo-5-oxo-L-norleucine decreases glutaminolysis, reduces right ventricular hypertrophy, and increases cardiac output in MCT-induced right ventricular hypertrophy. Cardiac glutaminolysis is associated with microvascular rarefaction/ischemia in the right ventricle of the MCT rat; capillary rarefaction is similarly found in the right ventricle of PAH individuals,

suggesting increased cardiac glutaminolysis (43). Overall, glutamine metabolism and glutamine-derived metabolites are linked to maladaptive remodeling in PAH right heart.

Increased GLS1 expression, GLS activity, and glutamine metabolism are reported in pulmonary arteries of PAH lungs (78). Increased glutamine levels in the circulation are related to severity of PAH. PAH PAECs but not PASMCs have increased glutamine consumption (12, 16, 76, 78) (**Table 5**; **Figure 2*c***). Genetic inhibition of *Gls1* (the main isoform of *Gls*) in endothelial cells or the genetic deletion in mice, or inhibition of *Gls1* in MCT rats, suppresses endothelial cell proliferation and angiogenesis and abrogates the severity of PH in these models (78, 79). These findings support the importance of glutamine metabolism in the proliferative endothelial phenotype of PAH.

Glutamine also has other metabolic fates, including conversion to Δ1-pyrroline-5-carboxylate (P5C) by Δ1-pyrroline-5-carboxylate synthetase (P5CS) encoded by the aldehyde dehydrogenase 18 family member A1 (*ALDH18A1*) gene, a major step in the biosynthesis of proline, ornithine, and arginine (80, 81). A proteomics study found higher levels of ALDH18A1 in PAH PAECs compared to control PAECs (12). Consistent with these results, there is increased expression of ALDH18A1 in PAH lungs (34). High levels of plasma glutamate, a substrate of ALDH18A1, and spermidine, downstream of ornithine, are found in PAH (12). Upregulated ALDH18A1 and consequently elevated spermidine in PAH are similar to findings in rapidly growing malignant cells. Altogether, these studies support a role for glutamine metabolism in cell survival and proliferation in PAH.

2.4. Arginine Metabolism

Arginine, a semiessential amino acid, is the substrate for both nitric oxide synthase (NOS) and arginase (ARG) (**Figure 2*d***). Although arginine may be taken into cells by cationic amino acid transporter proteins, de novo arginine synthesis is the major intracellular source for arginine (82, 83). Endothelial nitric oxide synthase (eNOS), the predominant NOS isoform in the pulmonary vasculature, converts arginine to NO and citrulline. NO is a potent vasodilator that is deficient in PAH (10, 17–20). Under certain in vivo conditions, arginine bioavailability may limit the production of NO, e.g., arginine utilized by other enzymes, such as ARG (30, 84, 85). ARG catabolizes arginine to ornithine and urea (30). ARG1 is present exclusively in the cytosol of hepatic cells as part of the urea cycle, but ARG2 is found in mitochondria of many tissues without a functioning complete urea cycle, including the lungs and heart.

PAH patients, animal models, and PAECs derived from human PAH lungs are deficient in NO production by eNOS (17, 30) (**Figure 2*d***). Loss of NO production in PAH and the underlying mechanisms have been identified: (*a*) phosphorylation inactivation of eNOS and (*b*) decreased eNOS substrate arginine bioavailability due to increased mitochondrial ARG2 (8, 10, 17, 19, 25, 26, 28, 30, 86). A recent study found differential expression of proteins in the eNOS pathway in PAH PAECs, confirming impaired eNOS in PAH (12).

While eNOS activity is low, serum arginase activity is higher in PAH patients compared to controls (8, 30). ARG2 is expressed at higher levels in PAECs from PAH than controls and is present at high levels in the endothelium of PAH lungs in vivo, particularly in plexiform lesions (8, 30, 87). Addition of excess arginine to PAH cells in culture does not recover NO synthesis unless ARG is inhibited by *S*-(2-boronoethyl)-L-cysteine-HCl (8). ARG inhibition results in arginine bioavailability and increased pulmonary NO production in neonatal rat lungs of a bleomycin-induced PH model (88). ARG inhibition in mouse and rat models of PH leads to lower RVSP, reduced lung tissue remodeling, and improved NO bioavailability (89, 90), suggesting a potential treatment strategy for PAH. Arginine and citrulline levels and the arginine-to-ornithine

ratio are significantly lower in plasma of PAH patients, confirming the substrate limitation for NOS in PAH in vivo (8, 12, 30). However, arginine metabolic fate impacts PAH beyond the loss of vasodilatory NO. Arginine metabolism to ornithine via ARG2 can provide glutamate and α-KG to fuel the TCA cycle (**Figure 2*d***). Increased mitochondrial ARG2 production of ornithine provides a steady supply of α-KG to TCA (10, 30), which affects cell metabolism and mitochondrial bioenergetics (electron transport chain and oxygen consumption) in PAH and the accumulation of HIF (11, 49, 91). Mitochondrial arginine metabolism is a biochemical link between the increased vasoconstriction, i.e., loss of NO production, defining the hemodynamic component of PAH with the abnormalities of the TCA cycle and bioenergetics that drive the proliferative phenotype of pulmonary vascular cells.

2.5. One-Carbon Metabolism and Glycine Metabolism

Rapidly proliferating cells upregulate other metabolic pathways, including one-carbon metabolism, which is essential for biosynthetic processes, including purine and thymidine synthesis and homocysteine remethylation, and is pivotal for REDOX balance during hypoxia (92, 93). While mitochondrial generation of one-carbon units from both serine and glycine is not a major metabolic process in nonproliferative adult tissues, one-carbon metabolism is required in proliferating cells. Increased one-carbon metabolism has been observed in the mouse model of PH (94, 95) and in human PAH (12) (**Figure 2*e***).

Serine catabolism is initiated by serine hydroxymethyltransferase (SHMT) activity, catalyzed in the cytosol by SHMT1 and in the mitochondrion by SHMT2. SHMT catalyzes a reversible reaction converting serine to glycine with concurrent 5,10-methylene tetrahydrofolate (5,10-methylene-THF) generation (**Figure 2*e***). THF is an essential precursor for purine and *N*-formylmethionine (fMet) synthesis. One-carbon transformations producing and consuming REDOX equivalents are also important in mitochondrial REDOX homeostasis (96, 97). In cancer cells, the transcription factors HIF1 and MYC cooperate to upregulate SHMT2 during hypoxia to prevent uncontrolled levels of hydrogen peroxide (H_2O_2) in the mitochondrial matrix (93, 98). Proteomic studies reveal that two key enzymes in the one-carbon pathway [SHMT2 and monofunctional C1-THF synthase (MTHFD1L)] are elevated in PAH PAECs (12). Global metabolomics of plasma from PAH patients reveal lower glycine levels, supporting the notion of higher enzyme activity of SHMT2. Global plasma metabolomics also identify an increase in purine metabolites in PAH, including fMet, guanosine, adenosine, inosine, xanthosine, and hypoxanthine. Further studies are needed, but these early studies point to one-carbon metabolism as a mechanism to support the proliferative angiopathy of PAH.

2.6. The Reducing and Oxidizing (REDOX) Cell Environment

ROS, including hydroxyl radicals (•OH), superoxide ($O_2^{\bullet -}$), and H_2O_2, are by-products of normal cellular metabolism. Mitochondria are the largest producers of ROS through the process of cellular respiration in which mitochondria catalyze one-electron reduction of oxygen to a superoxide radical, followed by formation of H_2O_2 in addition to the four-electron reduction of oxygen to water. Antioxidant enzymes include superoxide dismutases (SODs), catalase, glutathione peroxidases (GPx), glutathione *S*-transferase, and thioredoxin. The primary antioxidants for the removal of superoxide are the superoxide dismutases [MnSOD (SOD2) in the mitochondria and CuZnSOD (SOD1) in both cytosol and mitochondria] (99).

While the transition to aerobic glycolysis minimizes the creation of ROS through diminished oxidative metabolism, increased oxidative stress and decreased SOD activity are present in PAH

Table 6 Reduced antioxidants and increased reactive oxygen species (ROS) in pulmonary arterial hypertension

Sources	Parameters	References
Human data		
Pulmonary artery endothelial cells (PAECs) in vitro	↓Superoxide dismutase (SOD) activity by the rate of reduction of cytochrome c ↓SOD2 by Western blot analyses ↓SOD1 by global proteomics and Western blot analyses ↑ROS using CellROX staining	11, 12
Lung	↓SOD activity by the rate of reduction of cytochrome c ↓SOD2 by Western blot analyses ↓Glutathione peroxidase (GPx) activity by spectrophotometry ↑8-Hydroxy guanosine by immunohistochemistry	100, 103
Small pulmonary arteries	↓SOD2 by immunofluorescence ↓ROS by MitoSOX fluorescence staining ↓Hydrogen peroxide (H_2O_2) by Amplex Red	14, 15
Plasma	↑Oxidative stress by measure of levels of cysteine and its oxidized form, cystine glutathione (GSH) and glutathione disulfide (GSSG), using HPLC	105
Animal models		
Shunt lambs		
Lung	↓SOD2 by Western blot analyses ↑Superoxide by MitoSOX fluorescence staining ↑ROS by dihydroethidium (DHE) or dichlorodihydrofluorescein diacetate (H_2DCF-DA) fluorescence staining	40, 102
Chickens with pulmonary hypertension syndrome		
Lung	↓Mitochondrial GSH by HPLC ↑GSSG/GSH ratio by HPLC	51
Breast and heart muscle	↑H_2O_2 using 2′, 7′-dichlorofluorescin diacetate (DCFHDA)	50, 104
Fawn hooded rats		
Pulmonary artery smooth muscle cells (PASMCs)	↓SOD activity with a colorimetric assay ↓SOD2 by immunofluorescence and Western blot analyses ↓ROS by MitoSOX fluorescence staining ↓H_2O_2 by Amplex Red	14, 15
Small pulmonary arteries	↓SOD2 by immunofluorescence ↓ROS by MitoSOX fluorescence staining ↓H_2O_2 by Amplex Red	14, 15
Monocrotaline (MCT) rats		
Right ventricle	↓SOD activity by determination of percent inhibition of pyrogallol autooxidation ↓GPx and catalase activity by spectrophotometry	58

Abbreviation: HPLC, high-performance liquid chromatography.

lungs and PAECs and in animal models of pulmonary hypertension, and contribute to the pathophysiology of PAH (7, 11, 12, 14, 15, 40, 50, 51, 100–105) (**Table 6**; **Figure 2*f***). Excessive ROS generation and loss of SOD activity have been linked to HIF activation (11, 12, 14, 15, 49, 100, 103, 106).

In addition to reduced expression/activity of SOD, the antioxidants glutathione, GPx, and catalase are downregulated in PAH and animal models of PH (12, 50, 51, 58, 100, 105) (**Table 6**). A significant increase in myocardial lipid peroxidation along with a decrease in antioxidant reserve is found in the right ventricle in the late stage of MCT-induced PH (58).

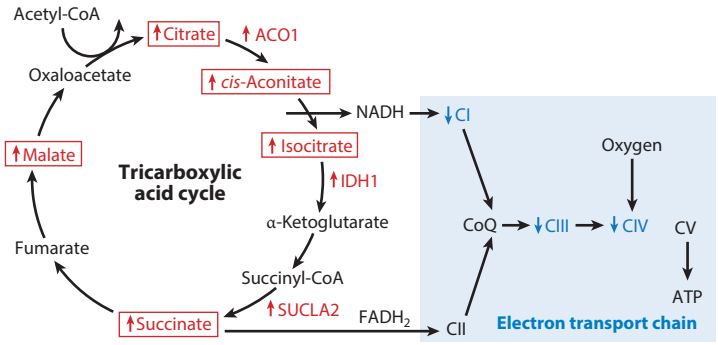

Figure 3

TCA cycle and ETC in PAH. Increased TCA intermediates and downregulated ETC complexes I, III, and IV. Abbreviations: Acetyl-CoA, acetyl coenzyme A; ACO1, aconitase 1; C, complex; CoQ, coenzyme Q; ETC, electron transport chain; $FADH_2$, flavin adenine dinucleotide; IDH1, isocitrate dehydrogenase 1; NADH, reduced nicotinamide adenine dinucleotide; PAH, pulmonary arterial hypertension; SUCLA2, succinate-CoA ligase beta subunit; TCA, tricarboxylic acid.

Taken together, the loss of antioxidant response contributes to greater capacity for ROS generation and increased oxidative stress in PAH. Targeted therapies to decrease ROS generation may be beneficial in PAH.

2.7. The TCA Cycle and Electron Transport Chain

Within the mitochondria, when metabolic processes are used for energy production, the final step is oxidative phosphorylation. The ETC, located in the mitochondrial inner membrane, consists of a series of complexes that receive reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) from TCA to generate ATP. Complexes I and II, along with the flavoprotein-ubiquinone oxidoreductase, transfer electrons from different sources to ubiquinone (coenzyme Q). Next, electrons are transferred sequentially to complex III, cytochrome c, complex IV, and finally to molecular oxygen, the terminal electron acceptor. Protons are pumped by complexes I, III, and IV into the mitochondrial intermembrane space, generating an electrochemical gradient. These protons are then used by mitochondrial F0F1 ATP synthase to synthesize ATP from ADP.

There is some evidence for deficiencies in the ETC in PAH. Expression and/or activity of mitochondrial complexes are reduced in PAECs (10) and small pulmonary arteries (14, 15) from patients with PAH, as well as in PH animal models (14, 15, 50–52) (**Table 3**; **Figure 3**). While CoQ levels in serum are similar between PAH patients and controls, CoQ levels rise to significantly higher levels in PAH patients with supplementation as compared to healthy controls. Supplementation with CoQ improves echocardiographic markers of right ventricular function (107), suggesting that therapies targeting ETC may benefit patients.

While the TCA cycle is important for the oxidation of substrates for energy production (ATP), it is also the biochemical hub that facilitates the traffic flow of carbon in the form of acetyl-CoA, receiving inputs from all metabolic pathways and providing the biomolecules necessary for cell function, proliferation, and signal transduction. Nontargeted metabolomics, proteomics, and transcriptomics analyses indicate that PAH PAECs have abnormal TCA cycle mitochondrial metabolic pathways (12). Metabolomics studies reveal higher TCA cycle metabolites such as citrate, isocitrate, *cis*-aconitate, succinate, and malate in PAH plasma and lung when compared to controls (12,

Table 7 Pathogenic mutations in pulmonary arterial hypertension

Gene	Location	Parameters	References
BMPR2	Chromosome 2	↑Citrate, ↑glutamine metabolism, ↑glycolysis, ↑HIF1α expression, ↑isocitrate dehydrogenase (IDH) activity, ↑lipid accumulation, ↑mitochondrial ROS ↓Acylcarnitines, ↓fatty acid β-oxidation, ↓malate, fumarate, and succinate	39, 56, 76, 108, 112–114
ALK1	Chromosome 12	Glucose and lipid metabolism	119
ENG	Chromosome 9	↓Hepatic triglyceride content, ↓insulin levels, ↓NO production (↓eNOS)	120, 121
SMAD9	Chromosome 13	Involved in the TGF-β/BMP signaling pathway	118
CAV1	Chromosome 7	↑Lipid uptake, ↑^{14}C-palmitate oxidation, ↑cAMP, ↑eNOS-derived NO synthesis and vasodilation ↓Triglycerides, fatty acids, and cholesterol	57, 125
KCNK3	Chromosome 2	↑cAMP, ↑lipolysis rate, ↓lipid accumulation	124

Abbreviations: *ALK1*, activin A receptor-like type 1; BMP, bone morphogenetic protein; *BMPR2*, bone morphogenetic protein receptor type 2; cAMP, cyclic adenosine monophosphate; *CAV1*, caveolin-1; *ENG*, endoglin; eNOS, endothelial nitric oxide synthase; HIF1α, hypoxia inducible factor 1α; *KCNK3*, potassium channel subfamily K member 3; NO, nitric oxide; ROS, reactive oxygen species; *SMAD9*, SMAD family member 9; TGF-β, transforming growth factor β.

34, 44) (**Figure 3**). The higher levels are accompanied by increased expression of genes of TCA cycle enzymes, such as aconitase 1 (ACO1), isocitrate dehydrogenase 1 (IDH1), and succinate-CoA ligase beta subunit (SUCLA2) (**Figure 3**) in PAH lung (34) and biopsies of right ventricles from individuals with PAH (39, 56). Proteomic studies of PAECs show lower levels of the mitochondrial citrate carrier solute carrier family 25 member 1 (SLC25A1) (12, 108). SLC25A1 exchanges mitochondrial citrate to cytosolic malate. Transporting citrate from the mitochondrial to the cytoplasmic compartment is essential to form acetyl-CoA to be used for fatty acid and sterol synthesis in the cell (109) (**Figure 2*c***). Finally, HIF accumulation is adjusted in response to nonhypoxic stimuli of TCA intermediates (49). Prolyl hydroxylases target HIF for proteasomal degradation using both oxygen and α-KG as substrates, while succinate and fumarate are able to compete with α-KG for binding to the catalytic center to decrease degradation of HIF. Thus, TCA metabolism and HIF activity are closely intertwined.

3. GENETICS AND METABOLISM IN PULMONARY ARTERIAL HYPERTENSION

Heritable PAH (HPAH) is defined by the World Symposium on Pulmonary Hypertension (WSPH 1.2) to include patients with an identifiable genetic mutation and/or a family history of the disease. Genetic mutations are found in 25–30% of idiopathic PAH (IPAH, WSPH 1.1) patients (2, 3) (**Table 1**). Heterozygous germline mutations of the *BMPR2* gene, a serine-threonine kinase receptor of the transforming growth factor (TGF)-β superfamily that signals to inhibit cell growth, are identifiable in approximately 75% of HPAH cases and in approximately 20% of IPAH cases (22, 110, 111). Human PAECs with genetic knockdown of *BMPR2* using siRNA have higher levels of glycolysis (112) (**Table 7**). Similarly, human pulmonary microvascular endothelial cells stably transfected with mutant *BMPR2* have greater glycolysis, pentose phosphate, nucleotide salvage, and polyamine biosynthesis pathways. In addition, the stably transfected cells have less carnitine metabolism, fatty acid β-oxidation, and TCA cycle intermediates, i.e., malate, fumarate, and succinate (108). Activity of the TCA cycle enzyme IDH is increased in cells stably expressing mutant *BMPR2* and importantly in the serum of PAH patients with HPAH or IPAH

(108). Increased glutamine metabolism is also present in *Bmpr2*-mutant pulmonary microvascular endothelial cells from genetically modified mice, suggesting that the *Bmpr2* mutation leads to abnormal metabolic pathways (76). Lung endothelial cells of *Bmpr2*-deleted mice have elevation of mitochondrial ROS (112). The right ventricle of the *Bmpr2*-mutant mouse has increased lipid accumulation, decreased acylcarnitines, and decreased fatty acid oxidation (39, 56). HIF1α accumulation is increased in lungs of the *Bmpr2*-mutant mice exposed to bleomycin (113), and in endothelial cells of *Bmpr2*-deleted mice with hypoxia-induced PH (114). All these studies link BMPR2, HIF1α, and metabolism in the pathophysiology of PH.

Other mutations are also associated with PAH (**Tables 1** and **7**), but there are few studies of associated metabolic abnormalities. Mutations in genes encoding other components of the TGF-β/bone morphogenetic protein signaling pathways include activin A receptor-like type 1 (*ALK1*), endoglin (*ENG*), and SMAD family member 9 (*SMAD9*, also known as *SMAD8*) (115–119). Compared with wild-type mice, mice deficient in *Eng* show decreased hepatic triglyceride content, lower insulin levels (120), and decreased NO production due to decreased levels of eNOS (121). Mutations in potassium channel subfamily K member 3 (*KCNK3*) (122) and caveolin-1 (*CAV1*) (123) also are associated with PAH. *Kcnk3*-deficient cells show increased membrane potential, lipolysis rates, and cAMP production (124). Mutation in *Cav1*, which encodes the predominant protein of caveolae plasma membranes, increases lipid uptake but decreases myocardial triglyceride, fatty acid, and cholesterol levels (57), and enhances eNOS-derived NO synthesis and vasodilation (125).

Recent work also supports a role for mitochondrial genetics in PAH. Mitochondria contain ∼1,200 proteins, most encoded by nuclear DNA and imported into the mitochondria (99). Mitochondria have their own circular DNA (mtDNA), which contains 13 genes that encode oxidative phosphorylation proteins crucial for bioenergetic function and that are transmitted to subsequent generations exclusively through the maternal lineage. Due to limited repair, mutations occur with greater frequency in mtDNA than in the nuclear genome (126, 127). Adding to the complexity of studying mtDNA mutations or variants, cells have many mitochondria, and each mitochondrion has ∼10 copies of mtDNA. Thus, mtDNA mutations can be heteroplasmic, when different mtDNA variants coexist within a cell, or homoplasmic, when mutations occur in all the mtDNA within the cell. Mitochondrial haplogroups are defined collections of homoplasmic mutations that are maternally inherited. While haplogroups do not cause disease, they are associated with increased or decreased risk of metabolic, degenerative, infectious, and autoimmune diseases and have been identified as at risk or protective from cardiovascular disease, type 2 diabetes mellitus, neurodegenerative diseases, and several types of cancer (27, 126, 128). Several mitochondrial haplogroups, i.e., the M macrohaplogroup and the HV, JT, and UK haplogroups of the N macrohaplogroup are associated with the risk of PAH (27). mtDNA copy number is associated with cardiovascular disease and human cancers (129, 130). In a meta-analysis comparing individuals with cardiovascular disease to controls, mtDNA copy number relative to nuclear DNA is a biomarker of mitochondrial function and could predict cardiovascular events and mortality in longitudinal studies; i.e., a lower mtDNA copy number in blood predicts more cardiovascular events (131). mtDNA copy numbers are decreased in PAH PAECs (10) but have not been evaluated in PAH patients.

The impact of nuclear and mitochondrial mutations on metabolism in PAH is relatively underexplored. The roles of mitochondrial haplogroups, hetero- and homoplasmy, mtDNA copy numbers, and the cross talk between the nuclear and mitochondrial genomes need further investigation. Discovering the connection between genetics and metabolism could be beneficial for further understanding the genetic mechanisms that underlie the disease.

4. METABOLISM AND POTENTIAL THERAPEUTICS FOR PULMONARY ARTERIAL HYPERTENSION

Metabolic changes in PAH suggest new metabolic avenues for therapeutics. Preclinical and early clinical data support targeting many of the mitochondrial pathways to improve disease severity and outcomes. Specifically targeting glucose metabolism has been proposed in the treatment of PAH and shows some success. Dichloroacetate, an inhibitor of pyruvate dehydrogenase kinase, improves right ventricular function in animal PH models, increases mitochondrial respiration, lowers the mean pulmonary arterial pressure and PVR, and improves functional capacity (6-min walk) in patients with PAH (36, 132, 133). Targeting the glycolytic enzymes enolase (ENO) and PFKFB3 decreases glucose metabolism and improves right ventricular function in rat PH models (35, 37). mTOR inhibitors, which target HIF1α and block glycolysis, may also be viable treatments for PAH (134–136). Carvedilol, an adrenergic receptor blocker, decreases expression of glycolytic enzymes and reverses right ventricular remodeling in a rat PH model (41, 137). Carvedilol also decreases right ventricular glycolysis measured by FDG uptake and improves cardiac function in patients with PAH (47, 48).

Decreasing long-chain fatty acid oxidation with trimetazidine and ranolazine leads to improved cardiac output and treadmill distance in a right ventricular hypertrophy rat model (138). Metformin, an antihyperglycemic agent, enhances fatty acid oxidation and reduces lipid deposition in the right ventricle of *Bmpr2*-mutant transgenic mouse models (39). The glutamine antagonist 6-Diazo-5-oxo-L-norleucine inhibits glutaminolysis, abrogates right ventricular hypertrophy, and improves cardiac function and treadmill distance in a right ventricular hypertrophy rat model (43). Inhibitors of *Gls1* decrease pulmonary vascular cell proliferation in the monocrotaline rat model of PH (78). Likewise, 2-hydroxyben-zylamine (2HOBA), which decreases glutamine metabolism, improves cardiac output and decreases RVSP in a *Bmpr2*-mutant mouse PH model (76). Arginase inhibitors reverse lung tissue remodeling, reduce RVSP, and improve NO bioavailability in animal PH models (89, 90). Oral arginine therapy lowers pulmonary artery systolic pressures in PH patients with sickle cell disease (139).

Improving REDOX balance is another highly interesting avenue for treatment. High dietary vitamin E decreases mitochondrial H_2O_2 production and mitochondrial oxidative stress in avian species with PH syndrome (104). Treatment with Mn(III)tetrakis (4-benzoic acid) porphyrin (MnTBAP), a synthetic SOD-mimetic, lowers mean pulmonary artery pressure and PVR and improves treadmill distance in a PH rat model (132). In humans, supplementation with coenzyme Q, critical in mitochondria functions and REDOX, promotes greater mitochondrial respiration and improves echocardiographic markers of right ventricular function in PAH (107).

These reports of metabolic intervention successes are encouraging. However, further work is needed to define whether metabolic interventions will improve survival and/or reverse the cardiopulmonary remodeling of the disease. A precision approach for care will almost certainly be needed for metabolic interventions, as well as close observation over time to ensure that there are no, or limited, adverse effects. There is also a gap in our knowledge of how to best combine metabolic therapies in the context of current PH therapeutic approaches. Given the continued morbidity and mortality of PAH despite available therapies, these new metabolic concepts are likely to provide further advances in the care of patients.

5. SUMMARY AND FUTURE DIRECTIONS

Metabolic abnormalities are found in human PAH and animal models of PH. Key hallmarks of PAH metabolism include a shift to glycolysis, increased glutamine utilization and one-carbon

metabolism, and decreased fatty acid oxidation. Increased ROS generation and changes in the TCA cycle intermediates may contribute to HIF stabilization, driving the metabolic changes seen. Arginine metabolism, which links NO production and the TCA cycle, has a key role linking the vasoconstrictive phenotype of the disease with the metabolic abnormalities. The metabolic phenotype of PAH is characteristic of proliferative cells such as cancer. Thus, therapies being used to target metabolism in cancer might have relevance in addressing the hyperproliferation seen in vascular cells in PAH.

The interconnectedness of metabolic pathways, genetics, and substrate availability and utilization is difficult to study in vivo and in vitro. Novel bioinformatics methods, including genomics, transcriptomics, proteomics, metabolomics, network analysis, and systems biology, are powerful tools for a deeper understanding of metabolism in silico. Big data and computational modeling will provide tools with which we can address the questions of how the metabolic abnormalities in PAH develop and determine which pathways are ideal therapeutic targets.

While traditional therapies targeting the vasodilator–vasoconstrictor imbalance in PAH have resulted in improved survival, they are insufficient to cure the disease. Fully understanding mitochondrial dysfunction and metabolic abnormalities of PAH over the course of the disease is the essential next step for developing therapies.

SUMMARY POINTS

1. Metabolic abnormalities in PAH vary by cell type (PAEC versus PASMC) and organ (heart versus lung).
2. It is uncertain whether metabolic derangements are causative or compensatory for the disease.
3. HIF stabilization is mechanistically linked to altered metabolism.
4. Arginine metabolic fate connects metabolism to the vasoconstrictive phenotype of PAH.
5. Nuclear and mitochondrial genetics influence metabolism and risks for PAH.

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