Although traditionally pulmonary arterial hypertension (PAH) has been described as a disease exclusively of the pulmonary arteries, there is now evidence that PAH involves many other organs (Figure 1). For example, the hearts (right ventricle [RV]) of patients with PAH decompensate and fail at a much faster rate compared with what one would expect extrapolating from the left ventricle, suggesting that there may be mechanisms intrinsic to the RV that promote RV failure in PAH. Even patients with idiopathic PAH, without coexistent autoimmune disorders, have circulating immune cells that are activated, producing a strong inflammatory environment and potentially contributing to the malaise and weakness that patients with PAH have. Recent evidence suggests that, perhaps, their skeletal muscles (the target organs in the metabolic syndrome) have abnormalities similar to patients with diabetes mellitus, obesity, and

**Compendium**

**Circulation Research Compendium on Pulmonary Arterial Hypertension**

Pulmonary Arterial Hypertension: Yesterday, Today, Tomorrow
Pulmonary Arterial Hypertension: The Clinical Syndrome
Current Clinical Management of Pulmonary Arterial Hypertension

**The Metabolic Theory of Pulmonary Arterial Hypertension**

Inflammation and Immunity in the Pathogenesis of Pulmonary Arterial Hypertension
The Right Ventricle in Pulmonary Arterial Hypertension: Disorders of Metabolism, Angiogenesis and Adrenergic Signaling in Right Ventricular Failure
The Genetics of Pulmonary Arterial Hypertension

Guest Editor: Evangelos Michelakis

**The Metabolic Theory of Pulmonary Arterial Hypertension**

Roxane Paulin, Evangelos D. Michelakis

**Abstract:** Numerous molecular abnormalities have been described in pulmonary arterial hypertension (PAH), complicating the translation of candidate therapies to patients because, typically, 1 treatment addresses only 1 abnormality. The realization that in addition to pulmonary artery vascular cells, other tissues and cells are involved in the syndrome of PAH (eg, immune cells, right ventricular cardiomyocytes, skeletal muscle) further complicates the identification of optimal therapeutic targets. Here, we describe a metabolic theory that proposes that many apparently unrelated molecular abnormalities in PAH do have a common denominator; they either cause or promote a mitochondrial suppression (inhibition of glucose oxidation) in pulmonary vascular cells; in turn, the signaling downstream from this mitochondrial suppression can also explain numerous molecular events previously not connected. This integration of signals upstream and downstream of mitochondria has similarities to cancer and can explain many features of the PAH vascular phenotype, including proliferation and apoptosis resistance. This suppression of glucose oxidation (with secondary upregulation of glycolysis) also underlies the abnormalities in extrapulmonary tissues, suggesting a global metabolic disturbance. The metabolic theory places mitochondria at the center stage for our understanding of PAH pathogenesis and for the development of novel diagnostic and therapeutic tools. Current PAH therapies are each addressing 1 abnormality (eg, upregulation of endothelin-1) and were not developed specifically for PAH but for systemic vascular diseases. Compared with the available therapies, mitochondria-targeting therapies have the advantage of addressing multiple molecular abnormalities simultaneously (thus being potentially more effective) and achieving higher specificity because they address PAH-specific biology. (Circ Res. 2014;115:148-164.)

**Key Words:** hypertension, pulmonary □ metabolism □ mitochondria

Although traditionally pulmonary arterial hypertension (PAH) has been described as a disease exclusively of the pulmonary arteries, there is now evidence that PAH involves many other organs (Figure 1). For example, the hearts (right ventricle [RV]) of patients with PAH decompensate and fail at a much faster rate compared with what one would expect extrapolating from the left ventricle, suggesting that there may be mechanisms intrinsic to the RV that promote
PAH: Pathology and Molecular Phenotypes

Pulmonary Arteries

Metabolic Remodeling in PAH Vascular Cells and Its Signaling Implications

PAH is characterized by an obliterative vascular remodeling in the lungs. Pulmonary artery smooth muscle cells (PASMC), endothelial cells, fibroblasts, and myofibroblasts, at the peak of the disease, exhibit a proliferative and antiapoptotic phenotype that results in obliteration of the lumen by several of the recognized lesions, ie, intima and media hypertrophy and plexogenic arteriopathy. The pathology of the disease is also characterized by infiltration of activated inflammatory and metabolic syndrome, conditions also characterized by generalized inflammation. The serum of patients with PAH has increased levels of lipids and insulin although they may be neither obese nor diabetic. These muscle abnormalities may also contribute to the weakness these patients have. Are all these abnormalities simply resulting from a primary problem in the pulmonary arteries, perhaps related to the decreased cardiac output resulting from RV failure, or are they a part of a syndrome with multorgan involvement?

Here, we propose a theory supporting the latter. We propose that there may be a global mitochondrial abnormality in many patients with PAH, which affects diverse tissues, including the pulmonary vascular cells, explaining the involvement of many organs in this disease. This suggests that treatments that may attack one of the foundations of the problem, ie, the mitochondria, might be more effective in terms of treating the patient as a whole and not just the pulmonary vessels. Another important implication of this theory is that the myriad molecular abnormalities that have been described in pulmonary vascular cells may have (at least many of them) a common denominator. After a cancer paradigm (where downstream signaling from diverse oncogenes converges to mitochondria, suppressing glucose oxidation with a secondary upregulation of cytoplasmic glycolysis, both contributing to the antiapoptotic and proproliferative phenotype of cancer cells), we propose that mitochondria can integrate abnormal signaling from several diverse causes and result in the PAH cellular phenotype as we know it today. This may simplify efforts to discover therapies because attacking the mitochondrial abnormalities may correct the effects of several primary molecular abnormalities, without having to attack every single one of them simultaneously.

Non standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>αKG</td>
<td>α-ketoglutarate</td>
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<tr>
<td>BMPR-II</td>
<td>bone morpogenic protein receptor II</td>
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<tr>
<td>DRP-1</td>
<td>dynamin-related protein 1</td>
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<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>FDG</td>
<td>18F-labeled deoxyglucose</td>
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<tr>
<td>HIF-1α</td>
<td>hypoxia-inducible factor 1α</td>
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<tr>
<td>MnSOD</td>
<td>manganese superoxide dismutase</td>
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<tr>
<td>nROS</td>
<td>mitochondria-derived reactive O₂ species</td>
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<tr>
<td>MS</td>
<td>mass spectrometry</td>
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<tr>
<td>NFAT</td>
<td>nuclear factor of activated T cells</td>
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<tr>
<td>PAEC</td>
<td>pulmonary artery endothelial cells</td>
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<td>PAH</td>
<td>pulmonary arterial hypertension</td>
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<td>PASMC</td>
<td>pulmonary artery smooth muscle cells</td>
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<td>PDH</td>
<td>pyruvate dehydrogenase</td>
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<td>PDK</td>
<td>pyruvate dehydrogenase kinase</td>
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<tr>
<td>PET</td>
<td>positron emission tomodraphy</td>
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<tr>
<td>PPARγ</td>
<td>peroxisome proliferator-activated receptor γ</td>
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<tr>
<td>RV</td>
<td>right ventricle</td>
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<tr>
<td>TNFα</td>
<td>tumor necrosis factor α</td>
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<tr>
<td>UCP</td>
<td>uncoupling protein</td>
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<td>VOC</td>
<td>volatile organic compound</td>
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immune circulating cells. Of all the cells within the remodeled pulmonary arteries, the PASMC have been studied the most although the pulmonary artery endothelial cell (PAEC) and fibroblasts are increasingly being studied.

The molecular phenotype of PAH-PASMC persists even in vitro (where the potential primary triggers like circulating factors or paracrine signals are absent), suggesting that there may be positively reinforcing feedback loops that sustain the

<table>
<thead>
<tr>
<th>Table. Molecular Abnormalities of Pulmonary Arterial Hypertension</th>
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<tbody>
<tr>
<td><strong>Not related to the metabolic theory</strong></td>
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<tr>
<td>Decreased expression/function of TGF-β superfamily of receptor/ALK1</td>
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<tr>
<td>Decreased VIP signaling</td>
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<tr>
<td>Decreased prostaglandins/prostacyclins/thromboxanes</td>
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<tr>
<td>Increased elastolytic activity</td>
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<tr>
<td>Increased leukotriene B4 signaling</td>
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<tr>
<td>Increased small GTPase RhoA/ROCK activation</td>
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<td>Increased serotonin activity</td>
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<tr>
<td><strong>Indirectly related to the metabolic theory</strong></td>
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<tr>
<td>Increased endothelin axis</td>
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<td>Increased renin/angiotensin system</td>
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<tr>
<td>Decreased NO-GC-cGMP-PDE5 axis</td>
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<td>Decreased apelin/APJ system</td>
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<td>Increased S100A4 axis</td>
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<tr>
<td>Increased RAGE activation</td>
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<tr>
<td>Sustained activation of the c-Src/STAT3/Pim1 by miR-204</td>
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<tr>
<td>Upregulation of miR-145</td>
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<tr>
<td>Decreased BMPR-II/SMAD axis</td>
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<tr>
<td>Impaired PPARγ/ApoE signaling</td>
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<tr>
<td>Increased Notch 3 signaling</td>
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<tr>
<td>Increased p38/MAPK</td>
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<tr>
<td>Suppressed tumor suppressors p53, p21, p27</td>
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<tr>
<td>Tyrosine kinase receptors activation</td>
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<tr>
<td>Histone regulation</td>
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<tr>
<td>Upregulation of miR-210</td>
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<tr>
<td>Iron homeostasis</td>
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<tr>
<td>Increased survivin expression</td>
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<tr>
<td>Decrease PDH activity/Warburg effect</td>
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<td>Increased HIF-1α signaling</td>
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<td>Decreased SOD2 activity</td>
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<tr>
<td>Increased expression and activation of NFAT</td>
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<tr>
<td>Repression of voltage-gated K+ (Kv) channels</td>
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<tr>
<td>Decreased PGC1α axis/ mitochondria biogenesis, fission, and fusion</td>
</tr>
<tr>
<td>Increased ER stress, ATF6/Nogo-B axis</td>
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<tr>
<td>Decreased UCP2 function</td>
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*ALK1 indicates activin A receptor type II-like 1; APJ, apelin receptor J; ApoE, apolipoprotein E; ATF6, activating transcription factor 6; BAX, B-cell CLL/lymphoma 2–associated X protein; BMPR-II, bone morphogenic protein receptor II; c-Src, v-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; ER, endoplasmic reticulum; GC, gas chromatography; GTPase, guanosine-5’-triphosphate hydrolase; HIF, hypoxia-inducible factor; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T cells; NO, nitric oxide; PDE5, phosphodiesterase 5; PDH, pyruvate dehydrogenase; PGC-1α, PPARγ coactivator-1α; PPARγ, peroxisome proliferator–activated receptor γ; RAGE, advanced glycosylation end product–specific receptor; RhoA, ras homolog family member A; ROCK, rho-associated, coiled-coil containing protein kinase 1; SMAO, mothers against decapentaplegic homolog; SOD2, manganese superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor β; UCP, uncoupling protein; and VIP, vasoactive intestinal peptide.*
phenotype. The PAH-PASMC have many features of cancer cells, often exhibiting activated oncogenes or expressing cancer markers. Like in cancer, a long and diverse list of molecular abnormalities has been described in the past 15 years in PAH. With the unintended risk of missing some important findings, we list many of these molecular abnormalities in the Table. At first, one is impressed by the diversity of these mechanisms. Although many of these abnormalities have been successfully targeted in animal models in vivo, an important question arises: which of these are the most important, worthy of clinical translation? Practically, one can envision a combination therapy in the future with 2 or maybe 3 classes of drugs, like in systemic hypertension. But which of these should be left out? And does this not mean that the treatment would be incomplete because many abnormalities will remain active? We have recently proposed that this diversity is one of the main reasons that effective translation of animal work into human is poor. We proposed that a comprehensive theory attempting to put many of these abnormalities under one roof might offer more effective therapies. The metabolic theory suggests that many of these factors directly or indirectly relate to the metabolic/mitochondrial phenotype of the disease (Table), which we describe below. In other words, such a theory predicts that a single therapy aiming to normalize a mitochondrial abnormality may be beneficial to a large number of abnormalities that, although on surface seem diverse, they may essentially be a result of abnormal signaling downstream of mitochondria.

PAH-PASMCs are hyperproliferative and resistant to apoptosis, 2 properties that may be because of mitochondrial remodeling. Mitochondria are the metabolic sensors of the cell, and as such decide for both life (ATP production) and death (apoptosis). Mitochondria-dependent apoptosis maintains metabolic fuel efficiency and homeostasis, and its suppression offers a survival advantage to proliferating cells. PAH-PASMC mitochondria from several models and human tissues are hyperpolarized, have suppressed glucose oxidation and respiration, and have upregulated glycolysis. This normoxic glycolytic phenotype (the Warburg effect) is essentially identical to that of cancer cells.

There are several important downstream implications of this suppression of mitochondrial function that all result in characteristic features of PAH: (1) suppressed apoptosis, (2) suppressed signaling that affects several proproliferative downstream redox-sensitive extramitochondrial factors including transcription factors and plasmalemmal Kv channels, (3) increased availability of nonoxidized sugars, lipids, and amino acids for the building blocks of proliferating cells, and (4) potential signals to the nucleus regulating epigenetic mechanisms or even inducing inflammation via the induction of mitochondria-based inflammasomes. Here is how this happens:

Suppressed apoptosis: Proapoptotic mediators (like cytochrome c or apoptosis-inducing factor) efflux into the cytoplasm from the mitochondria through the mitochondrial transition pore, a voltage- and redox-sensitive megachannel. Increase in the mitochondrial membrane potential promotes closing of this channel and thus establishes a state of relative resistance to apoptosis. PASMC from many animal models and human tissues have hyperpolarized mitochondria like cancer cells. The generation of mitochondrial membrane potential is regulated by several mechanisms, and it is closely linked to the influx of carbons (carbohydrates, lipids) that enter mitochondria for oxidation. There is suppression of carbohydrates entry in mitochondria in PAH (and cancer), largely because the key enzyme regulating the influx of pyruvate (the product of glycolysis in the cytoplasm) into the mitochondria where it becomes acetyl-CoA and feeds the Krebs cycle (ie, pyruvate dehydrogenase, PDH) is inhibited. This results in suppression of glucose oxidation. The decrease in the efficiency of ATP production (2 molecules of ATP produced per glucose molecule in the cytoplasm from glycolysis versus 36 in the mitochondria from glucose oxidation) triggers several mechanisms to increase glucose entry and upregulate glycolysis. As part of this switch to glycolysis, on both PAH and cancer, glycogen synthase kinase 3β is activated and translocates from the cytoplasm to the mitochondrial outer membrane, where it binds and inhibits the voltage-dependent anion channel, a component of the mitochondrial transition pore. This traps anions in the mitochondria, increasing the membrane potential in PAH-PASMC (Figure 2) is a key feature of the PAH phenotype and links the supply of fuel to the resistance to apoptosis that characterizes this disease. Finally, another mechanism that may contribute to the suppression of mitochondria-driven apoptosis in PAH-PASMC is the activation of the master transcription factor, nuclear factor of activated T cells (NFAT, discussed later on), which induces an increase in the antiapoptotic members of the bcl-2 family. The bcl-2 system regulates apoptosis not by the reversible opening/closure of the mitochondrial transition pore but by the rupture of the outer mitochondrial membrane, resulting in the release of proapoptotic mediators.

Disturbed downstream signaling: During the flow of electrons down the electron transport chain complex redox gradient, some electrons react with molecular oxygen and produce mitochondria-derived reactive O2 species (mROS), mostly superoxide. In the presence of manganese superoxide dismutase (MnSOD), superoxide is dismutated to the more stable and diffusible H2O2, which can reach extramitochondrial targets, including membrane K+ channels or cytoplasmic redox-sensitive transcription factors. For example, the inhibition of Kv channels (activated by increased mROS and inhibited by decreased mROS) causes plasmalemmal depolarization, resulting in increased influx of Ca2+, which promotes constriction and proliferation. H2O2 can also regulate redox-sensitive components of the transcription machinery of the proproliferative hypoxia-inducible factor 1α (HIF-1α). Similarly,
the Krebs cycle–derived α-ketoglutarate (αKG) can leak into the cytoplasm and regulate HIF-1α because it is a required co-factor for the prolyl-hydroxylases that destabilize HIF-1α.7,88 Thus, the primary changes in mitochondrial function occurring in cancer cells and idiopathic PAH-PASMCs decreases mROS and αKG production, resulting in HIF-1α stabilization (Figure 2) and activation, even in the absence of hypoxia, a well-described event in PAH.67,69 Although the biology of the PAH-PASMCS is different than that of a normal PASMC, it is important to note that controversy exists on how hypoxia regulates mROS versus extramitochondrial reactive O₂ species in normal PASMC. This controversy likely relates not only to the complexity of redox biochemistry and its dynamic nature but also to the many different techniques and models used by different groups.90-92 Nevertheless, HIF-1α can feedback and inhibit mitochondrial function by increasing the expression of pyruvate dehydrogenase kinase (PDK) an inhibitor of PDH, thereby sustaining it own activation.93 The increased cytoplasmic Ca²⁺ causes activation of the master transcription factor NFAT (a well-described feature of PAH)²²,66,69,74 by promoting its entry to the nucleus. The switch toward glycolysis that follows mimics the well-described feature of PAH22,66,69,74 by promoting its entry to the nucleus promoting its activation. NFAT inhibits the transcription of many mitochondrial proteins and the expression of Kv channels.95 Thus, 2 important transcription factors that play a critical role in PAH participate in sustained activating feedback loops, which may explain why isolated PASMC maintain the same phenotype when cultured out of the PAH environment and why it is so difficult to reverse many molecular abnormalities in PAH.

Citrates, αKG, and epigenetic mechanisms: These diffusible small molecule products of the Krebs cycle can regulate both methylation (αKG) and acetylation (citrate) of nuclear histones.57,58 For example, citrate is considered to be the only known source of acetyl-CoA in the nucleus. Because acetyl-CoA is impermeable to membranes, citrate that leaks out of the mitochondria can enter the nucleus where in the presence of ATP-citrate lyase can provide acetyl-CoA for histone acetylation.57 Although not studied in detail, a role of histone deacetylase inhibitors as potential PAH therapies has been proposed.59 The benefits of these drugs suggest that histone acetylation is decreased in PAH-PASMC, which is compatible with the decreased production of citrate from the Krebs cycle (although it is possible that citrate could also be synthesized in the cytoplasm by the glutamine pathway, a mechanism established in cancer but not adequately studied in PAH).²⁵

Inflammasomes: Although unexplored in PAH, we now know that mitochondria can induce inflammasomes, multiprotein complexes engaged by the nucleotide-binding oligomerization domain (NOD)-like family of cytoplasmic receptors. NOD-like family of cytoplasmic receptors continuously monitors the cytosol and on detection of cellular stress oligomerizes and exposes its effector domain for interaction with the adaptor apoptosis-associated speck-like protein, which in turn recruits procaspase-1. Pro-caspase-1 clustering leads to its activation via auto-processing, and active caspase-1 proteolytically cleaves a variety of cytoplasmic targets, including interleukin-1β (which is increased in PAH).²⁶ After an activation cascade, several other
Inflammatory cytokines are subsequently activated, also known to be increased in PAH (as discussed in another chapter of this compendium). The NOD-like family of cytoplasmic receptors inflammasome is activated by high glucose levels in β cells in the pancreas, leading to cleavage and secretion of interleukin-1β. In addition to cleaving interleukin-1β, caspase-1 also targets several key glycolytic enzymes, such as aldolase and pyruvate kinase, suggesting that the NOD-like family of cytoplasmic receptors inflammasome reciprocally regulates cellular metabolism under stress conditions.

Similar glycolytic phenotypes, for example, increased uptake of glucose and increased production of lactic acid, have also been described in PAH-PAEC. After the cancer paradigm, this is likely a result of a primary suppression of glucose oxidation. These mechanisms yet have not been studied in fibroblasts, to the best of our knowledge. Last, the many ways that the cancer stroma can regulate metabolic pathways in the cancer cells have not been studied in PAH, where the interstitial matrix nevertheless plays an important role in overall tissue remodeling.

In summary, many aspects of the PAH cellular phenotype (eg, hyperpolarized mitochondria, decreased mROS, activated HIF-1α and NFAT, inhibited Kv channels, increased cytoplasmic calcium, suppressed histone acetylation, and activated inflammation) can be potentially explained by an inhibition of mitochondrial oxidative phosphorylation (and specifically glucose oxidation). What is the cause of this inhibition? Below we describe several intramitochondrial and extramitochondrial causes that have been described in PAH.

**Causes of the Metabolic Remodeling in PAH**

**Vascular Cells**

Extramitochondrial causes: Regulation of PDH. A major regulatory mechanism for PDH is its tonic inhibition by PDK with which it forms a complex. There are 4 isoforms of PDK, with PDK1 and 2 being ubiquitously expressed, PDK3 being

![Figure 3. Example of 2 intramitochondrial causes of pulmonary arterial hypertension (PAH). A, Pyruvate dehydrogenase (PDH) inhibition characterizes many animal models and human tissues with PAH. The importance of this is shown by the fact that activation of PDH by the small molecule dichloroacetate (DCA; an inhibitor of pyruvate dehydrogenase kinase, which phosphorylates and inhibits PDH), reactivates mitochondria-dependent apoptosis and reverses PAH in many animal models. A representative example of pulmonary artery pressure (measured by Millar catheter advanced through the jugular vein in the rat) is shown before and after 3-week therapy of DCA (added in the drinking water). The pulmonary arteries of the treated rats show evidence of activated apoptosis in the vascular wall (measured by TUNEL shown by arrows). Reprinted from McMurtry et al with permission of the publisher. B, The mitochondrial protein uncoupling protein (UCP) 2 functions as a mitochondrial Ca²⁺ channel, facilitating Ca²⁺ entry into the mitochondria from the endoplasmic reticulum. Lack of UCP2 causes a decrease in mitochondrial Ca²⁺ levels, which in turn inhibits many calcium-dependent mitochondrial enzymes including PDH. Ucp2KO-mice spontaneously develop pulmonary hypertension and pulmonary vascular remodeling. A representative example of pressures recorded in the right atrium, right ventricle (RV), and pulmonary artery in a wild-type mouse and the pulmonary artery in a Ucp2KO-mouse. In the histology examples, small pulmonary arteries are stained with smooth muscle actin (green), the proliferative marker Ki67 (red), and nuclei stained in blue with DAPI. Note the media thickening and the presence of proliferating cells (arrow) in the arteries from the Ucp2KO-mouse. Reprinted from Dromparis et al with permission of the publisher. DAPI indicates 4',6-diamidino-2-phenylindole; PA, pulmonary artery; PAP, pulmonary arterial pressure; RA, right atrium; and TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.
expressed in the testes, and PDK4 being inducible mostly in muscle under metabolic stress. The expression of PDK1 is induced by HIF-1α, and its activity can be enhanced by tyrosine kinase phosphorylation (thus both conditions inhibiting PDH); both HIF-1α and tyrosine kinase axis activation are well-described features of animal and human PAH. The importance of PDH in PAH and cancer is supported by the fact that the small molecule inhibitor of PDK dichloroacetate activates PDH, reversing the glycolytic shift and the resistance to apoptosis of PASMC and both prevents and reverses PAH in several models of PAH in vivo. Dichloroacetate increases survival in rats with PAH and its ability to also improve RV contractility further enhances its therapeutic potential.

Another way that PDH activity can be inhibited is by the activation of fatty acid oxidation. The Randle cycle ensures that only 1 of the 2 mechanisms, ie, glucose versus fatty acid oxidation, can be used as a primary fuel source at any given time, thus inhibition of fatty acid oxidation enhances PDH activity. Drugs or molecular mechanisms that decrease the activity of the fatty acid oxidation regulator malonyl-CoA decarboxylase enhance PDH activity, preventing the mitochondrial remodeling under PAH stimuli. Thus, trimetazidine and knockout of malonyl-CoA decarboxylase cause reversal and prevention of PAH in rodent models in vivo.

Endoplasmic reticulum (ER) stress. PDH and several other mitochondrial enzymes (eg, αKG-dehydrogenase and isocitrate dehydrogenase) are calcium-dependent. Thus, a decrease in mitochondrial Ca²⁺ inhibits PDH. We now know that ER stress can decrease mitochondrial calcium, by causing functional disruption of the ER-mitochondria unit. This is because the ER is the major calcium supplier of mitochondria. Expression of the reticulon protein Nogo, under the ER stress-activated transcription factor 6 axis, alters the shape of the ER, disrupting the functional mito-ER unit at specialized contact points of the 2 organelles, where the exchange of calcium and lipids occurs (Figure 4). PASMC lacking Nogo do not develop the PAH phenotype under hypoxia in vitro, and Nogo knockout mice are resistant to the development of chronic hypoxia–induced pulmonary hypertension. ER stress is a result of many known triggers of PAH including bone morphogenic protein receptor II (BMPR-II) mutations (which can result in accumulation of unfolded protein aggregates), viral infections (like human immunodeficiency virus and herpes simplex virus), upregulation of Notch signaling, hypoxia, or inflammation. Increased Endothelin-1 signaling, a well-studied pathway in PAH, was also recently shown to induce ER stress. Inhibition of ER stress by the small molecule chemical chaperone phenylbutyrate reverses the decrease in mitochondrial calcium and the PDH inhibition and normalizes the cellular phenotype of PAH, whereas it prevents and reverses PAH in rodent models. ER stress in PAH-PASMCs has also been linked to increased production of cytokines like interleukin-6 and chemokine (C-C motif) ligand 2/ mast cell proteinase-1.

Figure 4. The disruption of the mitochondria/endoplasmic reticulum (ER) unit induced by ER stress results in suppression of mitochondrial function. ER stress is a result of many known triggers of pulmonary arterial hypertension (PAH) including bone morphogenic protein receptor II and other mutations (like in Kv channels; such mutations have been shown to disturb intracellular cell trafficking and induce the unfolded protein response), viral infections, hypoxia, or inflammation. At least in pulmonary artery, smooth muscle cells ER stress activates the activating transcription factor 6 (ATF6) that enhances the expression of the reticulon protein Nogo. Nogo alters the shape of the ER, disrupting the functional mito-ER unit at points where the exchange of calcium occurs, thus decreasing calcium supply for mitochondrial calcium-dependent enzymes and resulting in decreased glucose oxidation and an overall mitochondrial suppression. The ER is the largest supplier of calcium for the mitochondria. Animals lacking Nogo are resistant to the development of pulmonary hypertension, and ER stress inhibitors have been shown to reverse PAH in animal models, as discussed in the text. Pyruvate dehydrogenase (PDH), isocitrate dehydrogenase (IDH), and α-ketoglutarate dehydrogenase (αKGDeH) are all important mitochondrial enzymes that are dependent on intramitochondrial calcium levels. MCU indicates mitochondrial calcium uniporter; and TCA, tricarboxylic acid cycle.
which in turn promote the activation and recruitment of macrophages in the lung. Another inhibitor of ER stress, salubrinal, also reverses monocrotaline PAH in a PERK (protein kinase RNA-like endoplasmic reticulum kinase)-dependent manner, and this was also associated with decrease recruitment of macrophages in the lung. ER stress is a feature of PAH-PAEC as well. In fact, a structurally abnormal ER (suggesting ER stress) in PAEC was prominent in the early classic descriptions of PAH pathology by Smith and Heath in 1979.

Additional factors suppressing mitochondrial function in PAH. There are many other examples in which additional well-characterized pathways in PAH can, in addition to their primary effect, lead to mitochondrial suppression, including signal transducer and activator of transcription 3, suppressed BMPR-II signalling and peroxisome proliferator–activated receptor γ (PPARγ) signaling. For example, signal transducer and activator of transcription 3 can downregulate BMPR-II/PPARγ and activate NFAT (which, as discussed, decreases the expression of many mitochondrial enzymes and members of the bcl-2 family); the BMPR-II axis inhibition as discussed above can cause ER stress (in the case of mutated dysfunctional BMPR-II receptors that disturb cellular trafficking) or inhibit PPARγ signaling, which in turn inhibits mitochondrial biogenesis; PPARγ activators have shown promise as potential therapies of PAH in animal models. PPARγ also regulates the expression of Apelin, which has been shown to increase mitochondrial biogenesis, improve mitochondrial oxidative capacity, and enhance insulin-stimulated glucose uptake.

Mitochondrial function can also be suppressed by dysregulation of the processes that regulate the mitochondrial network in the cytoplasm (fission/fusion) and mitochondria numbers (mitogenesis and mitophagy), which optimize energy and signaling production particularly in critical microdomains in the cells, in response to stressful stimuli. There is evidence that both processes, in response to extramitochondrial triggers, are disturbed in PAH:

Fission/fusion. The fusion machinery consists of the dynamin-like proteins mitofusin 1 and 2 that promote the fusion of the outer mitochondrial membrane and optic atrophy 1, which is involved in the sequential fusion of the inner mitochondrial membranes. Mitochondrial fission, however, mainly results from the dynamin-related protein 1 (DRP-1), a large GTPase assembled into oligomeric rings around the outer mitochondrial membrane. The maintenance of mitochondrial network and shape depends on a constant equilibrium between fusion and fission events, and alterations of this balance can disrupt mitochondrial signaling and regulation of cell cycle progression or apoptosis. The mitosis-initiating kinase B1-CDK1 phosphorylates DRP-1 at serine 616, leading to fission and regulating an equitable distribution of mitochondria to daughter cells on cell division.

The mitochondrial suppression of PAH-PASMC has recently been linked to increased fission and increased activity of DRP-1. Suppression of DRP-1 by siRNA or the inhibitor Mdivi-1 prevented fission, decreased proliferation, and arrested PAH-PASMCs at the G2/M interphase. These antiproliferative effects were beneficial in several models of rodent PAH, and similar mechanisms were described in various cancers. Interestingly, Mdivi-1 has also been shown to improve the muscle insulin sensitivity in obese mice, suggesting, first, a role of mitochondrial dynamics in insulin resistance in skeletal muscle and, second, that Mdivi-1 may be beneficial on several aspects of the mitochondrial abnormalities seen in PAH in several organs.

Whether inhibition of fission leads to activation or inhibition of apoptosis is not clear because some controversy exists. For example, some but not all studies show that inhibition of DRP-1 may suppress apoptosis, which may seem to be in conflict with its beneficial effects in PAH. More studies are needed to clarify this issue because the regulation of apoptosis in PAH is complex and depends on the timing of the disease model and the cellular compartments (eg, early in PAH, there is increased apoptosis of endothelial cells although later on, there is decreased apoptosis of both PAEC and PASMC).

Decreased fusion and expression of mitofusin 2 and its transcriptional coactivator PPARγ coactivator-1α, were also recently shown to contribute to mitochondrial fragmentation and a proliferation-apoptosis imbalance in human and rodent PAH. Augmenting mitofusin 2 with replacement gene therapy showed benefit in human PAH-PASMC in vitro and rodent PAH in vivo, decreasing proliferation and promoting apoptosis.

Mitogenesis/mitophagy. Mitochondria are continuously renewed by a physiological balance between biogenesis and mitophagy, which selectively degrades mitochondria, particularly those that produce high levels of mROS. Interestingly, the loss of fusion while fission is active (through optic atrophy 1 overexpression, fission 1 [mitochondrial outer membrane] homolog RNAi, and DRP-1 dominant negative expression) has been associated with reduced levels of mitophagy, suggesting that there may be an accumulation of dysfunctional mitochondria in such states. The role of mitophagy in PAH has not yet been fully investigated.

In summary, extramitochondrial factors like the transcription factors, signal transducer and activator of transcription 3, NFAT, PPARγ, PPARγ coactivator-1α, or disregulated BMPR-II signaling, can all decrease the gene expression of mitochondrial factors and disrupt mitochondrial dynamics, contributing to an overall suppression of mitochondrial function in PAH vascular cells. In addition, inflammation, via the induction of ER stress, or direct inhibition of mitochondrial enzymes (like the tumor necrosis factor α [TNFα]-induced inhibition of PDH), also results in suppressed mitochondrial function in PAH vessels. These data are summarized in Figure 5.

Intramitochondrial causes: Uncoupling protein (UCP) 2. The mitochondrial protein UCP2, despite its name, does not have significant mitochondrial uncoupling properties, but rather functions as a Ca2+ uniporter, facilitating Ca2+ entry from the ER into the mitochondria. UCPs belong to the superfamiliy of mitochondrial ion transporters and are embedded in the inner mitochondrial membrane. With the exception of the prototypical UCP1, which causes a primary uncoupling of the mitochondria by functioning as a H+ channel, the effects of other UCPs in uncoupling have been challenged. UCP2 is expressed in many tissues including the lungs and inflammatory cells. Ucp2KO-PASMCs have lower mitochondrial
Ca\textsuperscript{2+} levels, mitochondrial hyperpolarization, lower activity of Ca\textsuperscript{2+}-sensitive mitochondrial enzymes (like PDH), and resistance to apoptosis, compared with PASMC from the wild-type controls.\textsuperscript{76,77} Normoxic Ucp2KO-mice spontaneously develop pulmonary vascular remodeling and pulmonary hypertension\textsuperscript{76,77} (Figure 3B). These data showed for the first time...
that an abnormality in a protein intrinsic to mitochondria is sufficient to induce PAH in the absence of other triggers. It is intriguing that loss of UCP2 has also been linked to predisposition to cancer in mice. In addition, UCP2 has been linked to the metabolic syndrome. Polymorphisms in the human UCP2 gene (that result in decreased expression/activity of the protein) have been associated with several diseases. The UCP2 G(-866)A (rs659366) polymorphism located in the promoter region of the gene, is associated with lower mRNA expression levels. This -866 G allele has been associated with increased risks of chronic inflammatory diseases and greater susceptibilities to autoimmune diseases and cardiovascular diseases, including the metabolic syndrome.

Iron homeostasis. Another intriguing but less studied observation is that patients with PAH show evidence of anemia and dysfunctional iron homeostasis. Iron is a critical substrate for mitochondria as Fe-S clusters (exclusively formed in mitochondria) are essential components of electron transport chain complexes and enzymes like succinate dehydrogenase or aconitase. A general mitochondrial dysfunction, which includes iron-overloaded mitochondria, characterizes the increasing list of human diseases that are caused by mutations that disrupt Fe-S clusters and iron homeostasis in mitochondria. The emerging evidence suggests that PAH may belong to this list of diseases.

MnSOD. Recent evidence suggests that the SOD2 gene that encodes for the mitochondrial protein MnSOD is hypermethylated, resulting in decreased expression of MnSOD, contributing to the observed decrease in \( H_2O_2 \) in PAH-PASMCs. Treatment with the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine restored MnSOD expression, decreased proliferation, and increased apoptosis in PAH-PASMCs. It is interesting that this epigenetic regulation seems to be because of a tissue-specific upregulation of DNA methyltransferase 1 and 3 in pulmonary but not systemic arteries.

Taken together, these data suggest that whether there is a primary nonmitochondrial signal with secondary effects on the mitochondria or a primary intramitochondrial abnormality, eventually mitochondrial function is suppressed in PAH. These data are summarized in Figure 5. Thus, mitochondrial suppression may either directly cause PAH or facilitate its progression.

Metabolic Changes in the PAH RV

RV failure is the primary cause of morbidity and mortality in patients with PAH. As an early and short adaptive response to increased afterload, the RV initially undergoes hypertrophy. With persistent increase in afterload, myocardial apoptosis, excessive fibrosis, or increased expression of noncontractile proteins, all contribute to decreased RV systolic function and then to dilatation and failure. A metabolic switch from mitochondrial oxidative phosphorylation to glycolysis has been described in the compensated phase of RV hypertrophy (ie, while cardiac output is maintained). RV mitochondria become more hyperpolarized, suppressing mitochondria-dependent apoptosis, perhaps as a response to the stress in an attempt to preserve the cardiomyocytes. There is also evidence of decreased mROS production and activation of HIF-1α and NFAT in the compensated phase of RV hypertrophy, pointing to an intriguing similarity to the biology of cardiomyocytes and that of cancer cells and idiopathic PAH-PASMCs. In addition, the hypertrophied RV shows increased glucose uptake measured by positron emission tomography, similar to the PAH pulmonary vessels. This similarity is emphasized in Figure 2. The decrease in mROS may also be seen as an attempt to minimize additional sources of stress in a myocardium that struggles to hypertrophy to maintain cardiac output. The HIF-1α activation promotes angiogenesis because increased blood/oxygen supply is needed to support the growing myocardial mass. Increased levels of mitophagy have been suggested in the RV, in a pulmonary artery constriction model of RV hypertrophy, with increased expression of autophagy/mitophagy markers Light Chain 3A/B and p62, whereas mitochondrial biogenesis may be impaired because of the decreased expression of PPARγ coactivator-1α.

However, recent evidence suggests that this potentially adaptive switch seems to be reversed on entrance into the decompensated phase of RV function (where the cardiac output decreases and mortality increases sharply), suggesting that the mitochondrial suppression maybe a beneficial and reversible event in RV hypertrophy. The increase in glucose uptake, the activation of HIF-1α, and the mitochondrial hyperpolarization are lost at this point. Because the mitochondria start to para-doxically function again, they resemble mitochondria from the normal RV; only now, the afterload remains high, and the myocardium is unable to defend. The loss of the angiogenic program causes a loss of microvessels, contributing to ischemia, and the loss of mitochondrial hyperpolarization may facilitate the apoptosis and thus the loss of myocardial cells that characterizes myocardial decompensation and failure. The trigger for the loss of this adaptive molecular and metabolic program is unknown. Because this entry to a decompensation phase occurs much earlier than it does in the left ventricle facing similar levels of increased afterload, it is likely that it is associated with a mechanism intrinsic to and specific to the RV because the 2 heart chambers have many and important differences in their embryology, structure, and molecular physiology.

Metabolic Changes in Activated Inflammatory Cells

Inflammation is strongly associated with the metabolic disturbances seen in diabetes mellitus and insulin resistance; the similar and unexplained resistance to insulin seen in patients with PAH may be worsened by the generalized inflammatory environment. Inflammation characterizes many animal PAH models and is an increasingly recognized feature of clinical PAH, also reviewed elsewhere in this manuscript. The inflammatory milieu in PAH is composed of resident and recruited macrophages, dendritic cells, T and B cells, and mast cells. Intriguingly, many immune cells become highly glycolytic on activation because of a suppressed glucose oxidation (ie, they have the exact same mitochondrial remodeling seen in cancer cells and in PAH vascular cells), suggesting that these cells may also be responsive to mitochondria-targeting therapies. Furthermore, many of the cytokines produced from these immune cells can also have direct effects on pulmonary artery mitochondrial function. For example, TNFα can directly inhibit PDH activity in PASMC,
resulting in activation of NFAT and HIF-1α, apoptosis resistance, and enhanced proliferation. Inhibition of TNFα by etanercept (a drug used in patients with rheumatoid arthritis) normalized the metabolic phenotype of PAH in vitro and reversed established monocrotaline-induced PAH in vivo. The TNFα antibody infliximab reduced pulmonary pressures and improved the quality of life in a patient with severe PAH secondary to advanced scleroderma. These data suggest that the metabolic and inflammatory theories in PAH overlap and may even potentiate each other.

**Metabolic Changes in the Skeletal Muscle**

A high frequency of insulin resistance and a metabolic syndrome-like picture have been described in patients with PAH, even in the absence of obesity or diabetes mellitus. In fact, indices of insulin resistance in the serum of patients with PAH are associated with worse outcomes.

Patients with PAH have reduced mRNA expression of PPARγ in the lung (a ligand-activated nuclear receptor and transcription factor that regulates adipogenesis and glucose metabolism) and apolipoprotein E (a protective factor known to reduce circulating oxidized low-density lipoproteins). Deficiency of both PPARγ and apolipoprotein E has been linked to insulin resistance and the metabolic syndrome. There are signs of mitochondrial abnormalities in the skeletal muscle (a tissue that exhibits signs of insulin resistance in the metabolic syndrome) of PAH animals. PPARγ coactivator-1α, nuclear respiratory factor 1 and transcription factor A mitochondrial mRNA transcripts were found decreased as early as 2 weeks post monocrotaline injection in the gastrocnemius muscle of rats, a time at which the pulmonary pressure is not significantly increased and the cardiac output has not started decreasing. It is still not clear, however, whether these metabolic abnormalities are causative or a consequence of PAH. Apolipoprotein E deficient mice that develop insulin resistance on a high-fat diet also develop PAH, RV hypertrophy, and pulmonary vascular remodeling. This response is attenuated or reversed by the PPARγ agonist rosiglitazone, showing that direct attenuation of insulin resistance can improve PAH. Insulin resistance seems to also be present as an early feature in mice overexpressing a human BMPR-II mutation. These mice have evidence of lipid accumulation in skeletal muscle (a classic sign of metabolic syndrome) even before the development of pulmonary vascular disease (Figure 1). Exacerbated insulin resistance through high-fat diet in these mice worsens pulmonary hypertension, implying a causal role in disease.

**Circulating Factors in the Metabolic Remodeling of PAH Tissues**

There are 2 possibilities for the explanation of the mitochondrial abnormalities seen in several organs in PAH. First is the presence of a generalized intramitochondrial abnormality (eg, a UCP2 loss-of-function polymorphism and a mutation in the Fe-S cluster formation) or a generalized extramitochondrial trigger that suppresses mitochondrial function (eg, suppression of BMPR-II signaling and ER stress because of enhanced inflammation) in all organs. Second, in addition to circulating inflammatory cytokines that can suppress mitochondrial function globally, there may be additional, yet unidentified, circulating factors that can cause mitochondrial suppression in many organs. Recent work has suggested that mitochondrial dysfunction in 1 organ can cause the production of mitokines that can circulate and disseminate mitochondrial suppression signals in remote organs. Durieux et al engineered transgenic worms, in which the gene cco-1 (worm homologue gene encoding for a subunit of the cytochrome c oxidase/cytochrome c oxidase 4) was disabled in a tissue-specific manner in the brain. They then found evidence of mitochondrial dysfunction in remote organs like the intestine. Although the nature of this signal remains unknown, it seems that it involves the mitochondrial unfolded protein response. The authors suggested that if a diffusible molecule, a mitokine, is released from certain tissues, broadcasting a mitochondrial signal to remote target tissues. It is tempting to speculate that mitokines can be circulating between different organs in PAH as well. This area of research, yet unexplored in PAH, may eventually prove to be important for diagnosis or therapy.

Additional examples of mitochondria-produced diffusible factors that can circulate in the blood and affect several organs include the Krebs cycle’s products succinate and αKG. These diffusible small molecules are also measurable in the blood of patients with vascular disease, suggesting that they may exert systemic effects. Although not tested in PAH, it was recently shown that they can bind to G protein-coupled receptors in vascular cells and regulate the angiotensin axis in systemic vascular disease models.

**Implications of the Metabolic Theory in Therapeutic and Biomarker Discovery Programs**

**Mitochondria-Targeting Therapies for PAH**

Several agents that hold promise in the preclinical treatment of PAH were discussed above: PDK inhibitors like dichloroacetate, malonyl-CoA carboxylase inhibitors like trimetazidine, PPARγ activators like pioglitazone or rosiglitazone, NFAT inhibitors like cyclosporine, TNFα antagonists like etanercept, ER stress inhibitors like phenylbutyrate, mitochondrial fission inhibitors like Mdivi-1, and MnSOD activators like MnTBAP or the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine, which increases MnSOD expression. Their site of action and how this relates to the metabolic theory of PAH is shown in Figure 5. The preclinical evidence for some of these metabolic modulators has been strong, as in the case of dichloroacetate discussed earlier. The fact that dichloroacetate and many of these drugs have been studied in humans with other conditions increases their translational potential. A challenge will be the fact that most of these drugs are generic, imposing funding challenges for clinical translation. Nevertheless, several investigator-driven clinical trials are now ongoing with some of the drugs in this list. These include a trial assessing the effects of dichloroacetate treatment in patients with advanced PAH, currently ongoing at the University of Alberta (Edmonton, Canada) and Imperial College of Medicine (London, UK; clinical trials #NCT01083524); ongoing trials from Stanford University studying the effects of PPARγ activators and NFAT inhibitors in patients with PAH (clinical trials #NCT00825266
and NCT01647945, respectively); and trials in the Imperial College (London, UK) and Cleveland Clinic where scientists are testing the efficacy of parenteral or oral iron supplementation in patients with PAH (NCT01447628, NCT01446848).

**Metabolic Biomarkers in PAH**

Because all the discussed mitochondrial abnormalities alter the production of diffusible metabolites, their systematic detection and profiling can be used to evaluate mitochondrial activity. Current imaging methods can also study and follow large metabolic shifts, like the enhanced glycolysis and glucose uptake that follows the suppression of mitochondrial glucose oxidation. For example, $^{18}$F-labeled deoxyglucose (FDG) is a radiotracer used for positron emission tomography, and because of its analogy to glucose, FDG is taken up in cells via the glucose transporter-1 (Glut1). However, because of the lack of a 2′-hydroxyl group preventing further metabolism, FDG accumulates and can be visualized. FDG uptake is increased in glycolytic conditions like cancer. In increased FDG uptake has also been identified in the lungs of patients with idiopathic PAH, but the precise origin of the signal was unclear, the technique not permitting to clearly separate inhomogeneous signals from airway versus pulmonary arterioles, or from PASMCs versus PAECs. However, studies in animal models suggest that the origin of the signal is in the vasculature, correlating with histology, hemodynamics, and metabolism. Increased RV FDG uptake has also been described in patients and rats with PAH, and the signal was reduced by therapies. Because the biology of both the pulmonary vessels and the RV myocardium is important for the prognosis and treatment of patients with PAH, the ability of positron emission tomography imaging to potentially simultaneously image the metabolic shifts in both tissues (Figure 2) makes this technique attractive although its cost and availability may restrict its use as an end point in clinical trials for now.

The measurement of metabolites is starting to be used in PAH after the cancer paradigm. Several available technologies can be useful in this direction, including magnetic resonance spectroscopy, nuclear magnetic resonance (mostly $^1$H-nuclear magnetic resonance), or mass spectrometry (MS). These may be used in combination with separation methods (liquid chromatography–MS and gas chromatography) as used in cancer, which—as discussed—causes essentially identical to PAH metabolic shifts in the affected tissues. Nuclear magnetic resonance permits the simultaneous detection and quantification of multiple metabolites but lacks sensitivity. In comparison, MS is sensitive, selective, automated, and costs less. Although the field is in its infancy, proof-of-principle early publications using MS for systematic metabolomic studies of animal PAH vascular tissues have confirmed the ability of this approach to detect the large-scale metabolic shifts that follow the suppression of mitochondrial function.

Gas chromatography–MS has been used in the detection of volatile organic compounds in the exhaled air of patients with cancer. This breath-printing approach has been rapidly extended to several other diseases including cardiopulmonary diseases. The proximity of the resistance pulmonary vessels to the small airways suggests that the detection...
of metabolic signatures in the breath of patients with PAH is theoretically possible and holds promise as a potential noninvasive metabolic biomarker.

**Conclusion**

The emerging metabolic theory of PAH proposes that several organs in animals and humans with PAH may share mitochondria-based metabolic abnormalities. This theory may facilitate our understanding of the disease because at least several molecular abnormalities that may be seen as diverse and isolated events may actually all be consequences of a primary mitochondrial defect. There is some evidence at least in mice models that these defects may be causative for PAH. It also seems that such defects may facilitate the progression of the disease because they promote proliferation and inflammation and suppress apoptosis. Thus, the many potential therapeutic targets and biomarkers that this theory reveals may open a new window in our approach to this complex disease.

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**Disclosures**

None.

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