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# The Bidirectional Gut–Lung Axis in Chronic Obstructive Pulmonary Disease

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## Abstract

Epidemiological studies indicate that chronic obstructive pulmonary disease (COPD) is associated with the incidence of changes in intestinal health. Cigarette smoking, as one of the major causes of COPD, can have an impact on the gastrointestinal system and promotes intestinal diseases. This points to the existence of gut–lung interactions, but an overview of the underlying mechanisms of the bidirectional connection between the lungs and the gut in COPD is lacking. The interaction between the lungs and the gut can occur through circulating inflammatory cells and mediators. Moreover, gut microbiota dysbiosis, observed in both COPD and intestinal disorders, can lead to a disturbed mucosal

environment, including the intestinal barrier and immune system, and hence may negatively affect both the gut and the lungs. Furthermore, systemic hypoxia and oxidative stress that occur in COPD may also be involved in intestinal dysfunction and play a role in the gut–lung axis. In this review, we summarize data from clinical research, animal models, and *in vitro* studies that may explain the possible mechanisms of gut–lung interactions associated with COPD. Interesting observations on the possibility of promising future add-on therapies for intestinal dysfunction in patients with COPD are highlighted.

**Keywords:** gut–lung axis; lung diseases; systemic inflammation; hypoxia; microbiota

Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation and chronic airway obstruction, leading to a progressive and irreversible decline in lung function (1). COPD is associated with exacerbations and comorbidities, resulting in a significant social and economic burden (2). There are several risk factors for COPD, including cigarette smoking, genetic factors, environmental pollution, and infections (3, 4).

A retrospective analysis of 1,228 patients with COPD showed that the majority of patients with COPD have at least one gastrointestinal symptom (5). Intestinal diseases, such as inflammatory bowel disease

(IBD) and irritable bowel syndrome, are commonly observed in patients with COPD (6). The incidence of IBD is higher in patients with COPD compared with healthy subjects (7). Patients with COPD have intestinal microbiome dysbiosis and increased intestinal permeability, which is associated with inflammatory cell infiltration (8). Moreover, enterocyte damage in addition to intestinal hyperpermeability in patients with COPD, but not in control subjects, indicates functional changes in the gastrointestinal tract (9). Although the gut and lungs are separate organs with different functions and environments, they have structural similarities and interact

in health and disease. There is growing interest in how lung health affects the function of the intestinal system and vice versa (10).

Cigarette smoke, as one of the leading causes of COPD, has detrimental effects on respiratory and gastrointestinal mucosal health. Cigarette smoke can induce inflammatory responses in the lung, which might “spill” into the systemic circulation, causing systemic inflammation and leading to (low-grade) inflammatory responses in the intestine (11). Cigarette smoke can cause oxidative stress and hypoxia in airway epithelium (12), while the impaired gas exchange induced by cigarette smoke in

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patients with COPD also leads to intestinal hypoxia and enterocyte damage and hence can result in an enhancement of intestinal permeability, disturbed immune function, and microbiome dysbiosis (8, 13, 14). In addition, soluble cigarette smoke particles may directly affect the gastrointestinal tract by entering the bloodstream after inhalation, or these particles may reach the gastrointestinal tract because of mucociliary clearance in the airways or direct swallowing of cigarette smoke (15).

In this review, we discuss recent advances in understanding the interactions between the lungs and the gut in COPD. Systemic inflammation, epithelial barrier dysfunction, oxidative stress, hypoxia, and gut microbiome dysbiosis are considered to play a major role in gut–lung interactions. Clinical studies and preclinical *in vivo* and *in vitro* experiments related to the gut–lung axis in COPD are discussed, as well as potential interventions and their modulation via the gut–lung axis aimed at treating or preventing the progression of COPD and its intestinal comorbidities.

## Gastrointestinal Tract Disorders Associated with COPD

There is an association between gastrointestinal disorders and COPD. Gastrointestinal diseases are more prevalent in patients with COPD than in healthy subjects. A population-based retrospective cohort study using the administrative health databases of Québec showed that the incidence of IBD (i.e., Crohn's disease [CD] and ulcerative colitis [UC]) was 55% and 30% higher in patients with COPD than in the general population, respectively. The highest incidence rate of IBD among patients with COPD was at 50–59 years of age (7). There is also evidence that up to 60% of patients with IBD have some degree of subclinical lung disease (16). In addition, both CD and UC were associated with an increased mortality in patients with asthma-related COPD (patients with COPD with mixed features of asthma and COPD), while UC also increased mortality among patients with COPD (17). Shared environmental risks (i.e., smoking and air pollution) are unlikely to fully explain this association, because cigarette smoke may have a protective role in UC (18). A meta-analysis presented that the

risk of developing UC is significantly lower in smokers compared with people who have never smoked (19), whereas early smoking is associated with an increased risk for CD (20). However, the benefits of nicotine treatments in patients with UC in the form of transdermal patches, chewing gum, and nicotine-based enemas are not yet conclusive (21–23). Moreover, IBD is associated not only with COPD but also with other airway manifestations, such as bronchiectasis, characterized by airway inflammation and high volumes of sputum production (24).

A large U.S. population-based study (1997–2003) revealed that the prevalence of gastrointestinal ulcer disease in current and former smokers (11.4% and 11.5%, respectively) is almost doubled compared with never-smokers (6.0%). In addition, smokers who have a large daily intake of tobacco have higher risk of developing peptic ulcers compared with never-smokers (25).

A case–control study using data from the Ewha Womans University School of Medicine, Mokdong Hospital (Seoul, Republic of Korea), the database showed that the prevalence of colorectal adenomatous polyps was 66% (54 of 82) in the COPD group and 39% (98 of 251) in the non-COPD group matched for both age and sex. This indicated that the risk of colorectal malignant potential in the COPD group was higher than in the non-COPD group (26).

Although COPD is a well-known risk factor for lung cancer, a retrospective analysis of the National Health Insurance Service–National Sample Cohort in Korea indicated that COPD may also be an independent risk factor for cancers developing outside the lungs, such as colorectal and liver cancers, irrespective of smoking status (27). A clinical trial of 666 subjects (256 patients with liver diseases and 410 patients without liver diseases) showed that the prevalence of COPD was higher in patients with liver diseases (28). Furthermore, the prevalence of aseptic inflammation of the gallbladder is associated with COPD exacerbations (29).

Gastroesophageal reflux disease (GERD) was one of the most common comorbidities of COPD and was associated with exacerbations in patients with COPD in a national cross-sectional cohort study that included data from 141,057 patients with COPD using the Korean National Health Insurance Database (30). A longitudinal cohort study in the United Kingdom, following two groups of patients for 5 years,

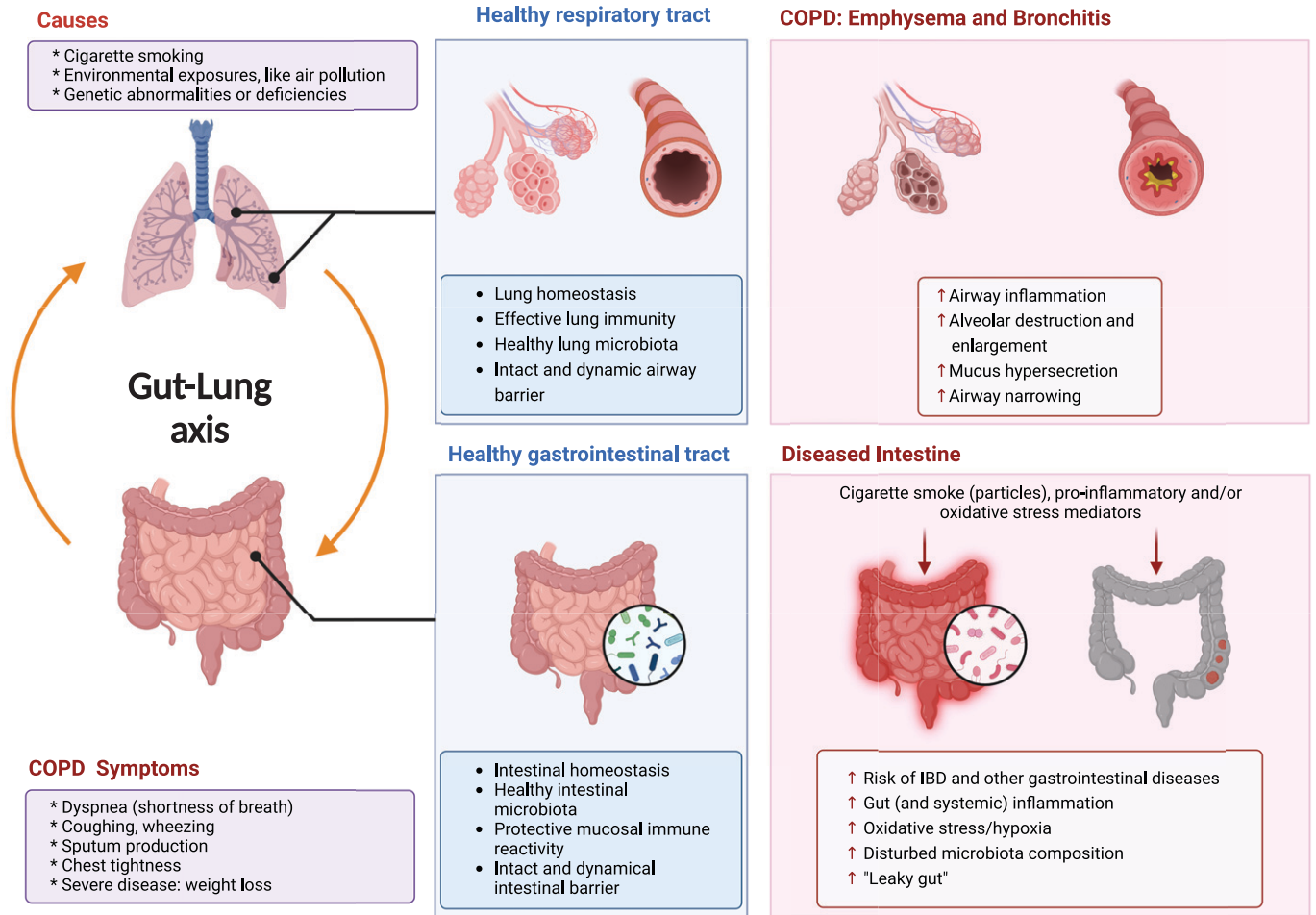
revealed that the relative risk of COPD diagnosis in patients with GERD was 1.17, and the relative risk of GERD diagnosis in patients with COPD was 1.46 (31). Cigarette smoking is a risk factor for both GERD and COPD in the general population (32, 33). Nicotine may affect esophageal sphincter tone and esophageal clearance and induce relaxation of the muscle of the esophageal sphincter, possibly increasing the duration of acid exposure and frequency of reflux events (34, 35). The rate of COPD exacerbations is twice as high in patients with GERD symptoms in comparison with those without GERD symptoms (36). A potential pathogenic mechanism for these COPD exacerbations might be related to the microaspiration of gastric contents and/or vagal irritation from gastroesophageal reflux, which may constitute airway irritants (37).

There is also an association between COPD and periodontitis. A meta-analysis demonstrated that patients with COPD have worse periodontal health status than patients without COPD, evidenced by more clinical attachment loss, gingival tissue inflammation, and bleeding (21). On the other hand, poor oral health is considered to be a contributing factor to the development of respiratory diseases (38).

In the next section, we discuss the interactions between the respiratory and gastrointestinal systems and their possible mechanisms of involvement in COPD, including changes in immunological and systemic inflammation, epithelial barrier function, hypoxia-related and oxidative stress processes, and the composition of the microbiota (Figure 1) (39). In addition, clinical evidence for the existence of the gut–lung axis in COPD is summarized in Table 1 (clinical part).

## Systemic Inflammation

Keely and colleagues showed that exaggerated innate immune responses, characterized by increases in systemic proinflammatory mediators such as IL-6 and TNF- $\alpha$  (tumor necrosis factor- $\alpha$ ), can contribute to the development of COPD (13). There is clear evidence that immune dysfunction precedes symptoms of intestinal disorders (40). The circulating proinflammatory mediators in patients with COPD may drive cross-organ inflammation; for example, systemic IL-6 together with pulmonary TGF- $\beta$  (transforming growth factor- $\beta$ ) can trigger T-helper cell type 17 (Th17)–polarized inflammatory responses in



**Figure 1.** Background of chronic obstructive pulmonary disease (COPD) and the gut–lung axis in COPD. COPD, including emphysema and bronchitis, causes shortness of breath, coughing, and sputum production and is triggered mainly by cigarette smoking. COPD is associated with gastrointestinal symptoms. External (e.g., cigarette smoking) and internal (e.g., proinflammatory and oxidative stress mediators) danger signals can induce gastrointestinal dysfunction, including IBD, and other gastrointestinal diseases, such as colorectal cancer, gastroesophageal reflux disease, periodontitis, and gallbladder disease. IBD = inflammatory bowel disease. Created with BioRender.com (39).

the intestine (13). A prospective case–control study showed that circulating concentrations of IL-6 and C-reactive protein (CRP) before diagnosis were associated with the risk of CD and UC (40). In addition, raised plasma concentrations of CRP and IL-6 are present in patients with COPD (41). TNF- $\alpha$  is believed to play a central role in the pathophysiology of COPD and IBD (13, 42). TNF- $\alpha$  inhibitors, such as infliximab, adalimumab, and etanercept, are currently in use to achieve remission, to avoid disease progression, and to minimize surgical resections in patients with IBD (43–45). Elevated concentrations of TNF- $\alpha$  are observed in peripheral blood, bronchial biopsy samples, induced sputum, and bronchoalveolar lavage fluid (BALF) of patients with COPD (42). Unfortunately,

contradictory results have been observed with anti-TNF therapy in COPD (46, 47).

Ahn and colleagues hypothesized that the spillover of exaggerated inflammation in COPD could lead to systemic consequences in other organs, such as carcinogenesis in the intestines (27). There is an intimate relationship between malignant cells and their inflammatory microenvironment (48). Cigarette smoke–induced chronic inflammation is associated with various proinflammatory cytokines, such as TNF- $\alpha$ , IL-1, and IL-6, and chemokines, such as CXCL8 (C-X-C motif chemokine ligand 8), which are capable of promoting tumor growth, tumor adhesion, and invasion (49). Cigarette smoke ingredients, such as nicotine, can directly activate the nicotinic acetylcholine receptors (nAChRs) on cancer

cells and induce the release of growth factors, such as VEGF (vascular endothelial growth factor) and IL-1 $\beta$ , into the tumor microenvironment, which can increase tumor angiogenesis and therefore promote tumor growth (49). These activated inflammatory mediators, like TNF- $\alpha$  and IL-1, are also observed in peptic ulceration induced by increased numbers of *Helicobacter pylori* bacteria, which may enhance COPD progression (50).

Vigliano and colleagues suggested that systemic inflammation, including TNF- $\alpha$ , also seems to play a role in the development of nonalcoholic fatty liver disease in patients with COPD (51). Moreover, IL-8 serum concentrations are significantly increased in patients with chronic liver disease (52) as well as in patients with COPD and smokers

**Table 1.** Clinical Evidence for Gut–Lung Axis in Chronic Obstructive Pulmonary Disease

Target Subjects	Study Design	Effects on Intestine/Mortality	References
Patients with COPD Non-COPD control subjects	Population of Québec, 2001–2006	The incidence of CD and ulcerative colitis was 55% and 30% higher in patients with COPD than in the general population, respectively The highest incidence rate for COPD cohort was at ages 50–59 yr	7
COPD Asthma–COPD Non-COPD control subjects	Health databases in Québec, age ≥ 65 yr, without a private drug plan as control Residents received one or more prescriptions for a specific respiratory medication from 1990 to 2007, age ≥ 41 Newly developed IBD in patients with COPD/asthma–COPD	In patients with asthma–COPD, IBD increased the risk of mortality from respiratory conditions In patients with COPD, IBD increased the risk of death of digestive conditions	17
Patients with COPD Non-COPD control subjects	National Health Insurance Database of Korea Patients received prescriptions for COPD medications at least twice in 2009	The prevalence of GERD in patients with COPD was 28% GERD increased the risk of hospitalization in patients with COPD	30
Patients with COPD Non-COPD control subjects	Smokers, former smokers, and nonsmokers of the Korean population in 2002; ages 40–79 yr	Higher incidence of colorectal cancer in patients with COPD regardless of smoking status	27
Patients with COPD Non-COPD control subjects	Database from the Ewha Womans University School of Medicine, Mokdong Hospital (Seoul, Republic of Korea), January 2003 to April 2012 Subjects ≥40 yr of age who underwent postbronchodilator spirometry Age and sex matched between groups	The prevalence of colorectal adenomatous polyps was 39% (98 of 251) in the non-COPD group and 66% (54 of 82) in the COPD group Among 54 patients with adenomatous polyps in the COPD group, 47 had tubular adenoma, and 7 had villous adenoma	26
Patients with COPD Non-COPD control subjects	Meta-analysis from PubMed and Embase	Patients with COPD had worse periodontal health status, including deeper periodontal pockets, high degrees of clinical attachment loss, worse oral hygiene, more inflammation and bleeding in the gingival tissue, and a lower number of remaining teeth	55
Patients with chronic diseases	No diagnosis of respiratory diseases; ≥40 yr of age	High COPD prevalence in patients with liver disease • Significantly high odds ratio (2.10 [95% CI, 1.23–3.57]; <i>P</i> = 0.006)	28
Patients with COPD Healthy control subjects	Moderate COPD (mean FEV <sub>1</sub> , 55 ± 3% predicted)	Functional alterations in the gastrointestinal tract • Intestinal permeability ↑	9
Patients with CD Healthy control subjects	Current smokers, former smokers, and never-smokers with CD Age and sex matched between groups	Current and former smokers with CD had higher systemic hypoxia and colonic remodeling than nonsmoker patients with CD • Systemic VEGF ↑ • Mucosal vasculature ↑	14
Patients with COPD Healthy controls	Patients with COPD with GOLD stages 1 and 2 severity Patients with COPD with GOLD stages 3 and 4 severity	Altered microbial diversity and composition • <i>Prevotella</i> ↑ Lower concentrations of short-chain fatty acids	72

(Continued)

Table 1. (Continued)

Target Subjects	Study Design	Effects on Intestine/Mortality	References
Patients with COPD Healthy controls	Patients with COPD with FEV <sub>1</sub> < 80% predicted and FEV <sub>1</sub> :FVC ratio < 0.7; age > 40 yr with history of smoking Healthy control subjects with FEV <sub>1</sub> > 80% predicted and FEV <sub>1</sub> :FVC ratio > 0.7; age > 40 yr without history of cardiac or respiratory disease and with normal lung function	Fecal microbiome and metabolome of patients with COPD were distinct from those of healthy individuals <ul style="list-style-type: none"> <li>• <i>S. sp000187445</i>, <i>S. vestibularis</i>, and multiple members of the family Lachnospiraceae correlated with reduced lung function.</li> <li>• Disease-associated network connecting <i>S. parasanguinis_B</i> with COPD-associated metabolites, including <i>N</i>-acetylglutamate and its analogue <i>N</i>-carbamylglutamate</li> </ul>	74

Definition of abbreviations: CD = Crohn's disease; CI = confidence interval; COPD = chronic obstructive pulmonary disease; GERD = gastroesophageal reflux disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease; IBD = inflammatory bowel disease; *S.* = *Streptococcus*; VEGF = vascular endothelial growth factor.

(53). IL-8 upregulation in bronchial biopsy samples from patients with COPD is associated with severe exacerbations and increased neutrophil recruitment (54).

Currently, there are no proper plasma or serum biomarkers to investigate the systemic effects in COPD-induced GERD (50).

Dysfunctional immune cells and inflammatory hyperreactivity in patients with COPD may prevent the host from effectively killing bacteria in the periodontium and thereby increase periodontal inflammation (55). In addition, COPD and periodontitis share common risk factors, including cigarette smoking (33). Zhou and colleagues recently found that periodontal treatment in patients with COPD improved lung function and decreased the frequency of COPD exacerbations (56). We assume that a disruption in the composition of the oral microbiota after periodontal treatment may play a role in the remission of COPD exacerbations (56, 57).

It can be hypothesized that excessive proinflammatory mediators produced in the lungs of patients with COPD affect the intestine through the systemic circulation, contributing to intestinal disease. Correspondingly, continuous intestinal inflammation can also exacerbate lung diseases by the presence of inflammatory mediators in the systemic circulation. During inflammation, the gut-associated lymphoid tissue regulates lymphocyte trafficking from intestinal tissue through the systemic circulation, which reflects the

bronchus-associated lymphoid tissue, and these lymphocytes from the intestine and the lungs migrate to other mucosal sites as part of their shared mucosal immune system (13). Moreover, inflammatory mediators, such as circulating TNF- $\alpha$ , have been strongly implicated in comorbidities associated with COPD (58) and have been implicated in the pathogenesis of both COPD and IBD (16).

In addition, the concentrations of acrolein and other aldehydes, harmful volatile constituents of cigarette smoke, in saliva and exhaled breath condensate of heavy smokers are about 10-fold higher compared with those in healthy persons (59, 60). Therefore, soluble cigarette smoke constituents may directly reach the gastrointestinal tract after swallowing or via the systemic circulation, which can cause gastrointestinal mucosal injury and a delay in ulcer repair (61). Many cytotoxic substances of tobacco smoke, such as nicotine, enter the bloodstream and affect the intestine. Lindell and colleagues showed that the nicotine concentration in gastric fluid was >50 times higher than in plasma on smoking days (62).

**Epithelial Barrier Dysfunction, Oxidative Stress, and Hypoxia**

The integrity of the epithelial barrier is essential for maintaining respiratory and intestinal mucosal health. Cigarette smoke impairs the airway epithelial barrier function and induces increased permeability, which was more common in smokers with COPD compared with smokers with normal lung function (12, 63). Further downregulation of apical junctional complex gene expression in

airway epithelium was observed in smokers with COPD compared with healthy smokers (63). Cigarette smoke can promote the production of endogenous reactive oxygen species (ROS) by airway epithelial cells and pulmonary immune cells (12). The ongoing production of ROS during cigarette smoke exposure and exacerbations will result in a substantial reduction in the general antioxidant capacity in patients with COPD (64), which may affect intestinal disorders.

The unmet metabolic demand (hypoxia) in patients with COPD during daily activities results in an increase in intestinal permeability and causes enterocyte damage (9). It has been described that chronic cigarette smoking changes microcirculation and significantly diminishes blood flow to the gastrointestinal mucosa, suggesting smoking-associated intestinal hypoxia, which may stimulate the development of inflammatory diseases (14, 20).

Elevated serum concentrations of VEGF, a marker of systemic hypoxia, have been reported in smokers as well as in patients with CD (65, 66). Fricker and colleagues showed the effects of smoking on increasing serum VEGF in patients with CD, which supports the hypothesis that smokers with impaired gas exchange and systemic hypoxia have a higher risk of developing CD (14).

In addition, patients with chronic hepatitis C who smoke may have more hepatic fibrosis, whereas cigarette smoke-induced hypoxia and associated increased VEGF and VEGF-D concentrations may be involved in the

molecular mechanisms of fibrogenesis (67). Furthermore, smokers appear to be at higher risk of becoming infected with *H. pylori*, a major factor in the pathogenesis of peptic ulcer disease. This increased risk may be related to the adverse effects of smoking on antioxidants or the immune system (68).

COPD-related systemic hypoxemia also negatively affects the status of patients with esophageal cancer by accelerating inflammation, undernutrition, and angiogenesis (69). Hypoxia stabilizes HIF-1 $\alpha$  (hypoxia-inducible factor-1 $\alpha$ ), leading to the expression of genes that are involved in tumor vascularization, metastasis and migration, cell survival, and chemoresistance, as observed in colorectal cancer (70).

Overall, we hypothesize that the toxic effects of cigarette smoke, inhaled ROS, and/or systemic hypoxia may extend systemically to the intestinal epithelium, resulting in intestinal epithelial dysfunction.

### Changes in Microbiota Composition

Gut microbiota dysbiosis has been observed in patients with COPD compared with healthy individuals (71). A clinical study with stool samples from a cohort of 73 healthy humans and 99 patients with COPD showed that patients with COPD had a relatively lower proportion of Bacteroidetes and a higher proportion of Firmicutes compared with healthy control subjects. At the family level, there was a difference in the relative abundances for Fusobacteriaceae, Prevotellaceae, and Bacteroidaceae (72). *Prevotella* were also enriched in the stool of patients with COPD. Fecal matter from these patients with COPD and healthy control subjects was homogenized for fecal microbiota transplantation in male C57BL/6 recipients and in C57BL/6 mice exposed to smoke from biomass fuel to induce COPD-like changes. Interestingly, mice receiving fecal microbiota from patients with stages 3 and 4 COPD showed reductions in body weight, higher pulmonary inflammation, and increases in IL-1 $\beta$  and TNF- $\alpha$  concentrations in plasma compared with control mice. In addition, a higher number of T lymphocytes and a lower number of B lymphocytes in blood were observed in mice that received fecal microbiota from patients with COPD. Moreover, accelerated declines in lung function and emphysematous changes were observed in the mouse recipients of fecal microbiota

from patients with COPD during biomass fuel smoke exposure. In conclusion, the altered gut microbiota of patients with COPD accelerated the development of COPD in mice (72).

Recently, a large multicenter study showed that the severity of COPD is associated with a decreased abundance of *Prevotella* and an increased abundance of *Moraxella* in the lung microbiome in concert with downregulation of genes promoting epithelial defense and upregulation of proinflammatory pathway responses (73). In addition, the abundance of *Streptococcus* in the airways was higher in patients with mild to moderate COPD than in healthy individuals (73).

The increased abundance of several *Streptococcus* species, including *S. parasanguinis\_B* and *S. salivarius*, was also found in the intestine in patients with COPD (74). However, there were no differences between the gut microbiome composition of nonsmokers with COPD and smokers with COPD, supporting that this is a disease-associated phenotype rather than a phenotype driven by the influence of cigarette smoking on the gut microbiome (74).

Translocation of gut microbiota may play a role in the gut–lung cross-talk in patients with COPD. Dysbiosis of the intestinal microbiota has been shown to disturb the composition of the respiratory microbiota through changes in circulating inflammatory cytokines and via translocation of intestinal microbiota to the airways (16). Translocation of gut microbiota to the airways might be related to the leaky gut induced by cigarette smoke, although this has not yet been confirmed in patients with COPD (16). Pathogens frequently colonize the airways, which is associated with exacerbations in patients with COPD, promoting COPD development by amplifying lung and systemic inflammation. We speculate that the disruption of the lung epithelial barrier function may lead to the translocation of pathogenic bacteria. Translocated pathogens and pathogen-associated virulence factors may also enter the gastrointestinal tract, causing intestinal inflammation and related diseases.

In addition, the coordination of ventilation and swallowing may be subverted in patients with COPD (75), possibly resulting in the simultaneous microaspiration of the pharyngeal or oral

microbiota into the respiratory and gastrointestinal tract. Another possible speculation is that the same embryonic origin and the structural similarities of gastrointestinal and respiratory tract may lead to similar responses and changes of the microbiota after systemic inflammation and immune responses caused by COPD (6).

### Changes in Microbial Metabolites

Components of the lung microbiome and metabolome have potential collaborative roles in COPD pathogenesis (76). Accumulating evidence indicates that short-chain fatty acids (SCFAs), derived from intestinal microbial fermentation of indigestible foods, are the most prominent immunomodulatory metabolites and well-studied bacterial metabolites in the gastrointestinal tract that exert their functions along the gut–lung axis (77). The effects of microbiota-derived SCFAs are not limited to the intestinal compartment, and SCFAs disseminate from the gut into the bloodstream, reaching the bone marrow to promote bone marrow hematopoiesis, leading to the resolution of airway inflammation and maintenance of healthy homeostasis in the lung (78). Moreover, as SCFAs produced in the gut can enter the circulation, these metabolites may have direct effects on the lungs or may even be produced by the lung microbiota (79). Patients with COPD have lower concentrations of fecal SCFAs compared with healthy individuals, and patients' COPD severity correlated with reductions in SCFA concentrations, including acetic acid, isobutyric acid, and isovaleric acid (72), which might be a subsequent event of a disrupted gut microbiota. Increasing SCFA concentrations and the abundance of beneficial gut microbiota by dietary fiber intake might be one of the solutions to mitigate COPD and its related comorbidities.

### Limitations

Although there is some clinical evidence for a connection between the lungs and the gut in COPD, additional clinical information regarding the severity of disease, controls, active smoking/passive smoking, and smoking rate and duration would improve the quality of trials in the future.

## Animal Studies: Gut–Lung Axis in COPD Models

### Immunological Changes and Systemic Inflammation

Besides the clinical evidence showing an interaction between the gut and the lungs in COPD, numerous *in vivo* studies have been undertaken to unravel the underlying mechanisms of this bidirectional connection.

Female Balb/c mice exposed to mainstream cigarette smoke for 72 days demonstrated gastrointestinal changes, including changes in intestinal histomorphology and immune network for IgA production (80). Another study showed that female C57BL/6J mice exposed to cigarette smoke for 2 months and treated with dextran sodium sulfate (DSS) exhibit exaggerated intestinal inflammation, with elevated Th17 cells, type 3 innate lymphoid cells, and neutrophil responses in the intestine. This might be due to the increased systemic susceptibility to inflammation by a higher number of Th17 cells and neutrophils in the circulation after cigarette smoke exposure (81). Moreover, there is an accumulation in the number of CD11b<sup>+</sup> (cluster of differentiation 11b) dendritic cells (DCs) in the intestinal Peyer's patches of male C57BL/6 mice after 24 weeks of cigarette smoke exposure (82). Interestingly, lung DCs, like CD103<sup>+</sup> mesenteric lymph node DCs, can stimulate the translocation of T cells into the gastrointestinal tract, probably via upregulating the gut-homing integrin  $\alpha_4\beta_7$  *in vivo* (83). In contrast, cigarette smoke seems to play a protective role in UC, as smoke exposure for 4 weeks reversed the CD4<sup>+</sup>/CD8<sup>+</sup> ratio in blood and colon and increased B cells in the colon of C57BL/6 mice with DSS-induced colitis (84). It has been described that cigarette smoke exposure for 27 days can reduce DSS-induced colitis severity in male C57BL/6 mice and modulates DSS-induced dysbiosis of specific bacterial genera, such as *Akkermansia*, *Intestinimonas*, and *Lactobacillus*, which may contribute to resolving intestinal inflammation or accelerate recovery. In addition, the activation of the cholinergic antiinflammatory pathway by nicotine might be one of the explanations for the protective role of cigarette smoke in UC (85). Qin and colleagues indicated that nicotine (subcutaneous injection of nicotine for 7 consecutive days) exerts its protective

action in murine DSS colitis by upregulating microRNA-124 expression and inhibiting STAT3 (signal transducer and activator of transcription 3) activation (86).

Possibly, these contradictory observations regarding the effect of cigarette smoke exposure on murine colitis might be related to the different procedure/duration of cigarette smoke exposure and DSS treatment.

Moreover, the controversial effects of nicotine appear to be site specific, as nicotine treatment through drinking water for 2 weeks exacerbated jejunum damage but reduced colonic inflammation in male C57BL/10 IL-10<sup>-/-</sup> mice (87).

Elevated systemic mediators, such as chemokines/cytokines, might play a role in the lung–gut cross-talk in COPD. Verschuere and colleagues showed that the upregulation of CCL9 (C-C motif chemokine ligand 9) and CCL20 induced by cigarette smoke exposure may play a role in the increased epithelial apoptosis in the Peyer's patches and the accumulation of CD11b<sup>+</sup> DCs and CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the Peyer's patches in the ileum (82). These results suggest that cigarette smoking causes alterations in the epithelium lining of the Peyer's patches, leading to changes in the composition of the underlying immune cell population, possibly via the CCL9–CCR1 (C-C motif chemokine receptor 1) and CCL20–CCR6 pathways.

### Epithelial Barrier Dysfunction, Oxidative Stress, and Hypoxia

Animal studies have demonstrated that cigarette smoke–induced intestinal barrier dysfunction and subsequent inflammation of the intestinal mucosa may be one of the mechanisms of intestinal damage in COPD (88, 89). Dysfunctional and structural changes in the intestinal mucosal barrier, including neutrophil infiltration, epithelial shedding, reduced tight junction protein expression (occludin and ZO-1 [zonula occludens 1]), and increased intestinal expression concentrations of TNF- $\alpha$ , IFN- $\gamma$ , and IL-8, were observed in Sprague-Dawley rats with emphysema after 6 months of side-stream cigarette smoke exposure (88). Male C57BL/6 mice exhibited elevated intestinal inflammation and reduced intestinal Paneth cell integrity after intragastric administration of cigarette smoke condensate (three times per week for 2 wk), resulting in reduced antimicrobial peptide production and bactericidal capacity (90). Mainstream cigarette smoke exposure for 24 weeks

induced intestinal inflammation in male C57BL/6 mice, which was related to altered epithelial mucus profiles, including increased mRNA expression of Muc2 (mucin 2, oligomeric mucus/gel-forming) and Muc3 in the ileum and Muc4 in the distal colon (91).

Impaired gas exchange (marked reduction in diffusion factor for carbon monoxide) induced by 8 weeks of mainstream cigarette smoke exposure caused systemic hypoxia, as measured by serum VEGF and intestinal hypoxia throughout the mucosal layer in female C57BL/6 mice, which drives angiogenesis and intestinal epithelial barrier dysfunction, resulting in increased risk and severity of CD (14). Systemic and local ischemia might be caused by oxygen-free radicals present in inhaled smoke, as described in the previous part (GASTROINTESTINAL TRACT DISORDERS ASSOCIATED WITH COPD). In addition, 14 weeks of whole-body cigarette smoke exposure elevated HIF-1 $\alpha$  expression in the male Wistar rat small intestine, which was associated with increased oxidative stress and apoptosis, leading to the destruction of intestinal tight junctions (92).

Increased ROS generation and HIF-1 $\alpha$  concentrations are also related to the development of colorectal cancer (93). Recently, a stress-activated mitochondrial protease (OMA1 [OMA1 zinc metallopeptidase]) has been found to promote colorectal cancer development by increasing ROS generation and stabilizing HIF-1 $\alpha$  under hypoxia. OMA1 knockout suppresses colorectal cancer development in murine azoxymethane (AOM)/DSS and xenograft models of colorectal cancer (94).

Moreover, exposure to cigarette smoke for 2 months caused decreased colonic diameter and an increased frequency of colonic contractions in male Balb/c mice; it is possible that nicotine contributed to these changes in gut function by acting on enteric neurons expressing nAChR to contract intestinal smooth muscle (95).

Antioxidants might have therapeutical potentials in gastrointestinal dysfunction in patients with COPD. The antioxidant ebselen prevents the reduction in colonic diameter in mice exposed to cigarette smoke for 2 months (95).

### Changes in Microbiota Composition

Papoutsopoulou and colleagues reviewed the deleterious influence of smoking on the human gut microbiota (96), but information is scarce in animal species. Cigarette smoke



exposure for 4 weeks reduces the relative abundance of Bacteroidales but increases the presence of Firmicutes in C57BL/6 mouse fecal microbiota, while a high-fat diet exacerbates the cigarette smoke–induced reduction in gut microbiota diversity (97). Interestingly, fecal microbiota from control, antibiotic-treated, ampicillin-treated, or vancomycin-treated mice reversed emphysema features of cigarette smoke–recipient C57BL/6 mice, in which the commensal bacterium *Parabacteroides goldsteinii* might play a key role. The LPS derived from *P. goldsteinii* exhibits antiinflammatory properties, which can ameliorate experimental emphysema by acting as an antagonist of the TLR4 (Toll-like receptor 4) signaling pathway (98).

An increased abundance of gut Lachnospiraceae species was found in male C57BL/6 mice exposed to cigarette smoke for 24 weeks, which might be one of the contributing factors leading to aberrant inflammation and reduction of cellular physiological activities in the colon (91, 98). Consistently, an increased abundance of Lachnospiraceae species was also reported in other chronic inflammatory diseases, such as IBD, irritable bowel syndrome with diarrhea, and obesity (74, 98). Fecal microbiota transplantation from healthy control mice or mice receiving a high-fiber diet attenuated emphysema development in C57BL/6 mice via local and systemic inhibition of inflammation and changes in gut microbiota composition, including the increased amounts of Bacteroidaceae and Lachnospiraceae (99).

Intraoral cigarette smoke exposure for 3 months reduced the abundance of potentially beneficial bacteria (i.e., *Clostridium*, *Turicibacter*) and increased the abundance of potentially harmful bacteria (i.e., *Desulfovibrio*, *Bilophila*) in rats (100). *Clostridium* and *Turicibacter* are important commensal bacteria, which have the ability to regulate the gut barrier and inflammatory/immune responses related to intestinal diseases, such as IBD (100–102).

The genera *Desulfovibrio* and *Bilophila* likely participate in the development of colorectal cancer by producing hydrogen sulfide and promoting chronic inflammation (103). Cigarette smoke exposure for 28 weeks can promote colon tumorigenesis by inducing gut microbiota dysbiosis in a carcinogen AOM-induced mouse model of colorectal cancer. This gut microbiota dysbiosis was characterized by enrichment

of *Eggerthella lenta* and depletion of *Parabacteroides distasonis* and *Lactobacillus* species and increased taurodeoxycholic acid, a bile acid metabolite, in the colon, resulting in gut barrier dysfunction and activation of oncogenic and proinflammatory signaling pathways. Moreover, transplanting stool from smoke-exposed mice into germ-free mice leads to enrichments in *E. lenta* and taurodeoxycholic acid, impaired colonic epithelium, and increased activation of proinflammatory pathways in recipient mice (104). Manipulation of the gut microbiota might represent a promising prophylactic strategy against intestinal disorders in patients with COPD.

### Changes in Microbial Metabolites

Cigarette smoke exposure for 4 weeks significantly decreased SCFA concentrations, including acetic acid, propionic acid, butyric acid, and valeric acid, in the cecal content of Wistar rats (105), whereas 3 months of smoking exposure reduced propionate as well as amino acid and carbohydrate metabolism in the cecal and colonic contents of Wistar rats (100).

Gut microbiota dysbiosis induced by cigarette smoke plays a protumorigenic role in colorectal cancer. AOM-treated C57BL/6 mice were exposed to cigarette smoke for 28 weeks, and the fecal metabolic profile in smoke-exposed mice was significantly different from that of control mice. Bile acid biosynthesis was the top enriched pathway in cigarette smoke–exposed mice compared with smoke-free mice. This study showed that smoke-induced gut microbiota dysbiosis altered gut metabolite profiles and impaired gut barrier function, which might activate oncogenic MAPK (mitogen-activated protein kinase)/ERK (extracellular signal-regulated kinase) signaling in colonic epithelium (104).

A whey peptide–based enteral diet inhibited elastase-induced emphysema and lowered the total cell counts in BALF of female C57BL/6 mice, which might be related to the increase in cecal SCFAs (106). Interestingly, a mixture of nondigestible oligosaccharides prevented the emphysemalike changes induced by LPS and decreased the LPS-induced neutrophil influx by >60% in Balb/c mice (107). Discontinuous feeding with a fiber-free diet accelerated emphysematous lesions after 8 weeks of cigarette smoke in spontaneous hypertension male rats, as observed by enlarged and disrupted alveolar walls compared with rats

fed the control diet. Moreover, the cigarette smoke–related decrease in total organic acid and acetic acid concentrations was also accelerated by discontinuous feeding with a fiber-free diet (108).

These studies confirm that shaping the gut microbiota composition and altering the production of microbial metabolites with specific dietary supplementation might have the potential for the treatment or prevention of COPD and its (intestinal) comorbidities.

### Limitations of Animal Models

Issues that are difficult or impossible to study in humans for ethical, safety, or logistical reasons can be addressed by using *in vivo* models. A major limitation of cigarette smoke exposure models is that these models exhibit relatively mild pathological changes of COPD, and none of the models shows the progressive disease observed in humans with Global Initiative for Chronic Obstructive Lung Disease stage 3 or 4 COPD (109). In addition, because of the differences in lung anatomy and physiology between mice and humans, it is difficult to capture both chronic bronchitis–related and emphysematous changes in a single model. An ideal animal model of COPD would reproduce a combination of the principal features of the human disease, namely, chronic bronchitis, mucus hypersecretion, small airway remodeling, emphysema, impaired lung function, and systemic comorbidities. Unfortunately, all of the described animal models meet only some of these criteria, and in the majority of studies described in this review, animal models of cigarette smoke–induced emphysema were used (110). Therefore, we must be careful with the translation of preclinical research to clinical practice.

### Lessons Learned from In Vitro Studies

Although it is difficult to integrate several organs (gut and lungs) in an *in vitro* system to mimic the physiological function of the human body, several *in vitro* studies have contributed to the knowledge of the underlying mechanisms of intestinal complications in COPD.

Human colorectal cancer (Caco-2) cells exposed to cigarette smoke extract for 3 hours showed reductions in cell necrosis, claudin-1, and E-cadherin expression and

**Table 2.** Preclinical Evidence for Gut–Lung Axis Using *In Vivo* and *In Vitro* Studies

<i>In Vivo</i> Studies			
Target Subjects	Experimental Design	Effects on Intestine/Intestinal Cells	References
Male Sprague-Dawley rats 8 wk old	Air/CS exposure 2 h/d 5 d/wk 6 mo	Dysfunctional and structural changes in the intestinal mucosal barrier <ul style="list-style-type: none"> <li>• Occludin and ZO-1 protein ↓</li> <li>• Epithelial shedding</li> </ul> Intestinal inflammation <ul style="list-style-type: none"> <li>• TNF-<math>\alpha</math>, IFN-<math>\gamma</math>, and IL-8 ↑</li> <li>• Neutrophil infiltration</li> </ul>	88
Male C57BL/6 mice 7–8 wk old	Intragastric CS condensate or PBS 3 times/wk 2 wk	Intestinal inflammation <ul style="list-style-type: none"> <li>• Intestinal Paneth cell integrity ↓</li> <li>• Antimicrobial peptide production ↓</li> <li>• Bactericidal capacity ↓</li> </ul>	90
Male C57BL/6 mice 8–9 wk old	Air/CS exposure 5 cigarettes/round 4 times/d 5 d/wk 24 wk	Intestinal inflammation <ul style="list-style-type: none"> <li>• <i>Cxcl2</i> ↑ and <i>IFN-<math>\gamma</math></i> ↓ in ileum</li> <li>• <i>Il-6</i> ↑ and <i>Tgf-<math>\beta</math></i> ↓ in the proximal colon</li> <li>• <i>Muc2</i> and <i>Muc3</i> in ileum ↑</li> <li>• <i>Muc4</i> in distal colon ↑</li> </ul> Altered bacterial activity and community structure <ul style="list-style-type: none"> <li>• Activity of Lachnospiraceae species ↑</li> </ul>	91
Female C57BL/6 mice	Air/CS exposure 12 cigarettes/round Twice/day 5 times/wk 12 wk	Systemic and intestinal hypoxia Epithelial barrier dysfunction	14
Male Wistar rats 6 wk old	Air/CS exposure 5 cigarettes Twice/day 7 d/wk 14 wk	Hypoxia and oxidative stress <ul style="list-style-type: none"> <li>• HIF-1<math>\alpha</math> expression in small intestine ↑</li> </ul> Increased intestinal permeability <ul style="list-style-type: none"> <li>• Dysregulation of TJ components</li> </ul> Apoptosis	92
Female C57BL/6J mice 6–8 wk of age	Air/CS exposure 3 cigarettes/d 5 d/wk 2 mo Plus DSS challenge	Enhanced intestinal pathological innate and adaptive immune responses <ul style="list-style-type: none"> <li>• Th17 cells, ILC3 cells, and neutrophils in intestine ↑</li> </ul>	81
Female Balb/c mice 11–13 wk old	Air/CS exposure 14 cigarettes/d Once/day 7 d/wk 72 d	Changes in proximal intestinal histomorphology Changes in intestinal immune network for IgA production in distal small intestine Increased fecal sIgA concentrations and enlargement of Peyer's patches	80
Male C57BL/6 mice 8–9 wk old	Air/CS exposure 5 cigarettes/round 4 times/d 5 d/wk 24 wk	Epithelial apoptosis Intestinal inflammation <ul style="list-style-type: none"> <li>• CD11b<sup>+</sup> DCs in small intestine ↑</li> <li>• CD4<sup>+</sup> and CD8<sup>+</sup> T cells in small intestine ↑</li> <li>• mRNA expression of CCL9 and CCL20 ↑</li> </ul>	82
Male C57BL/6 mice 6 wk old	Air/CS exposure 15-min CS exposure Twice/day 4 wk Plus normal-food diet/high-fat diet	CS exposure increased the accumulation of total cholesterol in liver Insulin resistance in the liver of mice with high-fat diet CS exposure altered the microbiota composition <ul style="list-style-type: none"> <li>• Bacteroidales ↓</li> <li>• Firmicutes ↑</li> </ul>	97
Female C57BL/6 mice 8–10 wk old	Air/CS exposure 12 cigarettes Twice/day 5 d/wk 12 wk	Altered microbiota composition <ul style="list-style-type: none"> <li>• Lachnospiraceae ↑</li> <li>• Erysipelotrichaceae ↓</li> <li>• Bacteroidales ↓</li> <li>• Ruminococcaceae ↓</li> </ul>	98

(Continued)

Table 2. (Continued)

In Vivo Studies			
Target Subjects	Experimental Design	Effects on Intestine/Intestinal Cells	References
	Plus fecal microbiota transplantation (~1 × 10 <sup>8</sup> cfu in 100 µl PBS) Oral gavage 12 wk	Fecal microbiota transplantation restored COPD pathogenesis <ul style="list-style-type: none"> <li>• Reduction of intestinal inflammation and enhancement of cellular mitochondrial and ribosomal activities in colon</li> <li>• Systematic restoration of aberrant host amino acids metabolism in sera</li> <li>• Inhibition of lung inflammation</li> </ul>	
Female and male Wistar rats 5 wk old	Air/CS intraoral exposure 20-min exposure/round Twice/day 3 mo	Altered microbiota composition <ul style="list-style-type: none"> <li>• Potentially beneficial genera (<i>Clostridium</i> and <i>Turicibacter</i>) ↓</li> <li>• Potential harmful genera (<i>Desulfovibrio</i> and <i>Bilophila</i>) ↑</li> </ul> Active smoking might impair amino acid, lipid, and propanoate metabolism function	100
Male C57BL6/cmdb mice 8 wk old	Colitis induction by DSS Plus air/CS exposure 20 cigarettes/round Once/day 5 d/wk 4 wk	Decreased severity of DSS-induced colitis <ul style="list-style-type: none"> <li>• Changes in CD4:CD8 ratio and B-cell number in the peripheral blood and colon</li> </ul>	84
Male C57Bl/6 mice 8–10 wk old	Air/CS exposure for 27 d Plus colitis induction by DSS	CS attenuated DSS-induced dysbiosis of specific bacterial genera CS resolved the inflammation and accelerated recovery	85
Male specific pathogen-free C57BL/6 mice 8–10 wk old	Air/smoke (produced by smoldering China fir sawdust) 40 g/exposure, 3-h periods Twice/day 5 d/wk 20 wk Plus fecal transplantation from patients with COPD Single oral administration of 100 µl per mouse twice/week 20 wk	Accelerated declines in lung function Severe emphysematous changes Airway remodeling Mucus hypersecretion	72

In Vitro Studies			
Cell Line	Exposures	Effects	References
Caco-2 cells	CSE (5% and 10%) Different time points (3 and 24 h)	Cell necrosis ↓ Claudin-1 and E-cadherin expression ↓ Cell permeability ↑	111
HT-29 cells	TNF-α (50 ng/ml) exposure plus nicotine (10 <sup>-11</sup> –10 <sup>-6</sup> M)	TNF-α-induced IL-8 release ↓	119

*Definition of abbreviations:* CCL = C-C motif chemokine ligand; CD = cluster of differentiation; COPD = chronic obstructive pulmonary disease; CS = cigarette smoke; CSE = cigarette smoke extract; *Cxcl2* = C-X-C motif chemokine ligand 2; DC = dendritic cell; DSS = dextran sodium sulfate; HIF-1α = hypoxia-inducible factor-1α; ILC3 = type 3 innate lymphoid; Muc2 = mucin 2, oligomeric mucus/gel-forming; PBS = phosphate-buffered saline; sIgA = secretory IgA; *Tgf-β* = transforming growth factor-β; Th17 = T-helper cell type 17; TJ = tight junction; TNF-α = tumor necrosis factor-α; ZO-1 = zonula occludens 1.

increases in microRNA-21 (a diagnostic marker for colorectal carcinoma), cytoskeleton rearrangement, cell motility, and invasiveness, which may contribute to colorectal cancer progression (111). Moreover, cigarette smoke condensate reduced cell viability and upregulated total ROS, mitochondrial ROS, and superoxide radicals in HCT-15 and HT-29 colon cells within 48 hours (112). Increased oxidative stress also contributes to reduced gene and protein expression of tight and adherens junctions in human bronchial epithelial cells, which may increase susceptibility to inhaled pathogens and environmental stressors (12, 113). The presence of exogenous ROS in cigarette smoke can induce the fragmentation of hyaluronic acid in human tracheobronchial epithelial cells, leading to impaired airway barrier integrity by binding to the

**Table 3.** The Effects of Intestinal Metabolites and Dietary Fiber in Chronic Obstructive Pulmonary Disease

Subjects	Treatment/Diet	Effect in the Lung	References
Patients with COPD 1,557 cases from the Swedish National Patient Register during follow-up (2002–2014)	Dietary fiber intake was assessed in 1987 and 1997 using a food frequency questionnaire	Long-term high dietary fiber intake ( $\geq 26.5$ vs. $< 17.6$ g/d) was associated with a 30% lower risk of COPD Current and ex-smokers with low long-term total fiber intake had a 33-fold or 10-fold higher risk of COPD compared with never-smokers	131
63 patients with COPD of GOLD stages 1 and 2 severity 30 patients with COPD of GOLD stages 3 and 4 severity 60 healthy control subjects	—	Total SCFA concentrations were significantly lower in stool samples of the COPD stages 3 and 4 group compared with healthy subjects and those with COPD stages 1 and 2 Acetic acid, isobutyric acid, and isovaleric acid concentrations were significantly lower in the subjects with COPD stages 3 and 4	72
Female C57BL/6 mice 8 wk old	Cigarette smoke exposure 5 d/wk for 4 wk Plus 50 $\mu\text{g}$ (1 $\mu\text{g}/\mu\text{l}$ ) of poly(I:C) via nasal aspiration twice a week at 3 and 4 wk High-fiber diet performed with a 4-wk study period	Mice receiving a high-fiber diet presented less severe emphysema <ul style="list-style-type: none"> <li>• Histological emphysema <math>\downarrow</math></li> <li>• Number of macrophages and lymphocytes in BALF <math>\downarrow</math></li> <li>• IL-6 and IFN-<math>\gamma</math> concentrations in BALF <math>\downarrow</math></li> </ul>	99
Male albino rats of Wistar Kyoto 10 wk old	Cigarette smoke exposure for 4 wk 30 cigarettes for 20 min 5 d/wk	Cecal concentrations of organic acids, such as acetic acid, propionic acid, butyric acid, and valeric acid, significantly decreased in the cigarette smoke-exposed mice	105
Wistar rats 5 wk old	Intraoral smoking exposure for 3 mo 20 min per time in the morning and afternoon	Impaired amino acid, lipid, propanoate metabolism function Microbiota associated with metabolism pathways for some antioxidants and vitamins, such as glutathione, lipoic acid, retinol, taurine, hypotaurine, riboflavin, and cofactors, was enriched in the active smoking group	100
Female C57BL/6 mice 6 wk old	Elastase-induced emphysema	The whey peptide-based enteral diet group exhibited protective effects in the lung <ul style="list-style-type: none"> <li>• Fewer emphysematous changes</li> <li>• Lower total cell counts in BALF</li> <li>• Higher cecal SCFA concentrations</li> </ul>	106
Male Balb/c by Jico mice 6 wk old	LPS-induced emphysema Diet with nondigestible oligosaccharides	Nondigestible oligosaccharides diminished disease progression <ul style="list-style-type: none"> <li>• <math>&gt;60\%</math> decrease in LPS-induced neutrophil influx</li> <li>• Prevented alveolar wall destruction</li> <li>• Attenuated the LPS-induced increase in right ventricular heart hypertrophy</li> </ul>	107

*Definition of abbreviations:* BALF = bronchoalveolar lavage fluid; COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease; poly(I:C) = polyinosinic:polycytidylic acid; SCFA = short-chain fatty acid.

hyaluronan receptor layilin and mediating the RhoA (Ras homolog family member A)/Rho kinase–dependent decrease in E-cadherin expression (114).

Papoutsopoulou and colleagues reviewed the deleterious consequences of smoking on various cell types and physiological processes within the intestinal epithelium, including the direct and indirect effects of cigarette smoking on various parameters, such as oxidative damage, impairment of intestinal barrier, and immune cell function (96).

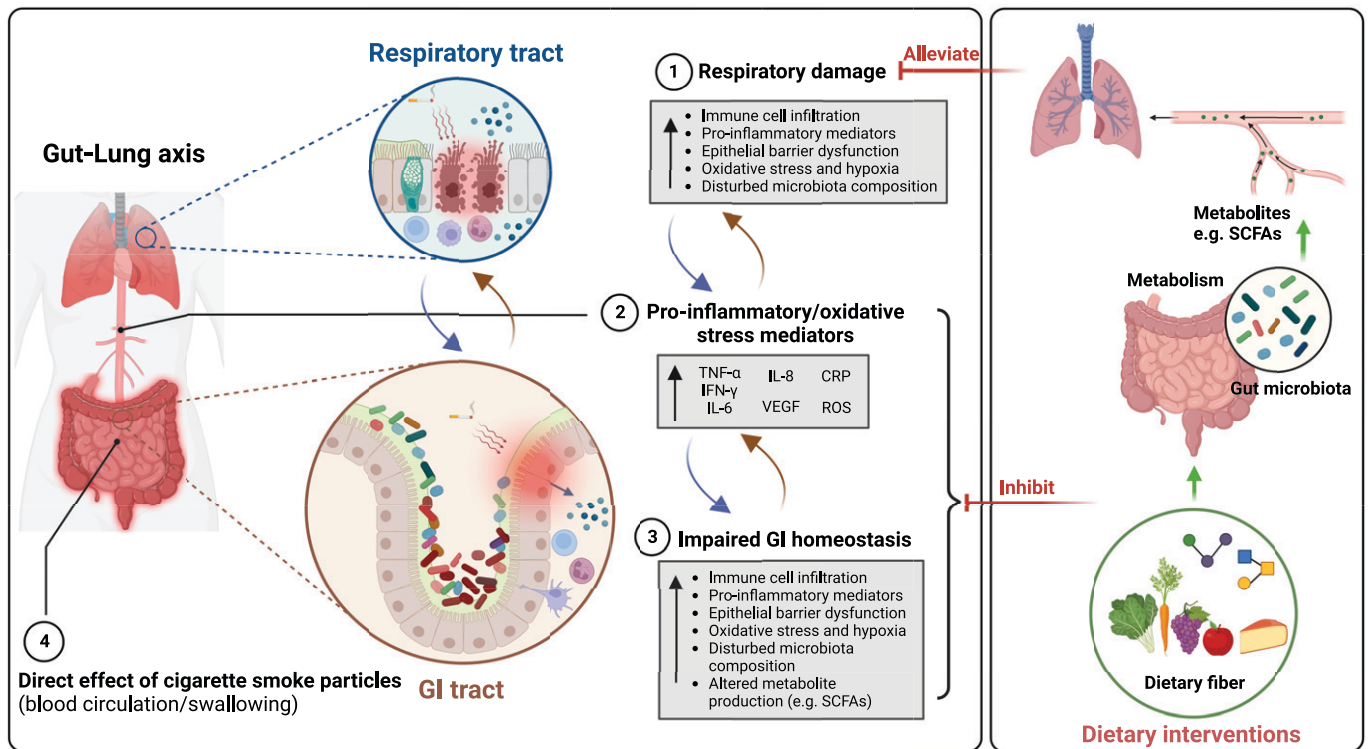
The biology of the oral epithelium is also altered by cigarette smoking, as observed by a cigarette smoke–induced reduction in the number of early apoptotic keratinocytes

and an increase in the number of late apoptotic cells (115).

Among all the components described, nicotine as the major and highly addictive component of cigarette smoke is of great interest in several *in vitro* studies. Nicotine-derived nitrosamine ketone, a highly carcinogenic tobacco-specific nitrosamine, stimulates colon cancer HT-29 cell proliferation through  $\beta$ -adrenoceptors, and the effect was abolished by  $\beta_1$ - or  $\beta_2$ -adrenoceptor antagonists (116). Moreover, nicotine was found to reduce TNF- $\alpha$  release by LPS-stimulated human leukemia (THP-1) cells *in vitro* (117) and to inhibit the *ex vivo* production of IL-2 and TNF- $\alpha$  by human peripheral blood lymphocytes stimulated

with phytohemagglutinin (118). An *in vitro* experiment using HT-29 cells showed that nicotine can inhibit TNF- $\alpha$ –induced IL-8 release, possibly via binding to the  $\alpha_7$  subunit of the nAChR (119). Nicotine may bind to nAChR and prevents NF- $\kappa$ B (nuclear factor- $\kappa$ B) signaling, thereby preventing the release of HMGB1 (high-mobility group box 1) and inhibiting the release of proinflammatory cytokines such as TNF- $\alpha$  (120). The protective effect of nicotine on epithelium may partly explain the protective effect of cigarette smoking on UC, as observed in clinical studies reviewed by Berkowitz and colleagues (20).

*In vitro* and *ex vivo* studies showed that SCFAs have the ability to improve the



**Figure 2.** Proposed connections between the gut and lungs in chronic obstructive pulmonary disease (COPD). COPD is characterized by immune cell infiltration, proinflammatory mediators in the lungs, impaired epithelial barrier function, oxidative stress, and hypoxia in the lungs. (1) Damaged and activated lung cells further stimulate the innate immune response through elevated proinflammatory mediators (e.g., TNF- $\alpha$ , IFN- $\gamma$ , IL-6, IL-8, CRP, ROS) (2). Those proinflammatory mediators (and cigarette smoke particles) can migrate to the gastrointestinal tract, (partly) via the systemic circulation, exacerbating intestinal impairments, including increased inflammatory immune infiltration, epithelial barrier damage, oxidative stress, and hypoxia in the gastrointestinal tract. Moreover, (long-term) cigarette smoking and inflammatory immune cell infiltration in the gut can change the microbiota composition, leading to decreased abundance of health-promoting commensal bacteria (3). In addition, soluble cigarette smoke particles may directly affect the gastrointestinal tract by entering the bloodstream after inhalation, or these particles may reach the gastrointestinal tract due to mucociliary clearance in the airways or direct swallowing of cigarette smoke (4). Impaired gut function not only increases the production and entry of proinflammatory mediators into the systemic circulation but also attenuates nutrient absorption, antioxidant capacity, and protection from pathogens and other environmental stimuli, further exacerbating COPD. Dietary intervention, such as fibers, can manipulate the gut microbiota composition to promote host health, increasing the abundance of health-promoting commensal bacteria, decreasing the permeability of intestinal mucosa, and enhancing the bacterial synthesis of immune-modulating compounds (e.g., SCFAs). This may decrease proinflammatory mediators and alleviate the symptoms in the lung. CRP = C-reactive protein; GI = gastrointestinal; ROS = reactive oxygen species; SCFA = short-chain fatty acid; TNF- $\alpha$  = tumor necrosis factor  $\alpha$ ; VEGF = vascular endothelial growth factor. Created with BioRender.com (39).

regulation of inflammation, carcinogenesis, intestinal barrier function, and oxidative stress (121). SCFAs are known to strengthen gut barrier function (122) and to regulate inflammation and oxidative stress via SCFA signaling mechanisms, such as the promotion of histone acetylation and activation of G protein-coupled receptors (78). The SCFA butyrate enhances the intestinal barrier by facilitating a tight junction in human colonic Caco-2 cells (123, 124) and small intestine porcine IPEC-J2 cells when exposed to inflammatory conditions (125). SCFAs even showed prophylactic and restorative effects on airway epithelial barrier function, which might be related to increased ZO-1 expression (126).

All preclinical (*in vivo* and *in vitro*) evidence for the connection between the lungs and the gut in COPD is summarized in Table 2, and relevant studies regarding the effects of intestinal metabolites and dietary fiber in COPD are summarized in Table 3.

## Conclusions

COPD is a complex disease and is often associated with comorbidities such as intestinal diseases. The concept of the “gut–lung axis” has gained attention with the discovery of the influence of gut health on lung immunity (127). Advances in understanding the potential mechanisms of gut–lung cross-talk have highlighted the bidirectional cross-talk between the gut and the lungs, showing the impact of the lungs on intestinal health and the importance of the intestine in maintaining immune homeostasis. In this translational review, this bidirectional cross-talk between the gut and the lungs in COPD is verified on the basis of clinical, *in vivo*, and *in vitro* studies. Overall, the findings of these studies support one another and demonstrate that COPD and/or cigarette smoking can cause intestinal inflammation, barrier disruption, hypoxia,

and oxidative stress, as well as microbiota dysbiosis and altered microbial metabolite production. The possible connections between the gut and the lungs and postulated mechanisms for how the gut–lung axis affects COPD are described in Figure 2. Systemic inflammation is considered one of the major causes of gut–lung cross-talk in COPD. Excessive inflammatory mediators generated from the lung can affect gastrointestinal health through the systemic and lymphatic systems. Correspondingly, inflammation in the gastrointestinal tract can also potentiate the development of lung diseases. Moreover, impaired gas exchange capacity in the lung and systemic hypoxia in COPD can lead to gastrointestinal epithelial integrity damage. In addition, gut microbiota dysbiosis in COPD can lead to disruption of the intestinal barrier integrity and can activate local and systemic immune responses, thereby aggravating the immunity and pathology in the lung. SCFAs are produced mainly by the gut microbiota through fermentation of dietary fibers and are known to beneficially influence host homeostasis (128). Although the function of intestinal microbiota in modulating local and systemic immune responses has been extensively studied, the impact of the lung microbiota and its products in regulating immunity has just begun, and further research is needed to better understand host immune–lung microbiome interactions.

The current management of COPD is focused mainly on suppressing airway inflammation and decreasing symptoms. The purpose of this translational review is not only to emphasize the interaction between the gut and the lung in COPD but also to highlight the possibility of targeting the intestine to improve clinical outcomes in patients with COPD. There is an urgent need for research into the primary prevention of COPD and deceleration of disease progression. The intestine might be a

promising target, through bidirectional gut and lung cross-talk, to improve lung health and minimize COPD and related comorbidities. An understudied area of COPD management might be nutritional interventions. On the basis of the literature presented in this review, understanding the importance of the gut–lung axis in COPD, it can be suggested that antiinflammatory and/or nutritional intervention strategies targeting the intestine might be used as a new add-on therapy to suppress the development of COPD and its comorbidities.

## Future Perspectives

The existence of the gut–lung axis in COPD has become increasingly clear. The gut microbiome plays an important role in profiling the intestinal and systemic immune system, thereby regulating lung health. Therefore, therapies focusing on gut microbiota manipulation might be considered in patients with COPD (and associated intestinal comorbidities). Dietary fibers, with their prebiotic and immunomodulatory properties, are also capable of increasing SCFA-producing bacteria (129) and exerting antiinflammatory effects in respiratory diseases, such as asthma (101). SCFAs are also potent antiinflammatory and barrier-protective molecules, which may reach the airways via the systemic circulation (130). Moreover, on the basis of a murine study showing that healthy fecal microbiota transplantation attenuates emphysema development via local and systemic inhibition of inflammation and changes in gut microbiota composition (99), fecal microbiota transplantation might represent a new paradigm for the treatment of COPD, and further research in this area is certainly needed. ■

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