Epithelial Dysfunction in Lung Diseases: Effects of Amino Acids and Potential Mechanisms

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Abstract

Lung diseases affect millions of individuals all over the world. Various environmental factors, such as toxins, chemical pollutants, detergents, viruses, bacteria, microbial dysbiosis, and allergens, contribute to the development of respiratory disorders. Exposure to these factors activates stress responses in host cells and disrupt lung homeostasis, therefore leading to dysfunctional epithelial barriers. Despite significant advances in therapeutic treatments for lung diseases in the last two decades, novel interventional targets are imperative, considering the side effects and limited efficacy in patients treated with currently available drugs. Nutrients, such as amino acids (e.g., arginine, glutamine, glycine, proline, taurine, and tryptophan), peptides, and bioactive molecules, have attracted more and more attention due to their abilities to reduce oxidative stress, inhibit apoptosis, and regulate immune responses, thereby improving epithelial barriers. In this review, we summarize recent advances in amino acid metabolism in the lungs, as well as multifaceted functions of amino acids in attenuating inflammatory lung diseases based on data from studies with both human patients and animal models. The underlying mechanisms for the effects of physiological amino acids are likely complex and involve cell signaling, gene expression, and anti-oxidative reactions. The beneficial effects of amino acids are expected to improve the respiratory health and well-being of humans and other animals. Because viruses (e.g., coronavirus) and environmental pollutants (e.g., PM2.5 particles) induce severe damage to the lungs, it is important to determine whether dietary supplementation or intravenous administration of individual functional amino acids (e.g., arginine-HCl, citrulline, N-acetylcysteine, glutamine, glycine, proline and tryptophan) or their combinations to affected subjects may alleviate injury and dysfunction in this vital organ.

Keywords

Amino acids · Lung dysfunction · Barrier integrity · Inflammatory response · Signaling pathways
### Abbreviations

| Abbreviation | Description                                    |
|--------------|------------------------------------------------|
| CaMKK2       | calcium/calmodulin-dependent kinase 2          |
| COPD         | chronic obstructive pulmonary disease          |
| IL-6         | interleukin 6                                  |
| Keap1        | Kelch-like ECH associated protein 1            |
| LPS          | lipopolysaccharide                             |
| NF-κB        | nuclear factor kappa B                         |
| NLR          | Nod-like receptors                             |
| NO           | nitric oxide                                   |
| Nrf2         | nuclear factor erythroid 2-related factor      |
| PAR          | protease-activated receptors                   |
| PRRs         | pathogen recognition receptors                 |
| RLR          | RIG-I-like receptors                            |
| ROS          | reactive oxygen species                        |
| Th           | T helper                                       |
| TJJs         | tight junctions                                |
| TLR          | Toll-like receptors                            |
| TNF-α        | tumor necrosis factor alpha                    |
| ZO           | zonulae occludens                              |

### 4.1 Introduction

The lungs are the foundational organs of the respiratory system, whose most important function is to facilitate gas exchange between the environment and the bloodstream (Zhang et al. 2018). In this process called respiration, oxygen in the ambient air enters the blood, whereas CO₂ (a product of nutrient metabolism) leaves the blood. Structurally, the bronchial monolayer epithelium in the respiratory system is responsible for preserving airway homeostasis in the lungs. Disruption of the barrier integrity by various stimuli, such as toxins, chemical pollutants, detergents, viruses, bacteria, microbial dysbiosis, and allergens, can contribute to the development of lung diseases (Budden et al. 2017; Georas and Rezaee 2014). Biochemically, these endogenous or exogenous risk factors activate an abnormal inflammatory response in the respiratory system, leading to the accumulation of reactive oxygen species (ROS), the breakdown of the epithelial integrity in the airways and alveoli, and ultimately reduced airflow capacity and lung dysfunction (Agusti and Hogg 2019; Guo and Ward 2007; Lang et al. 2002; Rahman et al. 2005; Zhang et al. 2018).

Despite significant advances in the pathogenesis of lung diseases in the last two decades, currently clinical therapies for lung diseases are largely dependent on the application of bronchodilators or glucocorticoids to improve airflow and ameliorate clinic symptoms in patients (Atto et al. 2019; Boskabadi et al. 2018; Bream-Rouwenhorst et al. 2008; Grainge and Rice 2010). Growing evidence has shown that the impairment of epithelial barrier in the alveoli is a critical step for the initiation and development of lung diseases (Steelant 2020). In our recent study, we found that dietary supplementation with L-arginine or glycine reduced immune cell infiltration, decreased mRNA levels for inflammatory cytokines and chemokines, and decreased the apoptosis of alveolar cells in LPS-challenged mice (Ma et al. 2019). These findings provide a new nutritional strategy to ameliorate lung injury through oral administration of functional amino acids. In this article, we reviewed recent progress in epithelial barrier biology and pathobiology related to lung disease. We also discussed potential mechanisms responsible for the beneficial effects of some amino acids in human patients and animal models.

### 4.2 Respiratory Barrier Integrity

The lung is made up of dozens of cell types and has evolved architecturally into a series of branching airways and alveoli to support an efficient permeable transfer of oxygen and carbon dioxide for the respiratory system and the whole body (Warheit-Niemi et al. 2019). An appropriate function of the lung is predominantly dependent on the alveolar epithelial cells, one of the predominant cells in the respiratory tract (Fig. 4.1). Under physiological conditions, the intracellular homeostasis and the normal function of the lungs are maintained through nutrient metabolism and
its regulation. However, following a severe or prolonged insult or injury to the alveolar epithelial cells, over-activation of immune responses leads to the accumulation of ROS, increased infiltration by immunocytes, and impaired function of the lungs (Kosmider et al. 2011; Steelant 2020).

The neighboring alveolar epithelial cell of the respiratory airway are held together by tight junctions (TJs) and adherens junctions, therefore forming a physical barrier against external particles and a first line of defense of the mucosal immunity (Georas and Rezaee 2014; Lambrecht and Hammad 2014). Similar to TJ proteins in the gas-

![Fig. 4.1](image)

Fig. 4.1 Functional amino acids activate Nrf2 survival signaling, while inhibiting NLRP3 inflammasome to alleviate lung injury. Air pollutants, cigarette smoke and bacterial or viral infections cause oxidative stress and inflammation in the lungs. Nrf2 activation induces the expression of cytoprotective genes to counteract the toxic effect of ROS and inhibits the transcription of proinflammatory cytokines, especially in macrophages, to reduce the recruitment of inflammatory cells into the lungs. Increased levels of ROS reduce Nrf2 and activate inflammasome, leading to the upregulation of IL-1β, IL-6, and TNF-α expression as well as the death of lung epithelial cells and consequently emphysema. Functional amino acids such as Arg, Gln, Gly activate the Nrf2 survival signaling to increase the expression of anti-oxidative genes possibly through modulating the NLRP3 inflammasome to inhibit the pro-inflammatory factors, thus improving the lung epithelial barrier. Arg, L-arginine; Gly, glycine; Gln, L-glutamine; IL, interleukin; ROS, reactive oxygen species; Nrf2, nuclear factor erythroid 2-related factor 2.

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4.3 Amino Acid Metabolism in the Lungs

Amino acids enter the lungs from the blood circulation via various sodium-dependent and independent transporters (Table 4.1). There is no de novo synthesis of citrulline, arginine, cysteine, three branched-chain AAs (BCAAs; isoleucine, leucine and valine), methionine, phenylalanine, taurine, threonine, tryptophan, tyrosine, histidine, or lysine in the lungs (Wu 2013). In addition, the lungs lack: (a) the transsulfuration pathway for converting methionine into cysteine (Berggren et al. 1984); (b) phenylalanine hydroxylase for converting phenylalanine into tyrosine (McGee et al. 1972); and (c) enzymes for converting taurine, threonine, tryptophan, tyrosine, histidine, or lysine into pyruvate, acetyl-CoA or the intermediates of the Krebs cycle (Wu 2013). However, the lungs can synthesize: (a) arginine from citrulline via argininosuccinate synthase and lyase (Wu and Morris Jr. 1998); (b) ornithine and proline from arginine via arginase-II, ornithine aminotransferase, and pyrroline-5-carboxylate reductase; (c) alanine, glutamate and glutamine from BCAAs plus α-ketoglutarate (α-KG) via BCAA transaminase, glutamate transaminase, and glutamine synthetase (Soubra et al. 1990); and (d) glutamate and aspartate from glutamine via phosphate-activated glutaminase, glutamate: pyruvate transaminase (alanine transaminase), and glutamate: oxaloacetate transaminase (aspartate transaminase); and (e) ornithine from proline via proline oxidase and ornithine aminotransferase (mitochondrial enzymes; Wu et al. 1997). These synthetic reactions also serve as metabolic pathways for the catabolism of arginine, alanine, glutamate, glutamine, aspartate, and proline in the lungs. In addition, serine and glycine are interconvertible in this organ through the action of serine hydroxymethyltransferase (present in both the cytosol and mitochondria), which plays an important role in both pulmonary health and the treatment of lung cancer (Amelio et al. 2014). Furthermore, in all cell types of the lungs (including macrophages, endothelial cells, epithelial cells, and smooth muscle cells), nitric oxide (NO; a major vasodilator and signaling molecule) and citrulline are generated from arginine by NO synthase (Folkerts et al. 2001), whereas ornithine decarboxylase decarboxylates ornithine to form putrescine (Hoet and Nemery 2000). The latter is converted into spermidine and spermine by decarboxylated 5-adenosylmethionine-dependent spermidine synthase and spermine synthase (cytosolic enzymes), respectively. The polyamines (putrescine, spermidine and spermine) are essential for DNA and protein syntheses and, therefore, play an important role in pulmonary health and diseases (including lung cancer; Agostinelli et al. 2020). During
sepsis or LPS infection, the lungs export an increased amount of glutamine by inducing the expression of glutamine synthetase at the transcriptional level partially mediated by glucocorticoid hormones (Lukaszewicz et al. 1997).

| Protein name | Gene name | Transport system | Transport mechanism | Major AA | Consequences of defects |
|--------------|-----------|------------------|---------------------|----------|--------------------------|
| EAAT3/EAAC1  | SLC1A1    | X⁻AG             | Na⁺                 | L-Glu, D/L-Asp, L-Cys | Metabolic disorders |
| ASCT1        | SLC1A4    | ASC              | Antiporter          | L-Ala, L-Ser, L-Cys | Metabolic disorders |
| ASCT2        | SLC1A5    | ASC              | Antiporter          | L-Ala, L-Ser, L-Cys | Metabolic disorders |
| rBAT         | SLC3A1    | HC-HAAT          | Exchanger           | Neutral and basic AAs | Cystinuria |
| GlyT1        | SLC6A9    | Gly              | Na⁺/Cl              | Gly      | NKHG |
| ATBα⁺        | SLC6A14   | Gly              | Na⁺/Cl              | Neutral AAs and cationic AAs | Obesity; abnormal |
| CAT-1        | SLC7A1    | y⁺               | Uniporter           | Basic AAs | Hypertension |
| CAT-2        | SLC7A2    | y⁺               | Uniporter           | Basic AAs | Inflammation |
| ORC1/ORNT1   | SLC25A15  | Orn/Cit carrier  | H⁺/antiporter       | Mit L-Orn/L-Cit exchange | HHH syndrome |
| GC2          | SLC25A18  | Glu carrier      | H⁺-coupled; OH⁻/antiporter | L-Glu | Metabolic disorders |
| Not assigned | SLC38A10  | A                | Na⁺                 | L-Gln, L-Ala | Metabolic disorders |
| LAT3         | SLC43A1   | L                | Uniporter           | Large neutral AAs | Metabolic disorders |
| Cystinosin   | SLC66A4   | LCT              | H⁺-coupled          | Efflux of Cys from lysosome | Metabolic disorders |

Adapted from Kanai and Hediger (1992), Kandasamy et al. (2018) and Wu (2013)

**HC-HAAT** heavy chain of heteromeric amino acid transporter, **HHH** hyperornithinemia-hyperammonemia-homocitrullinuria, **LCT** lysosomal cystine transporter, **MGT** mitochondrial glycine transporter, **it** mitochondrial, **NKHG** non-ketotic hyperglycemia, **rBAT** related to bo⁺⁺ amino acid transporters

**4.4 Functional Amino Acids, a Paradigm Shift in Protein Nutrition**

Besides serving as building blocks for proteins, which are the most fundamental component in tissues, amino acids have enormous physiological importance, such as the synthesis of low molecular-weight substances (e.g., nitric oxide, polyamines, creatine, carnosine, dopamine, serotonin, and glutathione), regulate metabolism and immune response, and maintain intestinal barrier function (Wu 2013; Wu et al. 2014). Based on studies on the fundamental effects of amino acids, as well as their metabolism and biological functions, a new concept of “functional amino acid” has been proposed (Hou and Wu 2017). In contrast to the traditional classification of nutritionally essential or non-essential amino acids, which were defined based on the criterion of growth or nitrogen balance, the con-
cept of “functional amino acids” is based on physiological functions of amino acids to improve survival, growth, development, lactation, reproduction, and health of humans and other animals (Wu 2014). This view advances our understanding of amino acid nutrition and metabolism, as well as dietary requirements for amino acids to maintain various functions of cells and tissues under both physiological and pathological conditions. Accumulating evidence has shown that functional amino acids are critical for the regulation of protein synthesis, gene expression, immune response, intestinal barrier function, and cellular fate decision (Hou et al. 2015; Wu et al. 2014).

4.5 Effects of Functional Amino Acids on Respiratory Barrier Function

The pulmonary epithelium is the predominant cells that prevent the entry of luminal contents into the blood circulation, while ensuring proper gas exchange. A relationship between low TJ protein abundance and high epithelial permeability has been reported for the gastrointestinal or respiratory epithelium of human patients and animal models (He et al. 2017). This observation supports a critical role of TJ proteins in epithelial integrity and prompts new search for molecules or compounds to reduce epithelial permeability and improve mucosal barrier function using both in vivo or in vitro models (Atto et al. 2019). A comparative study has identified differences in the metabolism of amino acids between patients with and without bacterial infection during the early stage of chronic obstructive pulmonary disease (COPD) (32). Specifically, COPD patients affected with bacterial infection had lung dysfunction, which was accompanied by decreased plasma levels of asparagine, citrulline, glutamine, histidine, methionine, serine, and threonine, compared with COPD patients without bacterial infection (Inoue and Ikeda 2019). This finding links lung injury with the abnormal metabolism of amino acids under pathological conditions.

L-Arginine (Arg), a product of glutamine (via the formation of glutamate) and proline metabolism via the intestinal-renal axis (Wu and Morris 1998), is a critical substrate for nitric oxide (NO) production by NOS, as noted previously. It has been shown that plasma Arg concentration is reduced in animal models of lung injury, including sheep (Murakami et al. 2007), rabbits (Chao et al. 2011; Yoshida et al. 1999), and rodents (Chu et al. 2005; Mabalirajan et al. 2010). This is likely due to increases in the expression of arginase-II in extrahepatic tissues and the leakage of hepatic arginase-I and extrahepatic arginase II into the blood, resulting in an excessive hydrolysis of arginine into ornithine plus urea. Supplementation with Arg leads to an increased bioavailability of Arg, which in turn, restores endothelial function, decreases inflammatory response, improves bronchial epithelial barrier, and mitochondrial dysfunction, therefore improving the lung function (Chao et al. 2011; Mabalirajan et al. 2010). L-Citrulline can be converted into L-arginosuccinate by argininosuccinate synthase, and L-argininosuccinate is subsequently converted into Arg by argininosuccinate lyase (Curis et al. 2005). These two enzymes are present in the lungs and other tissues in mammals and birds. Oral or intravenous administration of L-citrulline can enhance the circulating levels of Arg and systemic synthesis of NO, thereby attenuating hyperoxia-induced lung damage (Grisafi et al. 2012). Of particular note, dietary supplementation with arginine, which is safe for healthy adult humans (up to 30 g/day in divided doses; McNeal et al. 2018) as well as growing and adult pigs (up to 2% in the diet; Wu et al. 2016), prevents or alleviates pulmonary hypertension and injury in humans and farm animals under various pathological conditions (Wu 2020; Wu et al. 2000).

L-Glutamine (Gln) is the most abundant amino acid in the plasma of both humans and many other animals (Wang et al. 2015a). A critical function of Gln in maintaining intestinal mucosal barrier integrity has been described in various animal models (Jiao et al. 2015; Wu 2013; Wu et al. 2014). Depletion of plasma Gln is
associated with the impairment of intestinal barrier breakdown, which can be abolished by Gln supplementation in animals and human patients. We have investigated the physiological functions of amino acids in piglets, a well-known animal model for studying nutrition and metabolism. Our studies indicate that dietary supplementation with Gln, glutamate, or glycine attenuates weaning- or oxidative stress-induced epithelial barrier dysfunction by regulating the abundance and intracellular localization of TJ proteins (Fan et al. 2019; Wang et al. 2015b), as well as apoptosis (Fan et al. 2019; Jiao et al. 2015; Liu et al. 2018; Wang et al. 2014) and unfolded protein response (He et al. 2019) in piglets. Also, we found that Gln regulates the abundance of TJ proteins and intestinal barrier in a CaMKK2-dependent manner, thereby contributing to improvements in intestinal nutrient absorption and protein synthesis in weanling piglets (Wang et al. 2016; Wang et al. 2015b). It remains unknown whether these amino acids affect alveolar epithelium in virus- or endotoxin-challenged lungs.

We have conducted animal studies to address the foregoing issue. In our work, mice pretreated with aerosolized Arg, Gln, or glycine were exposed to aerosolized LPS to induce lung injury. We found that Arg or glycine pretreatment reduced LPS-induced collagen deposition, apoptosis of alveolar cells, decreased mRNA levels for inflammatory cytokines and chemokines, and reduced the accumulation of neutrophils and macrophages in the lung tissues of mice, thus contributing to an improved respiratory function (Ma et al. 2019). Gln administration reduced LPS-induced collagen deposition and inflammatory cytokines without affecting other parameters examined in the study (Ma et al. 2019). More studies are required to uncover underlying mechanisms responsible for these beneficial effects. In a previous study, Zhang et al. (2007) reported that Gln supplementation attenuated an LPS-induced increase in bronchoalveolar epithelial permeability and a concomitant decrease in the abundance of TJ proteins. The latter include occludin, zonula occludens (ZO)-1, and adherens junction protein E-cadherin. Clearly, increasing Gln availability protected the alveolar epithelium against barrier dysfunction and lung injury in rats (Zhang et al. 2007). These observations support the view that supplementation with functional amino acids, such as Arg, Gln, or glycine, may offer a novel nutritional strategy to reduce the deleterious effects of bacterial infection on alveolar function.

4.6 Effect of Amino Acid on Cellular Metabolic Programming and Lung Injury

Metabolic programming is one of the important mechanisms by which cellular responses are regulated under specific conditions (Vigeland et al. 2019). Dysregulation of metabolic reprogramming in lung diseases, such as asthma and COPD, might impair the innate function of immune cells (Michaeloudes et al. 2019). Gln utilization by lungs and other tissues increases under various stress conditions due to enhanced expression of mitochondrial phosphate-activated glutaminase, such that Gln becomes a conditional essential amino acid (Wilmore and Shabert 1998). Inhibition of glutamine metabolism with 6-diazo-5-oxo-L-norleucine that binds to glutamine-utilizing enzymes and transporters, accelerated recovery from LPS-induced acute lung injury, as shown by reduced immune cell infiltration and decreased protein levels for pro-inflammatory cytokines and chemokines (Vigeland et al. 2019). This metabolic programming is mainly due to the fact that immune cells, such as neutrophils, macrophages, and lymphocytes have a high metabolic rate and rely on Gln metabolism to support activation and immune response in response to stress or bacterial infection (Wang et al. 2015a). Also, a depletion of Gln in blood represents a risk for poor treatment outcomes and is associated with increased mortality in critical illness (Oudemans-van Straaten et al. 2001). Consistently, Gln supplementation reduces abdominal sepsis, or LPS-induced lung injury in animals models (Lai et al. 2014). Consistently, a deficiency of ASCT2 (a transporter of Gln and small neutral amino acids) impaired the induc-
tion of T helper 1 (Th1) and Th17 cells, attenuated activation of mTORC1 signaling, and inflammatory T cell responses in mouse models, further substantiating a functional role of cellular programming on immunocyte activation and inflammatory responses (Nakaya et al. 2014). Gln enhances expression of AST2 increases its availability in the cells to alleviate the adverse effects of general immune activation (Nakaya et al. 2014). In addition, Gln promotes tissue repair by enhancing the production of growth factors by immune cells. For example, EGF-like growth factor, amphiregulin (AREG), is a growth factor produced by macrophages, regulatory T cells, and type-2 innate lymphoid cells in the animal models of lung injury (Xu et al. 2016). Administration of an antibody to neutralize AREG has been reported to exacerbate LPS-induced lung injury (Ogata-Suetsugu et al. 2017; Xu et al. 2016). Inhibition of Gln metabolism with 6-diazo-5-oxo-L-norleucine increased the mRNA level of AREG in immune cells, which in turn promoted tissue repair and improved the function of the lungs in mice (Vigeland et al. 2019). These findings indicate a novel target for the prevention and treatment of lung injury by interfering with the metabolic programming of immunocytes. More studies are warranted to validate these effects in other inflammatory lung diseases and to elucidate the underlying molecular mechanisms.

4.7 Effects of Amino Acids on NLRP3 Inflammasomes and Lung Diseases

The innate immune system acts as the first line of defense in response to environmental risk factors. A serial of pathogen recognition receptors (PRRs), such as Toll-like receptors (TLR), RIG-I-like receptors (RLR), protease-activated receptors (PAR), Nod-like receptors (NLR), C-type lectin receptors, sense the pathogens in contact with the airway epithelium (Hartl et al. 2018). Nod-like receptors (NLRs), including NOD1, NOD2, and NLRP3, are intracellular pattern recognition molecules that can detect microbial- and danger-associated molecular patterns.

NLRP3 inflammasomes is a multiprotein large-cytoplasmic complex that is composed of NLRP3, apoptosis-associated speck-like proteins (ASC), and pro-caspase-1. Activation of NLRP3 has emerged as an important regulator of lung diseases (Xu et al. 2018). Both LPS and ROS have been reported to activate the NLRP3 inflammasome and caspase-1, promote the cleavage and maturation of pro-interleukin (IL)-1β (biologically the most active cytokine in the lungs of patients), resulting in damage to lung tissue (Xu et al. 2018). The resistance of NLRP3-deficient mice to polymicrobial sepsis-induced lethality validates a critical role of NLRP3 in the pathogenesis of lung disease (Chen et al. 2019; Fukumoto et al. 2013; Lee et al. 2017; Liu et al. 2019). We found that glycine administration reduced the LPS-induced accumulation of neutrophils and macrophages, as well as inflammatory responses and collagen deposition in the lung tissues of mice (Ma et al. 2019). Further study showed that
LPS-induced upregulation of NLPP3 and IL-1β expression was reduced by glycine supplementation (Zhang et al. 2020), indicating a regulatory effect of glycine on decreasing NLRP3 inflammasomes. Considering that ROS are activators of NLRP3 but glycine alleviates ROS-induced cellular damage by promoting the synthesis of GSH (an endogenous antioxidant) in intestinal porcine epithelial cells (Wang et al. 2014), it is plausible that administration of glycine might modulate the NLRP3/IL-1β signaling, therefore improving the lung epithelial barrier in LPS-challenged mice.

### 4.8 Effects of Amino Acid on the Nrf2 Signaling and Lung Injury

The nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of genes implicated in protection against oxidative damage in the lungs and other tissues (Cho and Kleeberger 2020; Mizumura et al. 2020). Under normal conditions, Nrf2 is continuously degraded in a Kelch-like ECH associated protein (Keap)1-dependent manner through the proteasome pathway. However, in the presence of elevated concentrations of ROS or electrophiles, Nrf2 is stabilized due to the disruption of Keap1-mediated repression. This leads to the accumulation of Nrf2 in the nucleus to activate the expression of anti-oxidative genes, such as catalytic and modulatory subunits of the GSH synthesizing enzyme glutamate-cysteine ligase (GCLC and GCLM), thioredoxin reductase 1, hemeoxygenase-1 (HO-1), NAD(P)H Quinone Dehydrogenase-1 (NQO-1), xCT, a subunit of cystine/glutamate transporter (Qian et al. 2018). Thus, Nrf2 plays an important role in alleviating oxidative damage and improving the function of the lungs. Consequently, Nrf2-knockout mice are more susceptible to LPS-induced lung inflammation than their wild-type counterparts, as indicated by neutrophils infiltration, as well as the elevated levels of proinflammatory cytokines (tumor necrosis factor-α and interleukin-6) and chemokines (macrophage inflammatory protein 2 and magnesium-dependent phosphatase 1) (Li et al. 2017; Thimmulappa et al. 2006).

In critical illness or in response to severe stress, serum Gln level is decreased which is accompanied by a modest therapeutic outcome and an increased mortality (Oudemans-van Straaten et al. 2001). Gln supplementation promotes the expression of subunits of the GSH synthesizing enzyme, GCLC and GCLM, in a Nrf2-dependent manner, leading to increases in the synthesis and concentration of GSH and reduced cellular damage in intestinal and lung tissues (Venooji et al. 2015). In a recent study, de Oliveira et al. (2019) found that Gln treatment reduced myeloperoxidase activity, decreased inflammatory responses, and improved both the morphological alteration and function in the lungs of LPS-challenged mice. We also observed that functional amino acids (e.g., glycine) activated Nrf2 survival signaling, while inhibiting NLRP3 inflammasome (Zhang et al. 2020), indicating a regulatory effect of amino acids on the Nrf2 signaling pathway. Note that glycine is highly abundant in meat (e.g., beef; Wu et al. 2016) but is relatively deficient in all plant-source proteins (Hou et al. 2019; Li and Wu 2020). Therefore, antioxidant agents or functional foods that modulate Nrf2 would be expected to potentially therapeutic options to alleviate lung injury by enhancing intracellular concentrations of antioxidant molecules (including enzymes) in the alveolar epithelium.

### 4.9 Effects of Amino Acids on the Lung Microbiota and Lung Disease

Healthy lungs were traditionally believed to be sterile due to their effective antimicrobial defenses (Budden et al. 2017). This view was challenged by the isolation of bacteria from the respiratory tract of healthy individuals with the use of culture-independent approaches for microbial community profiling (Faner et al. 2017). Compared with the gastrointestinal tract where more than 100 trillion microorganisms reside, the lungs of humans and animals harbor 10³–10⁵ CFU/g of tissue (Remot et al. 2017), with *Bacteroides*, *Firmicutes* and *Proteobacteria* being the predominant phyla commonly observed
(He et al. 2017). There is a relatively much lower abundance of bacteria in the lungs than in the gastrointestinal tract. However, increasing evidence indicates that lung microbiota dysbiosis is a critical environmental factor that interacts with host cells and contributes to the pathogenesis of multiple lung diseases through various mechanisms (Dickson et al. 2013; He et al. 2017; Wu and Segal 2017). First, microbes play an important role in shaping the normal and pathologic immune responses in lungs (He et al. 2017). Deregulation of microbiota in the lungs may predispose humans and other animals to the development of respiratory disease, and has a significant impact on clinical outcomes of respiratory disorders (Segal et al. 2014). Also, the microflora in the lungs can translocate to the gastrointestinal tract and other tissues through blood circulation, therefore triggering inflammatory responses (Sze et al. 2014). Additionally, intestinal bacteria influence the composition and diversity of microbiota in the lungs, therefore forming a bidirectional gut-lung axis (Budden et al. 2017; He et al. 2017). More studies are required to uncover the complicated crosstalk between the lungs and gut, as well as their impacts on health.

It is known that the intestinal microbiota affects the physiology, metabolism, and immunity of the host through: (a) interactions with enterocytes and immune cells of the gastrointestinal tract; and (b) the production of bacterial metabolites. Studies with pigs have shown that most of the amino acids in the intestinal lumen can be utilized by intestinal bacteria for protein synthesis and catabolism at various rates (Dai et al. 2012a; Dai et al. 2011), therefore affecting the proportions of dietary amino acids entering the portal vein, as well as the availability of amino acids for various cells in the lungs and colonized bacteria. This may be a reason why the intestinal microbiota affects the development of lung diseases as observed in clinical patients and experimental animals (O’Dwyer et al. 2016). Of note, dietary supplementation with functional amino acids (e.g., Gln, Trp and Arg) has been reported to regulate the bacterial ecosystem of the gut and improve the intestinal health of animals (Dai et al. 2012b, 2013; Liang et al. 2019; Wang et al. 2020). To extend this observation, we conducted an experiment involving mice pretreated with aerosolized arginine, glutamine, or glycine before exposure to aerosolized LPS (13). Each of these three amino acids was found to have beneficial effects on reducing LPS-induced lung injury (Ma et al. 2019). Although arginine supplementation can stimulate the production of NO (a potential oxidant) by LPS-activated macrophages, other benefits of this amino acid may contribute to its role in alleviating the infiltration of neutrophils into the lung tissues and in improving alveolar integrity and function. More studies are needed to explore whether the beneficial effect of the functional amino acids is associated with microbiota alterations in the lungs of animals.

### 4.10 Conclusion and Perspectives

Studies from clinical patients and animal models have provided enormous amounts of data on the development to lung injury (including inflammatory cell infiltration and morphological alterations), as well as the cellular and molecular events underlying the pathogenesis. Recent studies have shown that certain amino acids are critically important nutrients for maintaining the integrity and the functionality of the lungs by promoting the syntheses of proteins, bioactive compounds, and nucleic acids, which are required for cell proliferation, immune response, and cellular homeostasis. Accumulating evidence indicates that depletion of amino acids or deregulation of amino acid metabolism is associated with the development of multiple lung diseases. Amino acid supplementation improves lung homeostasis by regulating mucosal barrier function, inhibiting apoptotic cell death, and restoring the integrity of epithelial barrier via multiple signaling pathways. However, there is a long way to translate the basic research into clinical applications for the treatment of patients with lung disease. First, lung injury is a complicated pathological process and multiple types of cells (including endothelial cells, epithelium, smooth muscle cells, and fibroblasts) contribute to the dysfunction of the lungs. It remains unknown how these cells respond to...
amino acids under various physiological and pathological conditions. Second, amino acids have been reported to alleviate inflammatory responses and improve lung function. However, underlying mechanisms are not well defined and more studies are required to fulfill this gap of knowledge before their applications to clinical medicine. Third, most of our understanding and observations are based on studies with rodents. Despite a high degree of similarity in nutrition, physiology and immunology between mice and humans, it should be borne in mind that no animal models can fully mimic human disease. Thus, precautions should be taken not to simply extrapolate results from animals to humans. Also, data from various animal studies are not always consistent and sometimes are very different, due to differences in experimental designs, animal models, the duration and dosage of chemical exposure, methods for analysis, and the interpretation of results. Careful studies and a critical thinking are required to draw a convincing conclusion. Furthermore, because viruses [e.g., coronavirus (COVID-19, Shi et al. 2020) and influenza virus (Herold et al. 2015)] induce severe damage to the lungs, it is important to determine whether dietary supplementation or intravenous administration of individual functional amino acids (e.g., arginine-HCl, citrulline, N-acetylcysteine, glutamine, glycine, proline and tryptophan) or their combinations to affected subjects may ameliorate injury and dysfunction in this vital organ. Finally, because air pollution impairs the development of organs (particularly lungs; Wu et al. 2019), effects of environmental pollutants (e.g., PM₂.₅ particles) on amino acid metabolism in the lungs and nutritional methods involving the use of amino acid supplementation to alleviate respiratory disorders should be investigated.

Acknowledgments This work was supported by the National Natural Science Foundation of China (No. 31572423, and 31625025), the “111” Project (B16044), Jinxinnong Animal Science Development Foundation, and Texas A&M AgriLife Research (H-8200). Y.Y. and G.W. designed the review; J.C., Y.J., Y.Y., Z.W., and G.W. drafted the manuscript; Z.W., Y.Y., and G.W. revised and finalized the manuscript. Y.Y. and G.W. had primary responsibility for final content. All authors read and approved the final manuscript.

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