



Metabolic dysregulation in bronchopulmonary dysplasia: Implications for identification of biomarkers and therapeutic approaches

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ARTICLE INFO

Keywords:

Metabolic reprogramming
Bronchopulmonary dysplasia
Pulmonary hypertension
Neurodevelopmental abnormality

ABSTRACT

Bronchopulmonary dysplasia (BPD) is a common chronic lung disease in premature infants. Accumulating evidence shows that dysregulated metabolism of glucose, lipids and amino acids are observed in premature infants. Animal and cell studies demonstrate that abnormal metabolism of these substrates results in apoptosis, inflammation, reduced migration, abnormal proliferation or senescence in response to hyperoxic exposure, and that rectifying metabolic dysfunction attenuates neonatal hyperoxia-induced alveolar simplification and vascular dysgenesis in the lung. BPD is often associated with several comorbidities, including pulmonary hypertension and neurodevelopmental abnormalities, which significantly increase the morbidity and mortality of this disease. Here, we discuss recent progress on dysregulated metabolism of glucose, lipids and amino acids in premature infants with BPD and in related *in vivo* and *in vitro* models. These findings suggest that metabolic dysregulation may serve as a biomarker of BPD and plays important roles in the pathogenesis of this disease. We also highlight that targeting metabolic pathways could be employed in the prevention and treatment of BPD.

1. Introduction

The lung consumes as much as, or even more energy than any other organ to maintain cellular functions. This includes rearrangement of the cytoskeleton, cellular respiration, gene expression and replication, as well as several specialized energy-consuming processes such as airway clearance, bronchial gland secretion, constriction of airways and blood vessels, and production of pulmonary surfactant [1]. The catabolism and anabolism of glucose, lipids and proteins are key pathways for lung cellular metabolism. Accumulating evidence has shown that dysregulated metabolism disrupts lung homeostasis, leading to complex physiological and pathological changes. For instance, dysregulated metabolism causes abnormal ciliary function, polarization of alveolar macrophages, and induction of cellular senescence in the alveolar epithelium, all of which could result in the development of chronic pulmonary diseases, including bronchopulmonary dysplasia (BPD) [1–4].

BPD is a chronic lung disease in premature infants, which impacts

10,000 to 15,000 premature infants annually in the US. Although BPD is traditionally defined as requirement for supplemental oxygen at 36 weeks postmenstrual age, newer definitions take into consideration the level of invasive vs non-invasive mechanical support to classify disease severity rather than simply evaluating the need for supplemental oxygen [5,6]. The pathogenesis of BPD is complex and may result from interactions between antenatal and postnatal risk factors in premature infants with underlying genetic susceptibility. Lung pathology of BPD is characterized by alveolar and vascular simplification as well as dysmorphic vascular growth, resulting in defective gas exchange. Pharmacological management for BPD includes antenatal steroids, surfactant, caffeine, and vitamin A [7]. Although these therapies have greatly improved the survival of premature infants, they minimally reduce BPD prevalence and lung injury [8,9].

Infants who developed severe BPD often have growth failure and elevated metabolic expenditure, suggesting abnormal metabolic processes and substrate utilization. Using metabolomics assays, altered metabolism of glucose, lipids and amino acids was observed in the

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<https://doi.org/10.1016/j.redox.2021.102104>

Received 12 June 2021; Received in revised form 11 August 2021; Accepted 12 August 2021

Available online 13 August 2021

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exhaled breath condensate, amniotic fluid, urine and blood from preterm infants who developed BPD [2,10–14] (Table 1). Most human BPD survivors are weaned off oxygen within the first weeks to months of life, yet they may show evidence of persistent lung injury, vascular remodeling and neurodevelopmental abnormalities. Small clinical studies have also shown dysregulated cellular metabolism in preterm infants with pulmonary hypertension and neurodevelopmental abnormalities associated with BPD [14–17] (Table 2). Although much of the evidence in humans is derived from small single center trials, some of these concepts are verified in animal studies and therefore piqued our interest as to whether we can better understand the metabolic changes resulting from BPD. Here, we provide an overview of alterations in the major metabolic pathways described in BPD and associated complications, and discuss whether these can be used as potential biomarkers and help identify promising therapeutic options for BPD.

Table 1
Dysregulated metabolism in BPD and in related *in vivo* and *in vitro* models.

Model	Metabolism	Metabolic changes	Reference
BPD infants	Glucose	Increased lactate and reduced gluconate in urine	[13]
		Reduced Oxphos in HUVECs isolated from BPD infants	[45]
	Lipid	Reduced pulmonary surfactant in tracheal aspirates	[53]
		Increased unsaturated hydroxyl fatty acids, oxylipins, and fatty aldehydes in amniotic fluids	[12,14]
		Increased lyso-phosphatidylcholine in exhaled breath condensate	[11]
	Amino acid	Increased ceramide in tracheal aspirate	[59,60]
		Reduced docosahexaenoic acid and arachidonic acid in blood	[65]
		Increased FABP4 from peri-bronchial blood vessels of BPD patients	[76]
		Reduced S-adenosyl methionine and leucine acid in amniotic fluids	[12]
		Increased levels of histidine, asparagine, glutamic acid, citrulline, glycine and isoleucine in tracheal aspirate	[86]
<i>In vivo</i> model	Glucose	Increased glycolysis and PPP in hyperoxia-induced neonatal mice	[28]
		Reduced aconitase activity in baboons exposed to hyperoxia	[50]
		Reduced complex I activity and ATP production rate in mice exposed hyperoxia	[34,48]
	Lipid	Increased lung glycerophospholipid, sphingolipid and glycerolipid species in mice exposed to hyperoxia	[41]
		Increased sphingomyelin species, long chain and very long chain ceramides in bronchoalveolar lavage fluid from mice exposed to hyperoxia	[61]
		Reduced blood L-arginine and L-citrulline in rats exposed to hyperoxia	[95]
<i>In vitro</i> model	Glucose	Increased glycolysis in MLE-12 cells exposed to hyperoxia followed by air recovery	[33]
		Increased glycolysis and the PPP in lung endothelial cells exposed to hyperoxia followed by air recovery	[28]
		Reduced glycolytic capacity and glycolytic reserve in MLE-12 cells exposed to hyperoxia without air recovery	[34]
		Reduced Oxphos in MLE-12 cells exposed to hyperoxia	[51]
	Lipid	Reduced FAO in lung endothelial cells exposed to hyperoxia followed by air recovery	[49]
		Decreased monounsaturated and polyunsaturated fatty acids but increased saturated fatty acids in tracheal epithelial cells exposed to hyperoxia	[54]

Table 2
Dysregulated metabolism in BPD-associated complications.

Associated complication	Model	Metabolic change	Reference
Pulmonary hypertension	Human study	Increased glucose-6-phosphate and phosphoenolpyruvate in umbilical cord blood	[14]
		Increased asparagine and creatinine in umbilical cord blood	[14]
		Reduced phosphatidylcholines and sphingomyelins in umbilical cord blood	[14]
		Reduced lysine, ornithine and phenylalanine in umbilical cord blood	[14]
		Reduced total triacylglycerides, cholesterol esters, plasmalogen-phosphatidylcholines, lysophosphatidylcholines and plasmalogen-phosphatidylethanolamines in rats during hyperoxia-induced pulmonary hypertension	[118]
	<i>In vivo</i> model	Increased levels of intermediates in β -oxidation and the TCA cycle during hyperoxia-induced pulmonary hypertension	[118]
		Increased oxylipins and trimethylamine-N-oxide in rats during hyperoxia-induced pulmonary hypertension	[118]
		Reduced cerebral glucose metabolism in BPD infants	[15]
		Reduced gamma aminobutyric acid and glutamate in right frontal lobe of premature infants	[16]
		Decreased ratio of N-acetylaspartate-to-choline in hippocampus, cortex and subventricular zone of very low birth weight infants	[17]
Neurodevelopmental abnormality	Human study	Reduced glutamine synthetase activity and glutamine in brain of hyperoxia-exposed neonatal rats	[137]

2. Dysregulated glucose metabolism in BPD

Glycolysis generates the metabolite pyruvate. The latter is converted to lactate in the setting of oxygen deprivation, or enters into the mitochondria for utilization in the tricarboxylic acid (TCA) cycle towards ATP generation during oxidative phosphorylation in condition where sufficient oxygen is available [18–22] (Fig. 1). Mitochondria are the most critical organelles to provide cells with energy. Certain cell types, including endothelial cells, mostly rely on anaerobic glycolysis to generate the energy needed for cellular processes, which has been coined the Warburg effect [23]. Of note, the PPP shunts glucose-6-phosphate from glycolysis to utilize pentose residues for nucleotide synthesis as well as the generation of the reducing equivalent NADPH. The latter can be utilized for fatty acid synthesis and also acts as a vital cofactor for glutathione in maintaining cellular redox status [24, 25]. As the main electron donor, NADPH also reduces the selenoprotein thioredoxin reductase, which delivers electrons to the active site of thioredoxin [26]. Thioredoxin is a class of small redox proteins and plays important roles in redox signaling and combating oxidative damage. Therefore, the PPP serves a metabolic redox sensor and a regulator of the antioxidant response.

2.1. Glycolysis and the PPP

Premature babies exhibit high energy expenditure due to an

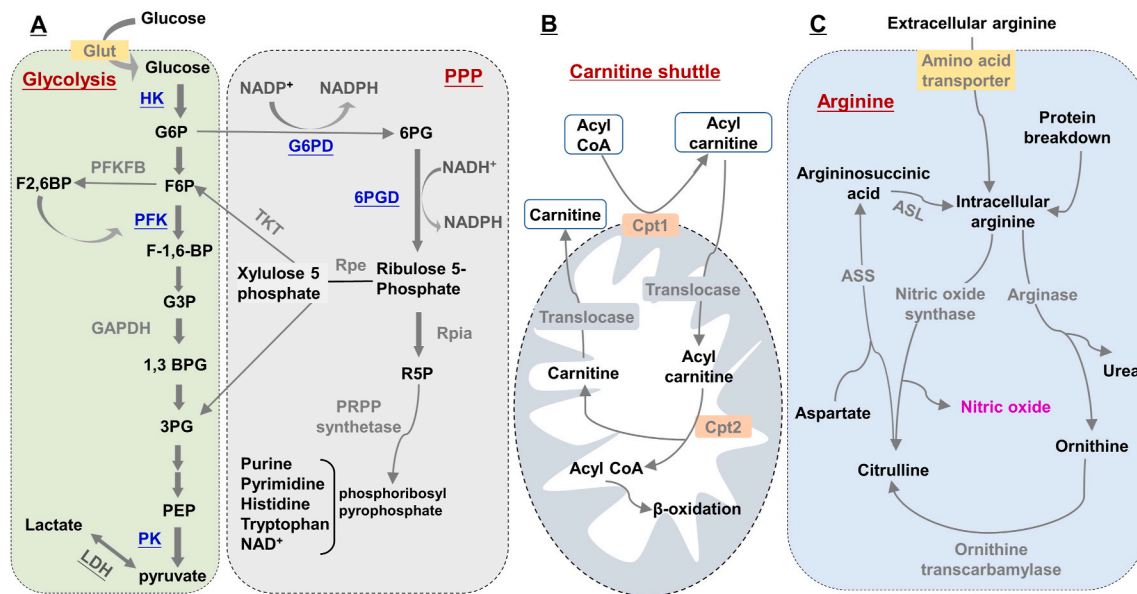


Fig. 1. Metabolic pathways of glucose, mitochondrial transportation of long-chain fatty acids and arginine. Schematic figure showing glycolysis and the pentose phosphate pathway (PPP) (A), the carnitine shuttle (B) and arginine metabolism (C). 6 PG: 6-phosphogluconate, ASL: argininosuccinate lyase, ASS: argininosuccinate synthetase, Cpt: carnitine palmitoyltransferase, F-1,6-BP: fructose-1,6-bisphosphate, F6P: fructose-6-phosphate, G3P: glyceraldehyde 3-phosphate, G6P: glucose-6-phosphate, HK: hexokinase, LDH: lactate dehydrogenase, PEP: phosphofruktokinase, PFK: phosphofruktokinase, PK: pyruvate kinase, R5P: ribose-5-phosphate.

accelerated growth rate. Intact glucose metabolism is needed to convert nutritional substrates into energy so as to maintain growth and promote normal development. During early postnatal life, infants born prematurely are at high risk of altered glucose homeostasis. They are prone to hypoglycemia due to limited glycogen stores. Hyperglycemia can also occur in these infants due to defective islet beta-cell processing of pro-insulin or insulin resistance [27]. As an example, urinary lactate was increased, while gluconate, a glucose derivative, was reduced in 18 infants born at less than 29 weeks gestation who developed BPD compared to 18 newborns born at a similar gestational age who did not develop BPD [13]. Although this is a small cohort, this suggests that infants with BPD have enhanced anaerobic glycolysis. Early identification of dysregulated glucose metabolism may serve as a marker for the development of BPD in premature infants.

Using metabolomics assays, we recently reported that lung glycolysis was increased in neonatal mice exposed to hyperoxia followed by air recovery [28] (Fig. 2). Allowing for air recovery after hyperoxic exposure mimicks clinical scenarios where infants are weaned off oxygen and tests whether the impact of hyperoxia is persistent. Protein levels of glucose transporter (Glut) 1, Glut4, 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase, isoform 3 (PFKFB3), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and pyruvate kinase muscle isozyme (PKM), involved in glucose uptake and glycolysis, were unchanged in the lungs of mice exposed to hyperoxia as neonates [28] (Table 3). Thus, further investigation is required to determine mechanisms underlying hyperoxia-induced increases in glycolysis. Glucose-6-phosphate dehydrogenase (G6PD) is the first enzyme of the PPP, which generates NADPH as a reducing equivalent. Lung levels of G6PD were unchanged in newborn mice exposed to hyperoxia as neonates and hyperoxia-exposed premature rabbits [28,29]. Phosphogluconate dehydrogenase (PGD), the second enzyme of the PPP, was increased in lungs of neonatal mice exposed to hyperoxia followed by air recovery [28]. In combination with metabolomics and metabolic flux assays, our report showed that the PPP was increased in neonatal mice exposed to hyperoxia followed by air recovery [28] (Fig. 2). These observations demonstrate that neonatal hyperoxia causes persistent changes in glycolysis and the PPP. Mechanistically, increased PPP generates dNTP, which contributes to DNA synthesis needed for lung endothelial cell

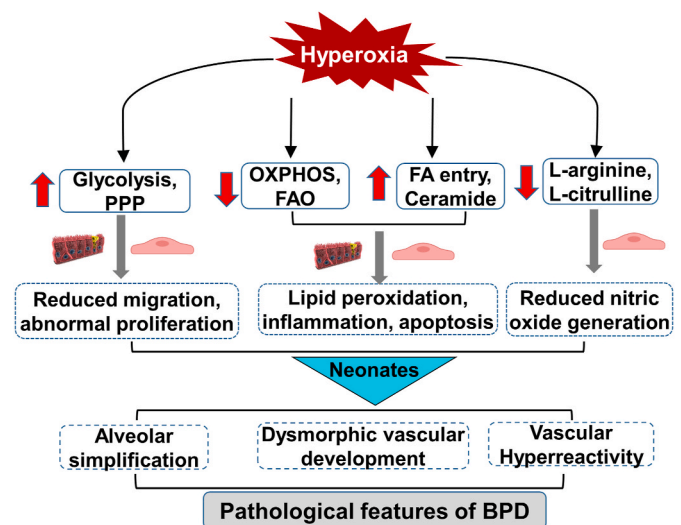


Fig. 2. Metabolic changes during neonatal hyperoxia-induced lung injury. Neonatal hyperoxia followed by air recovery increases glycolysis and the PPP, as well as reduces oxidative phosphorylation (OXPHOS) in lung epithelial cells and endothelial cells. Hyperoxia increases fatty acid (FA) entry, but reduces fatty acid oxidation (FAO) and L-arginine levels. These metabolic changes result in reduced migration, abnormal proliferation and increased apoptosis in lung cells, thereby leading to alveolar simplification and dysmorphic vascular development.

proliferation. Altered PPP activity may result in vascular dysgenesis in the lung interstitium of hyperoxia-exposed mice and in premature infants requiring mechanical ventilation [28]. It remains elusive whether the hyperoxia-induced increase in endothelial PPP persists into adulthood, leading to pulmonary vascular remodeling.

Several studies using cell lines have shown that glucose utilization increased in hyperoxia as a compensatory mechanism to the disruption of oxidative phosphorylation [30–32]. This is corroborated by our recent report showing that hyperoxia increased glycolysis in mouse lung

Table 3
Regulation of metabolic genes by hyperoxia in lung cells and neonatal lung tissues.

Metabolism	Gene targets	Tissues or cells	References
Glucose uptake	No changes in Glut1, Glut4,	Lung endothelial cells and neonatal mouse lungs	[28]
Glycolysis	No changes in PFKFB3, PKM, or GADPH Increased HKII, TIGAR, and PFKFB3	Lung endothelial cells MLE-12 cells	[28] [33]
PPP	Increased G6PD No changes in G6PD, but increased phosphogluconate dehydrogenase	Sheep lungs Lung endothelial cells and neonatal mouse lungs	[29] [28,37]
FAO	Reduced Cpt1a	Lung endothelial cells and neonatal mouse lungs	[49]
De novo synthesis of ceramide	Increased serine palmitoyltransferase long chain base subunit 1	Lung endothelial cells	[49]
Fatty acid	Increased cyclooxygenase and lipoxygenase	Rat lungs	[82,83]
Amino acid	Increased arginase activity	Rat lungs	[94]

epithelial cells (MLE-12), which was associated with increased expression of hexokinase II, PFKFB3, and PKM genes [33]. In contrast, others have reported that glycolytic capacity and glycolytic reserve were significantly decreased whereas the rate of the glycolysis remained unchanged in MLE-12 cells exposed to hyperoxia [34]. The discrepancies between these studies may be due to the specificity of the glycolytic rate assay versus glycolytic stress assay as well as the density of cells seeded in the Seahorse plate. MLE-12 cells express the SV40 large T antigen, which binds to p53 and inhibits p53-dependent transcription. It has been shown that p53 inhibits glycolysis and the PPP by reducing the expression of Glut1, Glut4, and phosphoglycerate mutase as well as by inhibiting G6PD activity [35]. Thus, changes in glycolysis need to be validated using primary lung epithelial cells. Although hyperoxia without room air recovery decreased Glut1 and Glut4 proteins in skeletal muscle from the hind limb of neonatal pigs and Glut4 expression in adult rat lungs [36,37], both Glut1 and Glut4 were not altered, nor was glucose uptake, in lung endothelial cells exposed to hyperoxia followed by air recovery [28]. The discrepancies in how hyperoxia modifies glucose uptake may be cell-specific or altered by air recovery.

Dysregulated glucose metabolism in response to hyperoxia could cause cell dysfunction, such as senescence, apoptosis, abnormal proliferation and collagen synthesis. Although hyperoxic exposure caused senescence accompanied by increased glycolysis in MLE-12 cells, inhibition of glycolysis did not prevent hyperoxia-induced senescence [33], suggesting that senescent cells are metabolically active as evidenced by their senescence-associated secretory phenotype. Hyperoxia also caused senescence in primary human fetal lung fibroblasts and fetal airway smooth muscle cells, which may contribute to abnormal repair in the lung following hyperoxia [38,39]. However, there are no reports demonstrating the impact of metabolism on these cells exposed to hyperoxia. Exposing human microvascular endothelial cells to hyperoxia without air recovery impaired cell viability and proliferation [40]. Using primary neonatal mouse lung endothelial cells, hyperoxia followed by air recovery increased proliferation, which was attributed to the observed increase in PPP [28]. Thus, endothelial cell function changes during the air recovery phase after hyperoxic exposure. Hyperoxia followed by air recovery also increased NADPH along with acetyl CoA, and citric acid in mouse lungs [41]. Increased PPP provides nucleotide synthesis and NADPH for fatty acid synthesis during proliferation in lung endothelial cells. Abnormal lung endothelial cell proliferation is one of the contributing factors leading to dysmorphic lung vascularity in premature infants with BPD, as well as in hyperoxia-exposed mice [28,42–44]. Further research is warranted to

explore whether glycolysis and the PPP are essential for modulating cell function in lung epithelial, endothelial, mesenchymal cells and/or immune cells, exposed to hyperoxia. Similarly, understanding whether these metabolic pathways impact lung cell function or cell fate in infants who develop BPD will be important.

2.2. Oxidative phosphorylation

Compromised mitochondrial oxidative phosphorylation has been implicated in the pathogenesis of BPD. This is associated with impaired mitochondrial function, as discussed previously [45–48]. Human umbilical venous endothelial cells from BPD patients had lower mitochondrial respiration as compared to cells from infants who did not develop BPD [45]. This study also suggests that decreased vascular endothelial mitochondrial respiration predicts death or adverse pulmonary outcomes in preterm infants. This is corroborated by our report showing that hyperoxia decreased mitochondrial respiration in lung endothelial cells without changes in mitochondrial mass or DNA content [49]. Endothelial ATP depletion may result in premature senescence and a reduction of endothelial cell proliferation. Whether reduced mitochondrial respiration decreases proliferation and causes senescence remains to be investigated.

Loss of lung activity of the TCA cycle protein aconitase was observed in baboons exposed to hyperoxia [50]. Hyperoxic exposure caused mitochondria dysfunction with significant suppression of Complex I activity and ATP production rate in mouse lungs [34,48]. In epithelial cells, a short hyperoxic exposure for 4 h reduced oxidative phosphorylation, respiratory complex I and IV activity, and utilization of mitochondrial metabolites [51]. Reduction in mitochondrial fuel utilization persisted even when cells were allowed to recover in air for 24 h after hyperoxic exposure [51]. This indicates that any exposure to clinical hyperoxia could lead to persistent metabolic dysregulation. Administration of pyridaben, a Complex I inhibitor, resulted in alveolar simplification in neonatal mice [34,48]. This further indicates a functional role for oxidative phosphorylation in maintaining lung homeostasis during hyperoxic exposure.

3. Dysregulated lipid metabolism in BPD

3.1. Glycerophospholipids

Pulmonary surfactant is composed of 80% phospholipids, mainly in the form of dipalmitoylphosphatidylcholine, and 10% neutral lipids. This substance is essential for decreasing surface tension at the air-liquid interface of the alveoli [52]. In premature infants, pulmonary surfactant was deficient in proportion to the immaturity of the lung [53]. In a small study, amniotic fluid from a group of infants delivered at term and who did not develop BPD (11 subjects) had higher levels of metabolites of phosphatidylcholine, the major surfactant lipid, than that from infants who developed BPD [12]. This is likely due to increased lung surfactant in the amniotic fluid of term infants compared to preterm infants. However, there may be an important role for these metabolites in mediating lung injury and repair.

Carraro et al. performed a metabolomic analysis of exhaled breath condensate in 20 adolescents who had been diagnosed with BPD as infants, compared to 15 children who had not [11]. They showed that lyso-phosphatidylcholine, an oxidized phospholipid found in inflamed tissues, could serve as a biomarker for BPD. Elevated lyso-phosphatidylcholine also implies that oxidative stress and neutrophilic inflammation persist in the lungs of prematurely born children and adolescents who had developed BPD as infants. This study also revealed that the compositional changes observed in surfactant may persist beyond infancy, although this would have to be documented in a larger trial [11]. Hyperoxic exposure decreased cellular content of monounsaturated and polyunsaturated fatty acids but increased levels of saturated fatty acids in cultured tracheal epithelial cells [54]. These

unsaturated phospholipids could impact the biophysical properties and absorption of surfactant and ultimately lung cell function.

Several studies using lipidomics demonstrated significant changes in the lung lipidome across developmental stages, from birth to adulthood in mice [55,56]. In newborn mice, a high content of monounsaturated lipid species was seen in the lungs, myristic and palmitic acids were observed at 2 weeks of age, while adult lungs were enriched with polyunsaturated lipid species [56]. This is corroborated by our findings that glycerophospholipid and glycerolipid species were augmented at postnatal day 14 compared to postnatal day 7, under normoxic conditions [41]. Glycerophospholipids may be used for pulmonary surfactant synthesis in proliferating type II cells during the expansion of the lung interstitium [57]. These lipidomic changes may result in alveolar simplification and dysregulated vascular development after neonatal hyperoxic exposure.

3.2. Sphingolipids

Sphingolipids regulate cell fates during lung development and the development of lung diseases [58]. Sphingolipid metabolites including ceramide were increased in tracheal aspirates from preterm infants with BPD. This was associated with augmented apoptosis [59,60]. This is validated in neonatal animals where hyperoxia for 4 weeks augmented sphingomyelin species (SM16:0, SM18:0, SM24:0 and SM24:1), long-chain and very long-chain ceramides in bronchoalveolar lavage fluid [61]. This is also in agreement with our findings that sphingolipids were generally increased in early alveolarization in mice exposed to hyperoxia [41]. In addition, hyperoxia increased the de novo synthesis of ceramides in lung endothelial cells, leading apoptosis [49]. Reducing ceramide production by D-sphingosine supplementation attenuated neonatal hyperoxia-induced alveolar simplification [61]. Thus, increased ceramide plays important roles in mediating apoptosis and hyperoxic lung injury in BPD.

Sphingosine kinase 1 phosphorylates sphingosine into sphingosine-1-phosphate, a major regulator of vascular and immune systems. Sphingosine kinase 1 was increased in the lungs of patients with BPD [62]. Hyperoxic exposure caused accumulation of sphingosine-1-phosphate, leading to activation of NADPH oxidase p47^{phox} and production of reactive oxygen species in lung endothelial cells [63]. Deficiency of sphingosine kinase 1 protected against hyperoxia-induced lung injury, which was associated with reduced generation of sphingosine-1-phosphate and reactive oxygen species [64]. Thus, decreasing sphingosine-1-phosphate using small molecule inhibitors of sphingosine kinase 1 may represent a novel therapeutic approach against hyperoxic lung injury.

3.3. Fatty acids

Decreased postnatal docosahexaenoic acid and arachidonic acid levels were associated with an increased risk for BPD in premature infants [65]. Similarly, neonatal hyperoxia also reduced docosahexaenoic acid and arachidonic acid in mouse lungs [41]. Docosahexaenoic acid is an omega-3 fatty acid, which suppressed apoptosis in the lungs of mice perinatally exposed to lipopolysaccharide/hyperoxia [66]. Docosahexaenoic acid and arachidonic acid are precursors of resolvin D1 and lipoxin A4, respectively. Administration of resolvin D1 and lipoxin A4 improved alveolarization and normalized septal wall thickness in a neonatal murine model of hyperoxia-induced lung injury [67]. Thus, nutritional supplementation of these fatty acids could be beneficial for preventing BPD.

Metabolites involved in fatty acid activation and fatty acid β -oxidation were decreased in tracheal aspirates of infants at risk for BPD [68]. Fatty acid oxidation (FAO) is a major pathway for the degradation of fatty acids and an important contributor to energy production. The mitochondrial membrane is impermeable to long-chain acyl-CoA, thus the carnitine cycle is needed to import long-chain fatty acids into the

mitochondrion for β -oxidation (Fig. 1). We have reported that neonatal hyperoxia reduced FAO in lung endothelial cells and mouse lungs, due to decreased activity of carnitine palmitoyltransferase 1a (Cpt1a), a rate-limiting enzyme in the carnitine shuttle [49]. Reducing FAO with a Cpt1 inhibitor or Cpt1a deficiency resulted in lung endothelial cell apoptosis, whereas enhancing FAO by L-carnitine protected against hyperoxia-induced lung alveolar simplification [49]. It is known that premature infants had lower tissue carnitine stores than term infants, particularly within the first two weeks of life [69–72]. This is corroborated by our study showing that neonatal hyperoxia decreased lung carnitine levels during early alveolarization in mice [41]. Although routine carnitine supplementation to parenterally fed premature neonates with BPD had no effect on weight gain, lipid utilization or ketogenesis [73], it decreased the duration of mechanical ventilation and surfactant requirement [74]. Therefore, infants diagnosed with or at risk for BPD would benefit from L-carnitine supplementation.

A recent study showed that neonatal hyperoxia inhibited proliferation and survival of atrial cardiomyocytes by suppressing fatty acid synthesis [75]. It is unknown whether neonatal hyperoxia also affects fatty acid synthesis in lung cells. The role of fatty acid synthesis in hyperoxia-induced alveolar and vascular simplification remains to be investigated.

Fatty acid binding protein (FABP) 4, a lipid chaperone responsible for transport and trafficking of fatty acids, was significantly increased in lung macrophages and endothelial cells from peri-bronchial blood vessels of infants who developed BPD [76]. The expression of FABP3 and FABP4 was also increased in a rat model of BPD induced by intra-uterine growth restriction, indicating enhanced entry of fatty acids. Under conditions of reduced FAO, increased fatty acid entry may result in lipid peroxidation or de novo synthesis of ceramides, leading to apoptosis [77]. It is known that FAO promotes de novo deoxyribonucleotide synthesis and the production of TCA intermediates as well as TCA cycle-derived amino acids including aspartate and glutamate, which eventually increases cell proliferation and vessel sprouting [78]. It is unclear whether decreased FAO is responsible for simplified vascularization by reducing lung endothelial cell proliferation in patients with BPD.

Androgen and estrogen modulate the uptake, lipolysis, oxidation and synthesis of fatty acids [79,80]. Differential enrichment of androgen and estrogen synthesis pathways was observed in infants at risk for BPD [68], suggesting that sexual dimorphism plays a role in the risk of developing BPD.

3.4. Oxidative lipid metabolites

Higher levels of unsaturated hydroxyl fatty acids (putative metabolite: 3-methoxybenzenepropanoic acid), oxylipins (putative metabolite: 4-hydroxy nonenal alkyne, 4-HNE alkyne a form of 4-HNE with a terminal alkyne), and fatty aldehydes (putative metabolite: muconic dialdehyde) were observed in 10 preterm infants who developed BPD [12]. 4-HNE is a major aldehydic product from the lipid peroxidation of ω -6 polyunsaturated fatty acids. Plasma concentrations of 4-HNE were higher in the first day of life in infants who developed BPD compared to those who did not [81]. This suggests that oxidative lipid metabolites may participate in the pathogenesis of BPD. Elevations in the oxylipins PGE1, PGE2, PGF2a, 9- and 13-HOTE, 9- and 13-HODE, and 9- and 13-KODE were positively associated with BPD and its severity [14]. As products of cyclooxygenase and lipoxygenase, these oxylipins may result in abnormal inflammatory responses in infants who develop BPD. Indeed, hyperoxia increased the activity and levels of cyclooxygenase and lipoxygenase in newborn rat lungs, and deficiency of cyclooxygenase 2 attenuated hyperoxia-induced lung inflammatory responses [82,83]. Therefore, reducing lipid peroxidation and oxylipins could be a potential therapy to prevent or mitigate the development of BPD.

4. Dysregulated amino acid metabolism in BPD

Amino acids are used for protein synthesis, the generation of nucleotides and neurotransmitters through the TCA cycle. Glutamine provides mitochondrial anaplerosis because of its role as a nitrogen and carbon donor. It traverses the cell membrane through amino acid transporters, ASCT2 and SN2, and is hydrolyzed to glutamate and ammonia (NH₃) via glutaminase [84]. Glutamate can be catalyzed by either glutamate dehydrogenase or aminotransferases to ammonium and α -ketoglutarate. Glutamate can also further be catalyzed by glutathione cysteine ligase to produce γ -glutamyl cysteine, a precursor of glutathione. The latter is a powerful antioxidant and plays an important role in the neutralization of reactive oxygen species and modulating immune defense, nutrient metabolism, and cellular functions [85].

Reduced levels of S-adenosyl methionine and leucine acid were observed in the amniotic fluid of premature infants compared to term infants [12]. S-adenosyl methionine is a precursor of the glutathione. Thus, reduced S-adenosyl methionine may contribute to increased oxidative stress in preterm infants who develop BPD. Histidine, asparagine, glutamic acid, citrulline, glycine and isoleucine levels were higher in tracheal aspirates at birth in infants who developed BPD [86]. Citrulline is the precursor of arginine, a substrate for nitric oxide (Fig. 1). Increased citrulline could be a compensatory response to generate more nitric oxide to prevent BPD-associated pulmonary hypertension, although this is highly speculative. In fact, inhaled nitric oxide was shown to prevent experimental BPD, but was not effective in preventing BPD in premature infants [87–91]. The effects of nitric oxide are often mediated via S-nitrosothiol formation. In addition to transporting large neutral amino acids, the L-type amino acid transporter-1 also imports S-nitrosothiol adducts [92]. Expression of the L-type amino acid transporter-1 was reduced in the lungs of infants who developed BPD and in experimental BPD in premature baboons [93]. Reduced L-type amino acid transporter-1 may affect S-nitrosothiol import, which could impair the effects of nitric oxide therapy for the management of BPD.

L-arginine and L-citrulline concentrations were reduced in the plasma of rats exposed to hyperoxia as neonates, and arginine supplementation or arginase inhibition attenuated impairment of relaxation of bethanechol-precontracted lung parenchymal strips seen in hyperoxia [94,95] (Fig. 2). In addition, L-citrulline treatment significantly attenuated hyperoxia-induced lung injury and pulmonary hypertension in rats [95]. This was associated with an increase in arginosuccinate lyase and L-arginine. The importance of arginine was further corroborated by a report showing that administration of arginyl-glutamine dipeptide attenuated hyperoxia-induced lung injury in neonatal mice [96]. Clinical studies are required to determine the efficacy of arginine supplementation in preventing pulmonary and vascular abnormalities in premature infants who develop BPD.

Studies have reported that human lung epithelial-like (A549) cells exposed to hyperoxia have improved survival and proliferation when supplemented with higher concentrations of glutamine [97,98]. This was associated with increased glutathione levels and reduced oxidative stress [99]. This cell line preferentially relies on the PPP to respond to NAD depletion and may exhibit constitutive overexpression of Nrf2-driven gene products such as heme oxygenase-1 that may promote resistance to oxidative stress [100,101]. Further experiments using primary human lung epithelial cells are warranted to investigate the effects of glutamine on proliferation in response to hyperoxic exposure.

Hyperoxic exposure suppressed global protein synthesis as indicated by reduced incorporation of phenylalanine and leucine in the newborn rat lung, particularly in a surface-active lung fraction [102,103]. This was associated with increased phosphorylation of eIF2 α in the lung [102]. These findings further reveal that lung epithelial cells, particularly within the airway, were susceptible to hyperoxia-induced eIF2 α phosphorylation. Whether such changes are adaptive, deleterious, or integral to the suppression of mRNA translation in response to hyperoxia remains to be investigated.

5. Metabolic dysregulation in BPD-associated complications

BPD is often associated with several comorbidities, which significantly increase mortality risk with this disease. These comorbidities include pulmonary hypertension and neurodevelopmental abnormalities.

5.1. BPD-associated pulmonary hypertension

Approximately 30% of patients with moderate to severe BPD develop pulmonary hypertension [104–109]. Although pulmonary hypertension can resolve with age in these patients, increased pulmonary arterial pressure at rest or during exercise could persist into young adulthood [110–112]. Pulmonary hypertension increases short- and long-term mortality in premature infants who develop BPD [113,114]. Pulmonary vascular and right ventricular remodeling are common pathological features for BPD-associated pulmonary hypertension. There are no curative therapies, and current management of this disease is limited to pulmonary vasodilators, minimizing further insults to the pulmonary vasculature, and optimizing respiratory status [108,115–117].

5.1.1. Glycolysis and the PPP

The glycolytic intermediates glucose-6-phosphate and phosphoenolpyruvate were increased in the umbilical cord blood of infants with BPD who developed pulmonary hypertension [14], suggesting that increased glycolysis promotes the development of pulmonary hypertension. We recently reported that neonatal hyperoxia increased glycolysis and the PPP as well as endothelial cell proliferation in mouse lungs [28]. It is unclear whether increased glycolysis and the PPP contribute to neonatal hyperoxia-induced pulmonary hypertension.

5.1.2. Lipid metabolism

Major choline-containing phospholipids, such as phosphatidylcholines and sphingomyelins, were reduced in the umbilical cord blood of infants with BPD who developed pulmonary hypertension compared to those without pulmonary hypertension [14], suggesting altered phospholipid metabolism. Similarly, in the rat lung, total triacylglycerides, cholesterol esters, plasmalogen-phosphatidylcholines, lysophosphatidylcholines and plasmalogen-phosphatidylethanolamines were reduced by neonatal hyperoxia (75% O₂, 14 days), a model to induce pulmonary hypertension [118]. It is unclear whether these lipids are oxidized under hyperoxic conditions, leading to pulmonary vascular thickening and the development of pulmonary hypertension [119].

Circulating nonadecanoic acid was increased in 21 infants with BPD who developed pulmonary hypertension [14]. Nonadecanoic acid is a 19-carbon saturated fatty acid, which inhibited tumor cell proliferation [120]. Further experiments are warranted to understand whether increased nonadecanoic acid has any effects on proliferation of lung endothelial cells and pulmonary vascular smooth muscle cells during the development of BPD-associated pulmonary hypertension.

Hyperoxia increases lung metabolic intermediates of β -oxidation and the TCA cycle during the development of pulmonary hypertension [118]. This may increase the generation of reactive oxygen species due to a higher rate of mitochondrial uncoupling. A previous report showed that reduced FAO by reduction or deletion of Cpt1a or Cpt2 resulted in enhanced endothelial-mesenchymal transition, an important cellular process that leads to the development of pulmonary hypertension [121]. We have shown that neonatal hyperoxia caused pulmonary hypertension, and this was associated with increased endothelial-mesenchymal transition [122]. Whether Cpt1a reduction contributes to neonatal hyperoxia-induced endothelial-mesenchymal transition and subsequent pulmonary hypertension remains unclear.

Oxylipins are a group of fatty acid metabolites generated from the oxygenation of polyunsaturated fatty acids. They play important roles in regulating inflammation, immunity, vascular tone, and coagulation during the development of pulmonary hypertension. In rats exposed to

hyperoxia, trimethylamine-*N*-oxide, a small amine oxide generated from choline, betaine, and carnitine by gut microbial metabolism, was increased as were oxylipins [118]. Indeed, neonatal hyperoxia decreased intestinal tight junction protein expression (occludin and ZO-1) and altered intestinal bacterial composition and diversity in early life [123]. Thus, gut microbial metabolism may also participate in the development of hyperoxia-induced pulmonary hypertension through alterations of important metabolites.

5.1.3. Amino acid metabolism

Circulating lysine, ornithine and phenylalanine were decreased in infants with BPD who developed pulmonary hypertension [14]. The amino acid asparagine was also elevated in the umbilical cord blood of infants with BPD who subsequently developed pulmonary hypertension [14]. The mechanisms underlying abnormal amino acid levels in these infants remain unclear. Although there were no differences in arginine, citrulline, or ornithine between the pulmonary hypertension-positive and negative groups, blood ornithine and tyrosine were negatively associated with the odds of developing persistent pulmonary hypertension of the newborn, a perinatal disease, while phenylalanine was positively associated [124]. Further studies are required to determine whether dysregulated metabolism of these amino acids also contributes to the pathogenesis of pulmonary hypertension in infants with BPD.

5.2. BPD-associated neurodevelopmental abnormality

BPD is strongly associated with neurodevelopmental abnormalities in children born preterm [125,126]. This includes cerebral palsy and other motor impairments, neurosensory impairments, cognitive delay, poor academic performance, and language delay [127,128]. Neonatal survivors with BPD have more adverse motor function, worse cognitive development and poorer academic progress than those without BPD. This may also be due to other risk factors such as postnatal hypoxia, hypercapnia and administration of corticosteroids [129].

Metabolism is important to provide energy for all cellular processes required for brain development and function. This includes ATP formation, synaptogenesis, synthesis, release and uptake of neurotransmitters and myelination [130]. Glucose and ketone bodies are vital substrates that meet the metabolic and energy needs of the brain during development [131]. As shown with F-18 fluorodeoxyglucose positron emission tomography images, cerebral glucose metabolism in infants who developed BPD was significantly reduced compared to infants without BPD [15]. In a cohort of 36 infants undergoing brain positron emission tomography scans and MRIs, preterm infants were observed to have lower γ -aminobutyric acid and glutamate in the right frontal lobe than term controls [16]. Reduced ratios of *N*-acetylaspartate-to-choline and *N*-acetylaspartate/myo-inositol are considered as potential risk markers for cognitive impairment and Alzheimer's disease [132,133]. In a prospective cohort of 43 extremely low birthweight infants, a decreased ratio of *N*-acetylaspartate-to-choline was observed in the hippocampus, cortex and subventricular zone compared to term infants measured at term-equivalent age, and a decreased *N*-acetylaspartate/myo-inositol ratio in the subventricular zone in these infants was associated with cognitive and language delay assessed at 18–22 months [17]. It would be intriguing to understand whether brain fatty acid and ketone metabolic changes are seen in infants with BPD-associated neurodevelopmental abnormalities. Altogether, these findings suggest that metabolic dysregulation appears to be associated with cognitive and language development in extremely low birth weight infants.

In animal models, neonatal hyperoxia impaired brain structure including the cortex, basal ganglia, hypothalamus, striatum, hippocampus, and white matter tracts [134–136]. This was associated with increased oxidative stress, reduced neurotrophin and inactivation of survival signals including increased expression of Ras protein, an important mediator of apoptosis and cell death. Glutamine synthetase activity and glutamine were reduced in the brain of hyperoxia-exposed

neonatal rats [137]. This suggests that hyperoxia decreases glutamine metabolism in the immature brain. A mechanistic explanation for these findings remains to be investigated. It is also unclear whether these changes are region-specific. In hypoxic ischemic encephalopathy, intrapartum hypoxia-ischemia disturbed the typical metabolic transition from anaerobic to aerobic metabolism in neonates, and increased the likelihood of low blood glucose concentrations through prolonged duration of anaerobic glycolysis and increased glucose utilization [138, 139]. Perhaps metabolomics and metabolic assays will reveal metabolic changes in specific regions of the brain in response to hyperoxic exposure.

6. Shared mechanisms between BPD and its associated comorbidities

Different mechanisms contribute to BPD and its comorbidities including abnormal inflammatory response and oxidative stress. One of the common mechanisms of injury in the lung and brain in premature infants with BPD could be disrupted or abnormal angiogenesis. Although numerous factors such as fibroblast growth factor (FGF), hepatocyte growth factor (HGF), bone morphogenetic protein (BMP), transforming growth factor (TGF)- β , and angiopoietins have been implicated as positive modulators, angiogenesis is predominantly regulated by vascular endothelial growth factor (VEGF). This factor has been shown to modulate endothelial cell metabolism including glycolysis, the PPP and FAO by upregulating the expression of Glut1, PFKFB3 and G6PD as well as and FABP4 [140–144] (Fig. 3). VEGF also increased mitochondrial biogenesis and mitochondrial respiration via AKT- and mTOR-dependent signaling pathways [145,146]. Metabolism of glucose, fatty acids, and amino acids drives neovascularization via the production of energy and biomass and the maintenance of redox homeostasis in vascular migratory tip cells, proliferating stalk cells, and quiescent phalanx cells [147]. For example, PFKFB3 drives glycolytic ATP and nucleotide production for VEGF-induced tip cell migration and stalk cell proliferation, respectively [143]. VEGF was measured in 189 tracheal aspirate samples and 24 plasma samples from 44 intubated extremely low birth weight preterm infants during their first postnatal week. VEGF levels were reduced in this group compared to infants of similar gestational age surviving without developing BPD [148]. This is corroborated by abnormal and ectopic lung vascular growth in infants with BPD and hyperoxia-exposed neonatal mice [28,42,43]. Whether abnormal distribution of VEGF signal contributes to dysmorphic vascular growth within the lung interstitium remains to be investigated. A recent study has shown that stunted alveolarization followed disordered microvascular development in neonatal mice exposed to hyperoxia [44]. Administration of soluble VEGFR1 (sFlt1) into the amniotic sacs of pregnant rats at 20 days of gestation, decreased the number of alveoli and pulmonary vessel density in pups at 14 days of life [149]. This was accompanied by right and left ventricular hypertrophy [149]. In contrast, VEGF overexpression attenuated alveolar simplification in newborn rats exposed to hyperoxia [150]. Thus, reduced VEGF signal may contribute to simplified vascularization in BPD and associated pulmonary hypertension. Although VEGF treatment attenuated lung fibrosis-associated pulmonary hypertension in rats [151], it is unclear whether VEGF therapy protects against BPD-associated pulmonary hypertension. In addition to VEGF, multiple angiogenic proteins (BMP10, HGF, angiopoietin-1, angiopoietin-2) were associated with the development of BPD, while early increases in BMP10 were strongly associated with the risk for BPD-associated pulmonary hypertension [152]. Further studies are required to determine the role of these factors in modulating angiogenesis in BPD and associated pulmonary hypertension.

With respect to the brain, VEGF-induced blood vessel growth and vessel-independent trophic roles are essential for nervous tissue growth during brain development. In the premature rat brain, VEGF-mediated angiogenesis stimulated the proliferation of neural stem cells and differentiation into neurons, astrocytes, and oligodendrocytes [153].

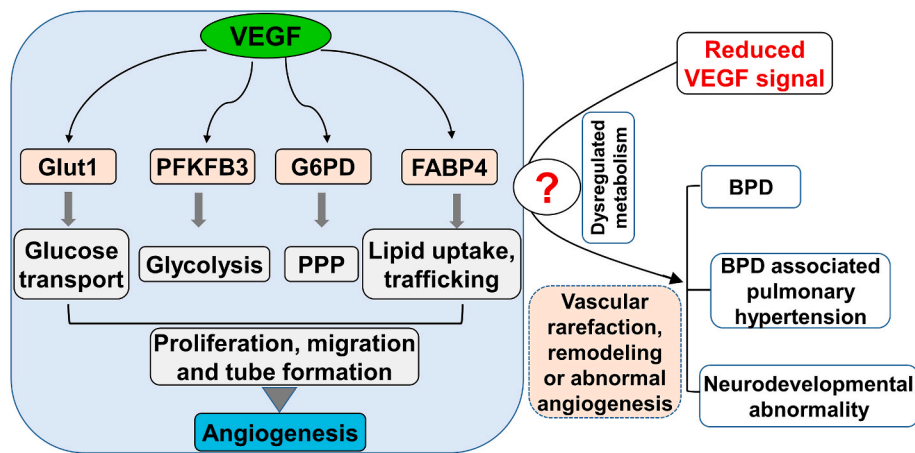


Fig. 3. VEGF-mediated metabolic reprogramming for angiogenesis and its involvement in BPD and associated comorbidities. VEGF upregulates the expression of glucose transporter 1 (Glut 1), PFKFB3, glucose-6-phosphate dehydrogenase (G6PD), and FABP4, leading to increased glucose uptake, glycolysis, activation of the pentose phosphate pathway (PPP), and lipid uptake and trafficking. This results in proliferation, migration, and tube formation of endothelial cells, which subsequently increases neovascularization. VEGF signal is reduced during the development of BPD and its associated pulmonary hypertension. It is altered in the brain as well, potentially leading to neurodevelopmental abnormalities.

Interestingly, increased VEGF expression was noted around foci of periventricular white matter injury in the brain of one extremely premature newborn with congenital heart disease [154]. Whether this isolated finding is relevant to infants with BPD is not clear. Altogether, angiogenic factors, including VEGF, play important roles in the development of the lung and brain, and abnormal VEGF signals may lead to BPD and its associated comorbidities. Therefore, studying metabolic dysregulation in endothelial cells and abnormal angiogenesis could enhance the understanding of pathogenesis of BPD and associated complications and identify therapeutic options for these diseases.

7. Future directions

Integration of metabolic profiling with multi-omics data, such as RNA-seq, genomics and proteomics, is a future approach for identifying pathogenic signaling, functional pathways, potential biomarkers and therapeutic targets in the lungs of infants who develop BPD. In addition to metabolomics, metabolic flux analysis, and positron emission tomography, single-cell RNA sequencing may provide clues as to metabolic dysfunction during the development of BPD and associated complications in a cell-specific manner [155]. For example, neonatal hyperoxia causes both proliferation and apoptosis in lung endothelial cells [28,49]. A study using single-cell RNA sequencing could answer the question as to whether these effects are specific to different vascular beds, subpopulations or lung endothelial cell states. Moreover, using a newly developed digital spatial profiling platform (a NanoString nCounter and GeoMX platforms) [156], temporal and spatial heterogeneity of metabolic molecules or pathways could be dissected. For instance, this platform could be helpful in identifying specific metabolic pathways leading to dysmorphic vascular growth in the lung interstitium or to simplified vascularization in the gas-exchange regions of the lung of premature infants with BPD. These exciting new techniques may lead to novel insights in the understanding of the pathogenesis of BPD and associated complications.

As to therapeutic approaches, enhancing FAO by providing oral supplemental L-carnitine for 6 months was beneficial in controlling moderate persistent asthma in 50 children [156]. Additionally, a single blind, randomized controlled trial on 130 infants at gestational ages 28–36 weeks, treated with L-carnitine resulted in a significantly increased serum carnitine levels at 7 days compared to untreated controls, and that this was associated with a decreased duration of mechanical ventilation and decreased surfactant requirement [74,157]. Additionally, in a four month, open-label study, dichloroacetate to inhibit pyruvate dehydrogenase kinase, was administered to patients with idiopathic pulmonary hypertension who were also on approved therapies, and led to reduced pulmonary arterial hypertension, albeit in a variable fashion [158]. Whether this would help infants with BPD

associated pulmonary hypertension is still unclear. Dehydroepiandrosterone functions as a metabolic intermediate in the biosynthesis of androgenic and estrogenic sex steroids. Dehydroepiandrosterone is also able to inhibit G6PD in the PPP, and a clinical trial to assess its utility in pulmonary hypertension was started in 2019 (NCT03648385). Further studies are required to identify metabolic pathways or targets to prevent or treat BPD and its associated comorbidities.

8. Conclusions

BPD is often associated with several comorbidities, including pulmonary hypertension and neurodevelopmental disabilities. Metabolic dysregulation is observed in epithelial cells, fibroblasts, airway smooth muscle cells and endothelial cells of premature infants with BPD and this correlates with disease severity in many cases. Dysregulated metabolism contributes to hyperoxia-induced apoptosis, abnormal proliferation and senescence, suggesting that these factors play important roles in hyperoxic lung injury. Early detection of dysregulated metabolism in premature infants could help identify individuals at risk for the development of BPD. Rectifying metabolic dysfunction is a promising approach to prevent simplified alveolarization and dysmorphic vascular dysgenesis seen in infants with BPD.

Author contribution

LY, XL and HY drafted the manuscript, and PAD and HY edited the manuscript.

Declaration of competing interest

The authors declare that they have no conflicts of interest with the contents of this article.

Acknowledgements

This work was supported by an Institutional Development Award (IDeA) from the NIGMS of NIH under grant #P20GM103652, the Dr. Ralph and Marian Falk Medical Research Trust Bank of America, N.A., Trustee, and the Warren Alpert Foundation of Brown University.

References

- [1] G. Liu, R. Summer, Cellular metabolism in lung health and disease, *Annu. Rev. Physiol.* 81 (2019) 403–428.
- [2] F. Piersigilli, V. Bhandari, Metabolomics of bronchopulmonary dysplasia, *Clin. Chim. Acta* 500 (2020) 109–114.
- [3] P.P. Ogger, A.J. Byrne, Macrophage metabolic reprogramming during chronic lung disease, *Mucosal Immunol.* 14 (2) (2021) 282–295.

- [4] J. Sabbatinelli, F. Prattichizzo, F. Olivieri, A.D. Procopio, M.R. Rippon, A. Giuliani, Where metabolism meets senescence: focus on endothelial cells, *Front. Physiol.* 10 (2019) 1523.
- [5] E.A. Jensen, K. Dysart, M.G. Gantz, S. McDonald, N.A. Bamat, M. Keszler, H. Kirpalani, M.M. Laughon, B.B. Poindexter, A.F. Duncan, B.A. Yoder, E. C. Eichenwald, S.B. DeMauro, The diagnosis of bronchopulmonary dysplasia in very preterm infants. An evidence-based approach, *Am. J. Respir. Crit. Care Med.* 200 (6) (2019) 751–759.
- [6] B. Thebaud, K.N. Goss, M. Laughon, J.A. Whitsett, S.H. Abman, R.H. Steinhorn, J. L. Aschner, P.G. Davis, S.A. McGrath-Morrow, R.F. Soll, A.H. Jobe, Bronchopulmonary dysplasia, *Nat. Rev. Dis. Prim.* 5 (1) (2019) 78.
- [7] Z. Michael, F. Spyropoulos, S. Ghanta, H. Christou, Bronchopulmonary dysplasia: an update of current pharmacologic therapies and new approaches, *Clin. Med. Insights Pediatr.* 12 (2018), 1179556518817322.
- [8] E.W. Mandell, P. Kratimenos, S.H. Abman, R.H. Steinhorn, Drugs for the prevention and treatment of bronchopulmonary dysplasia, *Clin. Perinatol.* 46 (2) (2019) 291–310.
- [9] E. Bancalari, D. Jain, Bronchopulmonary dysplasia: 50 Years after the original description, *Neonatology* 115 (4) (2019) 384–391.
- [10] D. Rastogi, J.L. Aschner, Airway metabolome and chronic lung disease of prematurity, *J. Pediatr.* 166 (2) (2015) 224–226.
- [11] S. Carraro, G. Giordano, P. Pirillo, M. Maretti, F. Reniero, P.E. Cogo, G. Perilongo, M. Stocchero, E. Baraldi, Airway metabolic anomalies in adolescents with bronchopulmonary dysplasia: new insights from the metabolomic approach, *J. Pediatr.* 166 (2) (2015) 234–239 e1.
- [12] E. Baraldi, G. Giordano, M. Stocchero, L. Moschino, P. Zaramella, M.R. Tran, S. Carraro, R. Romero, M.T. Gervasi, Untargeted metabolomic analysis of amniotic fluid in the prediction of preterm delivery and bronchopulmonary dysplasia, *PLoS One* 11 (10) (2016), e0164211.
- [13] V. Fanos, M.C. Pintus, M. Lussu, L. Atzori, A. Noto, M. Stronati, H. Guimaraes, M. A. Marcialis, G. Rocha, C. Moretti, P. Papoff, S. Lacerenza, S. Puddu, M. Giuffrè, F. Serraino, M. Mussap, G. Corsello, Urinary metabolomics of bronchopulmonary dysplasia (BPD): preliminary data at birth suggest it is a congenital disease, *J. Matern. Fetal Neonatal Med.* 27 (Suppl 2) (2014) 39–45.
- [14] M.R. La Franco, J.F. Fahrman, D. Grapov, T.L. Pedersen, J.W. Newman, O. Fiehn, M.A. Underwood, K. Mestan, R.H. Steinhorn, S. Wedgwood, Umbilical cord blood metabolomics reveal distinct signatures of dyslipidemia prior to bronchopulmonary dysplasia and pulmonary hypertension, *Am. J. Physiol. Lung Cell Mol. Physiol.* 315 (5) (2018) L870–L881.
- [15] J.H. Park, C.S. Kim, K.S. Won, J.S. Oh, J.S. Kim, H.W. Kim, Asymmetry of cerebral glucose metabolism in very low-birth-weight infants without structural abnormalities, *PLoS One* 12 (11) (2017), e0186976.
- [16] S.H. Kwon, D. Scheinost, C. Lacadie, J. Benjamin, E.H. Myers, M. Qiu, K. C. Schneider, D.L. Rothman, R.T. Constable, L.R. Ment, GABA, resting-state connectivity and the developing brain, *Neonatology* 106 (2) (2014) 149–155.
- [17] R. Bapat, P.A. Narayana, Y. Zhou, N.A. Parikh, Magnetic resonance spectroscopy at term-equivalent age in extremely preterm infants: association with cognitive and language development, *Pediatr. Neurol.* 51 (1) (2014) 53–59.
- [18] Z. Li, H. Zhang, Reprogramming of glucose, fatty acid and amino acid metabolism for cancer progression, *Cell. Mol. Life Sci.* 73 (2) (2016) 377–392.
- [19] R.J. DeBerardinis, C.B. Thompson, Cellular metabolism and disease: what do metabolic outliers teach us? *Cell* 148 (6) (2012) 1132–1144.
- [20] D.H. Tran, Z.V. Wang, Glucose metabolism in cardiac hypertrophy and heart failure, *J. Am. Heart Assoc.* 8 (12) (2019), e012673.
- [21] E. Brekke, T.S. Morken, U. Sonnewald, Glucose metabolism and astrocyte-neuron interactions in the neonatal brain, *Neurochem. Int.* 82 (2015) 33–41.
- [22] H. Zhao, P.A. Dennery, H. Yao, Metabolic reprogramming in the pathogenesis of chronic lung diseases, including BPD, COPD, and pulmonary fibrosis, *Am. J. Physiol. Lung Cell Mol. Physiol.* 314 (4) (2018) L544–L554.
- [23] M.G. Vander Heiden, L.C. Cantley, C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation, *Science* 324 (5930) (2009) 1029–1033.
- [24] K.C. Patra, N. Hay, The pentose phosphate pathway and cancer, *Trends Biochem. Sci.* 39 (8) (2014) 347–354.
- [25] A. Stincone, A. Prigione, T. Cramer, M.M. Wamelink, K. Campbell, E. Cheung, V. Olin-Sandoval, N.M. Gruning, A. Kruger, M. Tauqeer Alam, M.A. Keller, M. Breitenbach, K.M. Brindle, J.D. Rabinowitz, M. Ralser, The return of metabolism: biochemistry and physiology of the pentose phosphate pathway, *Biol. Rev. Camb. Phil. Soc.* 90 (3) (2015) 927–963.
- [26] C.G. Miller, A. Holmgren, E.S.J. Arner, E.E. Schmidt, NADPH-dependent and -independent disulfide reductase systems, *Free Radic. Biol. Med.* 127 (2018) 248–261.
- [27] D. Mitanchez, Glucose regulation in preterm newborn infants, *Horm. Res.* 68 (6) (2007) 265–271.
- [28] J. Gong, Z. Feng, A.L. Peterson, J.F. Carr, X. Lu, H. Zhao, X. Ji, Y.Y. Zhao, M.E. De Paeppe, P.A. Dennery, H. Yao, The pentose phosphate pathway mediates hyperoxia-induced lung vascular dysfunction and alveolar simplification in neonates, *JCI Insight* 6 (5) (2021) e137594-e137610.
- [29] L. Frank, I.R. Sosenko, Failure of premature rabbits to increase antioxidant enzymes during hyperoxic exposure: increased susceptibility to pulmonary oxygen toxicity compared with term rabbits, *Pediatr. Res.* 29 (3) (1991) 292–296.
- [30] C.B. Allen, C.W. White, Glucose modulates cell death due to normobaric hyperoxia by maintaining cellular ATP, *Am. J. Physiol.* 274 (1) (1998) L159–L164.
- [31] W.G. Schoonen, A.H. Wanamarta, J.M. van der Klei-van Moorsel, C. Jakobs, H. Joenje, Respiratory failure and stimulation of glycolysis in Chinese hamster ovary cells exposed to normobaric hyperoxia, *J. Biol. Chem.* 265 (19) (1990) 11118–11124.
- [32] R. Scatena, I. Messana, G.E. Martorana, M.L. Gozzo, S. Lipa, A. Maccaglia, P. Bottoni, F. Vincenzoni, G. Nocca, M. Castagnola, B. Giardina, Mitochondrial damage and metabolic compensatory mechanisms induced by hyperoxia in the U-937 cell line, *J. Biochem. Mol. Biol.* 37 (4) (2004) 454–459.
- [33] A.M. Scaffa, A.L. Peterson, J.F. Carr, D. Garcia, H. Yao, P.A. Dennery, Hyperoxia causes senescence and increases glycolysis in cultured lung epithelial cells, *Physiol. Rep.* 9 (10) (2021) e1483-e1495.
- [34] K.C. Das, Hyperoxia decreases glycolytic capacity, glycolytic reserve and oxidative phosphorylation in MLE-12 cells and inhibits complex I and II function, but not complex IV in isolated mouse lung mitochondria, *PLoS One* 8 (9) (2013), e73358.
- [35] C.D. Wiley, J. Campisi, From ancient pathways to aging cells-connecting metabolism and cellular senescence, *Cell. Metabol.* 23 (6) (2016) 1013–1021.
- [36] K.S. Bandali, M.P. Belanger, C. Wittnich, Does hyperoxia affect glucose regulation and transport in the newborn? *J. Thorac. Cardiovasc. Surg.* 126 (6) (2003) 1730–1735.
- [37] C.B. Allen, X.L. Guo, C.W. White, Changes in pulmonary expression of hexokinase and glucose transporter mRNAs in rats adapted to hyperoxia, *Am. J. Physiol.* 274 (3) (1998) L320–L329.
- [38] K. You, P. Parikh, K. Khandalavala, S.A. Wicher, L. Manlove, B. Yang, A. Roesler, B.B. Roos, J.J. Teske, R.D. Britt Jr., C.M. Pabelick, Y.S. Prakash, Moderate hyperoxia induces senescence in developing human lung fibroblasts, *Am. J. Physiol. Lung Cell Mol. Physiol.* 317 (5) (2019) L525–L536.
- [39] P. Parikh, R.D. Britt Jr., L.J. Manlove, S.A. Wicher, A. Roesler, J. Ravix, J. Teske, M.A. Thompson, G.C. Sieck, J.L. Kirkland, N. LeBrasseur, D.J. Tschumperlin, C.M. Pabelick, Y.S. Prakash, Hyperoxia-induced cellular senescence in fetal airway smooth muscle cells, *Am. J. Respir. Cell Mol. Biol.* 61 (1) (2019) 51–60.
- [40] I. Attaye, Y.M. Smulders, M.C. de Waard, H.M. Oudemans-van Straaten, B. Smit, M.H. Van Wijhe, R.J. Musters, P. Koolwijk, A.M.E. Spoelstra-de Man, The effects of hyperoxia on microvascular endothelial cell proliferation and production of vaso-active substances, *Intensive Care Med.* Exp. 5 (1) (2017) 22.
- [41] A.L. Peterson, J.F. Carr, X. Ji, P.A. Dennery, H. Yao, Hyperoxic exposure caused lung lipid compositional changes in neonatal mice, *Metabolites* 10 (9) (2020) 340–357.
- [42] M.E. De Paeppe, Q. Mao, J. Powell, S.E. Rubin, P. DeKoninck, N. Appel, M. Dixon, F. Gundogan, Growth of pulmonary microvasculature in ventilated preterm infants, *Am. J. Respir. Crit. Care Med.* 173 (2) (2006) 204–211.
- [43] J.D. Miller, J.T. Benjamin, D.R. Kelly, D.B. Frank, L.S. Prince, Chorioamnionitis stimulates angiogenesis in sacculus stage fetal lungs via CC chemokines, *Am. J. Physiol. Lung Cell Mol. Physiol.* 298 (5) (2010) L637–L645.
- [44] S.V. Appuhn, S. Siebert, D. Myti, C. Wrede, D.E. Surate Solaligie, D. Perez-Bravo, C. Brandenberger, J. Schipke, R.E. Morty, R. Grothausmann, C. Muhlfield, Capillary changes precede disordered alveolarization in a mouse model of bronchopulmonary dysplasia, *Am. J. Respir. Cell Mol. Biol.* 65 (1) (2021) 81–91.
- [45] J. Kandasamy, N. Olave, S.W. Ballinger, N. Ambalavanan, Vascular endothelial mitochondrial function predicts death or pulmonary outcomes in preterm infants, *Am. J. Respir. Crit. Care Med.* 196 (8) (2017) 1040–1049.
- [46] D. Shah, P. Das, V. Bhandari, Mitochondrial dysfunction in bronchopulmonary dysplasia, *Am. J. Respir. Crit. Care Med.* 197 (10) (2018) 1363.
- [47] L. Yue, H. Yao, Mitochondrial dysfunction in inflammatory responses and cellular senescence: pathogenesis and pharmacological targets for chronic lung diseases, *Br. J. Pharmacol.* 173 (15) (2016) 2305–2318.
- [48] V. Ratner, A. Starkov, D. Matsiukevich, R.A. Polin, V.S. Ten, Mitochondrial dysfunction contributes to alveolar development arrest in hyperoxia-exposed mice, *Am. J. Respir. Cell Mol. Biol.* 40 (5) (2009) 511–518.
- [49] H. Yao, J. Gong, A.L. Peterson, X. Lu, P. Zhang, P.A. Dennery, Fatty acid oxidation protects against hyperoxia-induced endothelial cell apoptosis and lung injury in neonatal mice, *Am. J. Respir. Cell Mol. Biol.* 60 (6) (2019) 667–677.
- [50] R.L. Morton, D. Ikle, C.W. White, Loss of lung mitochondrial aconitase activity due to hyperoxia in bronchopulmonary dysplasia in primates, *Am. J. Physiol.* 274 (1) (1998) L127–L133.
- [51] D. Garcia, J.F. Carr, F. Chan, A.L. Peterson, K.A. Ellis, A. Scaffa, A.J. Ghio, H. Yao, P.A. Dennery, Short exposure to hyperoxia causes cultured lung epithelial cell mitochondrial dysregulation and alveolar simplification in mice, *Pediatr. Res.* (2020). PMID: 33144707.
- [52] R. Veldhuizen, K. Nag, S. Orgeig, F. Possmayer, The role of lipids in pulmonary surfactant, *Biochim. Biophys. Acta* 1408 (2–3) (1998) 90–108.
- [53] P.L. Ballard, R.L. Keller, W.E. Truog, C. Chapin, H. Horneman, M.R. Segal, R. A. Ballard, I. Tolsurf, Surfactant status and respiratory outcome in premature infants receiving late surfactant treatment, *Pediatr. Res.* 85 (3) (2019) 305–311.
- [54] P.A. Dennery, C.M. Kramer, S.E. Alpert, Effect of fatty acid profiles on the susceptibility of cultured rabbit tracheal epithelial cells to hyperoxic injury, *Am. J. Respir. Cell Mol. Biol.* 3 (2) (1990) 137–144.
- [55] S.E. Dautel, J.E. Kyle, G. Clair, R.L. Sontag, K.K. Weitz, A.K. Shukla, S.N. Nguyen, Y.M. Kim, E.M. Zink, T. Luders, C.W. Frevert, S.A. Gharib, J. Laskin, J.P. Carson, T.O. Metz, R.A. Corley, C. Ansong, Lipidomics reveals dramatic lipid compositional changes in the maturing postnatal lung, *Sci. Rep.* 7 (2017) 40555.
- [56] S. Karnati, V. Garikapati, G. Liebisch, P.P. Van Veldhoven, B. Spengler, G. Schmitz, E. Baumgart-Vogt, Quantitative lipidomic analysis of mouse lung during postnatal development by electrospray ionization tandem mass spectrometry, *PLoS One* 13 (9) (2018), e0203464.
- [57] M. Yee, B.W. Buczynski, M.A. O'Reilly, Neonatal hyperoxia stimulates the expansion of alveolar epithelial type II cells, *Am. J. Respir. Cell Mol. Biol.* 50 (4) (2014) 757–766.

- [58] J. Lee, B. Yeganeh, L. Ermini, M. Post, Sphingolipids as cell fate regulators in lung development and disease, *Apoptosis* 20 (5) (2015) 740–757.
- [59] K.D. Hendricks-Munoz, J. Xu, J.A. Voynow, Tracheal aspirate VEGF and sphingolipid metabolites in the preterm infant with later development of bronchopulmonary dysplasia, *Pediatr. Pulmonol.* 53 (8) (2018) 1046–1052.
- [60] E. van Mastrigt, S. Zweekhorst, B. Bol, J. Tibboel, J. van Rosmalen, J.N. Samsom, A.A. Kroon, J.C. de Jongste, I.K.M. Reiss, M. Post, M.W. Pijnenburg, Ceramides in tracheal aspirates of preterm infants: marker for bronchopulmonary dysplasia, *PLoS One* 13 (1) (2018), e0185969.
- [61] J. Tibboel, S. Joza, I. Reiss, J.C. de Jongste, M. Post, Amelioration of hyperoxia-induced lung injury using a sphingolipid-based intervention, *Eur. Respir. J.* 42 (3) (2013) 776–784.
- [62] A.W. Ha, T. Bai, D.L. Ebenezer, T. Sethi, T. Sudhadevi, L.A. Mangio, S. Garzon, G. S. Pryhuber, V. Natarajan, A. Harijith, Sphingosine Kinase 1 Regulates Lysyl Oxidase through STAT3 in Hyperoxia-Mediated Neonatal Lung Injury, *Thorax*, 2021.
- [63] A. Harijith, S. Pendyala, D.L. Ebenezer, A.W. Ha, P. Fu, Y.T. Wang, K. Ma, P. T. Toth, E.V. Berdyshev, P. Kanteti, V. Natarajan, Hyperoxia-induced p47phox activation and ROS generation is mediated through S1P transporter Spns2, and S1P/S1P1&2 signaling axis in lung endothelium, *Am. J. Physiol. Lung Cell Mol. Physiol.* 311 (2) (2016) L337–L351.
- [64] A. Harijith, S. Pendyala, N.M. Reddy, T. Bai, P.V. Usatyuk, E. Berdyshev, I. Gorskova, L.S. Huang, V. Mohan, S. Garzon, P. Kanteti, S.P. Reddy, J.U. Raj, V. Natarajan, Sphingosine kinase 1 deficiency confers protection against hyperoxia-induced bronchopulmonary dysplasia in a murine model: role of S1P signaling and Nox proteins, *Am. J. Pathol.* 183 (4) (2013) 1169–1182.
- [65] C.R. Martin, D.A. Dasilva, J.E. Cluette-Brown, C. Dimonda, A. Hamill, A. Q. Bhutta, E. Coronel, M. Wilschanski, A.J. Stephens, D.F. Driscoll, B.R. Bistrrian, J.H. Ware, M.M. Zaman, S.D. Freedman, Decreased postnatal docosahexaenoic and arachidonic acid blood levels in premature infants are associated with neonatal morbidities, *J. Pediatr.* 159 (5) (2011) 743–749, e1–2.
- [66] M. Ali, K.M. Heyob, M. Velten, T.E. Tipple, L.K. Rogers, DHA suppresses chronic apoptosis in the lung caused by perinatal inflammation, *Am. J. Physiol. Lung Cell Mol. Physiol.* 309 (5) (2015) L441–L448.
- [67] C.R. Martin, M.M. Zaman, C. Gilkey, M.V. Salguero, H. Hasturk, A. Kantarci, T. E. Van Dyke, S.D. Freedman, Resolvin D1 and lipoxin A4 improve alveolarization and normalize septal wall thickness in a neonatal murine model of hyperoxia-induced lung injury, *PLoS One* 9 (6) (2014), e98773.
- [68] C.V. Lal, J. Kandasamy, K. Dolma, M. Ramani, R. Kumar, L. Wilson, Z. Aghai, S. Barnes, J.E. Blalock, A. Gagger, V. Bhandari, N. Ambalavanan, Early airway microbial metagenomic and metabolomic signatures are associated with development of severe bronchopulmonary dysplasia, *Am. J. Physiol. Lung Cell Mol. Physiol.* 315 (5) (2018) L810–L815.
- [69] J.E. Van Aerde, In preterm infants, does the supplementation of carnitine to parenteral nutrition improve the following clinical outcomes: growth, lipid metabolism and apneic spells?: Part B: clinical commentary, *Paediatr. Child Health* 9 (8) (2004) 573.
- [70] T. Van Aerde, In preterm infants, does the supplementation of carnitine to parenteral nutrition improve the following clinical outcomes: growth, lipid metabolism and apneic spells?: Part A: evidence-based answer and summary, *Paediatr. Child Health* 9 (8) (2004) 571–572.
- [71] R.H. Clark, D.H. Chace, A.R. Spitzer, Impact of L-carnitine supplementation on metabolic profiles in premature infants, *J. Perinatol.* 37 (5) (2017) 566–571.
- [72] J.P. Shenai, P.R. Borum, Tissue carnitine reserves of newborn infants, *Pediatr. Res.* 18 (7) (1984) 679–682.
- [73] P.A. Cairns, D.J. Stalker, Carnitine supplementation of parenterally fed neonates, *Cochrane Database Syst. Rev.* 4 (2000) CD000950.
- [74] M.A. Ozturk, Z. Kardas, F. Kardas, T. Gunes, S. Kurtoglu, Effects of L-carnitine supplementation on respiratory distress syndrome development and prognosis in premature infants: a single blind randomized controlled trial, *Exp. Ther. Med.* 11 (3) (2016) 1123–1127.
- [75] E.D. Cohen, M. Yee, G.A. Porter Jr., E. Ritzer, A.N. McDavid, P.S. Brookes, G. S. Pryhuber, M.A. O'Reilly, Neonatal hyperoxia inhibits proliferation and survival of atrial cardiomyocytes by suppressing fatty acid synthesis, *JCI Insight* 6 (5) (2021) e140785–e140803.
- [76] E. Ghelfi, C. Karaaslan, S. Berkelhamer, S. Akar, H. Kozakewich, S. Cataltepe, Fatty acid-binding proteins and peribronchial angiogenesis in bronchopulmonary dysplasia, *Am. J. Respir. Cell Mol. Biol.* 45 (3) (2011) 550–556.
- [77] E. Zana-Taieb, H. Pham, M.L. Franco-Montoya, S. Jacques, F. Letourneur, O. Baud, P.H. Jarreau, D. Vaiman, Impaired alveolarization and intra-uterine growth restriction in rats: a postnatal genome-wide analysis, *J. Pathol.* 235 (3) (2015) 420–430.
- [78] S. Schoors, U. Bruning, R. Missaen, K.C. Queiroz, G. Borgers, I. Elia, A. Zecchin, A.R. Cantelmo, S. Christen, J. Goveia, V. Heggermont, L. Godde, S. Vinckier, P. P. Van Veldhoven, G. Eelen, L. Schoonjans, H. Gerhardt, M. Dewerchin, M. Baes, K. De Bock, B. Ghesquiere, S.Y. Lunt, S.M. Fendt, P. Carmeliet, Fatty acid carbon is essential for dNTP synthesis in endothelial cells, *Nature* 520 (7546) (2015) 192–197.
- [79] M.S. Ojeda, N. Gomez, M.S. Gimenez, Androgen regulation of lung lipids in the male rat, *Lipids* 32 (1) (1997) 57–62.
- [80] B.T. Palmisano, L. Zhu, J.M. Stafford, Role of estrogens in the regulation of liver lipid metabolism, *Adv. Exp. Med. Biol.* 1043 (2017) 227–256.
- [81] T. Ogihara, K. Hirano, T. Morinobu, H.S. Kim, M. Hiroi, H. Ogihara, H. Tamai, Raised concentrations of aldehyde lipid peroxidation products in premature infants with chronic lung disease, *Arch. Dis. Child. Fetal Neonatal Ed.* 80 (1) (1999) F21–F25.
- [82] G.E. Hosford, K.S. Koyanagi, W.I. Leung, D.M. Olson, Hyperoxia increases protein mass of 5-lipoxygenase and its activating protein, flap, and leukotriene B(4) output in newborn rat lungs, *Exp. Lung Res.* 28 (8) (2002) 671–684.
- [83] R.D. Britt Jr., M. Velten, T.E. Tipple, L.D. Nelin, L.K. Rogers, Cyclooxygenase-2 in newborn hyperoxic lung injury, *Free Radic. Biol. Med.* 61 (2013) 502–511.
- [84] S.M. Jeong, S. Hwang, K. Park, S. Yang, R.H. Seong, Enhanced mitochondrial glutamine anaplerosis suppresses pancreatic cancer growth through autophagy inhibition, *Sci. Rep.* 6 (2016) 30767.
- [85] T. Li, A. Le, Glutamine metabolism in cancer, *Adv. Exp. Med. Biol.* 1063 (2018) 13–32.
- [86] F. Piersigilli, T.T. Lam, P. Vernocchi, A. Quagliaroli, L. Putignani, Z.H. Aghai, V. Bhandari, Identification of new biomarkers of bronchopulmonary dysplasia using metabolomics, *Metabolomics* 15 (2) (2019) 20.
- [87] A. Greenough, F. Decobert, D. Field, M. Hallman, H.D. Hummler, B. Jonsson, M. Sanchez Luna, B. Van Overmeire, V.P. Carnielli, J.L. Potenza, J.C. Mercier, Inhaled nitric oxide (iNO) for preventing prematurity-related bronchopulmonary dysplasia (BPD): 7-year follow-up of the European Union Nitric Oxide (EUNO) trial, *J. Perinat. Med.* 49 (1) (2020) 104–110.
- [88] J.C. Mercier, H. Hummler, X. Durrmeyer, M. Sanchez-Luna, V. Carnielli, D. Field, A. Greenough, B. Van Overmeire, B. Jonsson, M. Hallman, J. Baldassarre, E. S. Group, Inhaled nitric oxide for prevention of bronchopulmonary dysplasia in premature babies (EUNO): a randomised controlled trial, *Lancet* 376 (9738) (2010) 346–354.
- [89] S.A. ter Horst, F.J. Walther, B.J. Poorthuis, P.S. Hiemstra, G.T. Wagenaar, Inhaled nitric oxide attenuates pulmonary inflammation and fibrin deposition and prolongs survival in neonatal hyperoxic lung injury, *Am. J. Physiol. Lung Cell Mol. Physiol.* 293 (1) (2007) L35–L44.
- [90] Y.J. Lin, N.E. Markham, V. Balasubramanian, J.R. Tang, A. Maxey, J.P. Kinsella, S.H. Abman, Inhaled nitric oxide enhances distal lung growth after exposure to hyperoxia in neonatal rats, *Pediatr. Res.* 58 (1) (2005) 22–29.
- [91] J.R. Tang, N.E. Markham, Y.J. Lin, I.F. McMurtry, A. Maxey, J.P. Kinsella, S. H. Abman, Inhaled nitric oxide attenuates pulmonary hypertension and improves lung growth in infant rats after neonatal treatment with a VEGF receptor inhibitor, *Am. J. Physiol. Lung Cell Mol. Physiol.* 287 (2) (2004) L344–L351.
- [92] O.M. Granillo, M.V. Brahmajothi, S. Li, A.R. Whorton, S.N. Mason, T.J. McMahon, R.L. Auten, Pulmonary alveolar epithelial uptake of S-nitrosothiols is regulated by L-type amino acid transporter, *Am. J. Physiol. Lung Cell Mol. Physiol.* 295 (1) (2008) L38–L43.
- [93] E.L. Bao, A. Chytsiakova, M.V. Brahmajothi, M.E. Sunday, E.N. Pavlisko, M. F. Wempe, R.L. Auten, Bronchopulmonary dysplasia impairs L-type amino acid transporter-1 expression in human and baboon lung, *Pediatr. Pulmonol.* 51 (10) (2016) 1048–1056.
- [94] N.K. Ali, A. Jafri, R.B. Sopi, Y.S. Prakash, R.J. Martin, S.I. Zaidi, Role of arginase in impairing relaxation of lung parenchyma of hyperoxia-exposed neonatal rats, *Neonatology* 101 (2) (2012) 106–115.
- [95] A. Vadivel, J.L. Aschner, G.J. Rey-Parra, J. Magarik, H. Zeng, M. Summar, F. Eaton, B. Thebaud, L-citrulline attenuates arrested alveolar growth and pulmonary hypertension in oxygen-induced lung injury in newborn rats, *Pediatr. Res.* 68 (6) (2010) 519–525.
- [96] L. Ma, N. Li, X. Liu, L. Shaw, S. Li Calzi, M.B. Grant, J. Neu, Arginyl-glutamine dipeptide or docosahexaenoic acid attenuate hyperoxia-induced lung injury in neonatal mice, *Nutrition* 28 (11–12) (2012) 1186–1191.
- [97] S. Ahmad, C.W. White, L.Y. Chang, B.K. Schneider, C.B. Allen, Glutamine protects mitochondrial structure and function in oxygen toxicity, *Am. J. Physiol. Lung Cell Mol. Physiol.* 280 (4) (2001) L779–L791.
- [98] F. Ogunlesi, C. Cho, S.A. McGrath-Morrow, The effect of glutamine on A549 cells exposed to moderate hyperoxia, *Biochim. Biophys. Acta* 1688 (2) (2004) 112–120.
- [99] E. Roth, R. Oehler, N. Manhart, R. Exner, B. Wessner, E. Strasser, A. Spittler, Regulatory potential of glutamine—relation to glutathione metabolism, *Nutrition* 18 (3) (2002) 217–221.
- [100] M.H. Kweon, V.M. Adhami, J.S. Lee, H. Mukhtar, Constitutive overexpression of Nrf2-dependent heme oxygenase-1 in A549 cells contributes to resistance to apoptosis induced by epigallocatechin 3-gallate, *J. Biol. Chem.* 281 (44) (2006) 33761–33772.
- [101] Y. Xiao, M. Kwong, A. Daemen, M. Belvin, X. Liang, G. Hatzivassiliou, T. O'Brien, Metabolic response to NAD depletion across cell lines is highly variable, *PLoS One* 11 (10) (2016), e0164166.
- [102] W. Konsavage, L. Zhang, T. Vary, J.S. Shenberger, Hyperoxia inhibits protein synthesis and increases eIF2alpha phosphorylation in the newborn rat lung, *Am. J. Physiol. Lung Cell Mol. Physiol.* 298 (5) (2010) L678–L686.
- [103] G. Gacad, D. Massaro, Hyperoxia: influence on lung mechanics and protein synthesis, *J. Clin. Invest.* 52 (3) (1973) 559–565.
- [104] R. Bhat, A.A. Salas, C. Foster, W.A. Carlo, N. Ambalavanan, Prospective analysis of pulmonary hypertension in extremely low birth weight infants, *Pediatrics* 129 (3) (2012) e682–e689.
- [105] L. Ruffini, Pulmonary hypertension in a premature infant with bronchopulmonary dysplasia, *J. Pediatr. Health Care* 29 (5) (2015) 463–469.
- [106] J. Check, N. Gotteiner, X. Liu, E. Su, N. Porta, R. Steinhorn, K.K. Mestan, Fetal growth restriction and pulmonary hypertension in premature infants with bronchopulmonary dysplasia, *J. Perinatol.* 33 (7) (2013) 553–557.
- [107] S. Vayaltrikkovil, E. Vorhies, A. Stritzke, R.A. Bashir, K. Mohammad, M. Kamaluddeen, S. Thomas, E. Al Awad, P. Murthy, A. Soraisham, Prospective study of pulmonary hypertension in preterm infants with bronchopulmonary dysplasia, *Pediatr. Pulmonol.* 54 (2) (2019) 171–178.

- [108] G. Hansmann, H. Sallmon, C.C. Roehr, S. Kourembanas, E.D. Austin, M. Koestenberger, N. European pediatric pulmonary vascular disease, pulmonary hypertension in bronchopulmonary dysplasia, *Pediatr. Res.* 89 (3) (2021) 446–455.
- [109] S.G. Yallapragada, R.C. Savani, K.N. Goss, Cardiovascular Impact and Sequelae of Bronchopulmonary Dysplasia, *Pediatr Pulmonol.*, 2021. PMID: 33756045.
- [110] K.N. Goss, A.G. Beshish, G.P. Barton, K. Haraldsdottir, T.S. Levin, L.H. Tetri, T. J. Battiola, A.M. Mulchrone, D.F. Pegelow, M. Palta, L.J. Lamers, A.M. Watson, N. C. Chesler, M.W. Eldridge, Early pulmonary vascular disease in young adults born preterm, *Am. J. Respir. Crit. Care Med.* 198 (12) (2018) 1549–1558.
- [111] S.J. Cassady, A. Lasso-Pirot, J. Deepak, Phenotypes of bronchopulmonary dysplasia in adults, *Chest* 158 (5) (2020) 2074–2081.
- [112] S.S. Laurie, J.E. Elliott, K.M. Beasley, T.S. Mangum, R.D. Goodman, J.W. Duke, I. M. Gladstone, A.T. Lovering, Exaggerated increase in pulmonary artery pressure during exercise in adults born preterm, *Am. J. Respir. Crit. Care Med.* 197 (6) (2018) 821–823.
- [113] J.M. Lagatta, E.B. Hysinger, I. Zaniletti, E.M. Wymore, S. Vyas-Read, S. Yallapragada, L.D. Nelin, W.E. Truog, M.A. Padula, N.F.M. Porta, R.C. Savani, K.P. Potoka, S.M. Kawut, R. DiGeronimo, G. Natarajan, H. Zhang, T.R. Grover, W. A. Engle, K. Murthy, B.P.D.F.G. Children’s hospital neonatal consortium severe, the impact of pulmonary hypertension in preterm infants with severe bronchopulmonary dysplasia through 1 year, *J. Pediatr.* 203 (2018) 218–224 e3.
- [114] S. Arjaans, E.A.H. Zwart, M.J. Ploegstra, A.F. Bos, E.M.W. Kooi, H.L. Hillege, R.M. F. Berger, Identification of gaps in the current knowledge on pulmonary hypertension in extremely preterm infants: a systematic review and meta-analysis, *Paediatr. Perinat. Epidemiol.* 32 (3) (2018) 258–267.
- [115] N. Varghese, D. Rios, Pulmonary hypertension associated with bronchopulmonary dysplasia: a review, *Pediatr. Allergy Immunol. Pulmonol.* 32 (4) (2019) 140–148.
- [116] A. El-Saie, B. Shivanna, Novel strategies to reduce pulmonary hypertension in infants with bronchopulmonary dysplasia, *Front. Pediatr.* 8 (2020) 201.
- [117] S.H. Abman, G. Hansmann, S.L. Archer, D.D. Ivy, I. Adatia, W.K. Chung, B. D. Hanna, E.B. Rosenzweig, J.U. Raj, D. Cornfield, K.R. Stenmark, R. Steinhorn, B. Thebaud, J.R. Fineman, T. Kuehne, J.A. Feinstein, M.K. Friedberg, M. Earing, R.J. Barst, R.L. Keller, J.P. Kinsella, M. Mullen, R. Deterding, T. Kulik, G. Mallory, T. Humpl, D.L. Wessel, C.C.P. American Heart Association Council on Cardiopulmonary, Resuscitation, C. Council on Clinical, Y. Council on Cardiovascular Disease in the, R. Council on Cardiovascular, Intervention, S. Council on Cardiovascular, Anesthesia, S. the American Thoracic, Pediatric pulmonary hypertension: guidelines from the American heart association and American thoracic society, *Circulation* 132 (21) (2015) 2037–2099.
- [118] M.R. La Frano, J.F. Fahrman, D. Grapov, O. Fiehn, T.L. Pedersen, J.W. Newman, M.A. Underwood, R.H. Steinhorn, S. Wedgwood, Metabolic perturbations of postnatal growth restriction and hyperoxia-induced pulmonary hypertension in a bronchopulmonary dysplasia model, *Metabolomics* 13 (4) (2017) 32.
- [119] Y. Hoshikawa, S. Ono, S. Suzuki, T. Tanita, M. Chida, C. Song, M. Noda, T. Tabata, N.F. Voelkel, S. Fujimura, Generation of oxidative stress contributes to the development of pulmonary hypertension induced by hypoxia, *J. Appl. Physiol.* 90 (4) (1985) 1299–1306, 2001.
- [120] M. Fukuzawa, R. Yamaguchi, I. Hide, Z. Chen, Y. Hirai, A. Sugimoto, T. Yasuhara, Y. Nakata, Possible involvement of long chain fatty acids in the spores of *Ganoderma lucidum* (Reishi Houshi) to its anti-tumor activity, *Biol. Pharm. Bull.* 31 (10) (2008) 1933–1937.
- [121] J. Xiong, H. Kawagishi, Y. Yan, J. Liu, Q.S. Wells, L.R. Edmunds, M.M. Fergusson, Z.X. Yu, Rovira II, E.L. Brittain, M.J. Wolfgang, M.J. Jurczak, J.P. Fessel, T. Finkel, A metabolic basis for endothelial-to-mesenchymal transition, *Mol. Cell* 69 (4) (2018) 689–698 e7.
- [122] J. Gong, Z. Feng, A.L. Peterson, J.F. Carr, A. Vang, J. Braza, G. Choudhary, P. A. Denney, H. Yao, Endothelial to mesenchymal transition during neonatal hyperoxia-induced pulmonary hypertension, *J. Pathol.* 252 (4) (2020) 411–422.
- [123] Y.C. Lo, K.Y. Chen, H.C. Chou, I.H. Lin, C.M. Chen, Neonatal hyperoxia induces gut dysbiosis and behavioral changes in adolescent mice, *J. Chin. Med. Assoc.* 84 (3) (2021) 290–298.
- [124] M.A. Steurer, S. Oltman, R.J. Baer, S. Feuer, L. Liang, R.A. Paynter, L. Rand, K. K. Ryckman, R.L. Keller, L.L. Jelliffe-Pawlowski, Altered metabolites in newborns with persistent pulmonary hypertension, *Pediatr. Res.* 84 (2) (2018) 272–278.
- [125] B. Schmidt, E.V. Asztalos, R.S. Roberts, C.M. Robertson, R.S. Sauve, M. F. Whitfield, I. Trial of Indomethacin Prophylaxis in Preterms, Impact of bronchopulmonary dysplasia, brain injury, and severe retinopathy on the outcome of extremely low-birth-weight infants at 18 months: results from the trial of indomethacin prophylaxis in preterms, *J. Am. Med. Assoc.* 289 (9) (2003) 1124–1129.
- [126] A. Majnemer, P. Riley, M. Shevell, R. Birnbaum, H. Greenstone, A.L. Coates, Severe bronchopulmonary dysplasia increases risk for later neurological and motor sequelae in preterm survivors, *Dev. Med. Child Neurol.* 42 (1) (2000) 53–60.
- [127] L.J. Van Marter, K.C. Kuban, E. Allred, C. Bose, O. Dammann, M. O’Shea, M. Laughon, R.A. Ehrenkranz, M.D. Schreiber, P. Karna, A. Leviton, E. S. Investigators, Does bronchopulmonary dysplasia contribute to the occurrence of cerebral palsy among infants born before 28 weeks of gestation? *Arch. Dis. Child. Fetal Neonatal Ed.* 96 (1) (2011) F20–F29.
- [128] E.J. Short, N.K. Klein, B.A. Lewis, S. Fulton, S. Eisengart, C. Kerckmar, J. Baley, L. T. Singer, Cognitive and academic consequences of bronchopulmonary dysplasia and very low birth weight: 8-year-old outcomes, *Pediatrics* 112 (5) (2003) e359.
- [129] L.W. Doyle, J.L. Cheong, A. Burnett, G. Roberts, K.J. Lee, P.J. Anderson, G. Victorian Infant Collaborative Study, Biological and social influences on outcomes of extreme-preterm/low-birth weight Adolescents, *Pediatrics* 136 (6) (2015) e1513–e1520.
- [130] M.C. McKenna, S. Scafidi, C.L. Robertson, Metabolic alterations in developing brain after injury: knowns and unknowns, *Neurochem. Res.* 40 (12) (2015) 2527–2543.
- [131] P. Steiner, Brain fuel utilization in the developing brain, *Ann. Nutr. Metab.* 75 (Suppl 1) (2019) 8–18.
- [132] M. Waragai, M. Moriya, T. Nojo, Decreased N-acetyl aspartate/myo-inositol ratio in the posterior cingulate cortex shown by magnetic resonance spectroscopy may Be one of the risk markers of preclinical Alzheimer’s disease: a 7-year follow-up study, *J. Alzheimers Dis.* 60 (4) (2017) 1411–1427.
- [133] D. Ben Salem, P.M. Walker, Y. Bejot, S.L. Aho, B. Tavernier, O. Rouaud, F. Ricolfi, F. Brunotte, N-acetylaspartate/creatine and choline/creatine ratios in the thalami, insular cortex and white matter as markers of hypertension and cognitive impairment in the elderly, *Hypertens. Res.* 31 (10) (2008) 1851–1857.
- [134] A.W. Poon, E.X. Ma, A. Vadivel, S. Jung, Z. Khoja, L. Stephens, B. Thebaud, P. Wintermark, Impact of bronchopulmonary dysplasia on brain and retina, *Biol. Open* 5 (4) (2016) 475–483.
- [135] U. Felderhoff-Mueser, P. Bittigau, M. Sifringer, B. Jarosz, E. Korobowicz, L. Mahler, T. Piening, A. Moysich, T. Grune, F. Thor, R. Heumann, C. Bührer, C. Ikonomidou, Oxygen causes cell death in the developing brain, *Neurobiol. Dis.* 17 (2) (2004) 273–282.
- [136] U. Yis, S.H. Kurul, A. Kumral, S. Cilaker, K. Tugyan, S. Genc, O. Yilmaz, Hyperoxic exposure leads to cell death in the developing brain, *Brain Dev.* 30 (9) (2008) 556–562.
- [137] N.F. Schor, M. Ahdab-Barmada, E. Nemoto, Brain glutamine synthetase activity and hyperoxia in neonatal rats, *Brain Res.* 566 (1–2) (1991) 342–343.
- [138] P. Basu, S. Som, N. Choudhuri, H. Das, Contribution of the blood glucose level in perinatal asphyxia, *Eur. J. Pediatr.* 168 (7) (2009) 833–838.
- [139] J.P. Boardman, J.M. Hawdon, Hypoglycaemia and hypoxic-ischaemic encephalopathy, *Dev. Med. Child Neurol.* 57 (Suppl 3) (2015) 29–33.
- [140] W.L. Yeh, C.J. Lin, W.M. Fu, Enhancement of glucose transporter expression of brain endothelial cells by vascular endothelial growth factor derived from glioma exposed to hypoxia, *Mol. Pharmacol.* 73 (1) (2008) 170–177.
- [141] S. Pan, C.J. World, C.J. Kovacs, B.C. Berk, Glucose 6-phosphate dehydrogenase is regulated through c-Src-mediated tyrosine phosphorylation in endothelial cells, *Arterioscler. Thromb. Vasc. Biol.* 29 (6) (2009) 895–901.
- [142] H. Elmasri, K. Karaaslan, Y. Teper, E. Ghelfi, M. Weng, T.A. Ince, H. Kozakewich, J. Bischoff, S. Cataltepe, Fatty acid binding protein 4 is a target of VEGF and a regulator of cell proliferation in endothelial cells, *Faseb. J.* 23 (11) (2009) 3865–3873.
- [143] K. De Bock, M. Georgiadou, S. Schoors, A. Kuchnio, B.W. Wong, A.R. Cantelmo, A. Quaegebeur, B. Ghesquiere, S. Cauwenberghs, G. Eelen, L.K. Phng, I. Betz, B. Tembuysier, K. Brepoels, J. Welti, I. Geudens, I. Segura, B. Cruys, F. Bifari, I. Decimo, R. Blanco, S. Wynn, J. Vangindertael, S. Rocha, R.T. Collins, S. Munck, D. Daelemans, H. Imamura, R. Devlieger, M. Rider, P.P. Van Veldhoven, F. Schuit, R. Bartrons, J. Hofkens, P. Fraisl, S. Telang, R.J. Deberardinis, L. Schoonjans, S. Vinckier, J. Chesney, H. Gerhardt, M. Dewerchin, P. Carmeliet, Role of PFKFB3-driven glycolysis in vessel sprouting, *Cell* 154 (3) (2013) 651–663.
- [144] Y. Xu, X. An, X. Guo, T.G. Habetsion, Y. Wang, X. Xu, S. Kandala, Q. Li, H. Li, C. Zhang, R.B. Caldwell, D.J. Fulton, Y. Su, M.N. Hoda, G. Zhou, C. Wu, Y. Huo, Endothelial PFKFB3 plays a critical role in angiogenesis, *Arterioscler. Thromb. Vasc. Biol.* 34 (6) (2014) 1231–1239.
- [145] G.L. Wright, I.G. Maroulakou, J. Eldridge, T.L. Liby, V. Sridharan, P.N. Tschlis, R. C. Muise-Helmericks, VEGF stimulation of mitochondrial biogenesis: requirement of AKT3 kinase, *Faseb. J.* 22 (9) (2008) 3264–3275.
- [146] D. Guo, Q. Wang, C. Li, Y. Wang, X. Chen, VEGF stimulated the angiogenesis by promoting the mitochondrial functions, *Oncotarget* 8 (44) (2017) 77020–77027.
- [147] X. Li, A. Kumar, P. Carmeliet, Metabolic pathways fueling the endothelial cell drive, *Annu. Rev. Physiol.* 81 (2019) 483–503.
- [148] P. Lassus, A. Ristimaki, O. Ylikorkala, L. Viinikka, S. Andersson, Vascular endothelial growth factor in human preterm lung, *Am. J. Respir. Crit. Care Med.* 159 (5 Pt 1) (1999) 1429–1433.
- [149] J.R. Tang, S.A. Karumanchi, G. Seedorf, N. Markham, S.H. Abman, Excess soluble vascular endothelial growth factor receptor-1 in amniotic fluid impairs lung growth in rats: linking preeclampsia with bronchopulmonary dysplasia, *Am. J. Physiol. Lung Cell Mol. Physiol.* 302 (1) (2012) L36–L46.
- [150] B. Thebaud, F. Ladha, E.D. Michelakis, M. Sawicka, G. Thurston, F. Eaton, K. Hashimoto, G. Harry, A. Haromy, G. Korbut, S.L. Archer, Vascular endothelial growth factor gene therapy increases survival, promotes lung angiogenesis, and prevents alveolar damage in hyperoxia-induced lung injury: evidence that angiogenesis participates in alveolarization, *Circulation* 112 (16) (2005) 2477–2486.
- [151] L. Farkas, D. Farkas, K. Ask, A. Moller, J. Gaudie, P. Margetts, M. Inman, M. Kolb, VEGF ameliorates pulmonary hypertension through inhibition of endothelial apoptosis in experimental lung fibrosis in rats, *J. Clin. Invest.* 119 (5) (2009) 1298–1311.
- [152] S. Arjaans, B.D. Wagner, P.M. Mourani, E.W. Mandell, B.B. Poindexter, R.M. F. Berger, S.H. Abman, Early angiogenic proteins associated with high risk for bronchopulmonary dysplasia and pulmonary hypertension in preterm infants, *Am. J. Physiol. Lung Cell Mol. Physiol.* 318 (4) (2020) L644–L654.
- [153] J. Sun, B. Sha, W. Zhou, Y. Yang, VEGF-mediated angiogenesis stimulates neural stem cell proliferation and differentiation in the premature brain, *Biochem. Biophys. Res. Commun.* 394 (1) (2010) 146–152.
- [154] P. Wintermark, M. Lechpammer, B. Kosaras, F.E. Jensen, S.K. Warfield, Brain perfusion is increased at term in the white matter of very preterm newborns and

- newborns with congenital heart disease: does this reflect activated angiogenesis? *Neuropediatrics* 46 (5) (2015) 344–351.
- [155] M. Hurskainen, I. Mizikova, D.P. Cook, N. Andersson, C. Cyr-Depauw, F. Lesage, E. Helle, L. Renesme, R.P. Jankov, M. Heikinheimo, B.C. Vanderhyden, B. Thebaud, Single cell transcriptomic analysis of murine lung development on hyperoxia-induced damage, *Nat. Commun.* 12 (1) (2021) 1565.
- [156] C.R. Merritt, G.T. Ong, S.E. Church, K. Barker, P. Danaher, G. Geiss, M. Hoang, J. Jung, Y. Liang, J. McKay-Fleisch, K. Nguyen, Z. Norgaard, K. Sorg, I. Sprague, C. Warren, S. Warren, P.J. Webster, Z. Zhou, D.R. Zollinger, D.L. Dunaway, G. B. Mills, J.M. Beechem, Multiplex digital spatial profiling of proteins and RNA in fixed tissue, *Nat. Biotechnol.* 38 (5) (2020) 586–599.
- [157] M. Al-Biltagi, M. Isa, A.S. Bediwy, N. Helaly, D.D. El Lebedy, L-carnitine improves the asthma control in children with moderate persistent asthma, *J. Allergy* (2012) 509730, 2012.
- [158] E.D. Michelakis, V. Gurtu, L. Webster, G. Barnes, G. Watson, L. Howard, J. Cupitt, I. Paterson, R.B. Thompson, K. Chow, D.P. O'Regan, L. Zhao, J. Wharton, D. G. Kiely, A. Kinnaird, A.E. Boukouris, C. White, J. Nagendran, D.H. Freed, S. J. Wort, J.S.R. Gibbs, M.R. Wilkins, Inhibition of pyruvate dehydrogenase kinase improves pulmonary arterial hypertension in genetically susceptible patients, *Sci. Transl. Med.* 9 (413) (2017).