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Influence of dietary factors, nutrients, and the gut-lung axis on respiratory health

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Influence of dietary factors, nutrients, and the gut-lung axis on respiratory health

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Editorial: Influence of dietary factors, nutrients, and the gut-lung axis on respiratory health

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Editorial on the Research Topic
Influence of dietary factors, nutrients, and the gut-lung axis on respiratory health

Dietary habits and nutrients are gaining increasing recognition as modifiable factors influencing respiratory health, with the gut microbiota playing a pivotal role as a mediator of these interactions.

The mechanisms linking diet with gut microbiota modulation, which may in turn alter lung inflammation, are still to be unraveled. The interplay between diet, gut microbiota, and respiratory health appears to revolve around the immune system, making immune interactions a central topic of discussion. This Research Topic aims to address the links between diet/nutrition and respiratory health, and the relationship between diet and the gut-lung axis.

Several contributions used NHANES data to explore how dietary intake and nutrition-related indicators affect respiratory outcomes in adults, investigating potential biomarkers for predicting disease progression and even mortality. Wen et al. found no association between protein intake and blood eosinophil counts (BEOC) in asthmatic patients. However, serum albumin levels showed an inverted U-shaped correlation with BEOC, suggesting that the nutritional status of patients may influence immune system changes and asthma regulation. Zhuang et al. found no association between dietary protein intake and mortality, but revealed that higher serum albumin levels were inversely associated with all-cause mortality. Interestingly, each unit increase in serum albumin (g/L) was linked to a 13% reduction in mortality risk. Serum albumin could serve as a reliable predictor of long-term outcomes in asthmatic patients, surpassing the influence of dietary protein intake alone. Likewise, Lu et al. examined the relationship between dietary protein intake and all-cause mortality in COPD patients. While a U-shaped trend was observed, with the lowest mortality risk at 90.3 g/day, no association between protein intake and mortality was found. Although adequate protein intake is critical for muscle maintenance, higher intake did not improve survival in COPD patients. Xu et al. investigated multiple nutrition-related indicators, including the Geriatric Nutritional Risk Index (GNRI), Advanced Lung

Index (ALI), and Prognostic Nutritional Index (PNI), in relation to COPD risk and all-cause mortality. The study found that malnutrition, as indicated by low ALI and GNRI, and high CONUT scores, was linked to increased COPD incidence and mortality. Compared to other nutritional scores, ALI provides more effective predictive value for both risk and all-cause mortality. Jiang et al. found a correlation between higher Dietary Inflammatory Index (DII) scores and an increased risk of sarcopenia. Sarcopenia was linked to higher all-cause mortality in COPD patients, suggesting that dietary inflammation plays a critical role in disease progression and survival outcomes.

A review by Zhang et al. highlighted the gut-lung axis in asthma development, showing how gut microbiota (e.g., *Lactobacillus*) and short-chain fatty acids (SCFAs), modulate lung immunity by reducing inflammation and regulating immune responses. Early-life gut dysbiosis and microbial diversity are linked to asthma risk, while factors like rural living and diet shape microbiota resilience. The review calls for exploring interventions like probiotics, fecal transplants, dietary changes, and microbial engineering as strategies for asthma prevention and management.

Bezemer et al. investigated the effects of a symbiotic combination of *Bifidobacterium breve* and a prebiotic fiber mixture (short-chain galacto-oligosaccharides, long-chain fructo-oligosaccharides, low-viscosity pectin) on a preclinical mouse model for pulmonary neutrophilia. The synbiotic mixture improved lung resistance, pulmonary neutrophil-to-lymphocyte (NLR) ratio, and provided relief from pulmonary neutrophilia. Lipopolysaccharide (LPS) applied to the upper airways negatively impacted fecal SCFA when comparing to mice receiving PBS. Acetic acid production was increased in all unchallenged mice (PBS) which after LPS challenge was observed only in mice receiving the symbiotic mixture. Moderate and weak correlations were found between SCFAs and lung function or NLR. These results highlight the bidirectional gut-lung crosstalk, suggesting synbiotic-induced SCFAs beneficial role in lung health.

Holloman et al., explored the potential of Indole-3-carbinol (I3C), a compound found in cruciferous vegetables and used as a dietary supplement, to alleviate LPS-induced acute respiratory distress syndrome (ARDS) in mice. I3C reduced lung inflammation by decreasing the recruitment of CCR2+ monocytes, CXCR2+ neutrophils, and downregulated chemokines that are critical for immune cell trafficking, thereby reducing neutrophil-mediated tissue damage. The effects of I3C were mediated by aryl hydrocarbon receptor (AhR) activation, as mice lacking AhR in myeloid cells did not respond to treatment. These findings suggest that CCR2+ monocytes facilitate neutrophil recruitment during ARDS, and targeting AhR with dietary ligands like I3C could provide a novel therapeutic strategy for managing ARDS.

Schenzel et al. investigated the effects of a fiber-rich diet on exacerbations triggered by respiratory infections. β-hydroxybutyric acid levels were reduced in asthmatic children after exacerbations, potentially due to gut dysbiosis caused by respiratory infections. In a mouse model of asthma, the fiber-rich diet alleviated lung inflammation, reduced eosinophils expressing FcεRIα, suppressed GATA3+ Th2 cells, increased CD8+ effector memory T cells, and enhanced antiviral immunity. These findings suggest that dietary fiber and β-hydroxybutyric acid could help reduce airway inflammation, Th2-mediated responses, and eosinophil activation, offering potential as a supportive therapy for asthma, especially during exacerbations.

Shi et al. examined serum GDF15 in patients with acute exacerbations of COPD (AECOPD). Serum GDF15 levels in patients with malnutrition and AECOPD were higher than those in patients without malnutrition, whilst the serum albumin levels were lower than those in patients without malnutrition. Serum GDF15 levels were an independent risk factor for malnutrition in patients with AECOPD. The combination of GDF15 and serum albumin levels revealed a higher predictive ability for malnutrition in patients with AECOPD.

Overall, these studies highlighted how dietary factors influence the gut-lung axis and how this translates in respiratory health. Key findings include the role of serum albumin and serum GDF15, in managing respiratory diseases. These biomarkers are particularly significant in identifying malnutrition, a critical factor that exacerbates disease progression and increases mortality risk, however there is need for further robust evidence to support their clinical application. Pro-inflammatory diets, linked to sarcopenia, exacerbate COPD mortality, while interventions like fiber-rich diets, probiotics, and bioactive compounds (e.g., I3C) showed promise in reducing lung inflammation and enhancing immunity. Let's not forget the major influence of SCFA in mediating these effects. These findings underscore the importance of dietary and microbiota-focused strategies in managing and preventing respiratory diseases.

Author contributions

IB: Writing – original draft, Writing – review & editing. TS: Writing – original draft, Writing – review & editing. NA: Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Indole-3-carbinol attenuates lipopolysaccharide-induced acute respiratory distress syndrome through activation of AhR: role of CCR2+ monocyte activation and recruitment in the regulation of CXCR2+ neutrophils in the lungs

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Introduction: Indole-3-carbinol (I3C) is found in cruciferous vegetables and used as a dietary supplement. It is known to act as a ligand for aryl hydrocarbon receptor (AhR). In the current study, we investigated the role of AhR and the ability of I3C to attenuate LPS-induced Acute Respiratory Distress Syndrome (ARDS).

Methods: To that end, we induced ARDS in wild-type C57BL/6 mice, Ccr2gfp/gfp KI/KO mice (mice deficient in the CCR2 receptor), and LyZcreAhRfl/fl mice (mice deficient in the AhR on myeloid linage cells). Additionally, mice were treated with I3C (65 mg/kg) or vehicle to investigate its efficacy to treat ARDS.

Results: I3C decreased the neutrophils expressing CXCR2, a receptor associated with neutrophil recruitment in the lungs. In addition, LPS-exposed mice treated with I3C revealed downregulation of CCR2+ monocytes in the lungs and lowered CCL2 (MCP-1) protein levels in serum and bronchoalveolar lavage fluid. Loss of CCR2 on monocytes blocked the recruitment of CXCR2+ neutrophils and decreased the total number of immune cells in the lungs during ARDS. In addition, loss of the AhR on myeloid linage cells ablated I3C-mediated attenuation of CXCR2+ neutrophils and CCR2+ monocytes in the lungs from ARDS animals. Interestingly, scRNASeq showed that in macrophage/monocyte cell clusters of LPS-exposed mice, I3C reduced the expression of CXCL2 and CXCL3, which bind to CXCR2 and are involved in neutrophil recruitment to the disease site.

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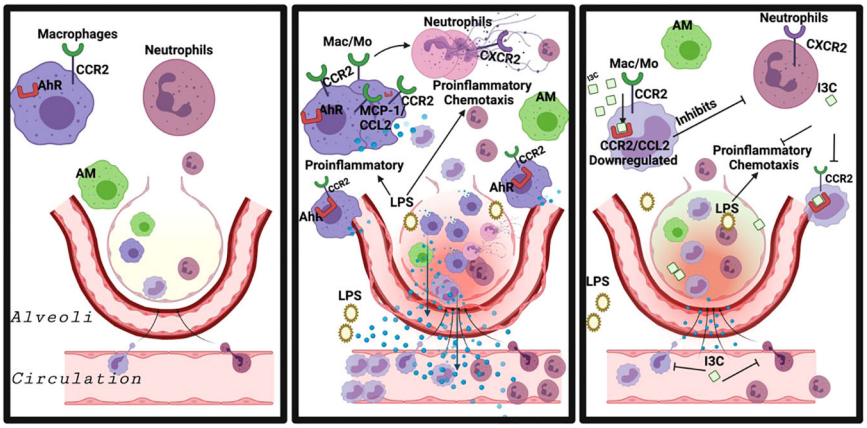
Discussion: These findings suggest that CCR2+ monocytes are involved in the migration and recruitment of CXCR2+ neutrophils during ARDS, and the AhR ligand, I3C, can suppress ARDS through the regulation of immune cell trafficking.

KEYWORDS

ARDS, LPS, CCR2, lung, inflammation, CXCL3, aryl hydrocarbon receptor, indole-3-carbinol



GRAPHICAL ABSTRACT

1 Introduction

Acute Respiratory Distress Syndrome (ARDS) is a form of respiratory failure that is caused by a variety of insults, including pneumonia, sepsis, trauma, and COVID-19 (1). It is estimated that ~33% of hospitalized COVID-19 patients develop ARDS and nearly 75% of such patients admitted to the ICU have ARDS (2). In patients, the severity of the disease is characterized by a systematic hyperimmune response, a cytokine storm, lung structure remodeling, decreased lung functionality, and systematic oxygen deprivation (3–6). While the ARDS caused by bacteria and viruses may differ in terms of the types of cells infected in the lungs, inflammation that ensues the infection can further cause significant damage to the lungs (1).

Severe damage to the lungs induced by ARDS results in severe hypoxemia and pulmonary edema. While there is no cure for this disease, significant advancements have been made in managing the symptoms and alleviating fatalities. The focus is on supportive care, targeted pharmacological interventions, and lung-protective ventilation strategies. Recent studies have illustrated the effectiveness of interventions like fluid management in improving oxygenation, neuromuscular blockade, and prone positioning, which help to improve mortality rates of ARDS patients (7, 8). Additionally, emerging therapies like extracorporeal membrane oxygenation (ECMO) have shown promise in cases of refractory hypoxemia, offering a potential lifeline for critically ill patients (9).

LPS-induced ARDS is one of the most commonly used rodent models to study ARDS (10, 11). ARDS in LPS-challenged mice is accompanied by lung remodeling, disrupted lung functionality, systematic oxygen deprivation, and a hyperimmune response (12–19). LPS-induced ARDS is a neutrophil-dependent lung injury model in which the progression of the disease depends on the activation and transmigration of neutrophils (11, 20). The sequence of neutrophil emigration to the lungs begins with blood neutrophil recruitment to the interstitium, followed by transepithelial migration to alveolar airspace (20). While for host defense and bacteria clearance, neutrophil recruitment into the lungs is necessary, the overzealous activation and accumulation of neutrophils causes damage to the lung tissue. Furthermore, neutrophil recruitment, the first leukocytes to migrate to the alveolus during lung injury, is speculated to be dependent on the activation of monocytes and macrophages in the lungs (21–24).

There are two main subsets of macrophages in the alveolus: residential alveolar macrophages (RAM) and recruited alveolar macrophages (RecAM). RecAM that migrate from the blood, also referred to as peripheral blood monocytes, are suggested to play a

role in the systemic inflammatory response and pathogenesis of LPS-induced ARDS. At the same time, RAM has an M2 phenotype and is considered to be immunosuppressive (21, 25–29). During ARDS, peripheral blood monocytes are recruited to the alveolar sac, where they differentiate into recruited alveolar macrophages with an M1 phenotype (proinflammatory macrophage subset) (25, 30). Neutrophils, transmigration is reported to be dependent on proinflammatory alveolar macrophages (AM) during ARDS (20, 21). The proinflammatory macrophage subset is universally known to have an M1 phenotype. Interestingly, as previously stated, the proinflammatory macrophage subset is suspected to be recruited to the alveolus during ARDS, which leads to the controversy surrounding the question of whether neutrophils or the macrophages are the first cells to be recruited to the lungs during ARDS.

Monocytes, a type of white blood cell derived from the bone marrow, play a pivotal role in immune surveillance and inflammatory responses. These cells exhibit remarkable plasticity, transitioning between circulating monocytes and tissue-resident macrophages in various organs, including the lungs. Pulmonary monocytes are integral components of the lung's immune defense system, contributing to both homeostasis and pathological processes such as acute lung injury and infection. Recent research has shed light on the diverse functions of pulmonary monocytes, highlighting their involvement in orchestrating immune responses, resolving inflammation, and promoting tissue repair within the lung microenvironment (31, 32). Furthermore, studies have elucidated the heterogeneity of pulmonary monocyte subsets, each with distinct phenotypic and functional characteristics, underscoring their complex role in lung immunity and pathology (33, 34). Understanding the dynamics of monocyte recruitment, activation, and differentiation within the pulmonary milieu is crucial for deciphering the mechanisms underlying lung diseases and devising targeted therapeutic interventions.

Peripheral blood monocytes entering the bloodstream during an inflammatory state express high levels of CCR2 (35, 36). CCR2+ monocytes egress and accumulate in the alveolus during LPS-induced ARDS which is dependent on CCL2 gradients in the inflamed lungs and circulation. CCR2-deficient mice treated with LPS lacked the alveolar monocyte accumulation (37–39). Some studies have also illustrated that pro-inflammatory T cells co-express multiple chemokine receptors, including CCR6 and CCR2, with distinct functions. CD4+CCR6+CCR2+ cells exhibit a pathogenic Th17 signature, produce inflammatory cytokines independent of TCR activation, and efficiently migrate through endothelial cells. While CCR6 mediates firm arrest of cells on endothelial surfaces, CCR2 is essential for transendothelial migration but not for cell arrest, indicating that these activities are mutually exclusive and depend on chemokine binding to different endothelial surfaces or forming transendothelial gradients (40). On the other hand, neutrophils recruited to the inflamed lungs express CXCR2. Interestingly, in a mouse model of polymicrobial septic peritonitis, blocking CCR2 diminished the neutrophil recruitment (41, 42). Notably, several studies have aimed to prevent neutrophil recruitment and chemotaxis and excessive neutrophil-mediated tissue damage in the lungs by inhibiting the chemokine receptor CXCR2. Navarixin (MK-7123/SCH 527123), and other CXCR2 inhibitors, have been proposed as a pharmacological intervention to treat COVID-19. However, common adverse effects include nasopharyngitis, headaches, and decreased neutrophil count, making the patients susceptible to respiratory and other infections (43–46).

The aryl hydrocarbon receptor (AhR) is a basic helix-loop-helix transcription factor that has been shown to play a critical role in the regulation of immune cell differentiation and modulation when ligated by an AhR ligand (47–49). There are several classes of AhR ligands that have been studied for their therapeutic effects against inflammatory diseases, which include endogenous (FICZ), synthetic (TCDD), and natural (Indoles) AhR ligands. Interestingly, different AhR ligands induce different immunological changes (50, 51). Indole-3-carbinol (I3C), a naturally occurring AhR ligand, has been shown to exhibit anti-inflammatory properties. I3C has been shown to downregulate LPS-induced proinflammatory gene expression of CCL2, the cytokine involved in the migration of CCR2 monocytes (52).

In the current study, we examined the role of AhR in the recruitment of proinflammatory CCR2+ monocytes and CXCR2+ neutrophils. Single-cell RNA seq t-SNEs analysis identified CCR2 as a critical receptor expressed by macrophages and monocytes populations and CXCR2 as a receptor exclusively expressed by neutrophils involved in the ARDS induction. In addition, the AhR gene was expressed by CCR2 expressing cells. Interestingly, I3C decreased the accumulation of CCR2+ monocytes and CXCR2+ neutrophils in the lungs of LPS-treated mice. In addition, the I3C-mediated decrease in CXCR2+ neutrophils was dependent on the CCR2 and AhR receptors.

2 Materials and methods

2.1 Mice

Eight-week-old female C57BL/6 and Ccr2gfp/gfp KI/KO mice (JAX stock #027619) were obtained from Jackson Laboratories (Bar Harbor, ME, USA). The Ccr2gfp/gfp KI/KO mice globally knock out CCR2 on all cell types and express GFP instead. Female LyZcreAhrfl/fl on a C57BL/6 background were bred in-house. Mice were housed at the University of South Carolina, School of Medicine AAALAC-accredited animal facility (Columbia, SC), where they were kept in temperature-controlled rooms under specific pathogen-free conditions and 12-h dark/light cycles. Mice were given ad libitum access to water and a standard chow diet, and had an average weight of 18 – 20 grams.

All animal experiments were approved by The University of South Carolina Institutional Animal Care and Use Committee.

2.2 LPS treatment

LPS-mediated ARDS in mice was induced as described (52, 53). Briefly, mice were anesthetized with isoflurane in combination with oxygen. These mice received 5 mg/kg of LPS (Escherichia coli,

serotype 055:B5; Sigma-Aldrich, St. Louis, MO) dissolved in PBS intranasally to induce ARDS. Mice were treated with vehicle control [DMSO] or I3C [65 mg/kg] three hours after LPS-exposure. After 48 hours, mice were euthanized for studies on ARDS. The dose, frequency, and route of I3C was standardized based on our previous studies (52, 53).

2.3 Tissue processing

Whole blood was collected from the portal vein of mice. 500 uL of whole blood was used for VetScan analysis, and the remaining blood was either used for flow cytometry analysis or was centrifuged at 8000 rpm for 8 minutes to collect serum. Serum was used to perform enzyme-linked immunosorbent assays (ELISA). Whole blood collected for flow cytometry analysis was resuspended in 10 uL EDTA and 2 mL of red blood lysis for 10 minutes and neutralized with 10 mL flow cytometry staining buffer (FACS buffer-1X PBS, 2% Fetal Bovine Serum (FBS), and 2mM EDTA). The cells were then centrifuged and resuspended in flow cytometry staining buffer. In addition, as previously described, lung mononuclear cells (MNCs) were isolated from the whole lung (4). In brief, euthanized mice were perfused with 10 mL of heparinized PBS. Next, the lungs were removed, and BALF was collected to perform ELISA. The lungs were dissociated and brought to a single-cell suspension. The lung single-cell suspension underwent red blood lysis before being passed through a 70 µM filter. Next, the cells were centrifuged and re-suspended in flow cytometry staining buffer or 10X buffer for single-cell RNA sequence analyses. The cells were counted using the TC20 Automated Cell Counter (BioRad, Hercules, CA) to check for the viability. Samples with 80% or higher viability were processed for flow-cytometry, single-cell RNA sequence analyses, and RNA extraction for qPCR.

2.4 Flow cytometry and sorting

For the detection of immune cells from the lungs and blood, fluorescently labeled monoclonal antibodies (mAbs) were used along with flow cytometry as described by previously (54) In brief, cells derived from the lungs (1 x 106) were incubated with TruStain FcX anti-mouse CD16/32 (Biolegend, San Diego, CA) and tagged with fluorescently labeled mAbs APC Cy7 labeled anti-CD45, AF 700 or BV785 labeled anti-CD11b, BV785 labeled anti-CD11c, BV605 labeled anti-CD11c, AF488 labeled anti-Ly6c, APC labeled anti-Ly6c, BV785 labeled anti-Ly6g, PE-labeled anti-Ly6g, BV510 labeled anti-Gr-1, AF488 labeled anti-F4/80, BV421 labeled anti-F4/80, AF647 labeled anti-CD 206, BV650 labeled anti-CD 206, Per Cp labeled anti-CCR5, FITC labeled anti-CCR2, and/or PE-labeled anti-CXCR2 purchased from Biolegend. UltraComp eBeads (Invitrogen, Waltham, MA) were used for compensation. Cells were analyzed using a BD FACSCelesta (Franklin Lakes, NJ), and FlowJo (BD Biosciences, San Jose, CA) was used to analyze FCS files.

2.5 RNA extraction and qPCR

As previously described (55), the remaining cells from the lungs were suspended and spun down in 700 uL of QIAzol. Samples were then stored at -80°C until isolation. Qiagen RNeasy kit was used to isolate total RNA. The Nanodrop 2000 (Waltham, MA) was used to check RNA quality and quality. Next, the miScript II RT Kit supplied by Qiagen (Germantown, MD) was used to make cDNA. The cDNA was used in combination with SsoAdvanced SYBR green supermix from Bio-Rad for the generation of mRNA. qRT-PCR was carried out on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Gene expression levels were normalized to β-actin expression. A detailed list of primers for genes is listed in Table 1.

scRNASeq was conducted as described previously (52, 56). The 10x Chromium instrument (10x Genomics, Pleasanton, California) was used to perform single-cell RNA sequencing. Cells (3000) were loaded onto the Chromium Controller [10x Genomics] that generated single-cell Gel Bead-In-Emulsions. Next, samples were processed into single-cell RNA-seq libraries using the Chromium v2 single-cell 3′ RNA-seq reagent kit [10x Genomics]. The libraries were sequenced with NextSeq 550 instrument (Illumina, San Diego, CA). Raw sequencing data were processed using Cell Ranger version 3.1.0 (10x Genomics). Downstream analysis of Cell Ranger output was completed using Loupe Browser 5.1.0. Cell cluster biomarkers were identified using differential gene expression in Loupe Browser.

2.6 Statistical analyses

Statistical analyses were performed using Graphpad software. All data shown in figure legends are presented as the mean ± standard error of the mean [SEM]. One-way ANOVA tests were used to calculate the statistical significance. The number of mice or samples tested were presented as individual dots in bar graphs. The level of statistical significance was determined using the following key: *p < 0.05, **p < 0.01, ***p < 0.001, ****p<0.0001.

TABLE 1 Primer sequences.

Gene name	F Primer Sequence	R Primer Sequence
CCR2	GCTGTGTTTGCCTCTCTACCAG	CAAGTAGAGGCAGGATCAGGCT
CXCR2	CTCTATTCTGCCAGATGCTGTCC	ACAAGGCTCAGCAGAGTCACCA

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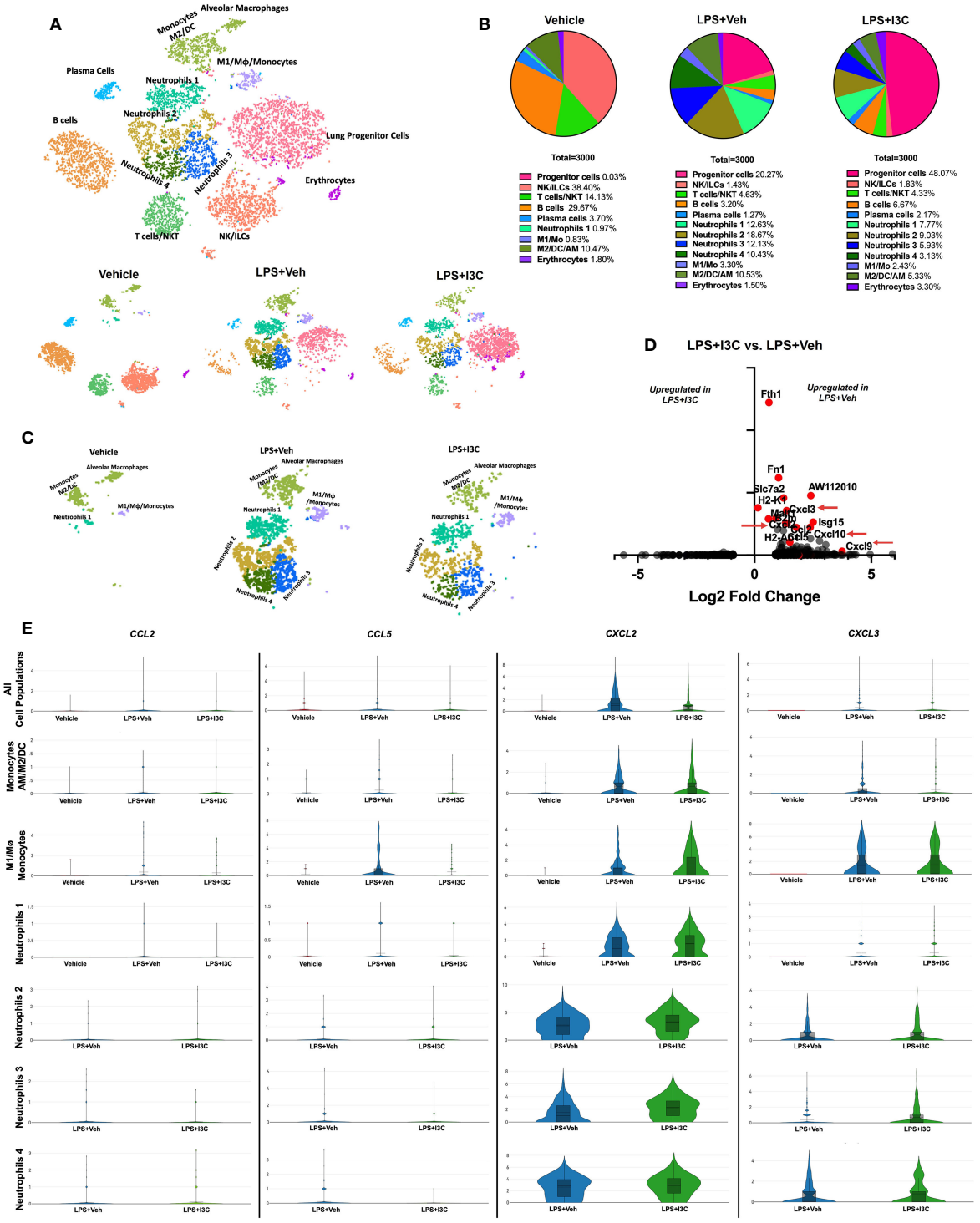

FIGURE 1
scRNAseq immune profiling of lung cells across treatment groups. Mice were exposed to LPS to induce ARDS followed by treatment with I3C as described in the Methods. The cells isolated from the lungs were screened as follows. **(A)** scRNAseq t-SNE colored by cell population. **(B)** Pie chart of cell populations by percentages. **(C)** scRNAseq t-SNE of myeloid cells. **(D)** Volcano plot of top differentiated genes in myeloid clusters in LPS+Veh *vs.* LPS+I3C samples. **(E)** Violin plots of chemokine ligand CCL2, CCL5, CXCL2, and CXCL3 gene expression across myeloid cell populations in treatment groups.

3 Results

3.1 scRNAseq immune profiling of lung-derived cells shows that I3C administration in LPS-treated mice induces gene dysregulation associated with cell migration

We have previously shown that I3C acts as an AhR ligand to attenuate LPS-mediated ARDS (44, 45). In the current study, we used scRNA-seq to determine lung immune cell profiles across treatment groups. Using lineage-defining genes, we identified several immune cell populations. Notably, Loupe Browser generated t-SNEs revealed considerable heterogenicity across immune cell populations in the control group *vs*. LPS+Veh and LPS+I3C groups. In LPS-challenged mice, there was an increase in clusters expressing myeloid cell gene signatures, while there was a decrease in clusters expressing lymphocyte gene signatures when compared to the control group (Figures 1A, B). Next, comparing the LPS+Veh group *vs*. LPS+I3C, we noticed a change in the cell profiles in the two groups (Figures 1A, B). Interestingly, lung progenitor cells were not present in the Vehicle-treated mice, started to appear during ARDS, and following treatment with I3C, there was a robust increase in these cells (Figure 1B). A further look into the myeloid cell clusters, we categorized four neutrophil populations using the clusters' transcriptional profiles. These were designated the neutrophil subgroups Neutrophils 1, Neutrophils 2, Neutrophils 3, and Neutrophils 4 determined by cluster gene signatures (SI appendix, Supplementary Figures 1A, B). These clusters were markedly upregulated following LPS treatment (Figures 1A–C). In contrast, the vehicle-alone group expressed only a small percentage of the Neutrophil 1 cluster (0.97%) and none of the other neutrophil clusters. Moreover, LPS+I3C group showed a decrease in all 4 neutrophil clusters when compared to LPS+Veh group (Figure 1A–C). When we compared the proportions of M2 *vs* M1 macrophages, this ratio was highest in Vehicle controls with more M2 than M1 macrophages. This ratio was decreased following LPS treatment and was further reduced in LPS+I3C group (Figures 1A, B). The proportions of lymphocytes such as T/NKT cells, B cells, and plasma cells were higher in the Vehicle group and were decreased in LPS or LPS+I3C groups. The NK/ILC population was highest in the Vehicle group and markedly decreased in LPS+Veh and LPS+I3C groups.

To determine the effect of I3C on myeloid cells, we investigated the differentially expressed genes amongst LPS+Veh and LPS+I3C groups. Interestingly, genes significantly dysregulated across the two treatment groups were associated with proinflammatory responses, antigen presentation, and immune cell migration. Comparing the LPS+Veh group *vs*. LPS+I3C, we noticed I3C treatment led to downregulation of chemokine transcripts for CCL2, CCL5, CXCL2, and CXCL3 associated with chemotaxis of myeloid cells (Figures 1D, E). Macrophages, dendritic cells, monocytes, and neutrophils secrete CCL2 and CCL5 proteins, which are ligands that bind to CCR2+ and CCR5+ monocytes leading to the emigration of monocytes from circulation to the tissue injury site. In addition, macrophages, dendritic cells, monocytes, and neutrophils secrete CXCL2 and CXCL3 proteins, which binds to the CXCR2 on neutrophils, leading to their migration to the site of inflammation. Interestingly, CCL2, CCL5, CXCL2, and CXCL3 genes were disproportionately expressed across myeloid cell clusters in Vehicle, LPS+Veh, and LPS+I3C groups. In LPS-exposed mice, there was an increase in CCL2, CCL5, CXCL2, and CXCL3 gene expression in most of the myeloid cell clusters compared to vehicle control mice. Next, comparing the LPS +Veh group *vs*. LPS+I3C, we noticed that I3C-mediated a downshift in CCL2, CCL5, CXCL2, and CXCL3 in most cell populations (Figure 1E). However, especially with CXCL2 expression, some cell clusters such as M1 macrophages and neutrophil subpopulations expressed slightly higher levels of in LPS+I3C *vs* LPS+Veh group (Figure 1E). Together these data suggested that when all lung cells were taken into consideration, the expression of CCL2, CCL5, CXCL2, and CXCL3 was upregulated following LPS exposure and I3C treatment significantly reduced their expression. However, scRNA-seq demonstrated that individual myeloid clusters

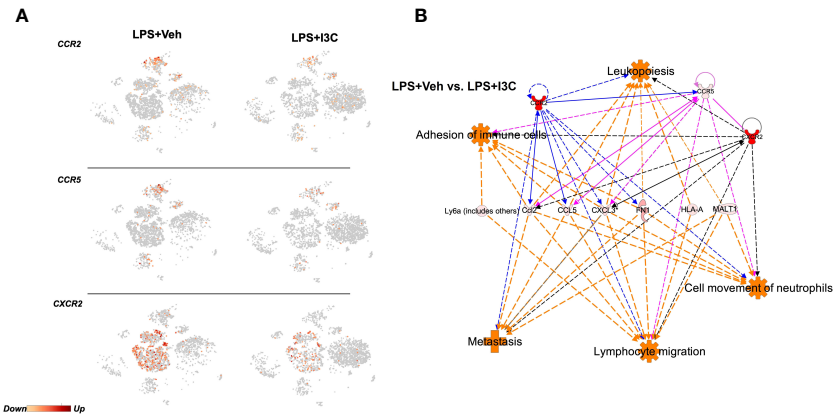

FIGURE 2
Ingenuity Pathway Analysis prediction of dysregulated chemoattractant ligand genes in LPS-exposed *vs* LPS+I3C treated mice. Mice were exposed to LPS to induce ARDS followed by treatment with I3C as described in the Methods and cells from the lungs were screened as follows. **(A)** scRNAseq t-SNE heatmap expression of CCR2, CCR5, and CXCR2. **(B)** IPA pathway prediction graphical summary of local gene dysregulation associated with immune cell trafficking functions when comparing LPS+Veh *vs*. LPS+I3C.

expressed differential levels of these chemokines and differential response to LPS or LPS+I3C treatment. Also, of the 4 chemokines, CXCL2 and CXCL3 expression was higher than the other two chemokines screened among the myeloid clusters. While these results may be inconsistent with what was expected, it is important to note that our understanding of the immunological landscape of the lung with and without disease will continue to change as we implement new techniques like scRNA-seq that allow us to evaluate cells with higher specificity. While this data focuses on transcript level of gene expression, there may be post-transcriptional changes that will cause changes in protein expression of these chemokines.

3.2 Ingenuity pathway analysis prediction of dysregulated chemoattractant ligand and receptor genes associated with CCR2+ monocytes and CXCR2+ neutrophils in LPS-exposed mice following I3C treatment

The recruitment of CCR2+ and CCR5+ monocytes and CXCR2+ neutrophils to the lungs during inflammation is heavily dependent on the ligand-receptor interactions. Therefore, we investigated whether the transcriptional activity of these receptors was altered in LPS-exposed mice following treatment with I3C. We found that I3C decreased the expression of CCR2, CCR5, and CXCR2 in myeloid cell clusters (Figure 2A). CCR2 and CCR5 are gene signatures of macrophages, monocytes, and dendritic cell clusters. On the other hand, CXCR2 is highly expressed by neutrophils (Figure 2A).

Next, ingenuity pathway analysis software was used to generate a pathway prediction of dysregulated genes in LPS+Veh *vs* LPS+I3C groups. This software allowed us to predict pathways due to the causal analysis algorithms utilized based on a master network derived from the Ingenuity Knowledge Base. Therefore predictions were made based on gene expression levels and validated results from previously published literature. Upon uploading our gene expression data for the most significant differentially expressed genes, we performed core analysis. Interestingly, IPA predicted dysregulated genes in LPS+Veh *vs* LPS+I3C groups to be associated with immune cell trafficking functions (Figure 2B). These immune cell trafficking functions included cell movement of neutrophils, lymphocyte migration, adhesion of immune cells, and metastasis. Towards this, IPA predicted CCL2, CCL5, and CXCL3 to be connected to immune cell trafficking functions. In addition, IPA predicted CCL2-CCR2/CCR5/CXCR2, CCL5-CCR2/CCR5, and CXCL3-CXCR2 relationships. Interestingly, the pathway prediction also depicted interconnected relationships between the receptors CCR2, CCR5, and CXCR2 (Figure 2B). Taken together, this suggested that neutrophils and monocytes may directly or indirectly play a part in the recruitment of one another during inflammation.

3.3 I3C prevents the influx of circulating inflammatory CCR2+ monocytes and CXCR2+ neutrophils in mice with ARDS

To further investigate if the changes in myeloid cell profiles in the lungs during ARDS are associated with the changes in the

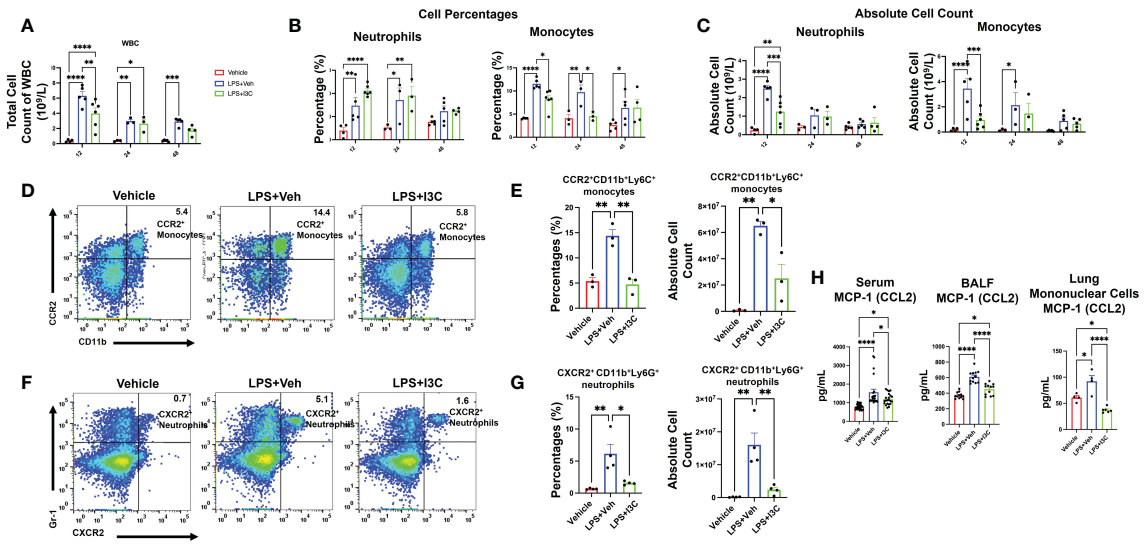

FIGURE 3
I3C decreases circulating inflammatory CCR2$^+$ monocytes and CXCR2$^+$ neutrophils in LPS-challenged mice. Mice were exposed to LPS to induce ARDS followed by treatment with I3C as described in the Methods. **(A)** Circulating white blood cell count at 12, 24, and 48 hours after exposure to LPS or LPS+I3C. **(B, C)** Cell percentages and absolute cell count of blood monocytes and neutrophils. **(D)** Representative flow cytometry plots of CCR2$^+$ monocytes at 12hrs. **(E)** Bar graph of CCR2$^+$ monocytes percentages and absolute cell count at 12hrs. **(F)** Representative flow cytometry plots of CXCR2$^+$ neutrophils at 12hrs. **(G)** Bar graph of CXCR2$^+$ neutrophils cell percentages and absolute cell count at 12hrs. **(H)** CCL2 protein level in bronchial lavage fluid, serum, and mononuclear cells supernatant detected by enzyme-linked immunosorbent assay. *p<0.05, **p<0.01, ***p<0.0002, ****p<0.0001.

monocyte and neutrophil populations in circulation during disease, we performed a differential white blood cell (WBC) count with blood samples. At 12 hours, there was an increase in the total count of WBCs in the blood of LPS+Veh *vs*. control mice (Figure 3A). Interestingly, I3C significantly decreased the total count of WBC in the blood of LPS-administered mice at 12 hours. At 24hrs, the WBC count in both LPS+Veh *vs*. LPS+I3C was lower than the 12 hour time point. However, WBC levels at 24 hours in LPS-challenged group was still significantly higher than baseline levels. Interestingly, the WBC levels in LPS+I3C group were returned almost to the baseline levels at 48 hours (Figure 3A).

Similar to the lungs, there was an increase in both monocytes and neutrophils in the blood of LPS-exposed animals *vs* Vehicle controls (Figure 3B). Furthermore, both neutrophil frequency and monocyte frequency were significantly increased at 12 and 24 hours in the LPS+Veh *vs* control group. At 48 hours, the neutrophil frequency was resolved, but there was still a significant increase in monocyte frequency at 48 hours in LPS+Veh *vs*. control group. Strikingly, there was a higher percentage of neutrophils in the blood of LPS+I3C mice *vs*. LPS+Veh and control mice at 12 hours and 24 hours. However, this may be due to I3C migration inhibitory capabilities, leading to an accumulation of neutrophils in the blood in diseased animals due to blocked cellular recruitment of the cells. However, at 48 hours, the frequency of neutrophils was similar across all treatment groups. We found that I3C attenuated the increased frequency of monocytes in LPS-exposed animals at 12 and 24 hours but monocyte percentages weren't significantly different at 48 hours in LPS+I3C mice *vs* LPS+Veh (Figure 3B). Taken together, these data suggested that a downshift in the percentages of monocytes coincides with an upshift in the frequency of neutrophils in the blood. However, the absolute cell counts of monocytes and neutrophils in the blood across treatment groups showed that at 12 hours, I3C-treated mice had a significant reduction in both monocytes and neutrophils. At 24 hours, the neutrophil total count in LPS-exposed animals was returned to baseline, and at 48 hours, the monocyte total count in diseased animals was returned to baseline (Figure 3C).

Furthermore, we phenotyped the immune cells to identify whether monocytes and circulation neutrophils expressed CCR2, CCR5, or CXCR2 chemokines receptors associated with myeloid cell trafficking. Flow cytometric analyses of blood at 12 hours after LPS exposure showed a significant increase in CCR2+ monocytes and CXCR2+ neutrophils in LPS+Veh *vs* Vehicle-only group (Figures 3D-G; Supplementary Figure 2). Interestingly, there was no significant increase in any immune cell population expressing CCR5 across treatment groups at 12-48 hours (data not shown). In the presence of I3C, CCR2+ monocytes and CXCR2+ neutrophils in circulation were decreased in LPS-exposed mice (Figures 3D-G; Supplementary Figure 2). Thus, the data showed that the increase in CXCR2+ neutrophils and CCR2+ monocyte populations during disease at 12 hours was attenuated by I3C.

Notably, at 24 hours, in LPS+Veh group, CXCR2+ neutrophils and CCR2+ monocytes were resolved to the levels of LPS+I3C. At 48 hours, CXCR2+ neutrophils and CCR2+ monocytes in LPS+Veh group were returned to baseline (data not shown). Furthermore, monocyte chemoattractant protein-1 (CCL2), the chemoattractant ligand for the CCR2, was increased in the serum and BALF at 48 hours in LPS +Veh mice *vs* the control group. Strikingly, I3C treatment significantly lowered this increase in LPS-exposed animals. Additionally, mononuclear cells isolated from the lungs at 48 hours from the LPS-exposed animals were producing more CCL2 than the control group. We found that the production of CCL2 by mononuclear cells from the LPS+I3C group was significantly lower than in the LPS+Veh group and Vehicle-only group (Figure 3H). Taken together, these data suggested that I3C partially blocks the upshift in CCR2 monocytes and CXCR2 neutrophils in circulation during ARDS.

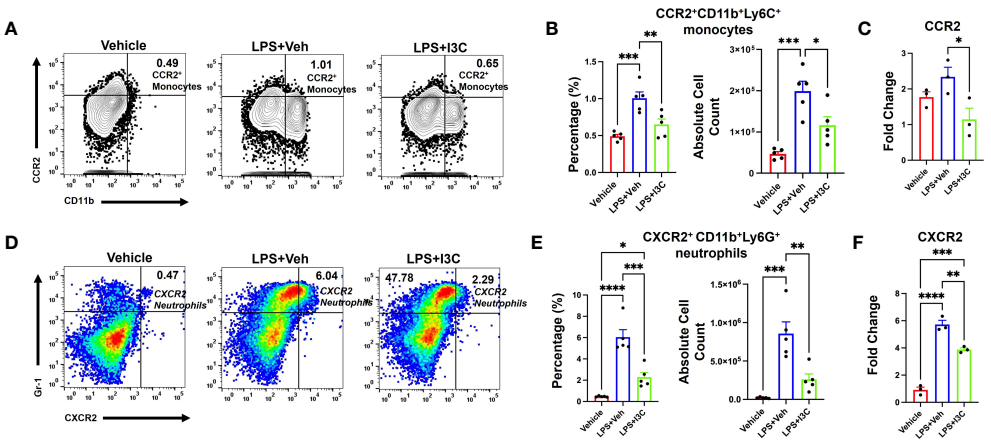

FIGURE 4
CCR2$^+$ monocyte and CXCR2$^+$ neutrophil populations increase following LPS exposure, which is attenuated by I3C. Mice were exposed to LPS to induce ARDS followed by treatment with I3C as described in the Methods. The cells isolated from the lungs were screened as follows: **(A)** Representative contour flow cytometry plots of CCR2$^+$ monocytes seen in the lungs at 48hrs. **(B)** Bar graph of CCR2$^+$ monocyte percentages and absolute cell numbers at 48hrs. **(C)** CCR2 mRNA expression in lung MNCs. **(D)** Representative flow cytometry plots of CXCR2$^+$ neutrophils at 48hrs. **(E)** Bar graph of CXCR2$^+$ neutrophil percentages and absolute cell numbers at 48hrs. **(F)** CXCR2 mRNA expression in whole lung cells. *p<0.05, **p<0.01, ***p<0.0002, ****p<0.0001.

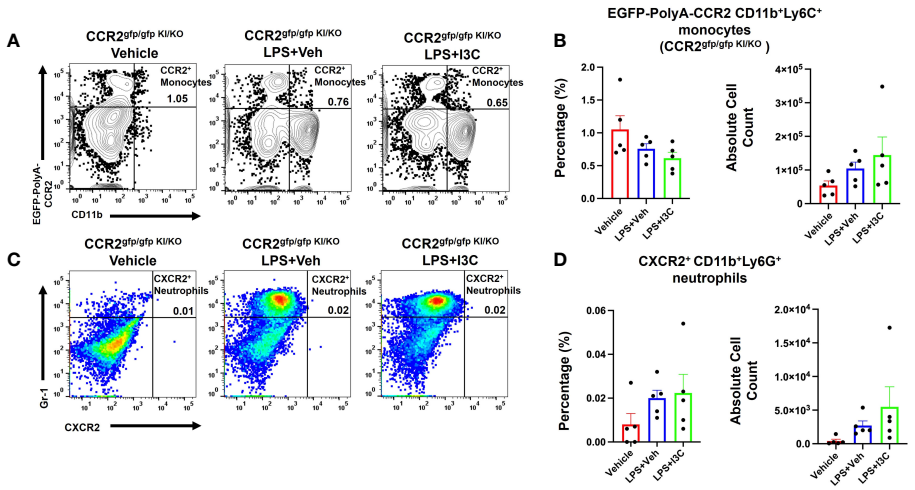

FIGURE 5
Cell percentages and numbers of CCR2^{+} monocyte and CXCR2^{+} neutrophil cell populations were not altered in CCR2$^{gfp/gfp}$ knock-in/knock-out mice following LPS treatment. Mice were exposed to LPS to induce ARDS followed by treatment with I3C as described in the Methods. The cells isolated from the lungs were screened as follows: **(A)** Representative contour flow cytometry plots of EGFP-Poly-A-CCR2^{+} monocytes in the lungs of *CCR2$^{gfp/gfp}$ KI/KO* mice at 48hrs. **(B)** Bar graph of EGFP-Poly-A-CCR2+ monocyte cell percentages and absolute cell numbers in *CCR2$^{gfp/gfp}$ KI/KO* mice at 48hrs. **(C)** Representative flow cytometry plots of CXCR2^{+} neutrophils at 48hrs in the lungs. **(D)** Bar graph of CXCR2^{+} neutrophils cell percentages and absolute cell count at 48hrs in the lungs.

3.4 I3C partially blocks the influx of CCR2+ monocytes and the recruitment of CXCR2 + neutrophils in the lungs of mice with ARDS

At 48hrs following LPS exposure, we analyzed the lung immune cells to investigate the migration of CXCR2+ neutrophils and CCR2+ monocytes population, and the ability of I3C to prevent the increase in these specific cell types. LPS-challenge resulted in an increase in CCR2+ monocytes in the lungs (Figures 4A, B). Interestingly, I3C treatment caused a decrease in CCR2+ monocytes in the lungs and blood, findings consistent with a suppressed myeloid response (Figures 3D, E; 4A, B). Further investigation into mononuclear cellular expression of the CCR2 gene showed that I3C downregulated the expression of the CCR2 gene in mononuclear cells of LPS-exposed mice (Figure 4C). Investigation into CXCR2+ neutrophils showed a significant increase in these cells in the lungs following LPS exposure and they were decreased in the LPS+I3C group (Figures 4D, E). The CXCR2 gene expression was decreased in the lung cells of LPS-exposed mice when treated with I3C (Figure 4F). These data further suggested that I3C suppresses CCR2+ monocytes and CXCR2+ neutrophil infiltration into the lungs during ARDS.

3.5 CXCR2+ neutrophil cell population frequency during ARDS is dependent on the CCR2+ monocytes

To investigate the role of infiltrating CCR2+ monocytes and their effects on CXCR2+ neutrophil recruitment during LPS-induced ARDS, we used a CCR2gfp/gfp knock-in/knock-out (KI/KO) mice. These mice have green fluorescent protein (EGFP) sequence followed by a polyadenylation signal inserted into the translation initiation site of CCR2 thereby abolishing its expression. In addition, CCR2gfp/gfp KI/KO mice lack peripheral blood Ly6chi monocytes (57). We observed that in CCR2gfp/gfp KI/KO mice, LPS+Veh group showed no significant increase in the percentage and total numbers of CCR2+ monocytes during LPS-induced ARDS when compared to the Vehicle group (Figures 5A, B). Also, this response was markedly attenuated when compared to the wild-type mice (Figures 4A, B). Also, CCR2gfp/gfp KI/KO mice treated with I3C showed no significant differences in the CCR2+ monocyte population when compared to the LPS-treated groups (Figures 5A, B). Next, we examined the CXCR2+ neutrophil response during ARDS in CCR2gfp/gfp KI/KO mice when CCR2 was not expressed on the cell surface of monocytes and macrophages. Strikingly, the LPS-induced ARDS-associated increase in the percentage and numbers of the CXCR2+ neutrophils in the lungs was markedly reduced in LPS-treated CCR2gfp/gfp KI/KO mice (Figures 5C, D) when compared to the WT mice (Figures 4D, E). I3C did not further significantly alter the CXCR2+ neutrophil population in LPS-treated CCR2gfp/gfp KI/KO mice (Figures 5C, D) suggesting that I3C-induced decrease in the CXCR2+ neutrophil population following LPS-exposure may result from a decrease in CCR2+ monocytes. Importantly, our studies suggested that infiltrating peripheral CCR2+ blood monocytes may be responsible for CXCR2+ neutrophil accumulation in the lungs during LPS-induced ARDS.

3.6 I3C-mediated decrease in the recruitment of CCR2+ monocytes and CXCR2+ neutrophils into the lungs during ARDS is dependent on AhR

Lastly, we investigated the role of AhR in I3C-mediated attenuation of CCR2+ monocyte and CXCR2+ neutrophil recruitment to lungs after LPS-challenge. To that end, we used

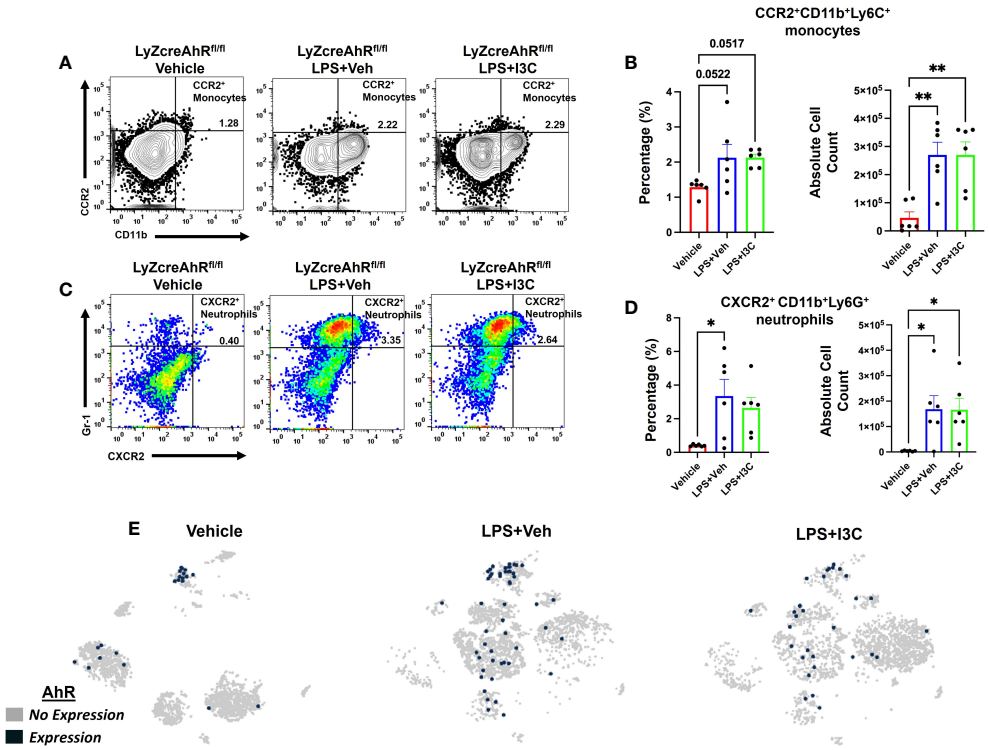

FIGURE 6
I3C-mediated attenuation of CCR2$^+$ monocytes and CXCR2$^+$ neutrophils in the lungs of LPS-exposed animals is dependent on the AhR. *LyZcreAhR$^{fl/fl}$* mice were exposed to LPS to induce ARDS followed by treatment with I3C as described in the Methods. The cells isolated from the lungs were screened as follows: **(A)** Representative contour flow cytometry plots of CCR2$^+$ monocytes at 48hrs. **(B)** Bar graph of CCR2$^+$ monocyte percentages and absolute cell counts at 48hrs. **(C)** Representative flow cytometry plots of CXCR2$^+$ neutrophils at 48hrs **(D)** Bar graph of CXCR2$^+$ neutrophil percentages and absolute cell counts at 48hrs. **(E)** scRNAseq t-SNE heatmap expression of AhR. *p<0.05, **p<0.01.

LyZcreAhRfl/fl mice, which do not express AhR on myeloid linage cells. Like the wild-type C57BL/6 mice with ARDS, there was an increase in CCR2+ monocytes in the lungs of LyZcreAhRfl/fl mice following LPS administration (Figures 6A, B). Interestingly, unlike LPS+I3C-treated wild-type mice (Figures 4A, B), I3C-mediated attenuation of CCR2+ monocytes in the lungs did not occur in LPS-exposed LyZcreAhRfl/fl mice (Figures 6A, B). In addition, CXCR2+ neutrophil populations were not decreased in LPS-exposed LyZcreAhRfl/fl mice following I3C treatment (Figures 6C, D). Furthermore, scRNA-seq t-SNEs showed that AhR is expressed by macrophage and monocyte cell clusters expressing CCR2 in wild-type mice (Figure 6E). Collectively, these findings demonstrated that AhR is essential for an I3C-mediated decrease in CCR2+ monocytes and CXCR2+ neutrophils.

4 Discussion

ARDS is a life-threatening pulmonary disease that affects almost 200,000 patients annually. The disease carries a ~40% mortality rate due to limited effective therapeutic modalities and different etiologies that induce ARDS (13, 58–60). Recently, with the advent of COVID-19, the number of patients suffering from ARDS will continue to rise (4). One of the clinical features seen in patients suffering from SARS-CoV-2 induced COVID-19 is elevated numbers of neutrophils in the blood and respiratory tract (61, 62). Neutrophil abundance coincides with COVID-19 severity (58, 63, 64). Severe forms of COVID-19 lead to ARDS (3). Besides COVID-19, ARDS is frequently associated with bacterial infections and thus, LPS-induced ARDS is a significant mouse model to study the recruitment of neutrophils in the blood and respiratory tract during ARDS (10, 11). Similar to the influx of neutrophils seen in patients infected with SARS-CoV-2 and their abundance being associated with ARDS, the LPS-induced ARDS mouse model is a neutrophil-dependent lung injury model in which disease progression is dependent on the activation and transmigration of neutrophils to the alveolar airspace (11, 20).

Monocyte and neutrophil interactions are essential for host defense against foreign invaders and the clearance of pathogens. However, if not tightly regulated, these interactions can have detrimental consequences. Neutrophils are non-residential cells deployed in circulation during inflammation when residential cells release chemoattractant into the circulation (65, 66). During ARDS, tissue activation of resident macrophages results in an influx of neutrophils to the lungs (37, 39, 67). Neutrophils augment monocytes' recruitment to the site of inflammation. It is also known that monocytes and neutrophils work in concert in response to pathogens (67, 68). However, both neutrophils and monocytes/macrophages have several different heterogeneous subsets, which may act in different ways thereby impacting the

degree of inflammation and tissue injury. In the current study we demonstrated that CCR2+ monocytes recruit CXCR2+ neutrophils during LPS-mediated ARDS.

Under inflammatory conditions, CCR2, a chemokine receptor, plays a critical role in the trafficking of bone marrow-derived monocytes to the inflamed lungs, and contributing to the lung injury during ARDS. CCR2+ monocytes are a subset of monocytes that express the chemokine receptor. These cells can facilitate the recruitment of other immune cells like neutrophils via the direct recruitment of CXCR2 (37, 69). CXCR2, is a key chemokine receptor expressed on neutrophils and is responsible for the recruitment and trans-endothelial and trans-epithelial migration of neutrophils to the lung injury site, contributing to the lung injury severity (14, 70). Research has shown that CCR2+ monocytes can directly recruit CXCR2-expressing cells, such as neutrophils, to the lungs through a mechanism involving chemokine gradients and cell-cell interactions. One study investigated the role of CCR2+ monocytes in lung inflammation and found that these cells were responsible for recruiting neutrophils to the lungs in a mouse model of acute lung injury. The researchers demonstrated that CCR2+ monocytes produced chemokines such as CXCL1 and CXCL2, which are ligands for CXCR2, and these chemokines were crucial for the recruitment of CXCR2-expressing cells to the lungs (71). Furthermore, another study examined the role of CCR2+ monocytes in recruiting neutrophils during pneumonia. The researchers found that CCR2+ monocytes produced high levels of CXCL1, which directly recruited CXCR2-expressing neutrophils to the lungs, contributing to the host defense against bacterial infection (72). Interestingly, several studies have examined the clinical benefits of Navarixin (MK-7123/SCH 527123) and other CXCR2 inhibitors, a pharmacological intervention to block neutrophil recruitment to treat COVID-19. But side effects of taking the CXCR2 inhibitors include nasopharyngitis, headaches, and decreased neutrophil count, leaving the patient susceptible to respiratory infections and other infections (43–45, 73). In endotoxin-mediated ARDS, bone marrow-derived blood-borne monocytes expressing CCR2 are involved in amplifying neutrophilic alveolitis (37, 38, 74). The goal of the current study was to explore the relationship between CCR2+ monocytes and CXCR2+ neutrophils during LPS-mediated ARDS while also exploring the role of AhR activation in immune cell trafficking and lung injury. Consistent with previous studies (14, 37–39, 70, 73), we found that there's an accumulation of CCR2+ monocytes and CXCR2+ neutrophils in the circulation and at the site of lung injury during LPS-mediated ARDS. The levels of chemokine CCL2 in the serum, BALF, and the production of CCL2 from mononuclear cells was increased in LPS-challenged animals. In the lungs of patients suffering from ARDS, CCL2 is upregulated (22, 67). The increase in CCL2 may be responsible for the increased frequency of CCR2+ monocytes during ARDS.

The AhR partially controls the activation and recruitment of CCR2+ monocytes during tissue injury (47, 75). Interestingly, I3C, a natural dietary AhR ligand, has been shown to inhibit CCL2 protein expression and CCR2+ monocyte recruitment to inflammatory sites (51). Consistent with these studies, we found that I3C decreased the recruitment and accumulation of CCR2+ monocytes under inflammatory conditions while regulating the frequency of CXCR2+ neutrophils in the circulation and the lungs during ARDS. We found that elevated counts of CCR2+ monocytes were associated with the elevated counts of CXCR2+ neutrophils in both the lungs and blood, suggesting a relationship between these myeloid cell subsets. Elevated counts of CCR2+ monocytes and CXCR2+ neutrophils in wild-type mice with ARDS significantly decreased following treatment with I3C but not abolished. Monocytes and neutrophils are involved in wound healing and recovery from tissue injury; therefore, abolishing monocytes and neutrophils or halting their recruitment may impact disease resolution and have dire consequences. This suggests that treatment with I3C may foster an environment for pathogen clearance and wound healing without the exaggerated response of CCR2+ monocytes and CXCR2+ neutrophils.

To examine if I3C directly affected CCR2+ blood-borne monocytes during ARDS, we used a CCR2gfp/gfp KI/KO mice, an EGFP knock-in, and CCR2 knock-out mice. CCR2gfp/gfp KI/KO mice lack peripheral blood Ly6chi monocytes. This allowed us to examine the residential macrophages that expressed the gene for CCR2 but not the receptor on the cell's surface. Interestingly, LPS-challenge in CCR2gfp/gfp KI/KO mice did not exhibit significant increase in the CCR2+ monocytes or CXCR2+ neutrophils population, suggesting that CCR2 was essential for the recruitment of CCR2+ blood-borne monocytes and, in return, was essential for CXCR2+ neutrophils emigration in the lungs. In addition, I3C didn't alter the CCR2 population or CXCR2+ neutrophils in CCR2gfp/gfp KI/KO mice. To further investigate if I3C-mediated attenuation of the accumulation of CCR2+ monocytes and CXCR2+ neutrophils was mediated through the AhR, we used LyZcreAhRfl/fl mice, with the loss of AhR on myeloid linage cells. These studies demonstrated that the infiltration of CCR2+ monocytes and CXCR2+ neutrophils was increased in LyZcreAhRfl/fl following LPS-challenge when compared to the controls. These results indicated that LPS was not causing the inflammatory response through the AhR on myeloid cells. However, unlike wild-type animals, I3C treatment did not decrease the frequency of CCR2+ monocytes and CXCR2+ neutrophils in the lungs of LyZcreAhRfl/fl diseased mice. This demonstrated that I3C-mediated attenuation of inflammatory myeloid cells in the lungs during ARDS was occurring through the ligation of the AhR. Thus, targeting the AhR ligand-receptor on myeloid linage cells may serve a potential intervention for ARDS.

5 Conclusions

In summary, disruption of CCR2 can interfere with monocyte-neutrophil cross-talk during ARDS. Our data support that CCR2+ monocytes are a prerequisite for the recruitment and accumulation of CXCR2+ neutrophils following LPS-induced ARDS. In addition, I3C can attenuate ARDS through activation of AhR I3leading to downregulation of CXCR2. Understanding the role of AhR ligands in the monocytes-neutrophil cross-talk may help with the development of novel therapeutics to treat patients suffering from ARDS

Data availability statement

The data presented in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through the GEO Series accession number GSE224938, on the link https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE224938.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

BH: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. KW: Writing – review & editing, Funding acquisition, Formal analysis, Data curation. AC: Writing – review & editing, Data curation. MN: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. PN: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1330373/full#supplementary-material

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Fiber rich food suppressed airway inflammation, GATA3 + Th2 cells, and FcεRIα+ eosinophils in asthma

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Background: Allergic Asthma is a disease presenting various endotypes and no current therapies act curative but alleviate disease symptoms. Dietary interventions are gaining increasing importance in regulating immune responses. Furthermore, short chain fatty acids (SFCA), as the main products of dietary fiber's fermentation by the gut bacteria, ameliorate the pathogenesis and disease burden of different illnesses including asthma. Nevertheless, the connection and crosstalk between the gut and lung is poorly understood.

Objective: In this work, the role of high fiber diet on the development of allergic asthma at baseline and after exacerbation of disease induced by respiratory viruses was investigated.

Methods: Hereby, SCFA in serum of asthmatic and non-asthmatic pre-school children before and after airway disease symptoms were analyzed. Moreover, the effect of high fiber diet *in vivo* in a murine model of house dust mite extract (HDM) induced allergic asthma and in the end in isolated lung and spleen cells infected *ex vivo* with Rhinovirus was analyzed.

Results: In this study, a decrease of the SCFA 3-Hydroxybutyric acid in serum of asthmatic children after symptomatic episodes at convalescent visit as compared to asthmatic and control children at baseline visit was observed. In experimental asthma, in mice fed with high fiber diet, a reduced lung GATA3 + Th2 type mediated inflammation, mucus production and collagen deposition and expression of Fc epsilon receptor Ia (FcεRIa) in eosinophils was observed. By contrast, the CD8+ memory effector T cells were induced in the lungs of asthmatic mice fed with high fiber diet. Then, total lung cells from these asthmatic mice fed with either standard food or with fiber rich food were infected with RV *ex vivo*. Here, RV1b mRNA was found significantly reduced in the lung cells derived from fiber rich food fed mice as compared to those

derived from standard food fed asthmatic mice. Looking for the mechanism, an increase in CD8+ T cells in RV infected spleen cells derived from fiber rich fed asthmatic mice, was observed.

Conclusion: Convalescent preschool asthmatic children after a symptomatic episode have less serum ß-Hydroxybutyric acid as compared to control and asthmatic children at baseline visit. Fiber rich diet associated with anti-inflammatory effects as well as anti-allergic effects by decreasing Type 2 and IgE mediated immune responses and inducing CD8+ memory effector T cells in a murine model of allergic asthma. Finally, *ex vivo* infection with Rhinovirus (RV) of total lung cells from asthmatic mice fed with fiber rich food led to a decreased RV load as compared to mice fed with standard food. Moreover, spleen cells derived from asthmatic mice fed with fiber rich food induced CD8+ T cells after *ex vivo* infection with RV.

Clinical implications: Dietary interventions with increased content in natural fibers like pectins would ameliorate asthma exacerbations. Moreover, respiratory infection in asthma downregulated SCFA in the gut contributing to asthma exacerbations.

KEYWORDS

fiber rich food, allergic asthma, Th2 cells, GATA3, FcεRI, antigen presenting cells, memory T cells

1 Introduction

Asthma is one of the most common chronic respiratory diseases associated with airway inflammation, mucus hyperproduction, remodeling and hyperresponsiveness (AHR) (1). These changes result in bronchoconstriction, thickening of airway walls, eosinophilic infiltration, and decreased lung function (2). Symptoms vary from wheezing and coughing to chest tightness and constant shortness of breath. With the increase of industrialization, more and more people are diagnosed with asthma every year (1). Approximately 300 million people worldwide are suffering from the symptoms of this disease, and the prevalence is increasing by 50% every decade. Moreover, worldwide 180.000 deaths related to asthma are reported annually (3). Although the prevalence of asthma is steadily rising and new therapies developed, the basic mechanisms of the disease are still largely unknown (4).

Asthma is divided into two sub-phenotypes: intrinsic (non-atopic) and extrinsic (atopic) asthma. Whereas atopic asthma is triggered by a range of allergens such as pollen, mold, and dust mites, non-atopic asthma normally occurs with cold weather, exercising, infections, or stress. Since atopic asthma is associated as a T helper 2 immune response driven disease, it is also described as T2-type asthma (4–7). The underlining mechanism has been attributed at dysregulated antigen presentation by DCs leading to pathologic T-cell differentiation into Th2 cells (8–11). These Th2 cells secrete proinflammatory cytokines like IL-4, IL-5, IL-9, and IL-13 and also the granulocyte-macrophage colony-stimulating factor (GM-CSF) (11) as well as chemokines which leads to the activation and recruitment of macrophages, eosinophils, basophils, and mast cells. Mast cells also secrete IL-4, IL-5, and IL-13, which mediate eosinophilic inflammation, IgE synthesis and thus promote Th2 immune responses (10). Specifically, the release of antigen-specific IgE is induced by class-switch of B cells which express the surface low affinity receptor FceRII. Moreover, IgE binds to high affinity receptor FceRI expressed on mast cells, basophils and APCs which eventually results in mast cell degranulation and the release of further pro-inflammatory mediators (12). Besides that, IL-5 mediates activation of eosinophils and IL-13 induces goblet cell hyperplasia and excessive mucus production in conducting airways which cause the symptoms of the allergic immune response (13). Up to 85% of asthmatic patients worldwide are allergic to house dust mites (HDM) (Dermatophagoides Pteronissinus) (14, 15). The allergenicity of HDMs is composed by several components, including their fecal pellets, dust, and mite-associated bacterial and fungal spores (14). Thus, this asthma model which includes several sensitization steps and HDM challenges either intranasally or intraperitoneally, might be important for a better understanding of the pathogenesis of Type 2 asthma and helpful for developing further therapies (15).

It is already known that the microbiota plays a fundamental role in the regulation and formation of the host immune system. Previous studies demonstrated that altered gut microbiota can affect the lung and the airways (16–18). Dysbiotic gut microbial composition can lead to a disruption of immune homeostasis inside and outside the gut. This is evident in patients with gastro-intestinal conditions such as the chronic irritable bowel syndrome. These patients are also at higher risk for developing respiratory diseases. Nevertheless, the crosstalk between lung and intestinal tract is still poorly understood. Furthermore, various external and internal factors affect the

Abbreviations: AHR, Airway hyperresponsiveness; SCFA, Short chain fatty acid; HDM, House dust mite; FcεR, Fc epsilon receptor; Ig, Immunoglobulin; DC, Dendritic cell; MHCII, MHC class II receptor; TEM, Effector memory CD8+ T cell; Th cell, T helper cell; Treg, T regulatory cell; TRM, Tissue resident memory CD8+ T cell; RV, Rhinovirus; NK cell, Natural killer cell.

composition of the intestinal microbiome. These enclose diet, genetics and also age of the host, which lead to altered immune response and disruption of lung homeostasis (19). Thereby, dietary intervention is important in modulating the immune system and therefore a promising therapy option (20). Dietary fibers are associated with lung immune response and have beneficial effects on lung function and inflammation. Dietary fibers are carbohydrates, which consist of soluble and insoluble components. The soluble components are further processed anaerobically through microbes in the intestine by fermentation. The most abundant metabolites made from dietary fiber, also called prebiotics, are termed as short chain fatty acids (SCFAs). SCFAs can locally affect the gut or modulate other organs by distribution to peripheral tissues through the blood system. Prebiotics can be ingested through high fiber diets such as fruits or whole grains and are subsequently degraded by microbes in the gut through carbohydrate active enzymes. SCFAs are then produced in pathways such as the propanediol, acrylate, and succinate pathway (21). Pectin as the main component of plant cell walls belongs to the non-starch polysaccharides, which needs to be digested by microbes in the gut and leads to microbiota-derived increased levels of SCFAs in the human body. With that, pectin shows indirect effects by fermentation and increasing levels of SCFAs, but also direct immunomodulatory effects (22). Human blood sample treated with modified pectin showed an activation of T-cells, B-cells, and NK-cells (23). It was also found that pectin dampens the activation of macrophages and delayed-type hypersensitivity reaction (24). Furthermore, modified pectin decreased IgE-Level production, but not IgG or IgM, in human peripheral blood mononuclear cells (PBMCs) *in vitro* (25) and suppressed hypersensitivity reaction in OVA-sensitized mice (26).

The most abundant SCFAs, which result from fermentation, are acetate, propionate, and butyrate (27–30). High levels of SCFAs are consumed by colonocytes, where some SCFAs enter the bloodstream by passive diffusion or active transport via the proton-coupled monocarboxylate transporter isoform 1 (MCT1) or Na^+-coupled monocarboxylate transporter 1 (SMCT1) and efflux into blood circulation via monocarboxylate transporters MCT3-5 (30). The molecular mechanisms of SCFAs affecting immune cells are binding of SCFAs to G-protein coupled receptors (GPCRs), diffusion channels, and inhibition of histone deacetyl transferases (HDACs). SCFAs connect to and are sensed by GPCRs such as GPR41 (FFAR3), GPR43 (FFAR2), GPR109A, and olfactory receptor 78 (Olfr78) with different sensitivities, which are coupled on signaling pathways (AMP-K, mTOR, STAT3, MAPK, and NF-kB). SCFAs can also be transported via diffusion channels, like the solute carrier family 16 member 1 (Slc) 16a1 and 5a8, directly into cytoplasm where they can interact with various signaling pathways. Via passive diffusion, SCFAs can directly influence HDAC and HAT enzymes and therefore directly regulate gene expression (5). Therefore, SCFAs can mediate anti-inflammatory effects by acting on different targets.

Thus, this study investigated the effect of an increased intake of dietary fiber on asthma pathogenesis. Moreover, in preschool children cohorts Predicta, SCFA in serum after a symptomatic episodes was analyzed. In a murine model of house dust mite induced asthma, the effects of dietary fiber rich food on immune cells, were analyzed. Moreover, lung cells from these asthmatic mice were infected *ex vivo* with Rhinovirus (RV) RV1b, which belongs to the RV-A species (31, 32). A decreased RV load in lung cells derived from fiber rich fed mice and immunological alterations in their *ex vivo* RV infected spleen was found. In summary, these studies would advance current understanding on the effect of dietary natural fibers on the immune system of asthmatics and control subjects at baseline and during asthmatic episodes of asthma exacerbations after respiratory virus infection.

2 Materials and methods

2.1 Pediatric cohort PreDicta

The European study PreDicta (Post-infectious immune reprogramming and its association with persistence and chronicity of respiratory allergic diseases) assessed two cohorts of healthy and asthmatic preschool children aged 4–6 years old. The study was approved by the ethics committee of the Friedrich-Alexander University (FAU) Erlangen-Nürnberg, Germany (Re-No 4435) and is registered in the German Clinical Trials Register (www.germanctr.de: DRKS00004914). The recruitment of the cohorts, the inclusion and exclusion criteria and the data collection were described previously (33). Characteristics of the children analyzed in this study are reported in Table 1. Starting 2011, 22 healthy control and 24 asthmatic children which fulfilled several inclusion criteria: apart from a written informed consent from the child's parents or guardians, the disease of asthma had to be diagnosed within the last 2 years and confirmed by a doctor of the Children's Hospital in Erlangen. The asthmatic disease should be mild to moderate with persistent severity according to the GINA guidelines (2005). Severe or brittle asthma lead to exclusion, as well as other chronic respiratory diseases (cystic fibrosis, bronchopulmonary dysplasia, and immunodeficiency) except allergic rhinitis. Other exclusion criteria were more than six courses of oral steroids during the previous 12 months, other chronic diseases except atopic eczema or chronic medication use. Moreover, children within the control group may not have a history of asthma or wheezing and atopic illness. For this study, only three control and five asthmatic children were analyzed at recruitment (B0). Additionally 11 asthmatic children were analyzed at convalescent visit (see Table 1).

2.2 Timescale

Predicta study is a longitudinal prospective evaluation over a 2-year period. At the baseline visit (B0), whole blood was drawn from the children and collected in heparin tubes for serum isolation (Figure 1A). Furthermore, asthmatic children were invited for additional visits within the next 2 days whenever they notice disease symptoms such as respiratory tract infection or cold, an exacerbation of asthma or a decrease of FEV1 > 15% or decreased PEF > 30% (symptomatic visits). Moreover, blood was collected also at the convalescence visit, 6–8 weeks after asthma exacerbations (C1, C2 Visit). In this study, we analyzed serum from selected children because serum was used for previous studies.

2.3 Experimental murine model of house dust mite induced allergic asthma

Female 4-week-old BALB/c wild-type mice were purchased from Janvier labs and acclimatized for one and a half weeks before the

TABLE 1 Clinical data of the children at the baseline (B0) and convalescent (C) visit controls at B0 (Recruitment visit).

Patient ID	Age at B0 (years)	Gender	Allergic rhinitis	Number of upper respiratory infections in the last 12 months
214	5	Male	No	5.5
218	4	Female	No	2
221	3	Male	No	3

Asthmatics at B0 (Recruitment visit)						
Patient ID	Age at B0 (years)	Phenotype (PRACTALL 2008)	Asthma control (GINA 2009)	Allergic rhinitis	Number of upper respiratory infections in the last 12 months	Treatment
207	5	v, a	Partly controlled	No	2	Steroid
210	6	v	Partly controlled	Yes	10	Non-Steroid
216	5	a,v	Uncontrolled	No	5	Steroid
217	6	a,e,v	Uncontrolled	Yes	4	Steroid
228	5	v	Controlled	Yes	2,5	Non-Steroid
Asthmatics at C1						
201	6	v	Controlled	No	1,5	Steroid
202	6	u	Partly controlled	Yes	5	Steroid
210	6	v	Partly controlled	Yes	10	Non-Steroid
212	5	e,v	Controlled	No	12	Steroid
216	5	a,v	Uncontrolled	No	5	Steroid
223	5	v	Controlled	Yes	8	Steroid
224	4	v	Controlled	Yes	12	Steroid
229	4	v	Controlled	Yes	10	Non-Steroid
230*C2	5	v	Controlled	Yes	4	Non-Steroid
238*C2	4	v	Controlled	Yes	6	Steroid
243	5	v	Uncontrolled	Yes	5	Steroid

A, Allergen induced; v, Virus induced; e, Exercise induced; and u, Unresolved.

beginning of the experiment. The mice were housed as four individuals per cage.

Starting on day 0, the mice were fed with two different diet foods: the control groups received standard maintenance diet for mice (1,324, Altromin Spezialfutter GmbH & Co. KG, Lage, Germany), while the test groups received a crude fiber rich diet modified with 30% Pectin (C1014, Altromin International, Lage, Germany) which resulted in a composition of 200.63 mg/kg crude fiber, 105.02 mg/kg Polysaccharides, and 162.59 mg/kg crude proteins. In contrast to this high fiber diet, standard diet contained 6.1% (60.74 mg/kg) Crude fiber, 391.216 mg/kg Polysaccharides, and 192.11 mg/kg crude proteins. The experiments were approved by the local animal ethics committee Regierung von Unterfranken (AZ: 55.2.2-2532-2-633).

For inducing asthma, the mice were sensitized first with 12.5 µg house dust mite extract (HDM) (Stallergenes Greer Laboratories Inc., Lenoir, United States) in 200 µL PBS intraperitoneally on day 12 and 19. The mice were challenged on days 26, 30, and 33 by applying 125 µg HDM intranasally after anesthetizing the mice briefly with isoflurane. The control groups received PBS instead. On day 35, the lung function was measured and the experiment was terminated.

2.4 Measurement of 3-Hydroxybutyric acid

The concentration of ß-Hydroxybutyric acid in serum samples was measured by using a kit from Dia Sys, diagnostic systems. Diagnostic reagent for quantitative *in vitro* determination of β-Hydroxybutyrate in serum or plasma on photometric systems (Cat. No. Kit size 1 3711 99 10 930 R1.;21 FS*) uses Enzymatic determination with β-Hydroxybutyrate-dehydrogenase. The absorbance at 340 nm is proportional to the β-Hydroxybutyrate concentration in the sample (Supplementary Table E1).

2.5 Flow cytometry staining

After cell isolation from the lungs, spleen, and lymph nodes, the cells were separated and pre-incubated with murine Fc-Block (Cat: 564220, BD Biosciences, Heidelberg, Germany) to prevent unspecific bindings. All staining's for surface markers were performed at 4°C for 15–20 min in the dark. For phenotyping, mast cells, dendritic cells, Th2 and Th1 cells, regulatory T cells, and memory T cells, the antibodies listed in repository Supplementary Table E2 were used.

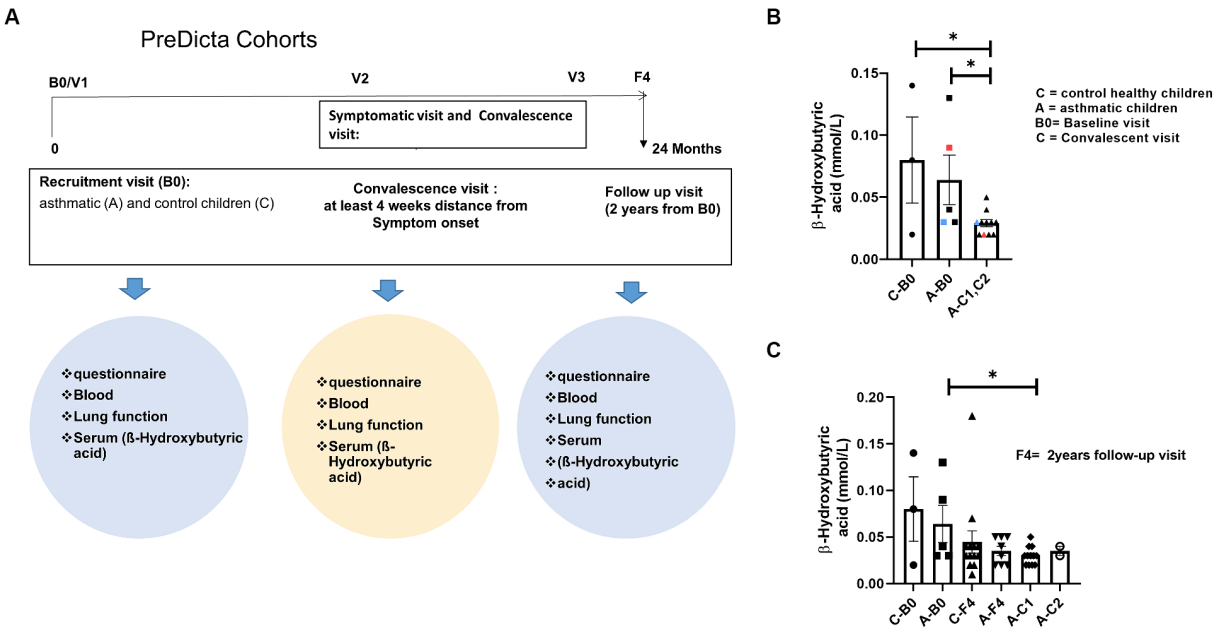

FIGURE 1
Reduced short chain fatty acid (SCFA) in serum of children with asthma after disease exacerbation. **(A)** Experimental design of the pediatric cohort PreDicta. **(B,C)** Measurement of β-Hydroxybutyric acid in the serum of healthy control children and asthmatic children at the baseline (B0) and convalescent visit (C1, C2) at 6–8 weeks after disease exacerbation (**A**—B0; $n = 5$) vs. **A**—C1, C2 ($n = 11$) $p = 0.0218$; **C**—B0 ($n = 3$) vs. **A**—C1, C2 ($n = 11$) $p = 0.0109$. An unpaired t-test was performed to compare the groups; $^*p < 0.05$. All data are shown as mean ± SEM.

For intracellular staining, surface-stained cells were fixed and permeabilized with Fixation/Permeabilization buffer Set (eBioscience, Thermo Fisher Scientific, Waltham, MA, United States) followed by the intracellular staining of the cells at RT for 30 min in the dark. The analysis of the cells was performed on a Flow Cytometer FACS Canto II (BD Biosciences, Heidelberg, Germany). The flow cytometry data were analyzed using FlowJo v10 software (TreeStar Inc., San Jose, CA, United States).

2.6 Histology

For histologic analysis, the lungs were isolated and fixed in formalin overnight. Afterward, the lungs were drained and embedded in paraffin at the pathology department at the university hospital in Erlangen. The samples were cut into 3 µm lung sections using a microtome (Leica, Wetzlar, Germany) and stained with H&E and PAS staining as well as collagen deposition by Sirius red and quantified (9, 34). The lung histologic sections, stained with H&E, were analyzed microscopically and evaluated in the pathology department by a pathologist and by the first author of this manuscript in a blinded manner according to the protocol of Doganci et al. (35). Therefore, a score of zero defines no detectable inflammation, a score of one is characterized by rare to occasional inflammatory cells around isolated peribronchial blood vessels, accumulations of scant inflammatory cells in more than one site is described as two, a score of three is defined as multifocal inflammation around peribronchial vessels, which are easily visible at ×4 magnification and four and as the most severe level, severe peribronchial inflammatory infiltrations at multiple sites (35). Lung sections stained with PAS and Sirius red were also analyzed microscopically and evaluated for mucus production in the bronchi of the mice' lungs and collagen deposition was evaluate semi quantitatively around the bronchi. For histologic analysis of the gut, parts of the colon were fixed it in formalin overnight. It was drained and embedded in paraffin and cut into sections (3 µm). The inflammation score with H&E staining was based on the method of Erben et al. (36). Briefly, the density of leukocytes in the lamina propria was classified as 1 (<10%), 2 (10–25%), 3 (26–50%), and 4 (>50%). The extent of expansion of leukocytes infiltration was counted as 1 (mucosal), 2 (submucosal), 3 (transmural), and the sum of both values was used as inflammatory score (36).

2.7 Rhinovirus infection

After the murine model of HDM induced asthma, lung and splenic cells were used for infection with RV1b. Therefore, 500 µL RV suspension was applied to 10^6 cells and incubated for 1 h at 33°C. After the incubation, cells were washed with 5 mL RPMI without supplements and up taken in cell culture medium. The cells were plated in a 48 well plate and cultured for 96 h. After harvesting the cells were used for RNA isolation and flow cytometry analysis. The mRNA expression of the virus was measured by qPCR with RV1b primers (5′-CCA TCG CTC ACT ATT CAG CAC-3′, 5′-TCT ATC CCG AAC ACA CTG TCC-3′) and normalized using the housekeeping gene hHPRT (5′-TGA CAC TGG CAA AAC AAT GCA-3′, 5′-GGT CCT TTT CAC CAG CAA GCT-3′).

2.8 ELISA

To analyze IL-5 levels in the serum mIL-5 ELISA set OptEIA (Cat. 555,236, BD Biosciences, Heidelberg, Germany) according to the

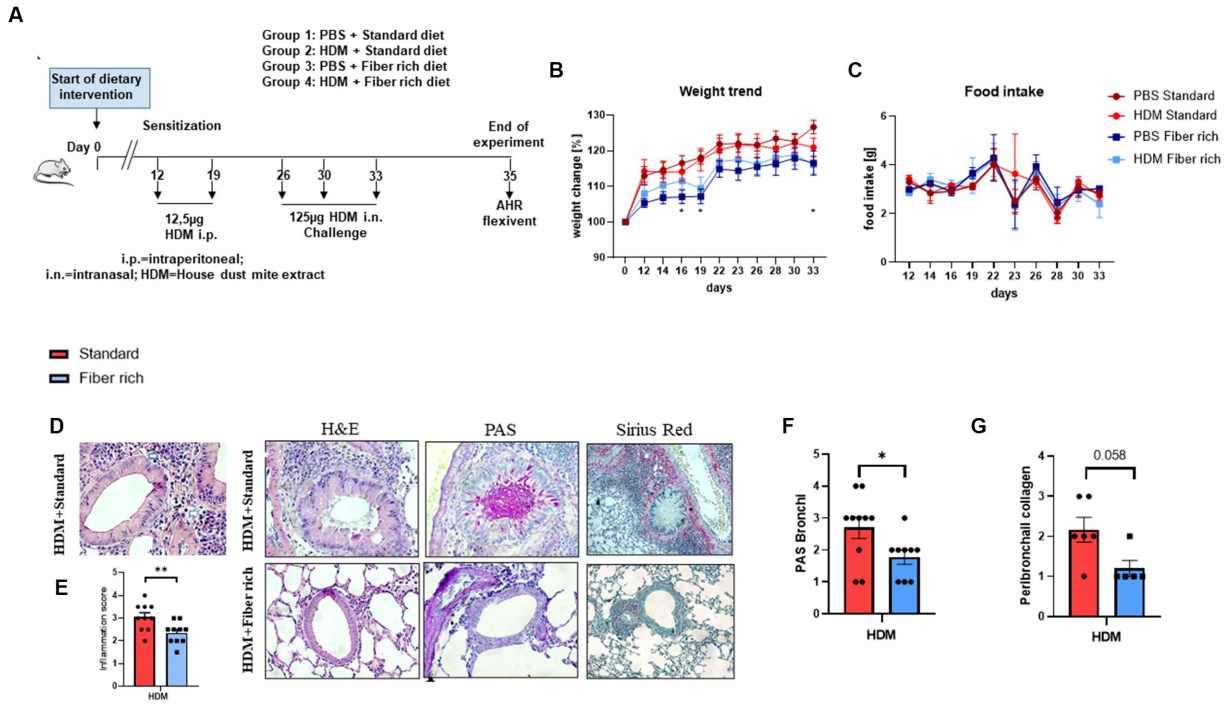

FIGURE 2
Asthmatic mice fed with fiber rich diet have significantly decreased lung inflammation, mucus production, and collagen deposition around the bronchi due to a high fiber diet. **(A)** Schematic presentation of the HDM-induced asthma model. Female 4-week-old Balb/c mice received different diets 1.5 weeks prior to the first HDM sensitization. On day 12 and 19, sensitization was performed by administrating intraperitoneally 12.5 µg HDM extract. On day 26, 30, and 33, the mice were challenged with 125 µg HDM intranasally. **(B)** Weight curves of all groups observed over the experimental time period as compared to the starting weight (100%) (PBS Standard vs. PBS Fiber rich: d16 $p = 0.0371$; d19 $p = 0.011$; d33 $p = 0.0226$; $^{*}p < 0.05$, $n = 10$). **(C)** Food intake of all groups monitored every second day by weighing the food of each cage ($n = 10$). **(D)** Representative histologic pictures taken in sections, stained with Hematoxylin and Eosin stain (left panels; 400×), PAS stain for mucus detection (middle panels; 400×), and with Sirius red-light-green (right panels; 200×). A representative picture for each experimental group is shown here. **(E)** Inflammation score according to Doganci et al. (35): "0: no inflammation; 1: rare occasional inflammatory cells around isolated peribronchial blood vessels; 2: accumulations of scant inflammatory cells around peribronchial vessels in more than one site; 3: multifocal inflammation around peribronchial vessels, easily visible at ×4 magnification; 4: severe peribronchial inflammatory infiltration at multiples sites" ($p = 0.0096$; $^{**}p < 0.01$, $n = 10$ animals per experimental group). **(F)** Lung tissue sections were stained with PAS and analyzed with a microscope at 400× magnification to detect mucus producing goblet cells. Semiquantitative analysis showed a significant reduction in mucus production by fiber rich food in asthmatic mice ($p = 0.038$, $^{*}p < 0.05$; $n = 10$, animals per experimental group). **(G)** Lung tissue sections were stained with Sirius red and fast green staining and analyzed with a microscope at 200× magnification to detect collagen deposition (in red) around the bronchi. Semiquantitative analysis showed a significant reduction in collagen deposition by fiber rich food in asthmatic mice ($p = 0.058$; $n = 6$ and 5). All data are shown as mean ± SEM.

manufacturer's protocol was used. For measuring the total IgE antibody concentration in serum samples, an ELISA with antibodies against murine IgE (Cat. 555,248, BD Biosciences, Heidelberg, Germany) was performed according to the manufacturer's protocol. Absorbance was measured with an ELISA Reader OPSYS MR (DYNEX Technologies, Denkendorf, Germany) at 450 nm.

2.9 Statistical analysis

Statistical analysis was performed using the GraphPad Prism software V8 (GraphPad, La Jolla, CA, United States). All data are shown as mean with the standard error of the mean (SEM). Groups were statistically compared by either two-way ANOVA or unpaired *t*-test. The variables used in this study include continuous variables for Figures 1, 2B,C, 3A,C, 4–7. The variables in Figures 2E,F,G, 3B are categorical variables.

Figure 2E unpaired *t*-test, normal distribution, equal SD $p = 0.0096$;

Figure 2F Mann–Whitney U test $p = 0.043$;

Figure 2G Mann–Whitney U test $p = 0.0584$.

For *post-hoc*, a Sidak's method test (two-way ANOVA) was used. Significant values are regarded at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

3 Results

3.1 Reduced SCFA in the serum of children with asthma after disease exacerbation

The effects of the gut microbiota on asthma are partially mediated by bacteria metabolites, which may influence immune responses in distal parts of the body. The most known metabolites with demonstrated protective properties in human airway inflammation are SCFAs. Children with high amount of butyrate in the feces at 1 year of age are less likely to develop asthma at the age of 3 and 6 (37).

Thus this study asked if SCFA could be detected also in the serum of pre-school children. Therefore, β-Hydroxybutyric acid was measured in serum of the cohorts of pre-school children with and without asthma at recruitment visit into the Study Predicta (Figure 1A).

Here, considering that, airway symptomatic episodes of asthma could affect the SCFA formation by dysregulating gut microbiota and thus at baseline (B0) and convalescent visit, 6–8 weeks after symptomatic episodes, serum SCFA was measured and found that, the asthmatic children at convalescent visit exhibited a significant decrease of SCFA β-Hydroxybutyric acid in serum as compared to asthmatic and control children at the baseline visit (Figure 1B) (A-B0; $n = 5$) vs. A-C1, C2 ($n = 11$) $p = 0.0218$; C-B0 ($n = 3$) vs. A-C1, C2 ($n = 11$) $p = 0.0109$. In colors, paired data from the same child are shown. Further, the Predicta children were recruited 2 years later (F4) and asthmatic children were additionally analyzed after symptomatic visit at convalescent visit (A-C1, AC2) (Figure 1C). Also in this case we could confirm a decrease of β-Hydroxybutyric acid in serum of the asthmatic pre-school children at convalescence visit as compared to control children (Figure 1C). These data indicate, that exacerbation of disease in the airway could contribute to dysregulate the gut microbiota.

3.2 Asthmatic mice fed with a fiber rich diet have decreased asthma airway features

To further investigate the importance of a high fiber diet in asthma *in vivo*, a murine asthma model with HDM in combination with different diets with different fiber composition was set up. Therefore, 4-week-old Balb/c female mice received either fiber rich food modified with 30% pectin, or a standard diet as a negative control, one and a half weeks before the asthma model started (Figure 2A). To record weight changes and the food intake of all experimental groups, the mice and the food were weighed every second day during the experimental period. To calculate the weight change, the weight on the first day of the experiment was set up as 100%. Body weight was calculated with current weight/start weight. Thereby, lower body weights for mice that received the fiber rich diet compared to the mice that received standard diet was observed (Figure 2B) (PBS Standard vs. PBS Fiber rich: d16 $p = 0.0371$; d19 $p = 0.011$; d33 $p = 0.0226$).

Considering the food intake, a regression on day 23 and 26 was observed for all groups excluding at day 23 for the HDM treated mice fed with standard diet (Figure 2C).

To classify the severity of the HDM induced airway inflammation, lymphocytes and granulocytes infiltrations in lung tissue were analyzed microscopically at higher magnification. Therefore, sections of lung tissue derived from PBS and HDM treated mice which received either standard or fiber rich diet were stained with Hematoxylin and Eosin (H&E) to determine the severity of inflammation (Figure 2D, left hand side panels). The HDM treated mice fed with the standard diet showed an increase in infiltration of eosinophils as can be appreciated (Figures 2D,E, upper left panel). Furthermore, the HDM treated mice which received fiber rich food showed significantly less inflammation than the HDM treated mice fed with standard diet (Figures 2D,E, lower left panel) ($p = 0.0096$; $^{**}p < 0.01$, $n = 10$ animals per experimental group). Looking at the mucus production in the lungs of the mice by staining lung tissue sections with PAS (Figure 2D, middle panels), a visible and also significant decrease in mucus producing cells in the lungs of HDM treated mice which were fed with fiber rich food was detected (Figures 2D,F) ($p = 0.038$, $^{*}p < 0.05$; $n = 10$ animals per experimental group). Finally, collagen deposition by Sirius red staining analysis showed a decrease of collagen around the bronchi of fiber rich fed mice (Figure 2D, right panel and Figure 2G) ($p = 0.058$ $n = 6$ and 5).

3.3 Dietary fiber rich food induced serum concentration of 3-Hydroxybutyric acid in asthmatic mice

Next, the differences between the two types of diet were analyzed in more detail. Both diets contain crude fibers (Figure 3A). Fiber rich diet increases with 130% crude fiber in comparison to standard diet. Also, crude fat increases to 140% and crude protein appeared to be 15% less in fiber rich diet. The specific ingredients of the fiber rich diet (C1014-diet-Altromin) with 30% pectin can be found online. This diet includes the following raw materials: Sojamin, Corn starch, Cellulose, Sunflower oil, Premix of minerals and trace elements, Premix of vitamins, Pectin. Additionally, the inflammation score in colon sections stained with H&E did not change with diet changes (Figure 3B).

To examine the effects of the increased intake of dietary fiber, a closer look at serum concentration of 3-Hydroxybutyric acid in asthmatic mice was taken. 3-Hydroxybutyric acid, also named as β-Hydroxybutyric acid, is a metabolite that derives from butyric acid which is produced in the liver. Fiber rich diet caused a significant increase of the concentration of 3-Hydroxybutyric acid (Figure 3C) ($p = 0.040$).

3.4 Fiber rich food significantly decreased FcεRIa+ eosinophils in the lungs of naïve and asthmatic mice

Moreover, a significant decrease in FcεRI on non-lymphocytes in the PBS and asthmatic group fed with fiber rich food was found (Figures 4A,B). Second antigen contact leads to crosslinking of IgE bound on mast cells, resulting in mast cell degranulation, with release of histamine and other preformed bronchoconstrictors resulting in asthma exacerbations. Mast cells were defined as non-lymphocytes and further gated by CD49b, CD11b, c-kit (CD117), and FcεRIa surface antibodies (Supplementary Table E2, Supplementary Figures S1A,B). No significant changes in mast cell numbers were observed between different diets in naïve and asthmatic mice. The IgE level in serum was found highly increased in HDM treated mice fed with standard as well as fiber rich diet compared to the control groups (Figure 4C). However, the number of eosinophils expressing FcεRIa significantly decreased in the lungs of fiber rich fed mice (Figure 4D) plots (PBS Standard vs. PBS Fiber rich $p = 0.0472$; HDM Standard vs. HDM Fiber rich $p < 0.0001$). However, IL-5 did not change in the serum of fiber rich fed asthmatic mice (Figure 4E). In summary, the fiber rich food resulted in decreased FcεRI in eosinophil expression. Further analysis on eosinophil function should be performed in future studies.

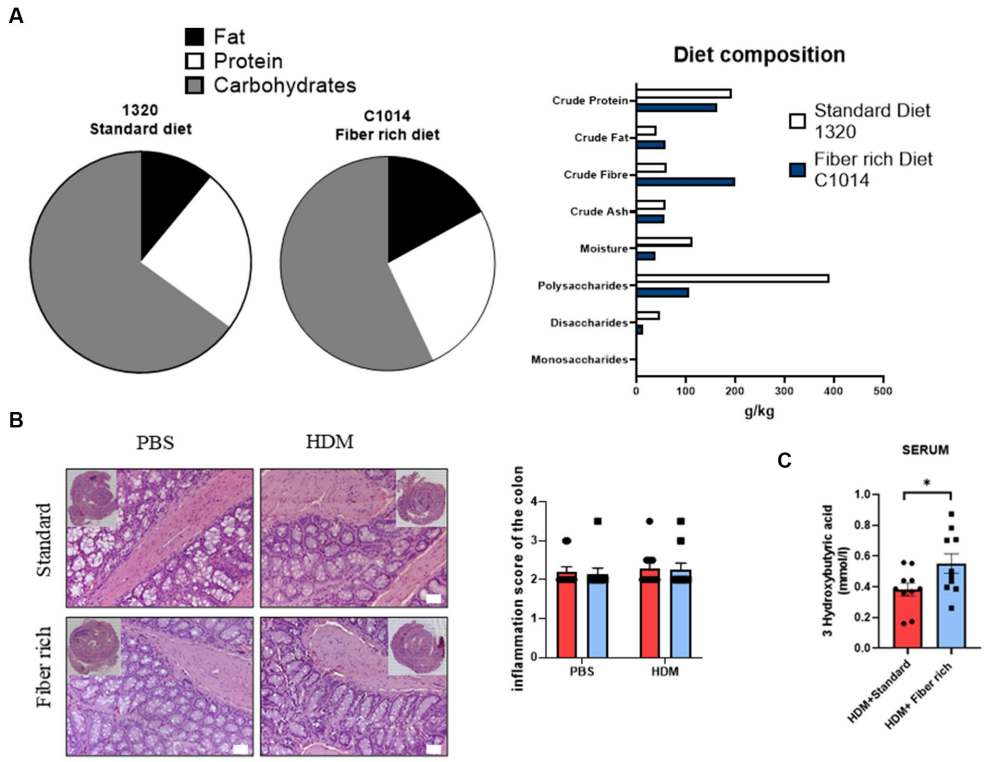

FIGURE 3
Asthmatic mice fed with fiber rich diet have induced SCFA (3-Hydroxybutyric acid) in serum as compared to asthmatic mice fed with standard diet. **(A)** Diet composition in accordance to the Altromin homepage informations. As standard control diet, the diet catalog number 1320 was used and for fiber rich diet C1004 was used. **(B)** Inflammation score of the colon analyzed according to Erben et al. (36). Representative pictures of Hematoxylin and Eosin stained gut sections are shown. **(C)** Measurements of 3-Hydroxybutyric acid in serum of asthmatic mice is shown ($p = 0.040$). Data are expressed as means ± SEM. The data were analyzed by unpaired t-test. *$p < 0.05$, $n = 10$ animals per group.

3.5 Fiber rich diet decreased the number of antigen presenting dendritic cells

Dendritic cells process antigens, present them to T effector cells and therefore regulate T cell activation including the activation of Th2 cells, which are key players in allergic immune responses. Here, classical dendritic cells of the lung stained with antibodies against F4/80, CD64, CD11c, CD8, and CD11b to differ between CD8+ and CD11b + DCs (Supplementary Table E2; Figure 5A) were analyzed. Here a significant decrease of CD11b + cDC2 in asthmatic mice fed with fiber rich food was found (Figure 5B). However, no differences in the numbers of CD8+ cDC1 could be detected (Figure 5C). These data indicate a reduced antigen presenting capability of lung DCs in asthmatic mice fed with fiber rich diet.

3.6 Fiber rich food significantly reduced the number of GATA3+ Th2 cells in HDM treated mice

Asthma shows a pathologic expansion of Th2 cells. To determine Th2 cells via flow cytometric analysis, cells were stained with antibodies against CD3, CD4, and GATA3 (Supplementary Table E2). It has also been reported that an imbalance of Th1/Th2 cells toward Th2 contributes to the pathogenesis of asthma (23–25). Here, although the CD4+ T cells did not change (Figure 6A), the number of GATA3+ CD3+ CD4+ cells was significantly decreased by fiber rich diet in asthmatic mice compared to HDM treated mice which received standard diet (Figures 6B,C) ($p = 0.00338$). Moreover, Th1 cells were analyzed as CD3, CD4, and Tbet+. However, there were no differences in Th1 cells due to fiber rich diet (Figure 6D). In conclusion, the anti-inflammatory effect observed in fiber rich diet is probably dependent on GATA3+ T cell downregulation in the airways of asthmatic mice.

3.7 Changes in CD4+CD25+Foxp3+ CD103+ Treg in the lymph nodes of HDM treated mice due to a fiber rich diet

T regulatory cells (Tregs) are important for the immunosuppressive anti-inflammatory immune response. To define Tregs in flow cytometric analysis, total lung isolates and lymph nodes were gated as CD3+, CD4+, CD25+ and FoxP3+ cells (Supplementary Table E1, Figure 6E and Supplementary Figure S2). However, no difference in T Tregs were observed among the four groups. Furthermore, we analyzed CD103+ regulatory T cells in lymph nodes (Figure 6F) ($p = 0.0139$). This subset is supposed to suppress airway inflammation by producing high levels of immunosuppressive IL-10.

In lymph nodes a decrease in CD4+ CD103+ Foxp3+ T cells was detected in the PBS group due to fiber rich diet (Figure 6F).

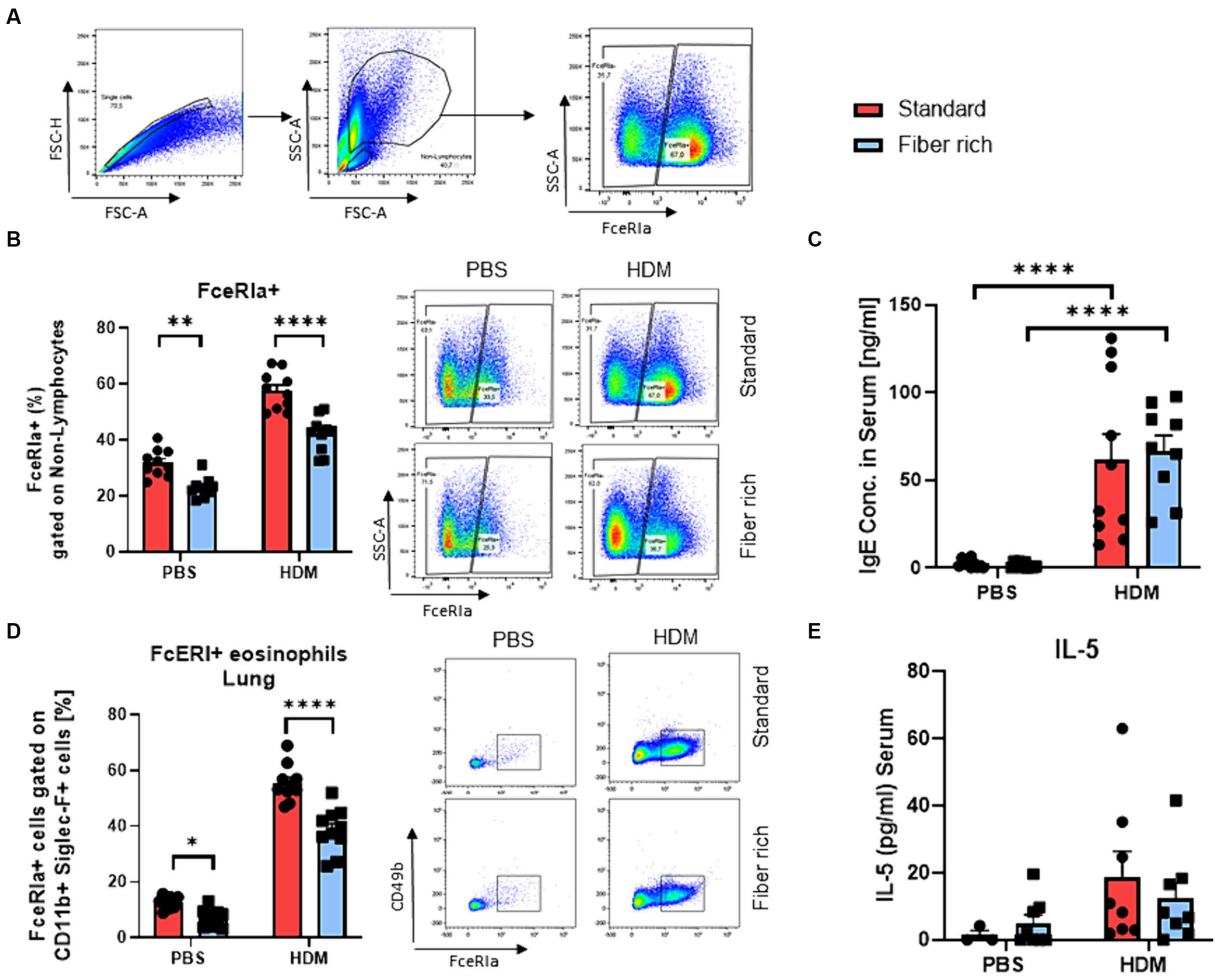

FIGURE 4
Fiber rich food significantly decreases the number of FcεRIa + Eosinophils in the lungs of naïve and asthmatic mice. **(A)** Gating strategy for flow cytometry analysis of lung mast cells. **(B)** Analysis of FcεRIa expression on non-lymphocytes via flow cytometry. (PBS Standard vs. PBS Fiber rich; $p = 0.02$, HDM Standard vs. HDM Fiber rich; $p = 0.0001$). A representative dot plot is shown. **(C)** ELISA analysis of total IgE in serum of PBS and HDM treated mice (PBS Standard vs. HDM Standard $p < 0.001$; PBS Fiber rich vs. HDM Fiber rich $p < 0.001$). **(D)** Analysis of lung FcεRIa+ eosinophils along with representative dot plots (PBS Standard vs. PBS Fiber rich $p = 0.0472$; HDM Standard vs. HDM Fiber rich $p < 0.0001$). **(E)** Serum IL-5 Elisa. Data are expressed as means ± SEM. The data were analyzed by a two-way ANOVA and Sidak's multiple comparisons test. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$, $n = 10$ animals per group.

3.8 T memory effector cells are induced by high fiber diet in asthmatic mice

Besides CD4+ effector T cells, also CD8+ effector T cells play an important role in the pathogenesis of asthma. To determine the subsets of CD8+ T cells, total lung cells were stained with antibodies against CD3, CD8, CD62L, CD197, and CD103 (Supplementary Table E2). CD103 is the determining marker for tissue resident memory CD8+ T cells (TRM) from effector memory CD8+ T cells (TEM). In general, a decrease in CD8+ T cell subsets in HDM induced asthma was observed (Figure 7A). Moreover, the number of pulmonary CD8+ T cells and TRMs did not change in asthmatic mice fed with fiber rich food (Figure 7B). By contrast, a significant induction of TEMs was detected in the lung of asthmatic mice fed with a fiber rich food compared to HDM treated mice which received standard diet (Figure 7C) ($p = 0.036$).

3.9 Fiber rich food diet controlled RV replication in the lung cells and induced CD8+ T cells in the spleen

In asthmatic children, symptomatic exacerbation of the disease are often associated with RV infection in their airways. Thus to mimic asthma exacerbation of disease, total lung cells from asthmatic mice fed with standard food or with fiber rich food were infected with RV (Figure 8A). Here, RV1b mRNA was significantly reduced in the lung cells from fiber rich food fed asthmatic mice as compared to those from standard food fed asthmatic mice (Figure 8B) ($p = 0.0009$). Looking for the underlying mechanism, an increase in CD8+ T cells in RV infected spleen cells from fiber rich fed mice was observed (Figure 8C) (HDM Standard RV vs. HDM Fiber rich RV $p = 0.0024$; HDM Fiber rich unst. vs. HDM Fiber rich RV $p = 0.0284$).

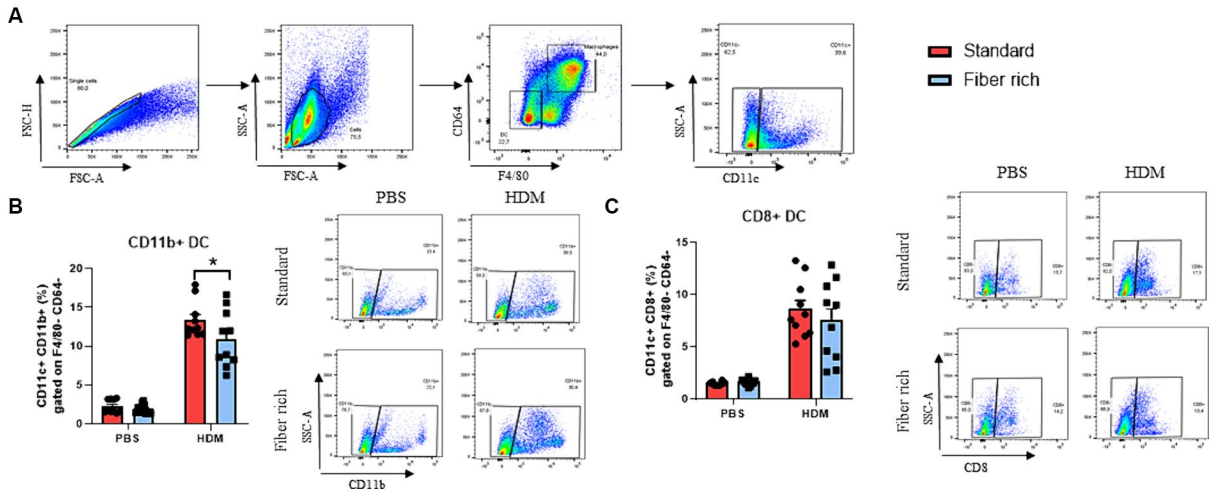

FIGURE 5
Fiber rich diet decreases the number of antigen presenting dendritic cells. **(A)** Gating strategy for flow cytometry analysis of lung dendritic cell subsets. **(B)** Percentages of CD11b + DC in the lungs of PBS and HDM animals ($p = 0.0427$). **(C)** Percentages of CD8+ DC in the lungs of PBS and HDM animals. Representative dot plots are shown. All data are presented as mean ± SEM. Representative dot plots are shown. The data was analyzed by two-way ANOVA, Sidak's multiple comparisons test. *$p < 0.05$, $n = 10$ animals per group.

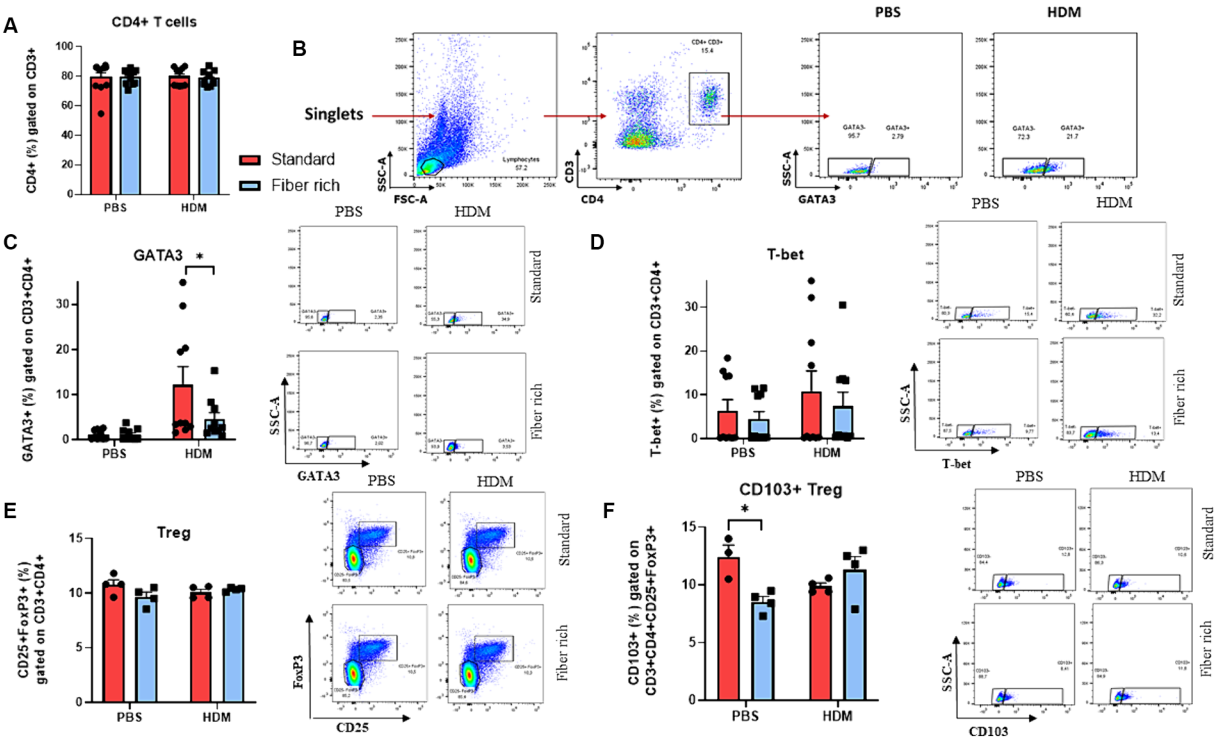

FIGURE 6
Suppression of GATA3+ CD4+ T cells in the lungs and changes in CD4 + CD25 + FoxP3 + CD103+ Tregs in the lymph nodes due to a fiber rich diet. **(A)** Analysis of CD4+ T cells in the lymph nodes. **(B)** Gating strategy of GATA3+ CD4+ CD3+ Th2 cells in the lung. **(C)** Flow cytometry analysis of GATA3+ CD4+ CD3+ Th2 cells in the lungs ($p = 0.00338$). **(D)** Flow cytometry analysis of T-bet+ CD4+ CD3+ Th1 cells in the lungs. **(E)** Flow cytometry analysis of CD4 + CD25 + FoxP3+ Tregs in the lungs of PBS and HDM mice. **(F)** Analysis of CD103+ Treg in the lymph nodes of asthmatic mice fed with high fiber diet or standard diet ($p = 0.0139$). All data are presented as mean ± SEM. Representative dot plots are shown. The data was analyzed by two-way ANOVA, Sidak's multiple comparisons test. *$p < 0.05$, $n = 4–10$ animals per group.

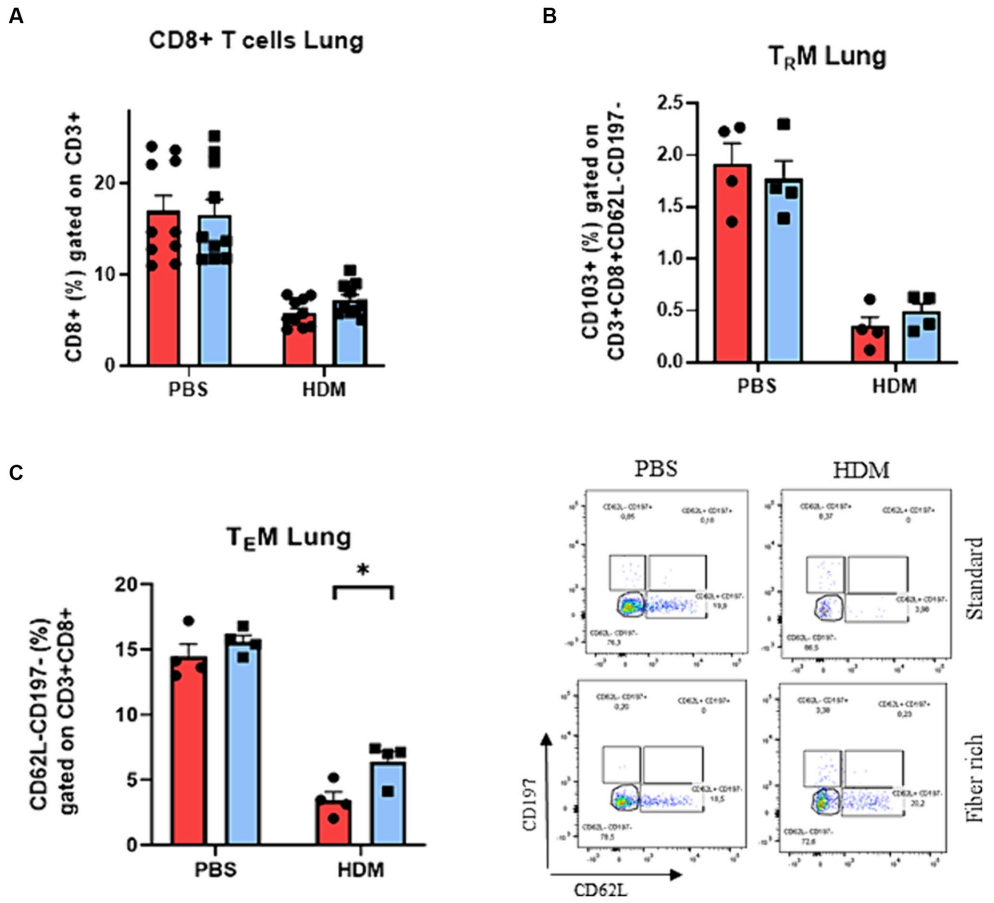

FIGURE 7
Increase of pulmonary TEMs of asthmatic mice fed with fiber rich diet. **(A)** Flow cytometry analysis of lung CD3 + CD8+ T cells. **(B)** Flow cytometry analysis of lung TRM characterized by CD103 expression (CD103 + CD62L-CD197-CD3 + CD8+). **(C)** Analysis of pulmonary TEMs (CD62L-CD197-CD3 + CD8+) via flow cytometry ($p = 0.036$). Representative dot plots are shown. All data are presented as mean ± SEM. Data are analyzed statistically by two-way ANOVA and Sidak's multiple comparisons test. *$p < 0.05$, $n = 4–10$ animals per group.

4 Discussion

It has been previously reported that dietary fibers may alter the gut microbiome and, thereby suppress systemic inflammation thus contributing to the resolution of asthma (21). Moreover, SCFAs which are produced by the gut microbiome by dietary fiber's fermentation, can mediate anti-inflammatory effects of fiber rich food and hence ameliorate the pathogenesis of a range of diseases including asthma and allergic diseases (27). To investigate SCFA influence in asthma during exacerbations, SCFA was measured in the serum of pre-school children before and after asthma exacerbation at baseline and after symptomatic visit at convalescent visit as well as at the follow up visit. Here, SCFA concentration dropped in the serum of asthmatic children after disease exacerbations as compared to their baseline levels and to the level of control children. Interestingly, all the asthmatic children analyzed had a virus induced asthma. This indicated to us a possible influence of the respiratory infection on the gut microbiome composition during symptomatic episodes of asthma. Specifically, the gut microbiome regulated by respiratory viruses would reduce the fermentation of dietary fiber by inducing gut dysbiosis and thus downregulating the SCFA production in serum. The limitation of this observation is the small number of children analyzed. Thus, additional studies in enlarged cohorts should be undertaken with detailed microbiome and metabolome analysis. In experimental model of disease, lung inflammation was decreased in fiber rich fed asthmatic mice. This observation is consistent with the findings of Trompette et al. (21). Microscopically, a reduction of eosinophils, mucus and collagen production surrounding the bronchial wall was observed in asthmatic mice fed with fiber rich food.

Further investigations on lung function with mice fed a high fiber diet thus would be useful. In our model, dietary fiber reduced the body weight. This is consistent with the findings of Drew et al., where the intake of dietary fiber prevented obesity in mice with high fat diet (38). Also in humans, the consumption of dietary fiber is associated with reduced body weight and body fat (39). Accordingly, SCFAs were shown to stimulate the intestinal hormone peptide YY (PYY) by active action of FFAR3 which induces a feeling of satiety and therefore leads to reduced food intake (40). Moreover, fiber rich food induces changes in the gut microbiome resulting in the release of SCFA in the blood which influence inflammatory reaction going on in the lung after allergen exposure. Consistently, in asthmatic mice fed with fiber rich diet, a significant increase in serum 3-Hydroxybutyric acid was found. 3-Hydroxybutyric acid, which is also named as β-Hydroxybutyric acid, was found to be the major ketone body produced in the liver

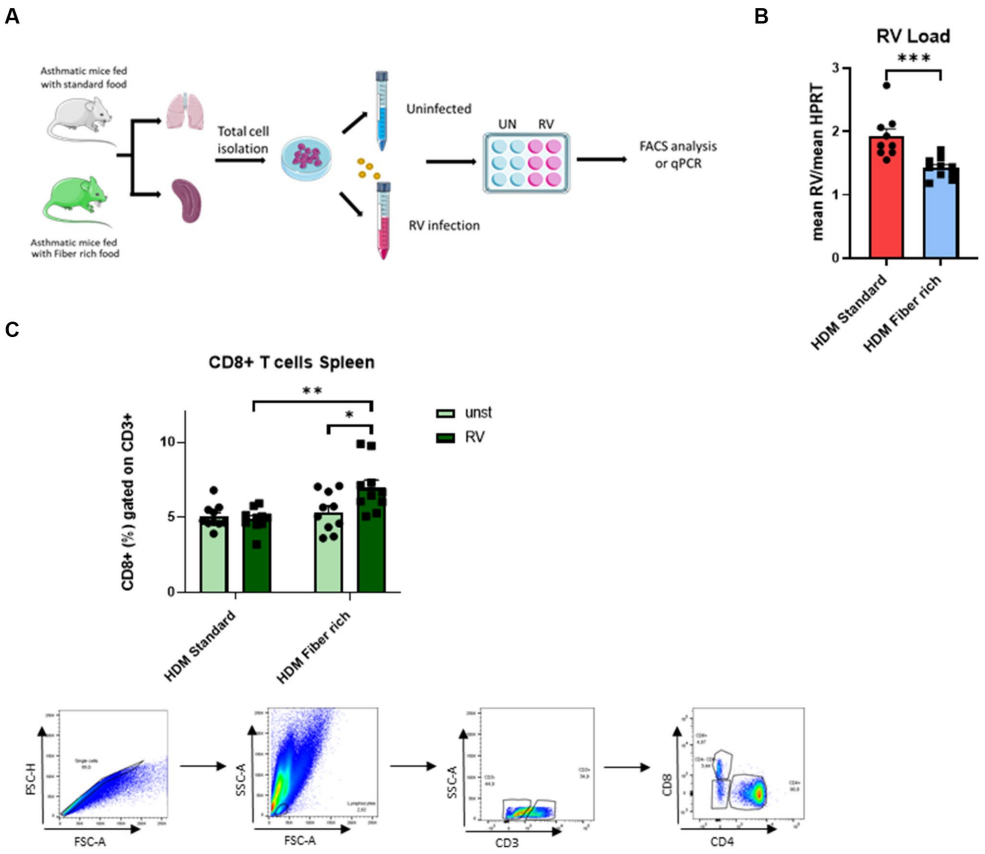

FIGURE 8
Induction of splenic CD8+ cells primed by fiber rich diet upon RV infection in asthmatic mice. **(A)** Experimental design for cell culture experiments. Lung and spleen cells from control or asthmatic mice fed with standard or fiber rich food were additionally infected with RV or remain uninfected and then incubated for 4 days at 37°C, 5% CO2. Afterward, the cells were harvested for flow cytometry analysis or RV mRNA analysis with qPCR. **(B)** RV mRNA in the infected lung cells was quantified by qPCR and normalized to reference gene HPRT ($p = 0.0009$). **(C)** Flow cytometry analysis of CD8+ T cells in total splenic cells infected with RV for 4 days or without infection (HDM Standard RV vs. HDM Fiber rich RV $p = 0.0024$; HDM Fiber rich unst. vs. HDM Fiber rich RV $p = 0.0284$) and gating strategy for flow cytometry analysis of spleen CD8+ T cells. The data were analyzed by two-way ANOVA, Sidak's multiple comparisons test. $*p < 0.05$; $**p < 0.01$, $***p < 0.005$; $n = 7–10$ per group.

from fatty acids as a result of low carbohydrate/low calorie diet, which modulates the gut microbiota into "keto microbiota" (41). The pathways for ketone bodies are mainly ketogenesis and ketolysis and thus, take place in the mitochondrial matrix of hepatocytes. Free fatty acids are released from tissue under low insulin conditions and are then degraded to ketone bodies as ß-Hydroxybutyrate and acetoacetate after they are released into extrahepatic tissues for ketolysis. Ketone bodies also seem to show epigenetic modulation such as working as HDACs and exert anti-inflammatory effects providing potential for therapy in asthma (42). This finding is consistent with the increased concentration of 3-Hydroxybutyric acid since free fatty acids lead to the production of ketone bodies by ketogenesis (42). It has also been reported a direct correlation between β-Hydroxybutyrate and SCFAs. Therefore, 3-Hydroxybutyrate does not only take part in regulating cellular processes but also in altering the gut microbiome and inducing butyrogenesis. Sasaki et al. reported, that the consumption of β-Hydroxybutyrate leads to an increased production of SCFAs by gut microbiota (43). These data are consistent with our human data which might indicate that symptomatic exacerbation of the disease is accompanied with a long-lasting effect on SCFA production systemically that might affect the resolution of the disease.

To uncover the SCFA target cells in the lung, the FcεRIa expression which mediates the IgE cross-linking on mast cells, but is also present in basophils and other cells of innate responses was analyzed (44). Wang et al. described a decrease in degranulated mast cells and the release of inflammatory mediators in weaned pigs by administration of butyrate (45). In our model, a reduction of FcεRIa expression on eosinophils in the lungs of asthmatic mice fed with fiber rich food was detected. Nevertheless, when looking at the total IgE in serum of the mice, no reduction by dietary fiber fed asthmatic mice was observed. This stands in contrast to previous findings of Trompette et al. where IgE levels in serum are significantly reduced in mice fed with pectin rich diet (21). The differences could relate to the different models. In fact, Tropette's studies were done in intranasal HDM challenge without intraperitoneal immunization. In conclusion, FcεRIa is significantly reduced by high fiber diet on eosinophils, whereas IgE remains unaltered. This might indicate decreased binding of IgE molecules to FcεRI receptor which is associated with less crosslinking and mast cell, basophil and eosinophil degranulation. In fact, crosslinking of FceRIa on eosinophils could lead to release of cytotoxic mediators responsible of the damage of the airway epithelial barrier seen in asthma. Thus, fiber rich food would also protect the airway epithelial barrier and thus reduce disease exacebations.Consistently

with previous reports, a significant suppression of GATA3, the main Th2 transcription factor, expressing CD3+ CD4+ T cells in the lungs of asthmatic mice which were fed with dietary fiber was found (46, 47). It can therefore be assumed that Th2 cell response is suppressed by dietary fiber as a result of less antigen presentation of DCs leading to reduced airway inflammation. Cait et al. also showed effects of SCFAs produced by fermentation of dietary fiber modulating immune responses by dampening Th2 response in OVA treated mice which received vancomycin and SCFA treatment (46). Previous studies of Kespohl et al. reported a decrease of GATA3 expression in T cells via qPCR by treatment of 1 mM butyrate (47). It can be therefore assumed that Th2 cell response is suppressed by dietary fiber as a result of less antigen presentation of DCs and induction of Tregs leading to enhanced airway inflammation. In our study, significant suppression of cDC2 (CD11b + CD11c+) DC subset by dietary fiber was observed. This is consistent with the published anti-inflammatory effects of dietary fiber and the derived SCFAs. Dietary fiber may suppress airway inflammation by alteration of the gut microbiome and therefore inhibit the function of DCs by increasing circulating SCFA levels (22). It has been also reported that the administration of butyrate, decreases the ability of DC to prime Th2 response in dysbiotic mice (46). Accordingly, dietary fiber seems to suppress especially the Th2 priming cDC2 fraction thus, might inhibit their migration to lymph nodes and therefore suppress the ability to activate T lymphocytes toward the Th2 lineage. It has been reported that a lack of Tregs or Treg dysfunction might not effectively suppress Th2 immune response, allergic diseases and thus asthma can develop (48). Tregs expressing the integrin CD103 have been identified as high IL-10 producing Tregs which also seem to demonstrate enhanced suppression for Th2 mediated allergic responses (49). In our studies, no changes in Tregs in asthmatic mice due to a fiber rich diet were detected.

Antigen presenting cells are required to prime CD8+ T cells for involvement in immune responses against pathogens and viral infections. Naïve CD8+ T cells are therefore activated and express T cell receptors. In our study, dietary fibers affected CD8+ effector cells and thus, influenced the capacity of CD8+ effector T cells to differentiate into CD8+ memory cells. TRMs unlike TEMs are located in specific tissues, especially mucus organs as the lungs or intestine, whereas TEMs recirculate through non-lymphoid tissues. TRMs promote the secretion of the pro-inflammatory cytokine IL-2. However, they are also able to produce IL-10, which leads to dampened inflammation and the resulting tissue damage. TEMs are at first case able to fight against infections which develop in peripheral tissues because of their ability to localize in those tissues and also due to their cytotoxicity (50). Nevertheless, Marsland et al. reported that CD8+ memory T cells may play an important role not only during viral infections, but also in allergic airway inflammation. CD8+ memory T cells therefore seem to inhibit allergen-induced airway inflammation by producing TGF-ß which acts suppressive against immune responses, reduce activation level, AHR and Th2 cytokine production (51). Previous studies, which investigated the dietary regulation on memory T cells, also showed that the microbiota altered by dietary fiber is also able to influence the development of memory CD8+ T cells. This was demonstrated by mice which received a 35% crude fiber diet. Mice with the high intake of dietary fiber showed an increased development of CD8+ memory T cells during a herpes-simplex virus infection (52). A consistent increase in TEMs in the lungs in asthmatic mice which was mediated by fiber rich diet was found. Finally, a decreased RV replication induced in the lung cells from fiber rich fed mice was detected. Further, this anti-viral effect in the lungs was associated to induction of cytotoxic CD8+ T cells. Regarding this, Trompette et al. reported a promotion of differentiation and activation of CD8+ T cell response by dietary fiber as well as derived SCFAs during influenza virus infection by activation of FFAR3 and altered metabolism in fatty acid oxidation (53). So dietary fiber or as well as derived SCFAs seem to increase antiviral immunity through CD8+ T cell activation. The ability of high fiber intake to modulate immune defense against respiratory viral infections also displays the measured virus load. Further, a significant decrease of virus load in lung after 4 days of infection which was mediated by a fiber rich diet was detected in this study. This is also consistent with the findings of Trompette et al., who described a decreased viral load in mice fed with high fiber diet after 8 and 10 days of influenza infection (53). This further indicates the beneficial effects of dietary fiber derived SCFAs which modulate immune response during allergic and also virus induced airway inflammation.

In summary, SCFA production might play an important role during virus mediated exacerbation of asthma in preschool children as this pathway is suppressed by the respiratory infection. This is a novel finding that needs to be followed up in enlarged cohorts. Consistently, fiber rich diet had resolving effects on airway inflammation, mucus production and collagen deposition and local Th2 response during experimental asthma development by reducing antigen presentation of cDC2, the GATA3 Th2 effector cells. Moreover, a significant downregulation of lung eosinophils carrying the FcεRIa was observed. Additionally, CD8+ effector memory T cells were boosted by fiber rich diet. This was also confirmed in a model of RV infection *ex vivo*. Although this study should be expanded to more specific cellular analysis, it demonstrates a need for clinical investigations on the effects of fiber rich diet on the asthmatic burden of human patients as a possible supportive therapy option especially during asthma exacerbations.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The European study PreDicta (Post-infectious immune reprogramming and its association with persistence and chronicity of respiratory allergic diseases) assessed two cohorts of healthy and asthmatic preschool children aged 4–6 years old. The study was approved by the ethics committee of the Friedrich-Alexander University (FAU) Erlangen-Nürnberg, Germany (Re-No 4435) and is registered in the German Clinical Trials Register (www.germanctr.de: DRKS00004914). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. The experiments were approved by the local animal ethics committee Regierung von Unterfranken (AZ:

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Author contributions

AS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. AG: Methodology, Writing – review & editing. EN: Methodology, Writing – review & editing. ZY: Formal analysis, Investigation, Methodology, Software, Writing – review & editing. SK: Formal analysis, Investigation, Methodology, Software, Visualization, Writing – review & editing. AL: Methodology, Writing – review & editing. A-KB: Methodology, Writing – review & editing. ST: Methodology, Writing – review & editing. SM: Methodology, Writing – review & editing. MR: Methodology, Writing – review & editing. CG: Methodology, Writing – review & editing. PT: Methodology, Writing – review & editing. KH: Methodology, Writing – review & editing. RR: Methodology, Writing – review & editing. OS: Methodology, Writing – review & editing. SZ: Formal analysis, Methodology, Writing – review & editing. SF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

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Association between protein intake, serum albumin and blood eosinophil in US asthmatic adults

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Background: Presently, numerous studies have indicated that protein consumption and levels of blood albumin serve as important biomarkers for a range of respiratory illnesses. However, there have been few investigations into the correlation between protein consumption, serum albumin, and asthma.

Methods: Our analysis incorporated 2509 asthmatics from the 2011–2018 NHANES dataset. The investigation employed three linear regression models and XGBoost model to investigate the potential link between protein intake, serum albumin levels, and blood eosinophil counts (BEOC) in patients with asthma. The trend test, generalized additive model (GAM), and threshold effect model were utilized to validate this correlation. As well, we undertook stratified analyses to look at the correlation of serum albumin with BEOC among distinct populations.

Results: In the univariable regression model, which did not account for any covariates, we observed a positive correlation between protein intake and BEOC. However, univariable and multivariable regression analyses all suggested a negative connection of serum albumin with BEOC in asthma populations. In Model C, which took into account all possible factors, BEOC dropped by 2.82 cells/uL for every unit increase in serum albumin (g/L). Additionally, the GAM and threshold effect model validated that serum albumin and BEOC showed an inverted U-shaped correlation.

Conclusion: Our investigation discovered there was no independent link between asthmatics' protein intake and BEOC. However, we observed an inverted U-shaped relationship between serum albumin levels and BEOC, suggesting a possible relationship between the overall nutritional status of asthmatics and immune system changes. Our findings provide new directions for future research in the field of asthma management and therapy.

KEYWORDS

protein intake, albumin, eosinophil, asthma, National Health and Nutrition Examination Survey (NHANES), XGBoost

1 Introduction

Asthma, which manifests as a clinical syndrome of inflammation, bronchial hyperresponsiveness, and reversible airflow obstruction, is a prevalent chronic airway disease (1). The prevalence of asthma has witnessed a significant rise in several nations over the last few decades. At present, the prevalence estimates for asthma among children and adults in the United States stand at 10.9% and 18.5%, respectively (2). Asthma was responsible for around 1.6 million visits to emergency departments and 183,000 hospitalizations in the United States in 2017, according to the survey (3). This caused a significant economic burden and a considerable number of missed school days.

Airway eosinophilia is a frequent clinical manifestation of asthma, a chronic inflammatory disease of the airways (4). Eosinophils play a role in multiple pathological processes, including airflow obstruction in asthma and chronic airway remodeling (5, 6). These processes include smooth muscle hypertrophy, neural plasticity, epithelial injury, and impaired tissue repair. The blood eosinophil count is a widely recognized and readily available biomarker that is of paramount importance in the management of asthma (7, 8). The correlation between elevated blood eosinophil levels and compromised disease control, as well as an increased susceptibility to severe asthma exacerbations, has been established by a multitude of studies (9–13). Additionally, blood eosinophils can be utilized to predict the response of asthma therapy and guide treatment decisions (14–17). In conclusion, blood eosinophils are an essential biomarker for asthma management and play a critical role in the onset, progression, and treatment of asthma.

Albumin is the predominant protein found in serum, making up over 60% of all blood proteins (18). It is solely produced in the liver and then released into the bloodstream. Serum albumin, with a half-life of 19 days, is crucial for regulating several physiological processes. Keeping the acid-base balance, transporting important molecules (like long-chain fatty acids, hormones, bilirubin, metal ions, etc.) through the bloodstream and to organs, stopping platelet function, Keeping the acid-base balance, transporting important molecules (like long-chain fatty acids, hormones, bilirubin, metal ions, etc.) through the bloodstream and to organs, stopping platelet function, keeping vascular permeability, and managing colloid osmotic pressure are some of these jobs (19). Furthermore, it demonstrates antioxidant properties and the ability to trap free radicals (20). Furthermore, serum albumin serves as an established clinical indicator for malnutrition (21). Asthmatic persons frequently experience malnutrition, which adversely affects their quality of life, exacerbation risk, duration of hospitalization, and overall healthcare costs (22, 23).

Recent research has shown that there is a correlation between low levels of albumin in the blood (hypoalbuminemia) and an extended duration of hospitalization in patients with acute exacerbations of chronic obstructive pulmonary disease (COPD) (24, 25). In individuals suffering from acute pulmonary embolism, low levels of serum albumin continue to serve as indicators of long-term mortality (20). However, there have been few investigations conducted to explore the connection between protein status and asthma thus far. Consequently, we utilized data from the National Health and Nutrition Examination Survey (NHANES) 2011–2018 cycles to examine the connection between protein intake, serum albumin and BEOC in patients with asthma.

2 Materials and methods

2.1 Study data and population

The data for this investigation were collected from the NHANES public database, which was a part of the Centers for Disease Control and Prevention (CDC) in the United States. The NHANES database was responsible for collecting vital and health statistics for the country. The NHANES survey was conducted using a sophisticated multistage stratified sample design to ensure that a diverse and accurate representation of the US population was obtained. The program included interviews, medical exams, and laboratory tests. The collected data was used to analyze the correlation between nutritional status and promoting health and preventing diseases. All participants underwent the necessary procedures for obtaining informed consent and comprehensive health tests. The NHANES study protocol received approval from the Research Ethics Review Board of the National Center for Health Statistics.

Between 2011 and 2018, the NHANES amassed a total of 39156 individuals of data. The following individuals were excluded from our investigation: (1) under 18 years old (n=15331); (2) missing BEOC (n=2147); (3) missing serum albumin or protein intake data (n=2191); (4) non-asthmatics (n=16486); (5) missing over one of following covariates (n=492): education, marriage, poverty to income ratios (PIR), body mass index (BMI), smoking state, alcohol intake, hypertension, diabetes, liver condition, COPD, cancer history, aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum creatinine, serum globulin, serum total protein, and urine albumin, steroid use. At last, a large, nationally representative group (n=2509) of asthmatic adults in the United States was collected for our investigation. The screening procedure's flowchart appears in Figure 1.

2.2 Measurement of serum albumin and blood eosinophil counts

The bichromatic digital endpoint technique (DcX800) is applied in order to determine the concentration of albumin in the serum. Throughout the procedure, bromcresol purple (BCP) reagent and albumin combine to form a complex. At 600 nanometers, the system logs any fluctuations in absorbance. The change in absorbance is directly proportional to the quantity of albumin present in the sample. The dietary protein intake of the participants was assessed using a 24-hour dietary recall approach. A skilled technician requested information regarding the specific kinds and amounts of food and medication consumed in a single

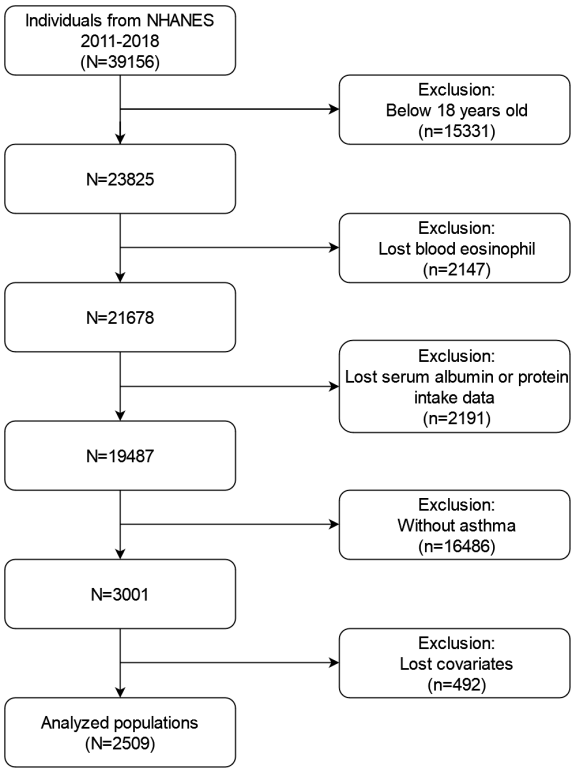

FIGURE 1
Flowchart for the studied individual's selection process.

day. This information was then recorded in the NHANES computer-assisted dietary survey system. A Beckman-Coulter MAXM analyzer was utilized to perform a complete blood count and 5-part differential on whole blood samples. For use in *post hoc* analyses, cell counts of lymphocytes, monocytes, segmented neutrophils, eosinophils, and basophils (1000 cells/uL) were obtained using the 5-part differential measure.

2.3 Covariates and asthma assessment

We incorporated numerous covariates in our investigation to lessen the potential impact of confounding variables. These factors were looked at: sex, age, race, education, PIR (low, middle, and high), marriage, BMI, history of smoking, alcohol use, high blood pressure, diabetes, liver disease, COPD, cancer, recent use of steroid drugs, AST, ALT, serum creatinine, serum globulin, serum total protein, and urine albumin. When people came in for their visit, standard surveys were used to confirm whether they had asthma. The intended evaluation question was: "Have you ever received a diagnosis of asthma from a doctor?" People who said "yes" were identified as having asthma.

2.4 Statistical analysis

The statistical analyses were accomplished via the R program (version 4.2.0). A p-value below 0.05 implied statistical significance. To address the complex sampling design of the NHANES, sample weights were incorporated. The BEOC was initially converted into quartiles. The p-value for categorical variables was calculated via the chi-square test, while for continuous variables, the Kruskal-Wallis rank sum test was deployed. Three distinctive linear regression models (Model A, Model B, and Model C) were utilized to evaluate the connection between protein intake, serum albumin, and BEOC while accounting for different covariates. And we employed trend analysis, the generalized additive model (GAM), and the threshold effect model to test the linearity or nonlinearity of the connection of serum albumin with BEOC. If a non-linear correlation was detected, a two-piecewise linear regression model was utilized to ascertain the threshold impact of serum albumin levels on BEOC. When the relationship between serum albumin and BEOC was obvious in a smoothed curve, the recursive technique automatically predicted the inflection point at which the greatest model likelihood would be adopted. As well, stratified analyses were conducted to investigate the connection of serum albumin with BEOC in various groups. Lastly, our investigation applied the XGBoost algorithm model to evaluate the relative significance of different indicators in connection to the impact of BEOC.

3 Results

3.1 Baseline characteristics of study individuals based on BEOC quartiles

We utilized BEOC quartiles to subdivide the weighted characteristics of the 2509 adults with asthma who participated in our investigation (1031 men and 1478 women) (Table 1). The research sample comprised non-Hispanic white individuals, with an average age of 47.1 years, representing the designated participants' demographic. The distributions of sex, age, race, BMI, hypertension, ALT, serum creatinine, serum total protein, and serum albumin varied significantly between the quartiles of BEOC. However, the distributions of education, marriage, PIR, smoking, alcohol consumption, protein consumption, diabetes, liver condition, COPD, cancer, steroid drug use, AST, serum globulin, and urine albumin did not differ significantly between the quartiles of BEOC. Participants whose BEOC was in the lowest quartile had elevated levels of serum total protein and serum albumin, in addition to lower values of age, BMI, ALT, and serum creatinine ($p < 0.05$), in comparison to the other groups.

3.2 Association between protein intake, serum albumin and BEOC

We applied both univariable and multivariable linear regression models to investigate the connection among asthma patients' protein intake, serum albumin, and BEOC. Only Model A, which did not account for any covariates (Supplementary Table 1), revealed a positive correlation of protein intake with BEOC. Nevertheless, we all identified with statistical significance in

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TABLE 1 The weighted characteristics of the study population were analyzed based on quartiles of BEOC.

	Q1 (0)	Q2 (100)	Q3 (200)	Q4 (300-2200)	P value
Sex (%)					0.0040
Male	33.96	34.15	40.38	45.51	
Female	66.04	65.85	59.62	54.49	
Age (years)	45.93 ± 1.82	43.39 ± 0.74	46.14 ± 0.83	46.97 ± 0.71	0.0021
Race (%)					0.0277
Mexican American	4.96	4.55	5.85	6.87	
Other Hispanic	5.58	5.75	6.07	6.41	
Non-Hispanic White	63.35	69.54	68.9	68	
Non-Hispanic Black	23.44	12.42	10.5	10.97	
Other Race	2.67	7.74	8.68	7.75	
Education (%)					0.2390
Below high school	14.65	10.31	13.29	12.61	
High school	17.18	19.16	20.39	23.62	
Above high school	68.17	70.52	66.32	63.78	
Marriage (%)					0.3450
Married	41.57	52.52	48.72	52.76	
Single	54	39.74	43	39.73	
Living with a partner	4.43	7.74	8.28	7.51	
PIR	2.51 ± 0.23	2.91 ± 0.09	2.74 ± 0.11	2.79 ± 0.1	0.1540
BMI (kg/m2)	29.32 ± 1.01	29.54 ± 0.32	30.85 ± 0.49	31.54 ± 0.42	0.0005
Smoking (%)					0.0779
Smoker	51.1	43.63	45.11	52.23	
Non-smoker	48.9	56.37	54.89	47.77	
Alcohol intake (gm)	14.62 ± 3.62	12.13 ± 1.41	12.31 ± 1.98	12.75 ± 1.69	0.8919
Protein intake (gm)	83.09 ± 9.7	78.76 ± 1.75	83.92 ± 1.98	84.72 ± 2.2	0.0780
Hypertension (%)					0.0031
Yes	38.55	30.04	37.49	41.05	
No	61.45	69.96	62.51	58.95	
Diabetes (%)					0.1215
Yes	15.91	8.12	12.93	13.89	
No	80.94	88.89	84.79	83.43	
Borderline	3.15	2.99	2.29	2.68	
Liver condition (%)					0.2336
Yes	4.29	4.45	7.09	4.6	
No	95.71	95.55	92.91	95.4	
COPD history (%)					0.1199
Yes	15.97	7.63	11.24	9.51	
No	84.03	92.37	88.76	90.49	
Cancer history (%)					0.4814

(Continued)

TABLE 1 Continued

	Q1 (0)	Q2 (100)	Q3 (200)	Q4 (300-2200)	P value
Yes	13.36	10.12	11.03	13.42	
No	86.64	89.88	88.97	86.58	
Steroid drugs use (%)					0.1958
Yes	20.74	14.22	15.91	18.77	
No	79.26	85.78	84.09	81.23	
AST (U/L)	22.95 ± 1.03	24.86 ± 0.89	25.59 ± 0.61	24.33 ± 0.54	0.1223
ALT (U/L)	24.27 ± 1.75	35.87 ± 1.25	35.45 ± 1.55	34.36 ± 1.16	<0.0001
Serum creatinine (umol/L)	73.4 ± 2.15	72.8 ± 0.73	77.57 ± 0.88	79.23 ± 1.1	<0.0001
Serum globulin (g/L)	28.58 ± 0.63	28.03 ± 0.19	28.06 ± 0.26	28.53 ± 0.21	0.0827
Serum total protein (g/L)	71.39 ± 0.57	70.63 ± 0.19	70.09 ± 0.23	70.44 ± 0.21	0.0367
Urine albumin (mg/L)	29.82 ± 8.75	31.99 ± 8.21	31.67 ± 11.05	33.97 ± 6.18	0.9830
Serum albumin (g/L)	42.81 ± 0.64	42.6 ± 0.15	42.03 ± 0.19	41.9 ± 0.15	0.0009

Continuous and categorical variable were displayed individually as weighted means ± SD or proportions. Q1-Q4: BEOC had been classified into quartiles. gm, gram; mg, milligram; mcg, microgram.

Models A, B, and C the inverse correlation of serum albumin with BEOC (Table 2). In Model C, which controlled for all covariates, BEOC decreased by 2.82 cells/uL for each extra unit of serum albumin (g/L). Furthermore, the outcomes of the trend test revealed statistical significance in Models A and B (p for trend < 0.05). However, no statistical significance was observed in the trend test of Model C (p for trend> 0.05), which suggesting a potential non-linear correlation of serum albumin with BEOC.

TABLE 2 Association between serum albumin and BEOC in asthmatics.

	Model A	Model B	Model C
	β (95% CI) P value	β (95% CI) P value	β (95% CI) P value
Serum albumin (g/L)	-2.87 (-5.14, -0.60) 0.0161	-4.64 (-7.03, -2.24) 0.0004	-2.82 (-5.52, -0.13) 0.0486
Serum albumin quartile			
Q1 (21-39)	Reference	Reference	Reference
Q2 (40-41)	1.20 (-30.03, 32.43) 0.9404	-5.03 (-35.75, 25.68) 0.7493	0.94 (-32.41, 34.29) 0.9563
Q3 (42-43)	-17.27 (-43.98, 9.43) 0.2098	-28.39 (-54.99, -1.79) 0.0414	-15.09 (-52.60, 22.41) 0.4368
Q4 (44-52)	-26.98 (-49.20, -4.75) 0.0206	-43.04 (-66.30, -19.79) 0.0007	-22.21 (-74.42, 30.00) 0.4115
P for trend	0.0032	0.0001	0.2895

Model A controlled for none. Model B controlled for sex, age and race. Model C controlled for sex, age, race, education, marriage, PIR, BMI, smoking, alcohol intake, protein intake, hypertension, diabetes, liver condition, COPD, cancer history, steroid drugs use, AST, ALT, serum creatinine, serum globulin, serum total protein and urine albumin. Q1-Q4: Seum albumin was grouped by quartile.

3.3 Generalized additive model and threshold effect model

The GAM and threshold effect models were highly effective in identifying whether the correlation demonstrated linearity or nonlinearity. We employed GAM to generate a smooth and continuous curve based on Model C. This enabled us to ascertain if there was a nonlinear correlation between serum albumin and BEOC (Figure 2). After accounting for all covariates except serum albumin, we observed an inverted U-shaped correlation of serum albumin with BEOC. As well, we performed a threshold effect analysis to compare the single-line regression model with the two-segment regression model. The log-likelihood ratio was less than 0.05, indicating that model I (the one-line model) was statistically distinct from model II (the two-piecewise linear regression model). Given the statistical significance of the inflection point (K = 36), the two-piecewise linear regression model was deemed more appropriate, as indicated in Table 3. The highest point of BEOC was seen when the serum albumin reached 36 g/L.

3.4 Stratified connection of serum albumin with BEOC

Stratified analyses were carried out to evaluate the connection between serum albumin and BEOC in various subgroups. Supplementary Table 2 displayed the results, stratified by sex, age, race, education, marriage, PIR, BMI, smoking, hypertension, diabetes, COPD, cancer, liver condition, and steroid use. We found that serum albumin was negatively linked to BEOC in women younger than 40, non-Hispanic Whites with low PIR, non-smokers, and people who did not have COPD, cancer, diabetes, liver disease, or use steroid. All stratified analyses showed no interaction (all p-values for interaction > 0.05).

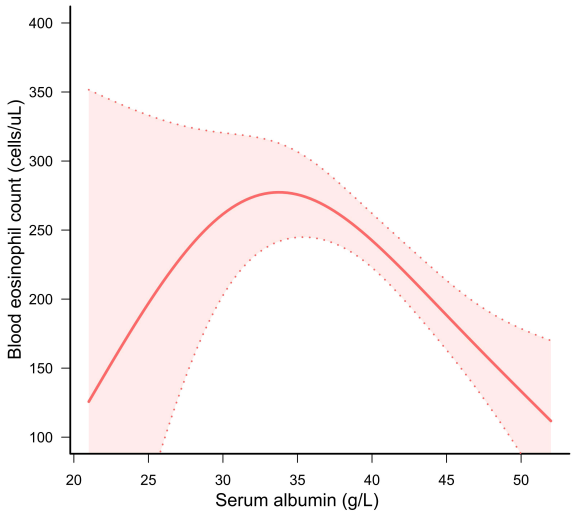

FIGURE 2
A solid red line displayed the correlation of serum albumin with BEOC. A red area indicated suitable 95% confidence ranges.

3.5 XGBoost model

When assessing the significance of the selected variable with regard to its impact on the BEOC, we adopted the XGBoost model. The selected variables included age, PIR, BMI, alcohol consumption, protein intake, AST, ALT, serum creatinine, serum albumin, serum globulin, serum total protein, and urine albumin. Ten variables, ranked in descending order of relative importance, primarily influenced the BEOC, according to the XGBoost model: urine albumin, protein consumption, BMI, serum creatinine, PIR, ALT, AST, age, serum globulin, and serum albumin (Figure 3).

4 Discussion

The investigation is the first and most extensive cross-sectional investigation to quantify the connection between protein intake, serum albumin, and BEOC in asthma patients, to the best of our knowledge. In our study, we found a positive correlation of protein intake with BEOC, but only in the univariable regression model.

TABLE 3 Threshold effect analysis of serum albumin with BEOC.

	β (95% CI) P value
Model I	
linear effect	-2.82 (-5.52, -0.13) 0.0486
Model II	
Inflection point (K)	36
Serum albumin< K	13.50 (1.71, 25.29) 0.0249
Serum albumin> K	-4.27 (-6.83, -1.71) 0.0011
Log likelihood ratio	0.0050

The model I and II controlled for all covariates.

However, in univariate and multivariate regression models, there was a negative correlation of serum albumin with BEOC in asthma people. In addition, we utilized the GAM and threshold effect model to verify the link between serum albumin and BEOC, which exhibited an inverted U-shaped correlation. The maximal BEOC occurred at a serum albumin concentration of 36 g/L. The XGBoost model proved that urine albumin, protein consumption, BMI, serum creatinine, PIR, ALT, AST, age, serum globulin, and serum albumin are the top 10 influential factors affecting BEOC. Our investigation gave unique insights into the connection of serum albumin with BEOC in patients with asthma.

Serum albumin is the most abundant plasma protein in the body, managing the distribution of vascular fluid and maintaining plasma colloid osmotic pressure. And serum albumin also possesses powerful anti-inflammatory and antioxidant properties due to its multiple binding sites, which provide an ideal substrate for free radical removal (26). In addition, it can still bind diverse inflammatory mediators and regulate the immune response during systemic inflammation (27). It has been postulated that serum albumin is responsible for over 70 percent of the total free radical–trapping activity, making it the predominant antioxidant in the circulatory system. Besides, albumin also has various biologic functions, such as the binding and transport of endogenous and exogenous molecules, endothelial stabilization, anti-thrombotic effects, and so on (26). Consequently, serum albumin is also a valuable biomarker for a variety of illnesses, which include obesity, diabetes, rheumatoid arthritis, and carcinoma, while it can be employed to treat various diseases, such as shock, trauma, hemorrhage, acute respiratory distress syndrome, hemodialysis, acute liver failure, chronic liver disease, hypoalbuminemia, and so on (28).

Additionally, serum albumin is also a valuable biomarker for a variety of respiratory disorders, including lung cancer, chronic obstructive pulmonary disease, acute pulmonary embolism, and bronchiectasis (20, 29–31). Meanwhile, some studies have reported the role of albumin in asthma, too. A case-control study in Nigeria involving 37 asthma cases and 30 controls has discovered that children with asthma have significantly lower serum albumin concentrations in comparison to controls and that there is a negative association between serum IgE and serum albumin, but that there is no significant association between serum albumin and blood eosinophil counts or eosinophil percentage (32). According to a Turkish case-control study involving 40 asthma cases and 40 healthy subjects, there is a significant decrease in serum albumin concentration among bronchial asthma patients compared to controls (33). Likewise, a Japanese investigation has observed that the serum albumin levels of asthmatic children are substantially lower than those of children without asthma, whereas the levels of children with wheezing symptoms or a history of allergic diseases are not significantly different (34). And another cross-sectional study in Australia has found that serum albumin is lower in people with more severe asthma and is positively related to lung function (35). Similarly, Khatri SB et al. have discovered that plasma albumin levels are substantially lower in asthmatics compared to nonasthmatics and are positively correlated with lung function (% forced expiratory volume in 1 second) in asthmatics (36). And a

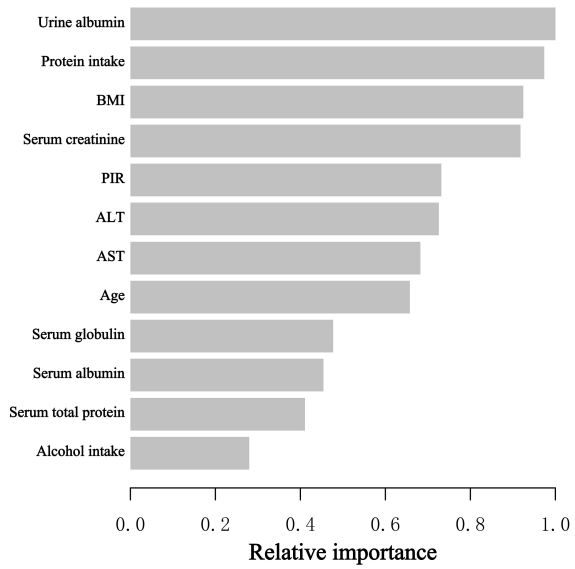

FIGURE 3
The XGBoost model provided the relative importance of each indicator on BEOC, along with the corresponding indicator importance score for every indicator.

prospective, multicenter study in Spain has demonstrated that the onset of asthma exacerbations is negatively correlated with serum albumin levels (37). However, AbdulWahab et al. have reported that there is no significant difference in serum albumin levels between asthmatic children and control groups (38). In the same way, another study in Turkey found that serum albumin levels are not significantly different between asthmatic children and control groups (39). As well, a few investigations have looked into the connection between protein consumption and asthma. Huang SL et al. reported that protein-rich and fat-rich foods of animal origin were associated with a higher prevalence of asthma in teenagers (40). However, Schwartz J. et al. found no connection between dietary protein consumption and wheezing or asthma in children in the USA (41). Another South Korean study proved that protein consumption was shown to be slightly but significantly connected with allergic rhinitis but not with asthma (42). And Han YY et al. reported no significant connection between protein food consumption and the prevalence of asthma in Puerto Rican kids (43). Due to the inclusion of various confounders in previous studies, inconsistent conclusions have been drawn. Our investigation found no independent connection between asthmatics' protein consumption and BEOC. But serum albumin and BEOC had an inverted U-shaped association.

In contrast to prior research, ours has some advantages. First, our inquiry provides a relatively large, nationally representative sample of adult asthmatics. Secondly, because confounders may influence the results, we use stratified analysis to determine the relationship of serum albumin with BEOC in various populations. Then, we employ the XGBoost model to determine the relative significance of selected indicators on BEOC. Lastly, the inflection point in the nonlinear relationship of serum albumin with BEOC is determined by using GAM and a two-piecewise linear regression model. But we have to acknowledge the inquiry's shortcomings. Cross-sectional studies cannot prove a causal link between serum albumin and BEOC. Additionally, there are certain potential confounding factors that we may disregard. The medications that influenced blood eosinophils in our investigation were predominantly cortisol medications and other anti-allergy medications, excluding biologics. Asthma diagnosis is dependent on questionnaires. Future research endeavors should explore the potential influence of serum albumin in regulating and managing asthma while also elucidating potential mechanisms of action.

5 Conclusion

Our investigation discovered there was no independent link between asthmatics' protein intake and BEOC. However, we observed an inverted U-shaped relationship of serum albumin with BEOC, suggesting a possible correlation between the overall nutritional status of asthmatics and immune system changes. Our findings provide new directions for future research in the field of asthma management and therapy.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: The official website of NHANES provides access to all available data (http://www.cdc.gov/nchs/nhanes/index.htm).

Ethics statement

The studies involving humans were approved by National Center for Health Statistics' Research Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

JW: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. JX: Data curation, Formal analysis, Writing – original draft. QH: Data curation, Formal analysis, Writing – review & editing. MG: Data curation, Formal analysis, Writing – review & editing. SG: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2024.1383122/full#supplementary-material

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Association of dietary inflammatory indices with sarcopenia and all-cause mortality in COPD patients

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Background: Sarcopenia frequently occurs as a comorbidity in individuals with COPD. However, research on the impact of Appendicular Skeletal Muscle Mass (ASM) on survival in COPD patients is scarce. Moreover, there is a lack of research on the association between dietary pro-inflammatory capacity and sarcopenia in COPD.

Methods: We analyzed data from the National Health and Nutrition Examination Survey (NHANES) covering the years 1999 to 2006 and 2011 to 2018. We aimed to investigate the relationship between the Dietary Inflammatory Index (DII) and sarcopenia prevalence among adults diagnosed with COPD in the United States. Furthermore, we sought to explore the relationship between sarcopenia, ASMI, and all-cause mortality. The study included a total of 1,429 eligible adult participants, divided into four groups based on quartiles of DII, with adjustments for sample weights. Methodologically, we used multivariable logistic regression analyses and to examine the association between DII and sarcopenia. Additionally, we used restricted cubic spline (RCS) tests to evaluate potential non-linear relationships. To assess the effect of sarcopenia on overall all-cause mortality, we used Kaplan–Meier models and Cox proportional hazards models. Moreover, we used RCS analyses to investigate potential non-linear relationships between ASMI and all-cause mortality. Subgroup analyses were conducted to confirm the reliability of our study findings.

Results: In our COPD participant cohort, individuals with higher DII scores were more likely to be female, unmarried, have lower educational attainment, and show lower ASMI. Using multivariable logistic regression models, we found a positive association between the highest quartile of DII levels and sarcopenia incidence [Odds Ratio (OR) 2.37; 95% Confidence Interval (CI) 1.26–4.48; $p = 0.01$]. However, analysis of RCS curves did not show a non-linear relationship between DII and sarcopenia. Throughout the entire follow-up period, a total of 367 deaths occurred among all COPD patients. Kaplan–Meier survival curves showed a significantly higher all-cause mortality rate among individuals with concurrent sarcopenia ($p < 0.0001$). Cox proportional hazards model analysis showed a 44% higher risk of all-cause mortality among COPD patients with sarcopenia compared to those without sarcopenia [Hazard Ratio (HR): 1.44; 95% CI 1.05–1.99; $p < 0.05$]. Additionally, our final RCS analyses revealed a significant non-linear association between ASMI levels and all-cause mortality among COPD patients, with a turning point identified at 8.32 kg/m^2. Participants with ASMI levels above this inflection point had a 42% lower risk of all-cause mortality compared to those with ASMI levels below it (HR 0.58; 95% CI 0.48–0.7).

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Conclusion: We observed a significant association between concurrent sarcopenia and an increased risk of all-cause mortality in COPD patients within the United States. Moreover, ASMI demonstrated a non-linear association with all-cause mortality, with a critical threshold identified at 8.32 kg/m^2. Our findings also revealed an association between DII and the presence of sarcopenia. Consequently, further investigations are warranted to explore the feasibility of dietary DII adjustments as a means to mitigate muscle wasting and enhance the prognosis of COPD.

KEYWORDS

COPD, sarcopenia, DII, ASMI, NHANES

Introduction

Chronic obstructive pulmonary disease (COPD) significantly contributes to the global disease burden, mortality rates, and healthcare resource utilization (1). Globally, it ranks as the fourth leading cause of death, affecting approximately 12% of the world's population (2). COPD comprises a range of chronic inflammatory respiratory conditions characterized by airflow obstruction and respiratory impairment. Primary causative factors often involve a history of smoking and exposure to environmental pollutants. Additionally, COPD often leads to various serious comorbidities, such as lung cancer, coronary heart disease, heart failure, renal dysfunction, hyperuricemia, and muscle wasting (3). These interrelated comorbidities, along with a diminished quality of life, impose additional burdens on patients, increasing the risk and mortality associated with these concurrent conditions (4).

Sarcopenia, known as muscle wasting, is a progressive systemic skeletal muscle disease that raises the risk of falls, fractures, and mortality rates, significantly contributing to physical disability and frailty (5). In 2010, the European Working Group on Sarcopenia in Older People (EWGSOP) established a universally acknowledged definition of sarcopenia (6). In early 2018, the group reconvened (EWGSOP2), providing updated guidelines and recommendations on sarcopenia definition, diagnosis, measurement, and associated risks, informed by scientific and clinical evidence from the past decade (7). The latest recommendations from EWGSOP2 aim to raise awareness of sarcopenia and its risks, advocating for early detection and treatment and encouraging additional research to prevent or delay adverse health outcomes for patients and healthcare systems. While sarcopenia has traditionally been linked with aging, it is now recognized to initiate early in life (8). Literature evidence associating birth weight, infant growth, and adult grip strength underscores the lasting impact of prenatal and postnatal muscle development, influencing muscle function via fiber size, quantity, and satellite cell activity (9). Currently, sarcopenia etiology is classified into primary (aging-related) and secondary (disease-related, lack of activity, and malnutrition). Sarcopenia can arise secondary to systemic diseases, notably those provoking inflammatory processes like malignancies, COPD, and cardiovascular diseases, among others. Moreover, inadequate physical activity and insufficient energy or protein intake can contribute to sarcopenia development (7). Due to the multifactorial nature of sarcopenia development, interactions among these factors can exacerbate its progression. As sarcopenia can be secondary to COPD and worsen COPD symptoms by affecting skeletal, smooth, and cardiac muscle function, diaphragmatic contraction, and patient activity levels, it can lead to respiratory failure and increase mortality among COPD patients (10). The interaction between COPD and sarcopenia creates a vicious cycle, where sarcopenia originating from COPD exacerbates COPD symptoms and accelerates disease progression. Considering this complex relationship, it is crucial to investigate the high-risk factors that predispose COPD patients to sarcopenia. This exploration offers the potential to reveal new insights into managing and treating COPD patients, improving their condition.

Targeted nutritional interventions, like high-protein diets, show promise in improving respiratory muscle strength, physical performance, overall health status, and quality of life in elderly COPD patients (11). These interventions can aid in the rehabilitation process (12). Conversely, age-related chronic low-grade systemic inflammation significantly contributes to sarcopenia onset, with dietary factors playing a crucial role (13). Certain nutrients, foods, and dietary patterns are associated with inflammation biomarkers. Yet, the complex interaction and mechanisms between inflammation and diet require further exploration (14). Considering these factors, the Dietary Inflammatory Index (DII) emerges as a valuable tool for assessing the inflammatory potential of dietary components (15, 16). The DII assesses the impact of nutrients and foods on six key inflammatory markers (IL-1β, IL-4, IL-6, IL-10, TNF-α, and CRP), computing pro-inflammatory and anti-inflammatory scores for each dietary parameter to generate an overall inflammation effect score (17). In recent years, the DII has gained considerable attention in clinical research, enabling investigations into the relationship between dietary inflammatory potential and various diseases (15). In a notable study by Gojanovic et al. (18), adherence to a pro-inflammatory diet was associated with reduced muscle mass and poorer muscle function. These dietary patterns may undermine muscle health and function, increasing the risk of sarcopenia. Remarkably, there is a lack of studies exploring the potential relationship between the dietary inflammatory index and sarcopenia in individuals with COPD. Therefore, exploring the association between the dietary inflammatory index and both all-cause mortality and sarcopenia-related mortality in COPD patients is clinically significant.

Abbreviations: COPD, Chronic obstructive pulmonary disease; DII, Dietary Inflammatory Index; DXA, Dual-energy X-ray absorptiometry; NHANES, National Health and Nutrition Examination Survey; CDC, Centers for Disease Control and Prevention; 95% CI, 95% confidence interval; HR, Hazard ratio.

The study utilized data from the National Health and Nutrition Examination Survey (NHANES), covering 1999 to 2006 and 2011 to 2018. The primary objective is to investigate the association between DII and sarcopenia, as well as the link between sarcopenia, muscle mass, and all-cause mortality in individuals with COPD. The overarching goal of this research is to provide new insights and empirical evidence to enhance survival outcomes in COPD patients by implementing dietary management strategies. This study aims to pave the way for more effective nutritional interventions tailored to the COPD population in the future.

Materials and methods

Study design and data source

The study utilizes NHANES data from 1999 to 2006 and 2011 to 2018. NHANES is administered by the National Center for Health Statistics (NCHS) under the guidance of the Centers for Disease Control and Prevention (CDC). Trained professionals conduct household interviews or administer examinations in mobile examination centers for data collection in NHANES. The survey includes cross-sectional interviews, physical examinations, and laboratory analyses collected from a complex, multi-stage, stratified, clustered probability sample representing the US population (19). The survey protocol is ethically overseen by the Institutional Review Board of the CDC, and informed consent is secured from all participants. The NHANES data are free and available on the Web (20). During NHANES cycles from 1999 to 2006 and 2011 to 2018, individuals aged 20 and above participated in research conducted by NHANES. NHANES methodology employs dual-energy X-ray absorptiometry (DXA) to measure body composition due to its speed, cost-effectiveness, and minimal radiation exposure (20). Individuals weighing over 136 kg or measuring over 192 cm were excluded due to DXA scanning limitations. Pregnant women and individuals with recent use of radiographic contrast agents or radiotherapy within the past 7 days were also excluded from the analysis.

Body composition, DXA, and definition of low muscle mass

NHANES surveys from 1999 to 2006 and 2011 to 2018 captured body composition parameters including height (cm), weight (kg), and waist circumference (cm). BMI was calculated using height and weight measurements. DXA scans were conducted on individuals aged 8 to 59 during NHANES 2011–2018 using the Hologic Discovery A densitometer. Within DXA scans, non-fat and non-bone mass components were classified as skeletal muscle, and Appendicular Skeletal Muscle Mass (ASM) represented the sum of lean soft tissue in the four limbs. According to EWGSOP2 recommendations (7), key assessments for identifying sarcopenia in clinical practice and research encompass: (1) Questionnaire screening (21). (2) Muscle strength evaluation, including grip strength measurement (22), chair stand test, etc. (3) Quantitative assessment of muscle mass, utilizing techniques like DXA for ASM measurement (23–25) and bioelectrical impedance analysis (BIA) for predicting total body skeletal muscle mass (SMM) or ASM (26–29). (4) Physical performance assessment, evaluating gait speed and conducting a 400-meter walk test to gauge walking ability and endurance (22), among others. Since muscle mass is correlated with body size, with larger individuals typically having greater muscle mass, quantifying muscle mass can be adjusted using various methods to normalize SMM or ASM absolute levels, such as height squared (ASM/height^2), body weight (ASM/body weight), or body mass index (ASM/BMI) (30). Despite ongoing controversy surrounding the adjustment of ASM for body size across diverse populations, we opted to use ALM/BMI for diagnosing sarcopenia. ALM/BMI defines low muscle mass with gender-specific thresholds: According to data from The Foundation for the National Institutes of Health(FNIH) Biomarkers Consortium Sarcopenia Project, low muscle mass is diagnosed if ALM/BMI is <0.789 in males, and < 0.512 in females (31). We then evaluated muscle mass using ASM/height^2 [Appendicular Skeletal Muscle Index, ASMI (32)] as a continuous variable for further analysis.

Definition of COPD

COPD diagnosis relies on participant self-reporting confirmed by a physician. COPD diagnosis is confirmed if participants answer positively to questions regarding physician-diagnosed COPD, related conditions like emphysema or chronic bronchitis. Participants answering "yes" are classified as COPD-positive, while those answering "no" are classified as COPD-negative. Those with an FEV1/FVC ratio less than 0.7 on pulmonary function testing are also classified as COPD-positive. The COPD group also encompasses individuals aged 40 and above with a history of smoking or chronic bronchitis who are using specific medications, such as selective phosphodiesterase-4 inhibitors, mast cell stabilizers, leukotriene modifiers, and inhaled corticosteroids.

DII definition

The method for calculating the DII was introduced by Shivappa et al. (15). In this study, DII served as the exposure variable, and dietary information was obtained via a 24-h dietary recall conducted on the initial day. DII calculation incorporated 26 dietary parameters, encompassing carbohydrates, protein, total fat, saturated fat, polyunsaturated fatty acids (PUFA), n-3 fatty acids, cholesterol, energy, alcohol, fiber, folate, iron, magnesium, zinc, selenium, monounsaturated fatty acids (MUFA), caffeine, niacin, riboflavin, thiamin, β-carotene, vitamins A/B6/B12/C/E (33). Initially, the mean intake of each nutrient among participants was calculated, then subtracted from the global mean intake, and divided by the standard deviation to compute z-scores. Next, z-scores underwent transformation into percentiles, doubled, and subsequently subtracted by "1" to center the data. Centered percentile values for each dietary parameter were multiplied by their respective inflammatory effect scores to yield "specific DII scores" for each food parameter. Lastly, these values were summed to determine individual "overall DII scores" (34).

Covariates

In this study, we used data collected from NHANES. Data in NHANES is collected through standardized questionnaire methods

(20), such as gender, age, race, education level, poverty index, marital status, diabetes, cardiovascular disease, and hypertension history, through household interviews. Physical data, such as weight, height, and blood pressure, were obtained during examinations at mobile examination centers. BMI was computed by dividing weight in kilograms by height in meters squared. Race categories included Mexican American, non-Hispanic white, non-Hispanic black, and other races. Education level was segmented into three groups: individuals without high school completion, those with high school completion or equivalent, and those with higher education. Family income was classified into low income, middle income, and high income brackets (PIR < 1.30, 1.30–3.49, ≥3.50). Marital status included categories of married/living with partner, never married, and widowed/divorced/separated. Diabetes diagnosis relied on self-reported history or medication use. Hypertension was diagnosed if systolic blood pressure was over 140 mmHg, diastolic blood pressure exceeded 90 mmHg, individuals were taking antihypertensive medications, or there was a self-reported history of hypertension. Cardiovascular disease (CVD) history was established by physician diagnosis of myocardial infarction, angina pectoris, coronary heart disease, or stroke.

Statistical methods

Analyses were performed using R software (version 4.1.1). Due to the NHANES survey's complex design, we adhered to NHANES recommendations and applied eight-year cycle weights, stratification, and clustering to our analyses. Categorical variables were expressed as numbers and weighted percentages, while continuous variables were presented as weighted means (standard errors). Participants were grouped into four quartiles based on DII scores. Weighted linear regression and design-adjusted chi-square tests were used to compare baseline characteristics between groups for continuous and categorical variables, respectively. Multivariable weighted regression analyses were conducted to evaluate the independent association between DII and sarcopenia, adjusting for potential confounders and obtaining odds ratios (OR) and 95% confidence intervals (CI). Model 1 was unadjusted; Model 2 adjusted for age, gender, race, education, poverty index, marital status; Model 3 further adjusted for history of diabetes, cardiovascular disease, and hypertension based on Model 2. Additionally, to explore the potential non-linear relationship between DII and sarcopenia, we used restricted cubic spline (RCS) models, adjusting for potential confounders. Kaplan–Meier curves and Cox regression models were used to assess the relationship between sarcopenia and all-cause mortality in COPD patients. RCS was also used to examine the non-linear relationship between ASMI and all-cause mortality. If the relationship was non-linear, a recursive algorithm was used to calculate ASMI and all-cause mortality separately. Two-piece Cox proportional hazards models were then applied to investigate the association between ASMI and all-cause mortality risk on both sides of the inflection point. Finally, subgroup analyses were performed to validate the above results. Subgroup analyses were based on the following considerations: (1) Previous studies have indicated differences between subgroups, including gender, age, and race, as demographic factors and lifestyle may influence muscle mass. (2) Various biological mechanisms, especially in chronic diseases like diabetes, hypertension and CVD, may play significant roles. Therefore, subgroup analyses were conducted among individuals with and without these conditions. A p-value <0.05 indicated statistical significance.

Results

Baseline characteristics of COPD participants

Among the initial 80,630 participants, individuals without a diagnosis of COPD were excluded (n = 77,309), along with those with missing muscle mass index (n = 1,846) and DII data (n = 46). A total of 1,429 participants met the inclusion criteria, and Table 1 presents the characteristics of these participants. Participants with higher dietary validation index scores comprised more females, unmarried individuals, individuals with lower education levels, sarcopenic individuals, and those with lower ASMI. In order to ensure the study's stability and eliminate the influence of confounding factors, we conducted additional subgroup analyses of ASMI using DII as both a continuous variable and a four-category grouped variable. The results indicated no interaction effect of DII on ASMI across all subgroups (Supplementary Table S1).

DII and sarcopenia in COPD

Three logistic regression models were developed, taking into account weighting considerations. Model 1 performed univariate analysis of DII and sarcopenia among COPD patients. Model 2 included variables such as age, gender, race, poverty index, education level, and marital status. Model 3 additionally incorporated histories of hypertension, diabetes, and cardiovascular disease, building on the variables in Model 2. All three models revealed a positive correlation between DII scores and sarcopenia prevalence in COPD patients. Specifically, in fully adjusted Model 3, logistic regression analysis showed that compared to DII quartile 1 (Q1), the multivariate adjusted OR and 95% CI for Q3 and Q4 were 2.32 (1.30, 4.15) and 2.37 (1.26, 4.48), respectively (Table 2). A clear dose–response relationship between DII scores and sarcopenia in COPD patients was identified (Figure 1). This analysis demonstrated a linear association between these variables, with a non-linear p-value of 0.98, indicating that as DII scores in dietary intake increase, sarcopenia prevalence rises accordingly.

Sarcopenia/ASMI and all-cause mortality in COPD patients

A total of 367 deaths were recorded over the entire follow-up period, with a median follow-up time of 106 months. Kaplan–Meier curves showed a significantly higher all-cause mortality rate in participants with sarcopenia (p < 0.0001) (Figure 2A). Further adjusting for covariates, the Cox proportional hazards model showed that COPD patients with sarcopenia had a 44% higher risk of all-cause mortality compared to those without sarcopenia (HR 1.44; 95%CI 1.05–1.99; p = 0.03) (Table 3). RCS results also showed a non-linear relationship between ASMI levels and all-cause

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TABLE 1 Baseline characteristics of participants with COPD according to DII.

Variable	Total (*N* = 1,429)	Q1 (*N* = 359) [−4.53,0.76]	Q2 (*N* = 355) [0.76, 2.27]	Q3 (*N* = 357) [2.27,3.19]	Q4 (*N* = 358) [3.19, 5.19]	*p*-value
Age, years, *n* (%)						0.24
≥60	388 (18.68)	108 (19.86)	97 (18.39)	92 (18.92)	91 (17.25)	
≥40and<60	668 (51.32)	150 (45.84)	171 (55.49)	167 (48.48)	180 (56.94)	
<40	373 (29.99)	101 (34.30)	87 (26.11)	98 (32.60)	87 (25.81)	
Gender, *n* (%)						< 0.0001
Male	543 (35.69)	180 (47.03)	145 (40.71)	117 (31.08)	101 (21.51)	
Female	886 (64.31)	179 (52.97)	210 (59.29)	240 (68.92)	257 (78.49)	
Race, *n* (%)						0.95
White	867 (76.58)	219 (77.25)	213 (75.83)	229 (77.76)	206 (75.16)	
Black	249 (8.87)	60 (8.18)	67 (9.85)	50 (7.70)	72 (10.04)	
Mexican American	144 (4.18)	45 (5.29)	30 (3.73)	31 (3.83)	38 (3.72)	
Others	169 (10.37)	35 (9.28)	45 (10.59)	47 (10.70)	42 (11.08)	
Marriage, *n* (%)						0.02
Married/Living with partner	763 (55.87)	208 (61.11)	181 (54.86)	193 (56.26)	181 (55.05)	
Widowed/divorced/separated	429 (27.15)	87 (20.67)	117 (31.58)	100 (25.63)	125 (34.46)	
Never married	215 (15.06)	55 (18.22)	50 (13.56)	62 (18.12)	48 (10.49)	
Poverty, *n* (%)						< 0.0001
High (>3.5)	302 (27.24)	98 (36.23)	86 (32.37)	67 (26.34)	51 (18.00)	
Middle (>1.3and ≤ 3.5)	501 (36.09)	140 (44.25)	122 (36.16)	128 (36.43)	111 (34.13)	
Low (≤1.3)	534 (31.64)	98 (19.52)	120 (31.48)	141 (37.23)	175 (47.87)	
Education, *n* (%)						0.01
>High school	656 (49.69)	188 (57.21)	159 (49.94)	156 (44.76)	153 (46.19)	
High school	350 (27.42)	90 (28.38)	81 (24.25)	98 (32.59)	81 (23.64)	
< High school	422 (22.79)	81 (14.42)	114 (25.81)	103 (22.65)	124 (30.17)	
Diabetes, *n* (%)						0.89
Yes	212 (12.00)	54 (11.58)	44 (11.07)	52 (12.12)	62 (13.50)	
No	1,216 (87.87)	304 (88.42)	311 (88.93)	305 (87.88)	296 (86.50)	
Hypertension, *n* (%)						0.11
Yes	728 (45.29)	173 (40.66)	195 (49.70)	185 (49.55)	175 (41.02)	
No	701 (54.71)	186 (59.34)	160 (50.30)	172 (50.45)	183 (58.98)	
CVD, *n* (%)						0.18
Yes	288 (15.39)	63 (12.25)	66 (14.25)	71 (17.60)	88 (17.90)	
No	1,141 (84.61)	296 (87.75)	289 (85.75)	286 (82.40)	270 (82.10)	
Sarcopenia, *n* (%)						0.02
Yes	246 (12.72)	47 (8.17)	56 (10.86)	66 (16.15)	77 (16.32)	
No	1,183 (87.28)	312 (91.83)	299 (89.14)	291 (83.85)	281 (83.68)	
ASMI, Kg/m^2, Mean (SD)	7.40 (0.06)	7.68 (0.11)	7.28 (0.10)	7.40 (0.11)	7.17 (0.14)	0.01

mortality in COPD patients (p non-linear <0.001) (Figure 2B). Threshold effect analysis suggested an ASMI threshold of 8.32 (Table 4). Below the ASMI threshold of 8.32, each unit increase was associated with a 42% reduction in all-cause mortality risk (HR 0.58; 95%CI 0.48–0.7; $p < 0.0001$), whereas no such association was observed above this threshold (HR 0.9; 95%CI 0.61–1.34; $p = 0.61$).

Stratified analysis

This study conducted a stratified analysis to investigate the impact of demographic characteristics and comorbidities on the relationship between DII and sarcopenia incidence, as well as ASMI and all-cause mortality rate among COPD patients. Sarcopenia incidence was similar across various subgroups stratified by age, gender, marital

TABLE 2 OR and 95% CI for sarcopenia according quartile of DII intake.

DII	OR (95%CI), *p*-value					
	Model 1		Model 2		Model 3	
Character	95%CI	*p*	95%CI	*p*	95%CI	*p*
Q1 [−4.53, 0.76]	ref		ref		ref	
Q2 [0.76, 2.27]	1.37(0.71,2.63)	0.34	1.39(0.75,2.58)	0.29	1.43(0.75,2.73)	0.27
Q3 [2.27, 3.19]	2.17(1.32,3.56)	**0.003**	2.48(1.40,4.39)	**0.002**	2.32(1.30,4.15)	**0.005**
Q4 [3.19, 5.19]	2.19(1.24,3.88)	**0.01**	2.28(1.22,4.26)	**0.01**	2.37(1.26,4.48)	**0.01**
p for trend		**0.002**		**0.003**		**0.005**

OR, odds ratio; CI, confidence interval; Q, quartile. Model 1: no adjustment. Model 2: adjusted for age, gender, race, poverty, education, marriage. Model 3: adjusted for age, gender, race, poverty, education, marriage, Diabetes, CVD, hypertension. The lowest quartile of dietary DII intake was used as the reference group. Bold values indicate statistically significant *p*-values.

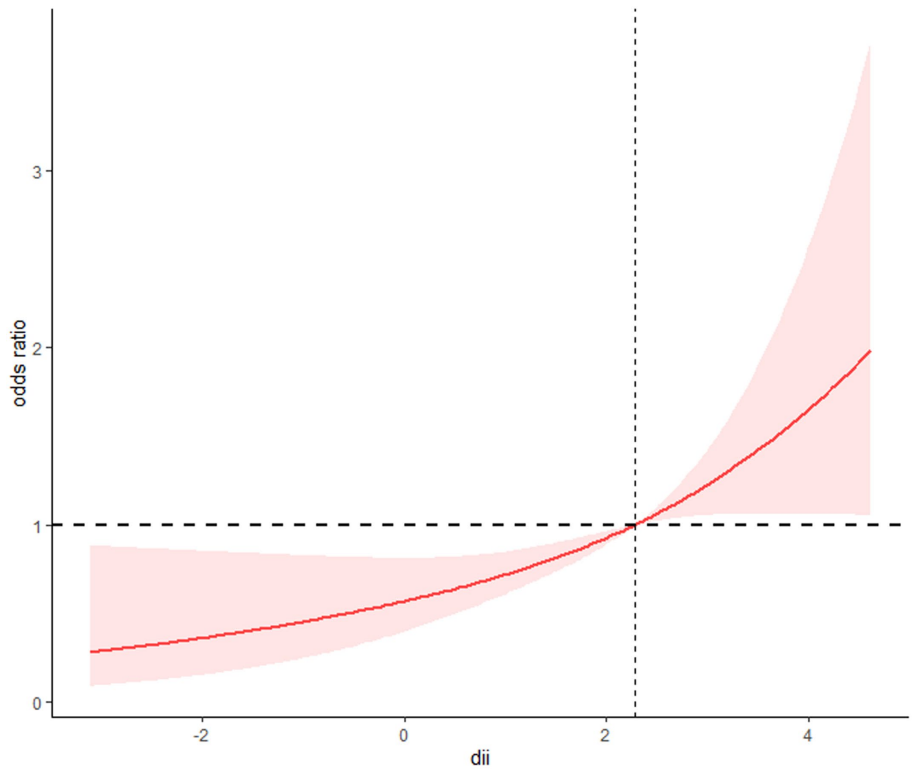

FIGURE 1
The restricted cubic spline model showed a dose–response relationship between dietary DII intake and sarcopenia. P for non-linearity = 0.98.

status, education, poverty, hypertension history, and diabetes history. No significant interaction was found between the DII index and stratification variables. However, interactions were observed between DII and race (Figure 3A). In terms of the relationship between ASMI and all-cause mortality, an interaction was observed between ASMI and history of diabetes, with no significant interactions found with other stratification variables (Figure 3B).

Discussion

Muscle loss has become a significant concern for quality of life in patients with various chronic diseases (35). While the association between COPD and sarcopenia has gained research attention in recent years (12, 36–38), to our knowledge, this study is the first to explore the link between DII and sarcopenia in COPD patients. It reveals that COPD patients with a pro-inflammatory diet are at higher risk of sarcopenia. Our study revealed a dose–response relationship between DII and sarcopenia in COPD patients, suggesting that higher DII index in dietary intake among COPD patients is associated with increased incidence of sarcopenia. Chen et al. (39) first reported the correlation between dietary inflammation index scores and muscle mass in older adults, confirming that an elevated DII index in the diet is linked to reduced muscle mass in the general population. Comparison with their data showed that in the highest quartile of DII index, COPD patients had significantly lower ASMI (7.17 ± 0.14) compared to the general population (7.40 ± 1.31), suggesting that a higher prevalence of pro-inflammatory diets among COPD patients

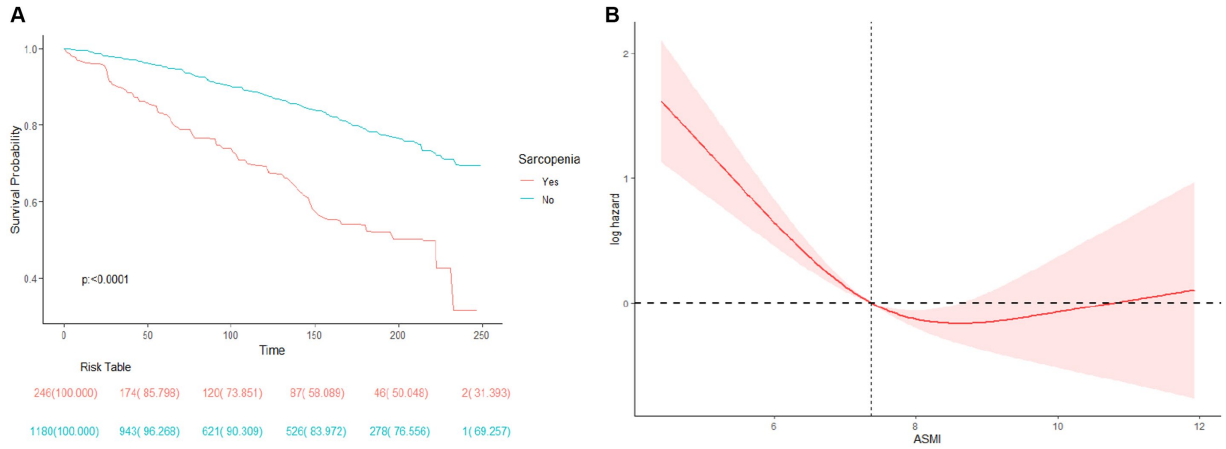

FIGURE 2
(A) Survival (Kaplan–Meier) curves for All-cause mortality according to history of Sarcopenia in COPD patients. **(B)** Association between ASMI levels and all-cause mortality in patients with COPD.

TABLE 3 HR (95% CI) for all-cause mortality according to COPD patients with concomitant sarcopenia.

Sarcopenia	HR(95%CI), *p*-value					
	Model 1		Model 2		Model 3	
Character	95%CI	*p*	95%CI	*p*	95%CI	*p*
No	ref		ref		ref	
Yes	3.18(2.36,4.28)	<0.0001	1.62(1.18, 2.23)	0.003	1.44(1.05, 1.99)	0.03
p for trend		<0.0001		0.003		0.03

Model 1: no adjustment. Model 2: adjusted for age, gender, race, poverty, education, marriage. Model 3: adjusted for age, gender, race, poverty, education, marriage, Diabetes, CVD, hypertension.

TABLE 4 Effect of ASMI level on survival: adjusted hazard ratios from segmented Cox (adjusted for age, gender, race, poverty, education, marriage, diabetes, CVD, hypertension).

Characteristic	Adjusted HR	95% CI1	*p*-value
ASMI≥8.32	0.9	(0.61, 1.34)	0.61
ASMI<8.32	0.58	(0.48,0.7)	<0.0001

may increase the risk of sarcopenia. Additional research on the association between sarcopenia and overall mortality in COPD patients showed that in the COPD population, for every unit increase when ASMI is below 8.32, the overall mortality risk decreases by 42%. This emphasizes the strong correlation between sarcopenia and mortality rates in COPD patients. Sepúlveda-Loyola et al.'s (40) meta-analysis results show that sarcopenia negatively affects various COPD-related clinical outcomes, underscoring the importance of studying COPD and sarcopenia. Our study findings suggest that modifying the pro-inflammatory diet in COPD patients can partially mitigate the decline in muscle mass, albeit with limitations. Further investigation into the factors influencing sarcopenia onset in COPD patients is needed for interventions aimed at enhancing patient quality of life.

COPD, as an inflammatory disease, has long been a focus of interest for exploring anti-inflammatory therapy (41). While current research has not conclusively shown the effective improvement of the disease process with anti-inflammatory drugs, inflammation remains a central focus of COPD pathogenesis research, and anti-inflammatory treatment retains significant research value (42, 43). Currently, COPD treatment primarily involves pharmacotherapy and non-pharmacological interventions. Commonly used drugs include β2 agonists, anticholinergic drugs, corticosteroids, and antibiotic prophylaxis (44). Non-pharmacological interventions mainly involve smoking cessation, long-term oxygen therapy, pulmonary rehabilitation, and nutritional support. However, current treatments can only alleviate symptoms and delay disease progression without curing COPD. Consequently, research focus has shifted to studying COPD-related extrapulmonary complications, including coronary heart disease, heart failure, renal insufficiency, and muscle wasting. As research progresses, extrapulmonary manifestations are increasingly recognized as significant contributors to functional decline in COPD patients (45). Functional impairment in COPD patients is linked to muscle weakness and weight loss (46, 47). Several assessment methods have shown reduced muscle mass in COPD patients (48). Furthermore, according to relevant literature, patients with moderate to severe COPD or those experiencing acute exacerbations are more prone to muscle mass and strength loss, particularly in the lower limbs (49). Severe sarcopenia can worsen COPD symptoms and increase the risk of death in patients.

Sarcopenia, also referred to as "muscle depletion," is characterized by the progressive decline in muscle strength, mass, and function, resulting in reduced physical capacity and heightened vulnerability to disability, falls, and mortality, frequently worsened by concurrent conditions like cardiovascular disease, COPD, and chronic kidney disease (50). Sarcopenia's underlying pathophysiological mechanisms are intricate, involving multifaceted interactions among various

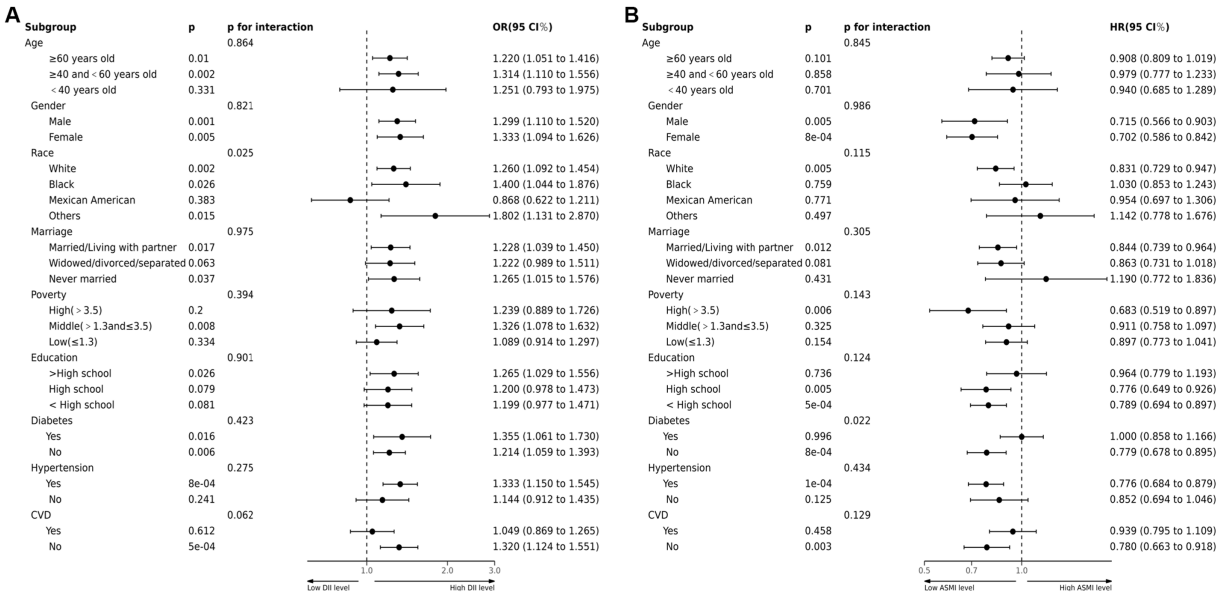

FIGURE 3
(A) Forest plots of stratified analyses of DII and sarcopenia in COPD. Age, gender, race, marriage, poverty, education and history of hypertension, CVD or diabetes were all adjusted except the variable itself. **(B)** Forest plots of stratified analyses of sarcopenia and all-cause mortality. Age, gender, race, marriage, poverty, education and history of hypertension, CVD or diabetes were all adjusted except the variable itself.

physiological systems (51), The principal mechanisms include: (1) Structural changes in skeletal muscle, characterized by disruptions in muscle homeostasis and neuronal degeneration leading to satellite cell aging, loss of type II fibers (hypoplasia), and diminished functional motor units (52, 53); (2) Muscle atrophy, attributed to metabolic disturbances such as skeletal muscle lipid or myocellular infiltration, alongside a pro-inflammatory state, insulin resistance, and glucose tolerance abnormalities (54); (3) Impedance of muscle homeostasis and synthesis metabolism, resulting from inhibition of muscle synthesis-related signaling pathways like serine/threonine kinase Akt/mTOR or disruptions in amino acid absorption, transport, and protein synthesis due to other metabolic diseases, leading to synthesis metabolic resistance, protein imbalance, and consequent muscle atrophy (55, 56); (4) Inflammation and mitochondrial dysfunction, marked by the chronic accumulation of pro-inflammatory and inflammatory factors such as CRP, IL-1, IL-6, and TNF-α, inducing skeletal muscle mitochondrial dysfunction, cellular degradation, heightened reactive oxygen species production, activation of the ubiquitin-proteasome cascade, and muscle protein hydrolysis, thereby causing muscle wasting. This mechanism is associated with an elevated risk of various chronic ailments, including heart failure, atherosclerotic heart disease, and COPD (57, 58); (5) Neurological pathways, where age-related degenerative alterations in the nervous system are implicated in muscle wasting development. The neuromuscular system's integrity significantly influences muscle contraction strength and speed. Although the precise mechanisms behind neurological system-induced muscle wasting are not fully elucidated, muscle wasting due to muscle weakness from impaired neuromuscular system integrity is deemed a pivotal mechanism of muscle wasting (59). Among elderly COPD patients, sarcopenia correlates with hastened COPD advancement, heightened mortality risk, increased falls, and diminished quality of life. Despite the intricate pathophysiological mechanisms, sarcopenia's fundamental etiology involves an imbalance between muscle synthesis and breakdown metabolism, sometimes accompanied by neuronal degeneration. Intrinsic molecular factors like aging, inflammatory response, chronic diseases, malnutrition, and reduced mobility contribute to sarcopenia development. Screening for sarcopenia, particularly in patients with chronic diseases like COPD, holds significant clinical relevance. Early identification of sarcopenia and timely intervention may mitigate the advancement of muscle disorders, thereby alleviating the adverse effects on COPD patient outcomes.

Inflammatory processes are a major contributor to accelerated muscle mass loss, particularly in COPD patients, where inflammation plays a crucial role in disease development and progression. Research by Buchmann et al. (57) has shown that elevated serum levels of inflammatory markers like IL-1β, IL-6, and CRP are linked to muscle weakness in critically ill patients, underscoring inflammation's significant role in sarcopenia development. Conversely, diet serves as a vital systemic regulator of inflammation, directly impacting muscle loss. Research findings from a six-year multicenter cross-sectional study by Abete et al. (60) suggest that adhering to the Mediterranean diet, consuming specific nutrients (e.g., vitamin C), and engaging in physical activity act as protective factors against sarcopenia development. This highlights the potential strategy of combining a healthy diet with exercise to counter age-related sarcopenia. Recent academic research indicates that dietary fiber intake may have therapeutic effects on inflammation (61). The DII serves as a valuable tool for assessing the overall inflammatory potential of the diet by considering the inflammatory properties of various dietary components. The inclusion of DII in relevant studies is becoming more common, providing valuable insights for informing clinical practice. Given that inflammation plays a critical role in COPD development, and current theories propose that a high dietary fiber

intake may attenuate age-related sarcopenia progression by reducing inflammation (62). However, prior to our study, research on the mediating role of DII in the relationship between COPD and sarcopenia risk among patients was lacking, and our study is the first to delve into this area.

This study has limitations. Firstly, cross-sectional studies allow us to investigate the association between DII and sarcopenia, not causal relationships. Further prospective studies or clinical trials are needed to confirm this. Secondly, sarcopenia involves a decrease in skeletal muscle mass accompanied by functional decline. In this study, we assessed sarcopenia solely through low muscle mass. Additionally, controversy persists regarding the adjustment of ASM for body size in different populations, and the diagnostic criteria for sarcopenia may vary, potentially affecting the diagnosis. Therefore, the diagnostic criteria for sarcopenia remain an area for future research. Moreover, due to the lack of relevant information in the NHANES dataset, muscle function was not assessed. Therefore, future research investigating the association between muscle function and DII is necessary. Thirdly, the DII score relies on food frequency questionnaires to obtain dietary intake data for calculation, which may be influenced by recall and misclassification biases. However, these biases may not differ between the COPD and non-COPD groups. Nonetheless, future studies may benefit from serum-based analyses to more accurately determine the direct relationship between DII levels and sarcopenia. Despite these limitations, the study has several strengths. Notably, it is the first to confirm the relationship between DII and sarcopenia in COPD patients based on the public NHANES database. The large and unstructured sample enhances the persuasiveness and applicability of our results. Additionally, we conducted multivariable regression and subgroup analyses to control for sociodemographic factors and some sarcopenia risk factors. Lastly, our study found an association between concomitant sarcopenia and higher all-cause mortality risk in COPD patients, suggesting the need for targeted improvements in muscle mass to enhance the quality of life and survival duration of these patients in the future.

Conclusion

Our study revealed that in COPD patients, the presence of sarcopenia is linked to an increased risk of all-cause mortality. Furthermore, ASMI demonstrates a non-linear association with all-cause mortality, with a threshold of 8.32 kg/m^2. Furthermore, a correlation exists between DII and the onset of sarcopenia in individuals with COPD. These results imply that lowering the DII score in the diet of COPD patients could offer potential benefits in preventing and managing the progression of COPD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

QJ: Conceptualization, Data curation, Software, Writing – original draft, Writing – review & editing. ZM: Conceptualization, Writing – original draft. JS: Writing – original draft. YL: Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1395170/full#supplementary-material

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Cross-domain microbiomes: the interaction of gut, lung and environmental microbiota in asthma pathogenesis

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Recent experimental and epidemiological studies underscore the vital interaction between the intestinal microbiota and the lungs, an interplay known as the "gut-lung axis". The significance of this axis has been further illuminated following the identification of intestinal microbial metabolites, such as short-chain fatty acids (SCFA), as key mediators in setting the tone of the immune system. Through the gut-lung axis, the gut microbiota and its metabolites, or allergens, are directly or indirectly involved in the immunomodulation of pulmonary diseases, thereby increasing susceptibility to allergic airway diseases such as asthma. Asthma is a complex outcome of the interplay between environmental factors and genetic predispositions. The concept of the gut-lung axis may offer new targets for the prevention and treatment of asthma. This review outlines the relationships between asthma and the respiratory microbiome, gut microbiome, and environmental microbiome. It also discusses the current advancements and applications of microbiomics, offering novel perspectives and strategies for the clinical management of chronic respiratory diseases like asthma.

KEYWORDS

gut-lung axis, respiratory microbiome, gut microbiome, asthma, short-chain fatty acids (SCFA)

1 Introduction

As microbiome research advances, it becomes increasingly clear that the gut microbiota interacts with various organs and systems in the human body, forming a complex network that collectively regulates many aspects of human metabolism. Studies have shown that disruptions in the gut microbiota are associated with a range of diseases, including respiratory disorders, metabolic conditions, and cardiovascular diseases, highlighting the microbiota's integral role in host physiology. Beyond the well-known gut microbiota, microbial communities in other regions of the body, such as the oral cavity, skin, reproductive tract, and respiratory tract, contribute to diverse functions in host regulation. These functions include pathogen inhibition, the secretion of bioactive metabolites, and immunomodulation. Furthermore, these microbiota can influence physiological processes in other parts of the body through the circulatory system (1). Although the functions of the gut and lungs differ significantly, they share a common developmental origin and possess similar

structural foundations. Both are covered by a thin mucosal layer that provides lubrication and defense against pathogens. Additionally, mucosal membranes are vital components of the integrated immune network that protects the body from infection. During respiratory diseases, the strictly regulated gut-lung axis may affect various molecular patterns, potentially exacerbating the severity of the disease and leading to dysfunction in normal functions. Research has revealed significant alterations in the respiratory microbiota, including bacteria, fungi, viruses, and their critical ligands and metabolites, in patients with chronic obstructive pulmonary disease (COPD) and allergic asthma, among other chronic respiratory diseases, compared to healthy individuals. However, the precise mechanisms by which these microbial communities influence disease development remain to be fully elucidated (2). Furthermore, the gut microbiota can potentially increase an individual's susceptibility to allergic airway diseases like asthma through the gut-lung axis. Investigating the interactions between respiratory microbiota and the host, as well as their associations with different disease phenotypes, can help identify biomarkers related to disease progression and risk stratification (3). These microbial communities inhabiting various body sites may also serve as potential targets for the treatment of chronic respiratory diseases.

Asthma represents a severe global health issue, being one of the most common chronic respiratory diseases in both children and adults. It's characterized by chronic airway inflammation, involving immune cells, inflammatory cells, airway epithelial cells, and various cellular components. Key features include airway inflammation, airway remodeling, and heightened airway responsiveness (4–6). Approximately 300 million people worldwide are affected by asthma (7), with prevalence rates ranging from 1 to 18% in different countries. In China, asthma incidence is on the rise, particularly among children. Reports indicate that asthma prevalence in individuals aged 20 and older in China is 4.26%, totaling 45.7 million patients (8). Asthma results from complex interactions between environmental factors and genetic susceptibility. The composition of the human microbiome is associated with increased rates of allergic diseases (9–11). One possible mechanism involves the presence of microbial communities in fetal placenta and meconium, suggesting that contact with bacteria occurs even before birth. Early fetal exposure to microbial communities and other allergens may contribute to inducing immune tolerance to these "harmless" antigens, thus preventing allergies in children. One possible mechanism involves the presence of microbial communities in fetal placenta and meconium, suggesting that contact with bacteria occurs even before birth (12). Furthermore, postnatal breastfeeding provides infants with a rich source of secretory IgA and prebiotic oligosaccharides, which aid in microbial colonization and enhance microbial diversity (13). The long-term stability of most microbial species begins at around the age of two. Therefore, studying the composition and function of early-life microbial communities can help unravel the development and occurrence of allergic diseases, as well as environmental risk factors that might induce these conditions. Microbial communities represent a potential therapeutic target for allergic asthma, serving as both preventive measures and adjunct therapies used in conjunction with oral allergen preparations or epidermal immunotherapy (14).

The human body hosts trillions of dynamic and diverse microbial communities collectively known as the "human microbiome" (15). These communities have the potential to regulate host metabolism, maintain immune system homeostasis, and prevent pathogen invasion. They are distributed throughout various parts of the body, with the majority residing in the gastrointestinal and respiratory tracts, commonly referred to as the gut microbiome and the respiratory microbiome. Over the past decade, significant advancements have been made in the field of microbiome research, propelled by initiatives such as the Human Microbiome Project, Human Microbiome Genomic Project, and the American Gut Project. The development of metagenomic techniques has shed light on the high diversity of microbial communities within the human body, both in terms of biology and functionality. The rapid progress of high-throughput sequencing technologies has enriched the ways in which researchers explore the interactions between microbial communities and their host, better facilitating the understanding of the roles, functions, and mechanisms of these communities in the human body and even in the global ecosystem. In the future, further in-depth research into the mechanisms mediated by microbial communities holds the promise of providing new perspectives and strategies for the prevention and treatment of a range of chronic respiratory diseases, including asthma.

2 Respiratory microbiome and asthma

For a long time, it was widely believed that the healthy human lung was a sterile environment. However, it has now been confirmed that the lungs are indeed colonized by a unique microbial community. This phenomenon is not limited to adults; even newborns have been found to have microbial communities in their lungs (16, 17). The lung microbiome exhibits two notable characteristics. First, the microbial density in the lungs is relatively low, with the bacterial genomes hidden in the human lung estimated to be around $2.2 \times 10^3/cm^2$ (18). This density is comparable to that found in the human duodenum, which contains $\sim 10^4$/mL microbes (19). Second, the lung microbiome is in a constant state of renewal and replacement, with the most common communities consisting mainly of the *phyla Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes* (3, 20–22). Similar to the gastrointestinal tract, *Actinobacteria* and *Firmicutes* are considered part of the "core" microbial communities in the lungs (23). The mucosal immune system, an integral part of the human immune network, interacts with the abundant microbial communities residing on the mucosal surfaces of the respiratory tract, contributing to the maintenance of the body's homeostasis and playing a crucial role in infection defense (24). The balance of the respiratory microbiota is constituted by the dynamic process in which the microbiome migrates into the lungs through "micro-aspiration" and the host defense mechanisms clear the microorganisms. When the composition and/or function of the microbial communities on the respiratory mucosa change, the critical ligands and metabolites of bacteria, fungi, and viruses in these microbial communities can impact the regulation of the host immune system (2). This can occur by upregulating inflammatory signals (such as NF-κB, Ras, IL-17, and PI3K) or inhibiting the

production of tumor necrosis factor (TNF) and IFN-γ in response to lower airway pathogens, ultimately leading to the development of chronic respiratory diseases. In mice, it has been validated that after aspiration of a mixture of oral commensals (*Prevotella melaninogenica, Veillonella parvula, and Streptococcus mitis*), lower airway immune function changes occur, leading to the activation of $CD4^+$ and $CD8^+$T cells and the recruitment of IL-17-producing T cells (25). However, it remains uncertain whether microbial dysbiosis is the cause of the disease or a result of the disease process. Studies have also indicated that during respiratory diseases, such as cystic fibrosis (26), chronic rhinosinusitis (27), and asthma (28), there is dysfunction of lung cilia, increased mucus secretion, and enhanced bacterial migration (e.g., gastroesophageal reflux). These conditions appear to exceed the airway's capacity to clear microorganisms, leading to increased microbial density and a disrupted balance of airway microbiota. In a prospective cohort study in Denmark conducted by Thorsen et al. (29), they analyzed the respiratory microbial community composition of 700 one-month-old children. They found that higher diversity in children's respiratory microbial communities and the high abundance of Streptococcus and Prevotella were associated with lower levels of TNF-α and IL-1β in the respiratory immune system, and elevated levels of CCL2 and CCL17. Changes in these cytokines are closely related to the development of asthma and are considered independent risk factors. This discovery reveals the relationship between the characteristics of the respiratory microbiome and the respiratory immune system, providing new evidence for the impact of early-life microbial communities and their interaction with immune factors on asthma risk.

While the precise mechanisms through which the respiratory microbiome regulates the development of diseases remain incompletely understood, extensive research has provided strong evidence linking dysbiosis in the respiratory microbiome to the onset and progression of asthma. The lungs, being a vital site that interfaces with the external environment, host a unique microbiome characteristic to lung tissues. DNA sequencing analyses have highlighted substantial differences in the microbial composition between healthy and diseased lungs (30). Compared to healthy control populations, individuals with asthma exhibit higher microbial diversity in their microbiome and altered composition, characterized by an increase in *Proteobacteria* and a decrease in *Actinobacteria*. Notably, Wang et al. (31) analyzed sputum microbiota in asthma patients, revealing a correlation between reduced abundance of Granulicatella and asthma, while confirming an increase in Streptococcus in the respiratory microbiota of asthma patients. Ariel Hernandez-Leyva et al. found that specific bacteria in the upper respiratory microbiome, including Haemophilus, Moraxella, and Streptococcus, were associated with an increased risk of wheezing and acute respiratory infections during childhood (32). Furthermore, a longitudinal multicenter cohort study conducted by Abdel-Aziz et al. analyzed the sputum microbiota from 100 severe asthma subjects, finding that individuals with a loss of core microbiota (reduced microbial richness and decreased α-diversity) exhibited more severe asthma phenotypes (33). Additionally, the composition of bronchial bacterial communities has been found to be associated with the degree of airflow obstruction and hyperresponsiveness in asthma subjects. In patients with mild asthma, bacterial diversity is negatively correlated with the degree of bronchial hyperresponsiveness (34). However, as asthma severity increases, the nature of these associations changes. It has been reported that in severe asthma, lower bacterial diversity is associated with more severe airflow obstruction, further suggesting different mechanisms of action of bacterial communities in the pathogenesis of the disease (35).

The multiple studies mentioned above unequivocally demonstrate an association between the composition and diversity of the respiratory microbiome and various asthma phenotypes (36, 37). Microbial communities can regulate immune responses in the lungs through different mechanisms, impacting the onset, phenotypic expression, severity, and treatment outcomes of asthma (5). These findings indicate that alterations in the respiratory microbiome offer potential targets for the treatment of chronic respiratory diseases. Therefore, researching specific bacteria and the structural characteristics of the respiratory microbiome contributes to the development of improved asthma diagnostics and prevention methods, offering valuable insights for translating research findings into practical applications.

3 Mechanisms of respiratory microbiota dysbiosis in asthma

Asthma is a complex chronic inflammatory disease that primarily affects the airways, leading to airway narrowing, difficulty breathing, and wheezing. In recent years, an increasing number of studies have shown that dysbiosis of the respiratory microbiome is closely related to the occurrence and development of asthma. Under normal circumstances, the respiratory microbiome functions to maintain immune balance and defend against pathogen invasion. However, when this microbiome becomes imbalanced, it can trigger a series of immune responses, leading to increased inflammation and airway hyperresponsiveness. Understanding the specific mechanisms of this dysbiosis is of great significance for developing new therapeutic strategies.

3.1 Immune system imbalance

Asthma is a chronic inflammatory disease where the immune system plays a critical role. In asthma patients, the immune response to respiratory microbiota is often abnormal, leading to excessive inflammation. Specifically, asthma is associated with an enhanced Th2-type immune response, resulting in the overproduction of IgE antibodies. These IgE antibodies bind to allergens, triggering mast cells and eosinophils to release inflammatory mediators such as histamines and leukotrienes, which further cause airway inflammation and narrowing. Moreover, the immune system in asthma patients may react inappropriately to certain microbes, complicating airway inflammation (38, 39).

3.2 Alterations in microbiota composition

Under normal conditions, the respiratory microbiota is diverse and balanced, maintaining a healthy airway environment.

However, in asthma patients, this balance is disrupted, leading to the overgrowth of specific pathogenic bacteria. For example, *Streptococcus pneumoniae* (40) and *Haemophilus influenzae* (41) are frequently increased in the airways of asthma patients. The overgrowth of these pathogens can directly cause infections and release toxins and other harmful substances, further exacerbating airway inflammation.

3.3 Imbalance of metabolic products

The metabolic products of the respiratory microbiota are crucial for maintaining airway health. Normally, the microbiota produces short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, which have anti-inflammatory properties and regulate the host immune response. In asthma patients, due to changes in microbiota composition, the production of these beneficial metabolites may decrease, leading to heightened inflammatory responses. Additionally, some pathogenic microbes may produce harmful metabolites, worsening asthma symptoms (42).

3.4 Compromised airway barrier function

Airway epithelial cells form the primary barrier of the respiratory tract, preventing pathogen invasion. In asthma patients, the airway epithelial barrier function is often impaired, characterized by reduced tight junction proteins and disrupted epithelial cell layer integrity. This impairment allows pathogens to enter and colonize the airways more easily, inducing and exacerbating inflammation. Dysbiosis of the microbiota further weakens the barrier function, creating a vicious cycle that makes the airways more susceptible to infections and persistent inflammation (43).

3.5 Host genetic factors

The genetic background of the host plays a significant role in the development and progression of asthma. Numerous studies have identified gene variations associated with asthma susceptibility. For example, variations in genes such as IL-4, IL-13, and ADAM33 (44) can affect immune responses and airway structure and function, influencing the microbiota composition and function. Genetic factors may also regulate the microbiota-host interaction by affecting the development and function of the host immune system, impacting the course of asthma.

3.6 Environmental influences

Environmental factors significantly impact the balance of respiratory microbiota. Allergens (e.g., pollen, dust mites), air pollutants (e.g., $PM_{2.5}$, vehicle exhaust), and lifestyle factors (e.g., smoking, diet) can alter the composition of respiratory microbiota (45, 46). Frequent antibiotic use, in particular, disrupts normal microbiota, leading to the overgrowth of harmful bacteria. Environmental factors, combined with genetic susceptibility, can trigger or exacerbate asthma (47).

3.7 Microbiota-host interactions

The interaction between respiratory microbiota and host cells is a critical regulatory mechanism in asthma. Microbes can influence the host immune response by interacting with receptors on the surface of host cells. Certain probiotics, for instance, can promote anti-inflammatory signaling pathways by binding to epithelial cell receptors, protecting the host from excessive inflammatory responses. In asthma patients, however, this microbiota-host interaction may be dysregulated, resulting in abnormal immune responses and persistent inflammation (48–50).

4 Gut microbiome and asthma (lung-gut axis)

The gut microbiome is the most densely populated microbial community within the human body (51), primarily composed of the *Firmicutes, Bacteroidetes, Actinobacteria*, and *Proteobacteria phyla* (52). It's noteworthy that the Lactobacillus genus within the *Firmicutes phylum* is one of the most significant probiotics in the gut microbiome. Increasing evidence suggests that the *Lactobacillus* genus and its constituents can engage in bidirectional immune signaling between the gastrointestinal tract and distant organs, such as the lungs. This interaction promotes anti-inflammatory responses and ameliorates asthma-related symptoms (53–55).

While the gastrointestinal and respiratory tracts are anatomically separate, they share a common embryonic origin and structural similarities, indicating that they may interact in various ways. This bidirectional communication hub between the gut and lungs is referred to as the "gut-lung axis" (56). It emphasizes the interactions and connections between gut microbiota and lung microbiota, which can influence the immune status of both organs (50), enabling bidirectional communication and regulation, and impacting an individual's susceptibility to allergic airway diseases like asthma (57, 58). Lee-Sarwar et al. (59) analyzed the gut microbiota composition and metabolites in 110 asthmatic children, discovering that children with frequent wheezing had an enrichment of the *Veillonella* genus and metabolites related to the histidine pathway in their gut. Conversely, bacterial abundances of other groups like Spirochaetaceae UCG-005, Holdemanella, and Mollicutes were negatively correlated with wheezing. These findings offer insights into further research on the mechanisms of the gut microbiota's role in asthma. Early-life dysbiosis in the gut microbiota of newborns is associated with an increased risk of developing asthma during childhood. The potential mechanism behind this is the elevated concentration of 12,13-diHOME produced by specific gut microbiota enzymes in newborns predisposed to asthma, which may reduce the number of lung Treg cells, inhibit dendritic cell anti-inflammatory factor secretion, and

lead to metabolic dysfunction, potentially increasing the risk of asthma (60). Similarly, children who develop asthma during school age were found to have lower gut microbiota diversity before the age of 1 month (61). Colonization of Veillonella (*Firmicutes phylum*) at 1 month of age was also associated with wheezing and asthma at 6–7 years of age (62). Although the direct causal relationship between gut microbiota and asthma is not yet established, these studies show that changes in early-life microbial communities and specific components can affect a child's susceptibility to asthma and may trigger specific responses later in development.

Current research has identified two primary categories of communication pathways within the gut-lung axis (63, 64): (i) Direct Impact of Gut Microbiota: This includes the presence of molecules like peptidoglycans and lipopolysaccharides (LPS), which can enhance the host's immune responses. (ii) Indirect Impact of Gut Microbiota: (a) Digestive Byproducts Entering the Circulatory System: Metabolites produced during digestion in the gut can enter the peripheral bloodstream and affect the development of immune cells. (b) Production of Short-Chain Fatty Acids (SCFA): Certain gut microbes metabolize dietary fiber to generate SCFAs, which can then trigger immune responses in the host's lungs. Unmetabolized SCFAs can also affect lung immune responses either by directly migrating to the lungs and enhancing G protein-coupled receptor (GPCR) activation (65) or by migrating to the bone marrow and promoting the differentiation of macrophages and dendritic progenitor cells into anti-inflammatory alternatively activated macrophages (AAMs). AAMs are a subtype of macrophages with immune-regulating capabilities that can reduce neutrophil recruitment and stimulate the production of anti-inflammatory cytokines (e.g., IL-10 and TGF-β) from Treg cells, thereby reducing lung damage and inflammation (54, 58). Besides, SCFAs can also modulate the metabolic pathways of alveolar macrophages exposed to LPS, a process critical for maintaining pulmonary immunometabolism (66). Furthermore, the detection of SCFAs in sputum can confirm the connection between the gut and the lungs (67, 68). A deeper understanding of the intricate mechanisms by which SCFAs, derived from gut metabolism, exert their complex effects holds promise for the development of more effective therapeutic interventions for chronic respiratory diseases (69). However, further extensive experimental and clinical validation is still needed. (c) Migration of Gut Immune Cells: Immune cells from the gut can directly migrate to the lungs via the peripheral circulatory system, influencing lung immune activities. Studies have shown that interleukin-25 (IL-25) can induce type 2 innate lymphoid cells (ILC2) to migrate from the gut to the lungs, participating in Th2-type immune responses and contributing to lung inflammation (70, 71). (d) Exchange via Lymphatics: Both gut and lung microbiota can communicate through lymphatic circulation (23, 58, 64, 71). This bidirectional communication is facilitated by chemical signals generated directly by microbiota or because of immune triggers. These signaling molecules can circulate systemically through blood and lymphatic fluids, regulating the host's immune system (72, 73). A comprehensive understanding of the complex mechanisms underlying the effects of SCFAs on the gut-lung axis can aid in the development of more effective interventions for chronic respiratory diseases. Nevertheless, further experimental and clinical validation is still required to explore these interactions (Figure 1).

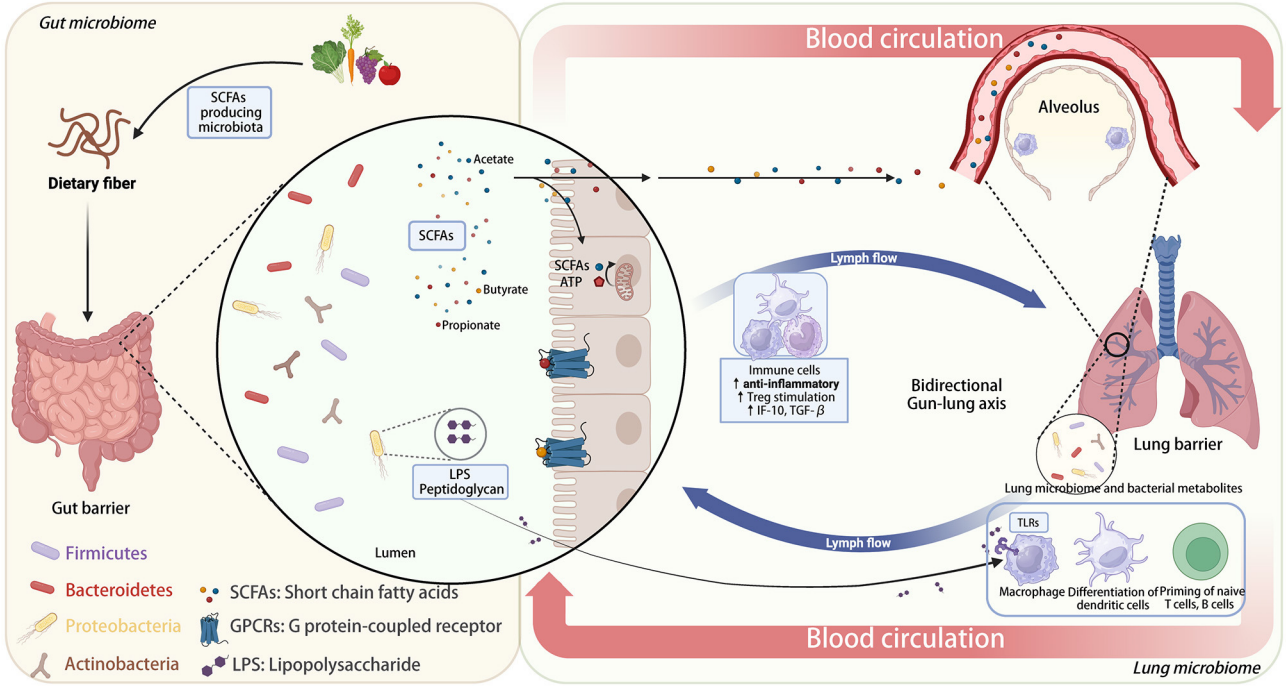

FIGURE 1
Bidirectional gut-lung axis: the role of dietary fiber and microbiota-derived SCFAs in immune modulation.

5 Environmental microbiome and asthma (external intake)

The hygiene hypothesis posits that reduced exposure to microbes in early childhood due to increased sanitation and hygiene practices may contribute to the rise in allergic diseases, including asthma. Studies have shown that children who grow up on farms or in rural environments, where they are exposed to a diverse range of environmental microbiota, have a lower prevalence of asthma compared to children in urban settings. The exposure to various microorganisms is thought to help in the proper development of the immune system, reducing the risk of developing asthma (74–76). The microbial flora colonized in human respiratory tract and environmental microbial flora are closely related to the prevalence of allergic asthma (77, 78). One prospective cohort study involving nearly 400 children and spanning a 10-year follow-up period found that the composition and richness of indoor microbiota in homes were inversely correlated with children's asthma risk. Furthermore, there were significant differences in the indoor microbiota composition between asthmatic and non-asthmatic children. The abundance of Streptococcus was positively correlated with asthma incidence, while the abundance of Actinomycetales was negatively correlated with asthma and could largely explain the association between indoor microbiota richness and asthma (79). Farm environments have served as a model for studying determinants of early-life allergies. Epidemiological research conducted in these settings suggests that prenatal exposure may be related to the early activation of the fetal immune system. Specifically, sustained maternal exposure to microbial compounds during pregnancy appears to be associated with a reduced risk of atopic sensitization and disease outcomes in offspring (80, 81). Exposure to pets, especially during early childhood, has been associated with a reduced risk of asthma. Studies suggest that children exposed to pets such as dogs and cats have a more diverse gut and respiratory microbiota, which may help in modulating the immune response and protecting against asthma (82, 83). Similarly, exposure to livestock on farms has been shown to have a protective effect against asthma and allergies (84).

The interaction between the human gut and its microbial communities is not only regulated by environmental changes but is also closely associated with dietary habits. These two factors together lead to a wide array of bacterial selection (85). This selection may result in changes in the composition and abundance of bacterial communities, affecting the ecological functions and interactions of microbes in different environments. Research suggests that dietary factors play a significant role in the development of allergic diseases. High-fat diets that induce obesity can trigger or exacerbate asthma. In contrast, diets rich in dietary fiber can modulate lung immunity. The intake of high dietary fiber not only alters gut microbiota but also affects the microbial composition in the lungs, indicating the regulatory role of nutritional factors in maintaining lung immune homeostasis by inhibiting the activation of Th2 effector cells to alleviate allergic airway inflammation (86). Moreover, diverse gut microbiota is associated with better health outcomes. Increasing microbial diversity or the abundance of certain beneficial bacteria can reduce the occurrence of allergic symptoms (87). These findings underscore the potential benefits of diverse diets, highlighting the pivotal role of both diet and gut microbiota composition in shaping lung immune responses. Common protective dietary factors include polyunsaturated fatty acids (Omega-3, eicosapentaenoic acid, and docosahexaenoic acid), dietary fiber, SCFAs, vitamins A and D, and zinc (88–90). These components play a certain role in the prevention or alleviation of allergic asthma (91). By studying lung microbiota, the interaction between lung and gut microbiota, and their relationship with different disease phenotypes, we can identify biomarkers for disease progression and risk stratification.

In sum, other epidemiological evidence strongly supports the role of environmental microbiome exposure in the development and regulation of asthma. Factors such as urban vs. rural living, exposure to pets and livestock, antibiotic use, mode of birth, breastfeeding, air pollution, and diet all play significant roles in shaping the respiratory and gut microbiomes, thereby influencing the risk of developing asthma. Understanding these associations can help design preventive strategies and interventions to reduce the burden of asthma.

6 Application of microbial sequencing technology in asthma

In microbiome research, the analysis of microbial community composition primarily relies on two high-throughput approaches: amplicon sequencing (16S/18S/ITS) and whole metagenome shotgun sequencing (WMS) (92). Among these, metagenomics and meta-transcriptomics are advanced techniques using shotgun sequencing methods. They employ Microbiome-Wide Association Studies (MWAS) to reveal the taxonomic composition, functional metabolic profiles, and active expression profiles of the entire microbial community comprehensively and precisely at the DNA/RNA levels. Compared to amplicon sequencing based on genes like 16S, 18S, or ITS, metagenomics genuinely explores the ecological roles and underlying mechanisms of microbial communities in ecosystems. It delves deeper into microbial ecological questions at the functional level and represents the cutting-edge technology and essential methodology in microbiome research.

The 16S and ITS rRNA (Internal Transcribed Spacer ribosomal RNA) sequencing are common amplicon sequencing methods used to identify bacteria or fungi in a given sample. The 16S rRNA gene is prevalent in bacterial cells and is located in the ribosomal small subunit (~1,540 base pairs), which comprises nine variable regions and ten conserved regions. The conserved regions are used to design primers for targeted amplification, while analysis of the variable regions enables the identification of genera or species in different microbial communities, allowing for systematic evolutionary classification. Currently, regions used for deep sequencing of the 16S rRNA gene include V4, V3-V4, and V4-V5 (93), making it well-suited for bacterial phylogenetic studies and species identification. The ITS1 region of the rRNA subunit is a common DNA marker used to identify fungal species in metagenomic samples (94). Next-generation sequencing (NGS) of ITS and 16S rRNA genes allows for systematic phylogenetics

TABLE 1 Common microbiome sequencing techniques.

Technology	Sequencing content	Sequencing depth	Application scope	Advantages	Disadvantages
16S rRNA Sequencing	16S rRNA gene fragments	Relatively shallow	Microbial community structure analysis, taxonomy	Low cost, simple operation, well-established databases	Limited resolution, amplification bias, off-target amplification, cannot distinguish at the species level
Metagenomics	All microbial genes	Deep sequencing (tens of Gb to several Tb)	Functional analysis of microbial communities, metabolic pathway studies	High resolution, identifies microbial species and functional genes	High cost, complex data analysis
Metatranscriptomics	mRNA from environmental samples	Moderate to deep	Gene expression and functional activity of microbial communities	Reveals dynamic functional activities of microbes	RNA stability issues, prone to degradation, complex data processing

and classification comparisons of complex microbiomes or samples that are otherwise challenging or impossible to study, making them mature techniques in this application field. However, amplicon sequencing does have its limitations, such as amplification biases, off-target amplification, and low species resolution, and it typically cannot simultaneously detect bacteria, archaea, and fungi. Shotgun metagenomic sequencing, on the other hand, can comprehensively detect all genes from every organism present in a given complex sample, allowing for the assessment of bacterial diversity and detection of microbial abundance across various environments. Moreover, it can also be employed to study unculturable microorganisms, which are challenging to analyze using other methods. NGS-based shotgun metagenomic sequencing can combine multiple samples in a single sequencing run, each achieving high sequencing coverage, enabling the detection of extremely low-abundance organisms within microbial communities. Due to the characteristic of directly fragmenting gene sequences, this technique effectively mitigates amplification bias. Moreover, theoretically, it does not limit the analysis to specific species, potentially yielding information on new genes and even new species. In conclusion, the advantages of meta-transcriptomics include moving beyond the confines of DNA, allowing for real-time insights into active genes and pathways contributing to community functions. Nevertheless, shotgun sequencing demands high DNA quality and quantity from samples, making it difficult to analyze highly degraded or severely contaminated specimens, with relatively higher sequencing costs. To overcome the core limitations of amplicon sequencing and shotgun sequencing, some studies have proposed a simplified shotgun metagenomics technology called 2bRAD-M, which can efficiently isolate microbial samples with minimal, highly degraded, or heavily contaminated from the human body and the environment. The principle behind 2bRAD-M involves processing total DNA samples from microbial communities, amplifying and sequencing cut fragments with IIB enzymes. Then, it uses the theoretical enzyme cleavage sites on various microbial genomic sequences to infer microbial community structures (95). Metatranscriptomic sequencing involves large-scale high-throughput sequencing of transcripts from all microbial communities in a specific environment at a specific time. This approach directly captures transcriptome information from both culturable and non-culturable microorganisms in the environment. Not only does this technology possess all the advantages of metagenomics, enabling the detection of active microorganisms, active transcripts, and active functions in the environment, but it also allows for the comparison of differentially expressed genes and functional pathways across different environments. This comparison reveals the adaptation mechanisms of microorganisms under various environmental pressures and explores the interaction mechanisms between the environment and microorganisms (Table 1).

Traditional microbial diagnostic techniques include the use of culture media (substrates and agar), serological examinations for pathogen-related antibodies, and the use of PCR technology to detect microbial genetic material (DNA or RNA). However, these techniques have significant limitations regarding their scope of application and detection sensitivity. Specifically, traditional microbial culture techniques typically require bacteria or fungi to be cultivated in media-rich environments, potentially rendering them unable to culture certain microorganisms present in extraneous environments. Furthermore, some microorganisms exist at extremely low concentrations in natural environments, making them challenging to detect using conventional culture methods. In some cases, culturing bacteria or fungi may take a significant amount of time, which is impractical for situations that require the rapid determination of the presence of pathogens, such as in infectious disease diagnostics. Hence, the next generation of RNA sequencing (RNA-Seq) for bacteria, viruses, and other microorganisms has become the standard method for analyzing transcriptome and meta-transcriptome information. RNA-Seq technology does not depend on culture or specific DNA sequences but directly detects RNA molecules within microbial samples. In addition, RNA-Seq provides critical information about microbial activity, metabolic pathways, and gene expression, which is vital for understanding microbial ecology, physiology, and pathogenicity. Furthermore, microbial transcriptome and meta-transcriptome information is crucial for predicting antibiotic resistance, understanding host-pathogen immune

interactions, quantifying changes in gene expression, and tracking disease progression.

7 Conclusions and prospects

At present, the exact mechanisms underlying the pathogenesis of allergic asthma remain incompletely understood. Clinically, patients often experience recurrent and episodic symptoms, necessitating long-term use of symptomatic drugs such as corticosteroids, antihistamines, and bronchodilators to manage the condition. However, these symptomatic treatments do not provide long-term effectiveness and carry potential risks of drug resistance and side effects (96). Hence, there is an urgent need for in-depth research into the pathogenesis of allergic asthma to identify new therapeutic targets. Over the past decade, there has been a growing consensus that respiratory, gut, and environmental microbiota play pivotal roles in the development and progression of asthma and allergic diseases. Targeting respiratory and/or gut microbiota offers potential avenues for the prevention and management of allergic asthma and other chronic respiratory diseases (97). Notably, the integrating multi-omics analysis techniques is beneficial for uncovering the functional effects and potential mechanisms of gut microbiota in chronic respiratory diseases. This aids in exploring the intricate gut-lung axis and accelerates research into the complexity of interactions between resident microbiota and mucosal immune systems. The highly diverse microbial ecosystem within the human gastrointestinal tract exerts profound influences on the host's immune, metabolic, endocrine, and other physiological processes, all of which are interconnected. Nonetheless, the mechanisms involving host cell functional or genomic changes induced by gut microbiota and the potential involvement of gut microbiota metabolites in the pathophysiology of lung diseases remain largely unknown. The causality between gut microbiota and most lung diseases is yet to be determined. Further research in this domain is needed to unveil these complex interactions.

Therefore, by advancing our understanding of the role of microbiota in the development of asthma, it becomes possible to further evaluate the critical roles played by microbiota in human chronic airway inflammation. This has the potential to lead to the development of novel therapeutic strategies involving dietary interventions (SCFA), probiotics (*Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*), fecal microbiota transplantation, or selective bacterial manipulation to modify microbiota (98). This review posits that investigating the relationship between microbiota changes and the development of lung diseases provides an avenue to identify new therapeutic targets. This is poised to yield significant breakthroughs in targeted screening models and microbiota-based therapies, offering fresh perspectives and strategies for clinical practice in the management of chronic respiratory diseases.

Author contributions

JZ: Writing – original draft. XZ: Writing – original draft. WL: Writing – review & editing. BS: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A synbiotic mixture of *Bifidobacterium breve* M16-V, oligosaccharides and pectin, enhances Short Chain Fatty Acid production and improves lung health in a preclinical model for pulmonary neutrophilia

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Introduction: Pulmonary neutrophilia is a hallmark of numerous airway diseases including Chronic Obstructive Pulmonary Disease (COPD), Neutrophilic asthma, Acute Lung Injury (ALI), Acute Respiratory Distress Syndrome (ARDS) and COVID-19. The aim of the current study was to investigate the effect of dietary interventions on lung health in context of pulmonary neutrophilia.

Methods: Male BALB/cByJ mice received 7 intra-nasal doses of either a vehicle or lipopolysaccharides (LPS). To study the effect of nutritional interventions they received 16 intra-gastric doses of either a vehicle (PBS) or the following supplements (1) probiotic *Bifidobacterium breve* (*B. breve*) M16-V; (2) a prebiotic fiber mixture of short-chain galacto-oligosaccharides, long-chain fructo-oligosaccharides, and low-viscosity pectin in a 9:1:2 ratio (scGOS/lcFOS/lvPectin); and (3) A synbiotic combination *B. breve* M16-V and scGOS/lcFOS/lvPectin. Parameters for lung health included lung function, lung morphology and lung inflammation. Parameters for systemic immunomodulation included levels of fecal short chain fatty acids and regulatory T cells.

Results: The synbiotic supplement protected against the LPS induced decline in lung function (35% improved lung resistance at baseline $p = 0.0002$ and 25% at peak challenge, $p = 0.0002$), provided a significant relief from pulmonary neutrophilia (40.7% less neutrophils, $p < 0.01$) and improved the pulmonary neutrophil-to-lymphocyte ratio (NLR) by 55.3% ($p = 0.0033$). Supplements did not impact lung morphology in this specific experiment. LPS applied to the upper airways induced less fecal SCFAs production compared to mice that received PBS. The production of acetic acid between day −5 and day 16 was increased in all unchallenged mice (PBS-PBS $p = 0.0003$; PBS-Pro $p < 0.0001$; PBS-Pre, $p = 0.0045$; PBS-Syn, $p = 0.0005$) which upon LPS challenge was only observed in mice that received the synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin ($p = 0.0003$). A moderate correlation was found for butyric acid and lung function parameters and a weak correlation was found between acetic acid, butyric acid and propionic acid concentrations and NLR.

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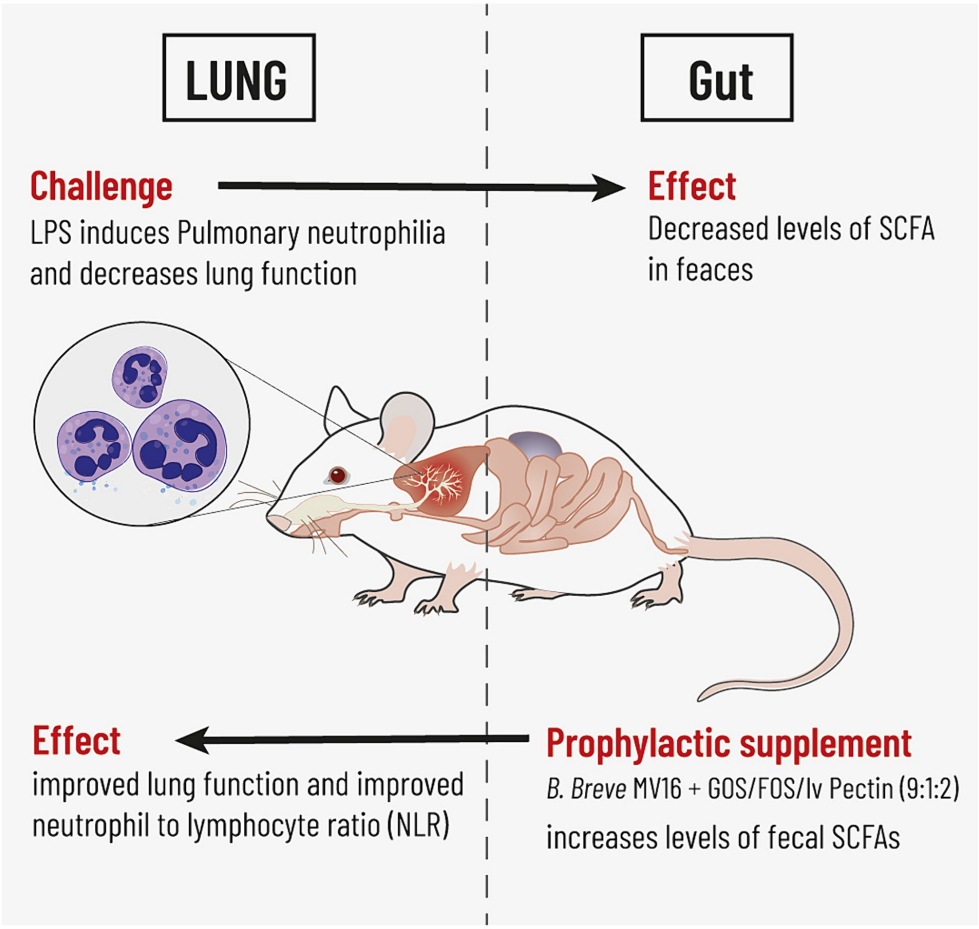
Conclusion: This study suggests bidirectional gut lung cross-talk in a mouse model for pulmonary neutrophilia. Neutrophilic lung inflammation coexisted with attenuated levels of fecal SCFA. The beneficial effects of the synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin on lung health associated with enhanced levels of SCFAs.

KEYWORDS

pulmonary neutrophilia, gut-lung axis, nutraceuticals, synbiotics, neutrophil to lymphocyte ratio, Short Chain Fatty Acids, acetate, butyrate

1 Introduction

Neutrophils are innate immune cells that play a key role in the first line of defense among others against invading pathogens. In a balanced situation, neutrophils undergo programmed cell death after their defensive actions, which when dysregulated, contributes to neutrophil accumulation, mucus hypersecretion, tissue destruction, airway remodeling and poor prognosis of certain lung diseases (1–4). Pulmonary neutrophilia is a hallmark of numerous airway diseases including, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, Acute Lung Injury (ALI), Adult Respiratory Distress Syndrome (ARDS) and certain types of severe persistent asthma (5–14). Such chronic respiratory diseases remain the leading causes of prevalence, mortality and disability-adjusted life years (DALY) with a high socioeconomic burden (15–17). COVID-19 is another recent example of an infection induced illness in which increased neutrophil to



GRAPHICAL ABSTRACT
This study highlights bidirectional gut lung cross-talk in a mouse model for pulmonary neutrophilia. Repeated pulmonary challenge with LPS induced neutrophilic lung inflammation which was associated with a reduction in levels of fecal SCFA. Intra-gastric supplementation with the synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin induced enhanced levels of SCFAs and a positive impact on lung health indicated by improved lung function and improved neutrophil to lymphocyte ratio (NLR).

lymphocyte ratio (NLR) correlates with disease severity and mortality (18–22). With rising prevalence of chronic respiratory diseases and uncontrolled respiratory infections posing high socioeconomic burden, cost-effective solutions for maintaining respiratory health and reducing neutrophilic hyperinflammation are of utmost importance.

Throughout the last decades, evidence is accumulating that diet and gut health play important roles in maintaining effective and balanced immune responses and homeostasis in and beyond the gastro-intestinal tract (23, 24). The gut resides relatively high numbers of bacteria, from which a total microbial to human cell ratio of 1.3:1 can be estimated (25). The intestinal microbiota is crucial for human health and require human diet as chief source of energy for their growth (26). Diet has a large and temporal effect on gut microbiota composition which has implications for inflammation and autoimmune diseases. Pro-, pre-, and synbiotics, are considered appealing cost-effective gut microbiota modulators that could aid the management of allergic and infectious airway diseases, via the so-called "gut-lung axis" (27–30). Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer health benefits on the host." (31). Dietary prebiotics are defined as "selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host cells" (26). The definition of a synbiotic was updated May 2019 to "a mixture comprising live microorganisms ánd substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host," in other words a combination of pro and prebiotics (32). Although the interaction between diet and immunology seems promising it is also complex and requires more information before disease-specific recommendations can be made (33).

Current scientific literature illustrates that modulation of the microbial gut ecosystem with pro-, pre- and synbiotics has a multi-facetted impact on pulmonary immune responses. Dampening of the immune system is broadly proven in the context of allergic eosinophilic asthma and boosting of the immune system is shown to enhance defense against respiratory infections (34). These apparently contradictory anti- and pro-inflammatory effects of gut microbiota changes can in part be explained Toll Like Receptor (TLR) activation (35). TLRs regulate the balance between different immune responses by recognizing specific pathogen associated molecular patterns (PAMPS) and other microbial-, or commensal associated molecular patterns (MAMPS or CAMPS) (35–40). Commensal gut bacteria boost gut-lung mediated innate immune activation in part via the activation of TLR4, which is demonstrated in defense against *E. coli* pneumonia (41). Although the benefit of TLR4 activation during certain infections is evident, the overzealous pulmonary activation of the TLR4 pathway is linked to excessive neutrophilic inflammation, locally and systemically (35, 42, 43). In the current study a TLR4 activating compound, Lipopolysaccharides (LPS), was used as a model agent to induce such hyperactivation of innate immunity leading to pulmonary neutrophilia (44). This model simulates TLR4 mediated neutrophilic lung inflammation which is illustrative of pathogens and cigarette smoke (45–53), ozone, nitrogen dioxide and traffic related air pollutants (54–57) and TLR4 mediated consequences in severe neutrophilic COVID-19 (58–64).

Relatively little is known about the immune modulating capacity of the gut lung axis in the context of a disease state that is characterized by such overzealous activation of innate immune pathways. Literature covering the effect of gut microbiota modulation on neutrophilic lung disorders is relatively scarce compared to the evidence supporting anti-allergic and pro-defensive (anti-infective) effects (65). It has previously been reported that supplementation with different Lactobacilli strains can increase an immune response in neutrophils indicated by enhanced neutrophil respiratory burst enzymes and nitric oxide production over a period of 60 days, which was stated to be strain dependent and to reach a maximum capacity within a window of stable health (66). In chronic diseases however, the interplay between microbiota and neutrophils is highly contextual and has been mentioned to have both disease worsening as well as improving capacity (67). A more recent review elaborated on the strain specificity of probiotics toward neutrophil recruitment, indicating an advanced need for thorough examination of probiotic effects on neutrophils in specific disease contexts (68). A careful consideration of the right ingredient for the right disease context asks for a more thorough investigation of gut modulating ingredients in context of pulmonary neutrophilia.

In the current study *Bifidobacterium breve* (*B. breve*) M16-V was selected which is a strain that originates from the gut of an infant and has emerged as a commercial probiotic supplement to help establish a favorable microbial gut ecosystem of infants (69). Colonization of mucosal surfaces by microbiota in early life occurs in parallel to the development and education of the mucosal immune system which has long-standing consequences on inflammatory diseases (70). Since the effects of microbiome modulation stretch into adulthood it reasons further investigations for more broad applications of dietary concepts originating from studies for infant health (71). *B. breve* strains can indeed help to fight acute respiratory infections and shorten the illness period (72). Existing data is however also supportive of the hypothesis that *B. breve* could have added value in the specific context of TLR4 mediated hyperinflammation. *B. breve* compared to other broadly used Lactobacilli strains, has been shown to exert immune inhibitory effects by activating TLR9, and TLR2 but not TLR4 explaining its relatively low pro-inflammatory profile (73, 74). It has also been shown that Bifidobacterium, including *B. breve* M16-V, has the ability to reduce the incidence of necrotizing enterocolitis (NEC) in part by down-regulating TLR4 signaling (69, 75).

This supports further investigation of the capacity of *B. breve* to dampen excessive LPS mediated innate immune activation. A previous study already revealed that *B. breve* can prevent the development of alveolar damage and right ventricle heart hypertrophy in an LPS induced mouse model for COPD (76). However, in that specific COPD model, *B. breve* did not have an effect on the numbers of neutrophils in broncho alveolar lavage fluid (BALF) in contrast to prophylactic treatment with a prebiotic scGOS/lcFOS formula, that was shown to reduce alveolar damage, heart hypertrophy as well as neutrophilic inflammation (76).

A beneficial effect of *B. breve* M16-V along the gut-lung axis has also been shown in murine allergic models illustrating its ability to dampen chronic eosinophilic airway inflammation to the same extend as the widely used inhalation corticosteroid, budesonide (73). *B. breve* M-16 V combined with short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (scGOS/lcFOS) resulted in a preventive effect on asthma-like symptoms in infants and possibly on subsequent development of asthma (77, 78). This scGos/lcFos formula was originally developed to mimic the function and structure of oligosaccharides from breast milk and has proven benefits on baby's

health including the reduced occurrence of infections and reduced incidence of allergic symptoms (77, 79).

In the current study design a prebiotic fiber mixture was included consisting of scGOS/lvFOS further supplemented with lvPectin (mimicking the acidic fraction of human milk oligosachcharides) which are plant cell-wall polysaccharides that can be metabolized by commensal bacteria in the gut, including *Bifidobacterium* (80). To test the value of such a mixture in context of pulmonary neutrophilia, a synbiotic formula of *B. breve* M16-V combined with scGOS/lcFOS/lvPectin was included in the study design as well. Furthermore, a functional parameter for lung health was added next to immunological and morphological lung parameters. Finally, the current study elaborated on associations between gut and lung by analyzing the effect of inhaled LPS and of the dietary supplements on Short Chain Fatty Acid (SCFA) production. SCFA are gut microbe-derived metabolites that act as key mediators affecting the direction of the local and systemic immune system (30). Breastmilk and/or formula with probiotics/prebiotics could modulate toward more favorable microbial species producing different amounts of SCFAs exerting anti-inflammatory effects which has been linked to improve microbial dysregulation associated with increased TLR4 signaling in preterm infants (81). SCFAs have previously also been linked to a protective effect of enteral diets in context of elastase-induced lung inflammation and emphysema (82).

2 Materials and methods

2.1 Animals

136 Male BALB/cByJ mice, 6–8 weeks of age were obtained from Charles River Laboratories. Mice were group housed under controlled conditions (temp 20°C, humidity 40–60%, and an inversed 12-h light–dark cycle). Cage bedding was enriched with tissue and non-toxic pvc pipes. Mice were given *ad libitum* access to standard food (801730 CRM (E) Expanded Special Diets Services, England) and water. Mice were randomly divided over the different study groups and were allowed to adapt for 2 weeks before the start of the experiments. Clinical appearance was checked throughout the study by measuring body weight at least 3 times per week and by scoring vital signs daily. Scoring categories included: normal indicated as "0"; moderate discomfort indicated as "1"; and severe discomfort indicated as "2." Scoring indicators included any changes in behavior (abnormal breathing/chest tightness, excessive salivation, immobility, shaking and tremors, continuous convulsion, inability to respond to stimuli, changes in social grooming, self-mutilation) and external appearance (pilo erection fur, abnormal posture, injuries). All animal studies were approved by the Utrecht Universities Committee on Animal Research (DECnumber: 2011.II.02.044) and comply with the principles of 3R. The animal experiments are carried out in accordance with (inter) national guidelines of animal experiments.

2.2 Experimental design LPS model and intra-gastric supplements

An overview of the experiment is presented in Figure 1. Male BALB/cByJ mice were instilled intra nasally (i.n.) under isoflurane anesthesia with 50 µL phosphate-buffered saline (PBS) or LPS (5 µg/mouse/challenge, dissolved in 50 ul PBS) purified from *Escherichia coli* O55:B5 (Sigma-Aldrich, Zwijndrecht, the Netherlands) which was administered 7 times in total, spread over a period of 15 days. Starting 1 week prior to the first LPS exposure until the end of the experiment, mice received 5 oral gavages per week to provide intra-gastric supplementations. Control groups (12 to 20 mice per group) received 200 ul of a vehicle (PBS) and study groups (20 mice per group) received 200ul of a solution (pH7) comprising one of the 3 dietary formula: (1) 25 mg of a prebiotic fiber mixture of GOS:FOS:lvPectin in a 9:1:2 ratio; (2) 50 mg of the probiotic *B. breve* M16-V (5×10^9 cfu/dose); and (3) A synbiotic combination of 25 mg GOS:FOS:lvPectin 9:1:2 and 50 mg *B. breve* M16-V (5×10^9 cfu/dose). Formulations were prepared fresh daily, short before enteric administration because of the oxygen sensitivity of *Bifidobacteria*. At the end of the experiment, at day 16, mice were euthanized, with an overdose of 600 mg/kg body weight sodium pentobarbital, i.p. (Nembutal™, Ceva Santé Animale BV, Naaldwijk, the Netherlands) and exsanguinated by cardiac puncture after which biological samples were obtained for further analysis.

2.3 Measurement of basal lung function *in vivo*

Per study group, 12 mice were reserved for the analysis of airway function, which was measured using an invasive EMKA plethysmography system (EMKA technologies) as described previously (83). Briefly, 24 h after the final exposure, mice were anesthetized via i.p. injection with a mix containing Ketamine (Vetoquinol S.A., France; 125 mg/kg) and Medetomidine (Pfizer, Netherlands; 0.4 mg/kg). Body temperature was kept at 37°C by placing the mice on a heating pad. Mice received a trachea cannula for mechanical ventilation with O2/air (1, 2). Mimicking spontaneous breathing, ventilation frequency was set at 150 breaths/min and tidal volume at 0.3 mL. Mice were placed in individual body boxes equipped with a pressure transducer (EMKA Technologies, Paris, France). For measuring transpulmonary pressure, the signal was obtained via a probe inserted alongside the trachea via the esophagus. Airflow and tidal volume (V_t) were determined using a flow transducer able to measure flow fluctuations inside the body box. V_t is expressed in ml. The lung resistance (R_L) was obtained by dividing the transpulmonary pressure by airflow at isovolume points (measured for 3 min) (cmH2O/ml/s). After measurement of basal lung function, 7 increasing doses of methacholine (acetyl-β-methyl-choline chloride, Sigma) (0–25 mg/mL, 10% puff for 10 s) were administered by aerosol generated in a nebulizer (EMKA Technologies, Paris, France) connected in between the animal in the body box and the ventilator (EMKA Technologies, Paris, France). After each dose of methacholine, V_t and R_L were measured for 3 min. After the final dose of Metacholine, mice were euthanized by an intraperitoneal overdose of sodium pentobarbital (Nembutal™, Ceva Santé Animale, Naaldwijk, The Netherlands, 600 mg/kg BW) followed by exsanguination by heartpuncture.

2.4 Measurement of cellular and cytokine parameters in BALF

Directly after airway function analysis and exsanguination, the lungs of 12 mice per experimental group were lavaged 4 times *in situ*

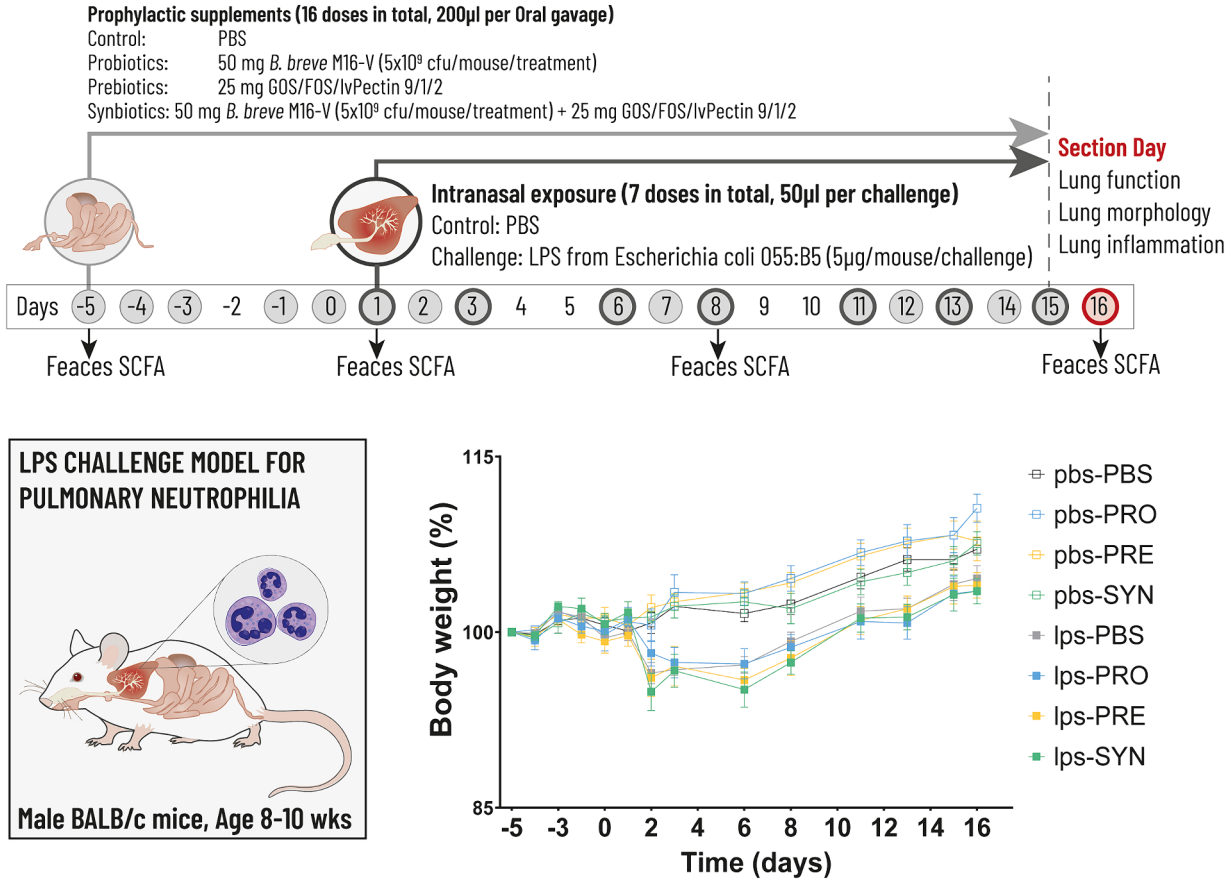


FIGURE 1
The timeline (above) depicts the experimental design for the LPS induced mouse model for pulmonary neutrophilia. The timeline shows the days on which the airways were challenged with either LPS or PBS control (dark grey circles) and the days on which the intragastric prophylactic supplements were provided (solid light grey circles). The arrows indicate the 4 timepoints at which feces was collected for the analysis of Short Chain Fatty Acids (SCFA). Lung function, lung morphology and lung inflammation was analyzed at day 16 (red circle). The graph (below) depicts body weight which was analyzed throughout the experiment as key indicator for mouse well-being. Stunted growth as a result of the first LPS challenge remained within acceptable levels of discomfort reaching a max of 3.5%, the day after the first LPS challenge (n.s.).

with each 1 mL of pyrogen-free 0.9% w/v saline (37°C) via a cannula that was inserted into the trachea. The first ml of lavage fluid was supplemented with a protease inhibitor cocktail (Complete mini, Roche applied sciences) to avoid degradation of protein components. All BALF samples were kept on ice until cells were pelleted at 400 g for 5 min at 4°C. Supernatants were discarded except from the first lavage, which was aliquoted and stored at -80°C until further analysis for keratinocyte-derived chemokine (KC), Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), Macrophage Inflammatory Protein-1α (MIP1a), mouse Interleukin-23 (p19/p40) (IL-23 P19/P40) using a Cytometric Bead Array (CBA) flex set according to standard instruction manual (BDTM CBA flex sets: instrument Setup, Data acquisition, and analysis) using BD FACSCanto. The BALF cell pellets were resuspended in 150 µL of cold 0.9% w/v normal saline and pooled per animal. Total cell numbers and viability were assessed by trypan blue exclusion using a Burker-Türk chamber. For differential cell counts, 5×10^4 cells per BALF sample were cytospinned for 5 min at 400 g, then airdried and stained with a Diff-Quik staining set (Dade A.G., Düdingen, Switzerland). A total of 200 cells were counted from each slide for differential cellular categorization in macrophages, neutrophils and lymphocytes based on morphological differences.

2.5 Measurement of FoxP3+ regulatory T cells

An exploratory analysis of the presence of FoxP3+ regulatory T cells (FoxP3 Treg) in thoracic – and mesenteric lymph nodes and in spleen was performed by multi-parameter flow cytometry. Freshly isolated spleens and lymph nodes were weighed after which in 5 mice per group the cells were isolated using a cell strainer. Red blood cells were lysed and cells were washed after which they were suspended in standard Fluorescence Activated Cell Sorting (FACS) buffer (PBS/1% Bovine serum albumin (BSA)). Cells were incubated at a density of 1×105 cells/100 µL per well with the following anti-mouse antibodies from ebioscience: anti-mouse FoxP3-APC, CD4-FITC and CD25-PE. Isotype controls were used as indicated in the manufacturers protocol: Rat Immunoglobulin G (IgG)2b, k and IgG2a, k (ebioscience 17-4321-41), Armenian hamster IgG Isotype (ebioscience 11-4888-81 and 12-4888-81). Cells were washed to remove the unbound antibodies and resuspended in PBS/1%BSA prior to analysis on a FACS Canto II using CellQuest software (BD Biosciences, the Netherlands). For the analysis of forward angle light scatter, side angle light scatter, and cell surface receptor expression, data were acquired

in real time as percent (%) positive-expressing cells and geometric mean fluorescence intensity (MFI).

2.6 Lung morphometric and histological analysis

Since both lung function analysis and BALF retrieval influence the airway architecture, separate mice, (8 per study group plus 1 PBS-PBS control group), were used for purposes of lung morphometric analysis. After exsanguination, a cannula was placed in the trachea and the lungs were taken out en bloc. The lungs were inflated with neutral 10% formalin under constant pressure (25 cm fixative) for five minutes. Tracheas were sutured and lungs were immersed in fresh fixative until completely degassed after which the lungs were changed to 70% ethanol. Left lungs were further dehydrated and embedded in paraffin blocks. Five µm sections were cut and samples at various tissue depths (200 µm, 400 µm, 600 µm and 800 µm) were mounted on coated slides after which the tissue was rehydrated and stained with Mayer hematoxylin/eosin (H&E). In order to analyze changes in tissue morphology, six photo-microscopic images were made per section at a total magnification of 10 times10x. Inter alveolar distance was measured by the mean linear intercept (Lm). Microscopic images were projected onto a reference grid using Image Pro MC Plus 7.0 software. Lm was expressed as the total grid length in µm divided by the number of alveolar wall-grid line intersections. A higher Lm-score reflects more disruption of alveolar walls which is an indication for emphysematous damage.

2.7 Short chain fatty acids (SCFAs)

At 4 time points throughout the experiment, 7 mice per experimental group were shortly separated for individual dry fecal pellet collection (Figure 1). Urine was absorbed using tissue as cage bedding. Two pellets per animal were dissolved immediately after collection at a dilution of 0.11 g feces/ml PBS and stored at −80 until quantitative SCFA analysis by a Varian 3,800 gas chromatograph (GC) (Varian, Inc., Walnut Creek, U.S.A.) equipped with a flame ionization detector as described before (84). Briefly, 350 µL of fecal suspension was mixed with 200 µL 5% (v/v) formic acid, 100 µL 1.25 g/L 2-ethylbutyric acid (as internal standard, Sigma-Aldrich, Zwijndrecht, The Netherlands) and 350 µL MilliQ water. The samples were centrifuged for 5 min at 16,000 × *g* to remove large particles. The supernatant was collected of which, 0.5 µL was injected at 80°C in the column (Stabilwax, 15 m × 0.53 mm, film thickness 1.00 µm, Restek Co., USA) using helium as carrier gas (3.0 psi). Next, the oven was heated at a speed of 16°C/min to 160°C, followed by heating at a speed of 20°C/min to 220°C at which the temperature was maintained for 1.5 min. The temperature of the injector and the detector was 200°C. Levels of acetic acid, propionic acid and butyric acid are expressed in µmol/g of fecal weight.

2.8 Statistical analysis

Data are expressed as mean ± standard error of mean (SEM). Comparisons between data were tested using GraphPad Prism version 9.1. Methods used included descriptive statistics; ordinary one way analysis of variance (1 way ANOVA) with Bonferroni's multiple comparisons test; Tukey's multiple comparison test, with individual variances computed for each comparison (2 way ANOVA); Simple lineair regression; and Unpaired t test; and Pearson correlation. Data were considered statistically significant with an p value smaller than 0.05. To label the strength of the Pearson associations, the r values between, 0–0.3 (or −0.3) are considered as weak, 0.3–0.7 (or −0.7) as moderate, 0.7–1.0 (or −1) as a strong positive (or negative) correlations (85).

3 Results

3.1 Animal weights and vital signs

Mouse vital signs were checked on a daily basis and weights were measured at least 3 times per week. During the adaptation phase, minor fight wounds (score of "1" in the well-being diary) occurred in 2 groups, which stabilized and did not reason for individual housing. During the gavage procedure damage was induced to the esophagus in 2 individual cases resulting in weight loss exceeding 15% of total body weight and a score of "2" in the well-being diary due to changes in social grooming and abnormal posture to avoid unnecessary further pain or distress these mice were euthanized immediately by cervical dislocation. Apart from these 2 cases, vital signs remained normal (score "0") in all study groups throughout the experiment. The intranasal LPS challenge induced an attenuation of the normal body weight increase in all groups, which is a signal for the expected illness driving effect of LPS (Figure 1). The drop in body weight reached 3.5%, the day after the first LPS challenge, after which growth resumed. The impact of LPS on bodyweight was not significant and remained within reasonable levels of discomfort. The intra-gastric supplementations did not influence vital signs or bodyweight, which is a supportive indication for the tolerability of the oral supplements.

3.2 Airway function

Lung function was analyzed in anesthetized and mechanically ventilated mice, by an EMKA plethysmography system. At baseline, LPS induced a modest decrease in tidal volume of 16.2% compared to mice that received i.n. PBS ($p = 0.0019$) (Table 1). The difference between LPS and PBS remained at a modest decrease of 15.7% ($p = 0.0019$) after exposure to the highest concentration of Metacholine. Tidal volume was not impacted by any of the treatments at basement level nor at increased levels of Methacholine. At baseline, LPS induced a robust effect on lung resistance (41.5% increase, $p < 0.0001$) compared to mice that received i.n. PBS (Table 2; Figure 2A). The synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin significantly dampened the negative effect of LPS on lung resistance by 34.9% at baseline ($p = 0.0099$). Metacholine dose response did trigger further worsening of the LPS induced adverse effect on lung resistance, reaching a 62% increase compared to the PBS control group ($p < 0.0001$, Figure 2A). The synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin was the only supplement that provided a significant decrease of the LPS induced deterioration in airway resistance up to the highest concentration of metacholine

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TABLE 1 Overview of airway function, tidal volume data.

Tidal volume										
	PBS–PBS		LPS–PBS		LPS–PRO		LPS–PRE		LPS–SYN	
Basal function	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
	0.32	0.021	0.27	0.019	0.29	0.024	0.26	0.017	0.31	0.023
Challenge effect (Difference PBS-PBS)	-		−16.2%** $p = 0.0019$		-					
Treatment effect (Difference LPS-PBS)	-		-		8.21$^{n.s.}$		−4.46$^{n.s.}$		13.76$^{n.s.}$	
Metacholine (mg/mL; 10% puff; 10s)										
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	0.31	0.020	0.27	0.018	0.28	0.022	0.25	0.016	0.30	0.022
0.38	0.30	0.021	0.26	0.016	0.27	0.022	0.24	0.016	0.29	0.022
0.75	0.30	0.021	0.25	0.018	0.27	0.020	0.24	0.016	0.29	0.022
1.56	0.29	0.021	0.24	0.017	0.26	0.020	0.24	0.016	0.28	0.022
3.13	0.28	0.022	0.23	0.019	0.25	0.019	0.22	0.015	0.27	0.024
6.25	0.27	0.022	0.23	0.019	0.24	0.019	0.21	0.015	0.26	0.024
12.5	0.26	0.023	0.22	0.019	0.23	0.018	0.20	0.015	0.24	0.025
25	0.25	0.022	0.21	0.019	0.23	0.017	0.20	0.017	0.23	0.025
Max Challenge effect (Difference PBS-PBS)	-		−15.66% n.s.		-					
Max Treatment effect (Difference LPS-PBS)	-		-		9.00$^{n.s.}$		-6.19$^{n.s.}$		11.43$^{n.s.}$	
Simple linear regression metacholine dose response										
Equation	Y = −0.00895*X + 0.313		Y = −0.00777*X + 0.264		Y = −0.00777*X + 0.282		Y = −0.00824*X + 0.255		Y = −0.00927*X + 0.303	
Goodness of Fit (R squared)	99.35%		99.42%		99.23%		98.63%		96.74%	
Challenge effect (Difference PBS-PBS)	-		$p = 0.0093$		-					
Treatment effect (Difference LPS-PBS)	-		-		n.s.		n.s.		n.s.	

ns $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$.

($p < 0.0002$, Figure 2A). Simple linear regression analysis confirmed the change between the tidal volume metacholine dose–response curves LPS-placebo and PBS-placebo ($p = 0.0093$) and absence of a treatment effect within the LPS challenged groups (Figure 2B). Simple linear regression analysis also confirmed a strong significant change between the lung resistance dose–response curves of the PBS-placebo and LPS-placebo groups ($p < 0.0001$) as well as a treatment effect of synbiotics within the LPS challenged groups ($p = 0.001$) (Figure 2C). The linear regression analysis furthermore pointed toward indications for a treatment effect of probiotics, $p < 0.0001$ and prebiotics $p = 0.0042$ on altering the effect of the methacholine stressor compared to mice that received a placebo supplement.

3.3 Lung morphology

Lung morphometric analysis was analyzed in distinct groups of mice, to avoid damage of the airway architecture as a result of lung ventilation or lavage procedures. H&E-stained lung tissue sections of all LPS challenged groups showed areas of inflammation and remodeling of the lung parenchyma which was absent in mice that received i.n. PBS. Representative micrographs of the lung parenchyma of 3 different mice per group are shown in Figure 3A. Images of the LPS challenged groups depict intraluminal-, peribronchial-, and perivascular cellular infiltrates, thickening of the bronchial-, and alveolar walls and detachments of mucosal epithelium. Figure 3B furthermore shows a selection of representative images of the distal alveolar airspaces of 3 different mice per group, depicting alveolar wall breakdown in the LPS challenged groups. The calculated Lm score, as indicator for emphysematous damage revealed little alterations between the different groups (Figure 3C). Lm was lowest in the PBS-PBS control group with a mean of 44.11 µm. The window of average Lm in the LPS groups ranged between 46.04 µm and 47.65 µm. An unpaired t test with Welch's correction provided a modest difference between PBS-PBS and LPS-PBS ($p = 0.0469$). Of all tested treatments, the Synbiotic group showed the lowest Lm-Score (mean 46.04 µm), which was however not significantly different from the LPS-PBS group.

TABLE 2 Overview of airway function, lung resistance data.

Lung resistance										
	PBS–PBS		LPS–PBS		LPS–PRO		LPS–PRE		LPS–SYN	
Basal function	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
	0.82	0.056	1.16	0.12	0.92	0.097	0.89	0.08	0.76	0.041
Challenge effect (Difference PBS-PBS)	-		41.47% **** $p < 0.0001$		-					
Treatment effect (Difference LPS-PBS)	-		-		−20.67 n.s.		−23.17 n.s.		−34.94*** $p = 0.0002$	
Metacholine (mg/ml; 10% puff; 10s)										
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	0.89	0.06	1.29	0.13	1.27	0.22	1.23	0.21	0.94	0.12
0.38	0.95	0.07	1.51	0.17	1.37	0.25	1.34	0.22	1.03	0.16
0.75	1.04	0.07	1.63	0.18	1.42	0.20	1.42	0.25	1.23	0.21
1.56	1.24	0.10	1.87	0.20	1.55	0.21	1.55	0.26	1.32	0.21
3.13	1.32	0.11	2.05	0.21	1.74	0.27	1.71	0.26	1.49	0.21
6.25	1.45	0.13	2.34	0.23	1.86	0.26	1.98	0.29	1.75	0.20
12.5	1.55	0.14	2.54	0.24	1.93	0.25	2.20	0.30	1.92	0.21
25	1.72	0.14	2.80	0.31	2.12	0.30	2.35	0.31	2.10	0.23
Max Challenge effect (Difference PBS-PBS)	-		62.77%**** $p < 0.0001$		-					
Max Treatment effect (Difference LPS-PBS)	-		-		−24.41% n.s.		−16.08% n.s.		−24.98%*** $p = 0.0002$	
Simple linear regression										
Equation	Y = 0.1203*X + 0.8483		Y = 0.2146*X + 1.252		Y = 0.1223*X + 1.228		Y = 0.1668*X + 1.139		Y = 0.1693*X + 0.8811	
Goodness of Fit (R squared)	98.94%		99.36%		98.61%		97.18%		98.75%	
Challenge effect (Difference PBS-PBS)	-		$p < 0.0001$							
Treatment effect (Difference LPS-PBS)	-		-		$p < 0.0001$		$p = 0.0042$		$p = 0.001$	

ns $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$.

3.4 Pulmonary inflammation

Intraluminal cellular and protein components were quantified in BALF (Figure 4). Challenging the airways with LPS induced significant increase in total numbers of cells, which was 10.7 fold higher compared to PBS control ($p < 0.0001$) (Table 3; Figure 4A). The synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin significantly dampened the negative effect of LPS on total influx of cells by 28.6% ($p < 0.01$). Differential analysis performed based on morphological differences of Diff-Quik stained BAL cells (Figure 4B) showed that LPS induced a significant upregulation of all quantified cells with a 4.3 fold increase in macrophages ($p < 0.0001$); 7.8 fold increase in lymphocytes ($p < 0.0001$); and 77.7 fold increase in neutrophils ($p < 0.0001$) compared to mice that received i.n. PBS (Figure 4C). In unchallenged mice, none of the analyzed enteric supplements influenced the differential amount of BAL cells. In the challenged mice however, the pro- pre- and synbiotic groups all presented lower neutrophil counts than the LPS-placebo group but a significant reduction in pulmonary neutrophilia was only observed in the synbiotic group. These mice that received *B. breve* M16-V and GOS:FOS:lvPectin had 40.7% less neutrophils ($p < 0.01$), 20.7% less macrophages (n.s.) and 30.6% more lymphocytes (n.s.) than the LPS-placebo group. These numbers are reflected in significantly improved pulmonary neutrophil-to-lymphocyte ratio of 55.3% ($p = 0.0033$) (Figure 4D). Figure 4E furthermore shows that the proinflammatory challenge effect of LPS was reflected by the analyzed cytokine profile showing a 55.3% increase in MIP ($p = 0.0451$) and a 49.6% increase in KC ($p = 0.0081$). The treatment effect was only in part reflected by the analyzed cytokine profile showing a significant decrease in MIP (32.3%, $p = 0.0451$) but only a slight non-significant decrease in KC of 9.3%. Table 3 provides a full overview of the lung inflammation data.

3.5 FoxP3+ regulatory T cells

Figure 5 shows that prebiotic supplementation with GOS:FOS:lvPectin 9:1:2 had a high count in mesenteric lymphnodes of unchallenged mice (mean 12.5 with a PBS reference mean of 3.72) but not in challenged mice (mean 3.2 with a PBS reference mean of 5.48).

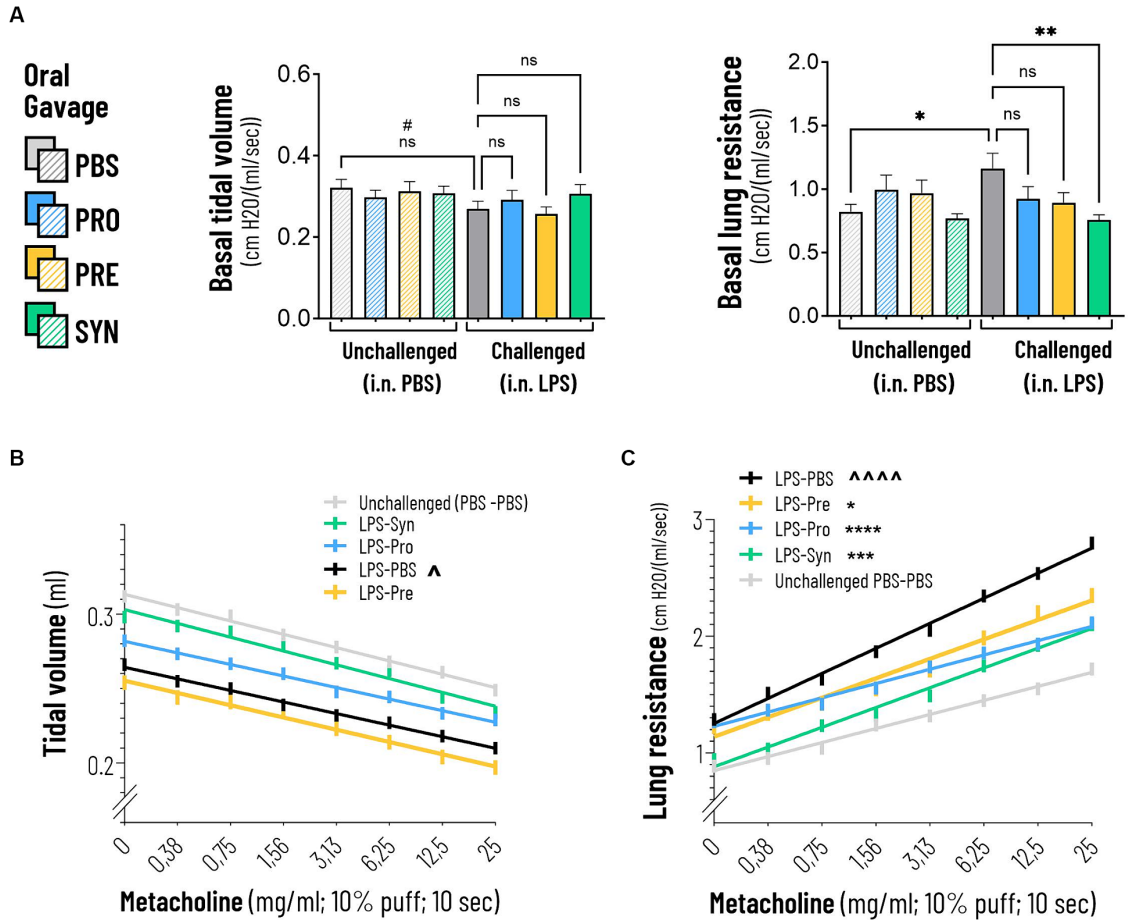

FIGURE 2
(A) Basal tidal volume was not impacted by intranasal LPS challenge. Lung resistance was negatively impacted by the intranasal LPS challenge at baseline and at increasing doses of Metacholine (indicated by ****$p < 0.0001$). The synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin significantly dampened the negative effect of LPS on lung resistance by 34.9% at baseline and 25% at peak challenge (indicated by ***$p < 0.001$ throughout the challenge). **(B)** The linear regression graph presents the pattern that step-wise methacholine challenge induces on the tidal Volume. A significant change (indicated by ^$p = 0.0093$) was seen between the effect of methacholine on tidal volume of the LPS-placebo and PBS-placebo. A significant treatment effect on tidal volume was not observed. **(C)** Simple linear regression analysis confirmed a strong significant change between the lung resistance dose–response curves of the PBS-placebo and LPS-placebo groups (indicated by ^^^^$p < 0.0001$). A treatment effect of synbiotics was also observed within the LPS challenged groups (indicated by ***$p = 0.001$). The lineair regression analysis furthermore pointed toward indications for a beneficial treatment effect of Probiotics, $p < 0.0001$ and Prebiotics $p = 0.0042$ on altering the effect of methacholine compared to mice that received a placebo supplement.

High numbers of Tregs were also counted in the mice that received the synbiotic formula of *B Breve* M16-V and GOS:FOS:lvPectin 9:1:2 in the thoracic lympnodes (unchallenged mean 4.4; LPS challenged mean 0.862) with PBS reference mean of 0.5 in unchallenged mice and 0.3 in LPS challenged mice. Treg counts in spleen in the synbiotic group were also higher in unchallenged mice (mean 1.8) than in LPS challenged (mean 0.9), with a PBS reference mean of 0.6 in unchallenged mice and 0.5 in challenged mice. Statistical analysis did not reveal any significant results. Table 4 provides a full overview of the Treg data.

3.6 Short Chain Fatty Acids

SCFA are suggested to support beneficial gut-lung crosstalk, therefore we analyzed concentrations of SCFA at 4 time points throughout the experiment (Figure 6). The first thing that stood out was that unchallenged mice showed a significant increase in the production of acetic acid between day −5 and day 16 (PBS-PBS $p = 0.0003$; PBS-Pro $p < 0.0001$; PBS-Pre, $p = 0.0045$; PBS-Syn, $p = 0.0005$) which in LPS challenged mice was only the case in the mice that received the synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin ($p = 0.0003$). Levels of acetic acid were 30.5% higher in LPS-synbiotic group compared to the LPS-placebo group. The fractional difference between day −5 and day 16 was 1.17 in the unchallenged placebo group and 0.73 in the challenged placebo group. Table 5 provides a full overview of the SCFA data. Similar patterns were observed for the amount of fecal butyric acid and propionic acid. All the analyzed enteric supplements induced higher levels of fecal butyric acid than the LPS-placebo group. On day 16, the levels of butyric acid in the synbiotic supplement group were 223.4% higher than day −5 ($p < 0.0001$), followed by 93.2% for the prebiotic group ($p = 0.0229$). The probiotic supplement group reached a 47.3% increase (n.s.). The levels of propionic acid were also significantly higher in the synbiotic group between day −5 and day 16 (65.9%, $p = 0.0253$) but

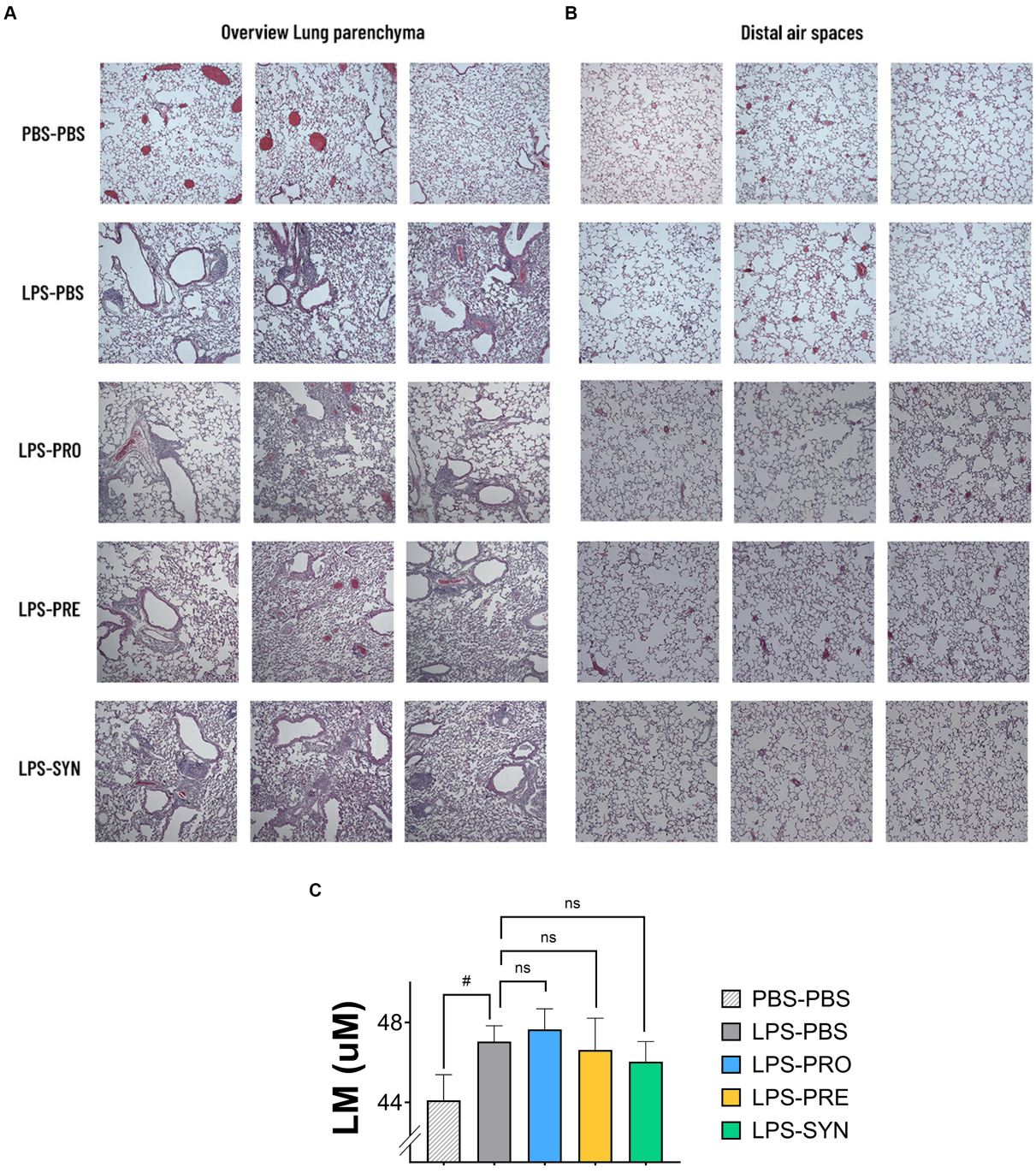

FIGURE 3
(A) Representative micrographs of H&E-stained sections of lung parenchyma of 3 different mice per group are shown. All LPS challenged groups showed areas of inflammation and remodeling of the lung parenchyma which was absent in mice that received i.n. PBS. The LPS effect includes cellular infiltrates intraluminal-, peribronchial-, and perivascular; thickening of bronchial-, and alveolar walls; and detachments of mucosal epithelium. **(B)** Representative micrographs of H&E-stained sections of the distal alveolar airspaces of 3 different mice per group are shown. The images depict larger open spaces in the LPS challenged groups which can be a signals for alveolar wall breakdown. **(C)** The graph depicts the calculated Lineair mean intercept (Lm score) as a measure of the disruption of alveolar walls. Lm was lowest in the PBS-PBS control group. The unpaired t test with Welch's correction provided a *p*-value of 0.0469 for the difference between PBS-PBS and LPS-PBS. Of all tested treatments, the synbiotic group showed the lowest Lm-Score, which was however not significantly different from the LPS-PBS group.

not in any of the other LPS groups. To gain further insight into the meaning of observed fecal SCFA results, Pearson correlations were performed for mice in which both lung health and SCFA was measured simultaneously ($n = 4$). A moderate correlation was found between levels of butyric acid and improved lung function parameters (Figure 7A). Levels of acetic acid were weakly correlated to improved lung resistance and tidal volume and propionic acid was moderately correlated to tidal volume and weakly to lung resistance. Levels of acetic acid and butyric acid both showed a moderate negative correlation with pulmonary NLR (Figure 7B), which should be read

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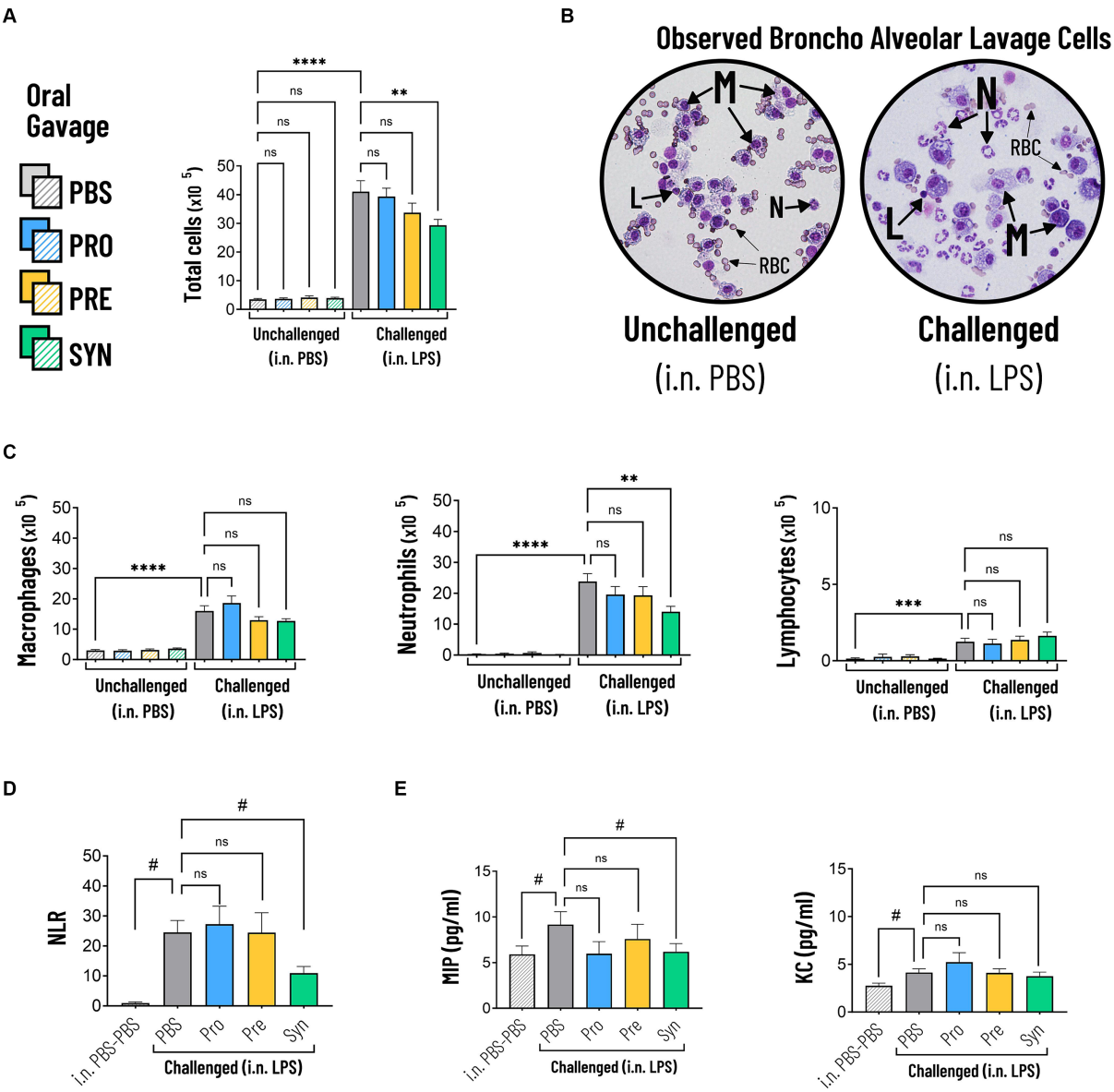

FIGURE 4
(A) The total amount of cells in BALF were significantly increased in mice that were challenged with LPS compared to PBS (indicated by ****$p < 0.0001$). The synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin significantly dampened the negative effect of LPS on total influx of cells by 28.6% compared to placebo (indicated by *$p < 0.01$). **(B)** Representative micrographs of Diff-Quik stained BAL cells showing the morphological differences between Macrophages (M), Lymphocytes (L), Neutrophils (N). Challenged mice showed high numbers of neutrophils which appear as cells with a typical multi-lobed nucleus and neutral-stained cytoplasmic granules. Red Blood Cells (RBC) were observed but not quantified. **(C)** LPS induced a significant increase in macrophages, neutrophils and lymphocytes compared to mice that received i.n. PBS (indicated by ****$p < 0.0001$). The synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin significantly reduced the numbers of neutrophils (indicated by **$p < 0.01$). **(D)** LPS induced a significant increase in the BALF Neutrophil to Lymphocyte Ratio (NLR) (indicated by #$p < 0.0001$, analyzed by *t* test). The synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin provided a significantly lower NLR compared to placebo (indicated by #$p < 0.01$). **(E)** BALF Cytokine analysis showed that LPS induced a 55.3% increase in MIP1a (indicated by #$p = 0.0451$) and a 49.6% increase in KC (indicated by #$p = 0.0081$). The synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin significantly dampened MIP1a (indicated by #$p < 0.05$) but not KC.

as low levels of acetic acid and butyric acid corresponding to a higher pulmonary NLR.

4 Discussion

This study was focused on gaining more insight into gut-lung crosstalk in context of pulmonary neutrophilia. Based on existing literature describing immune activating and inhibitory effects along the gut-lung axis, both via innate TLR pathways, one could argue for health promoting as well as disease promoting potential of gut modulation in context pulmonary neutrophilia (35). *B. breve* however has been described as having a low pro-inflammatory profile, by not exerting TLR4 activation (74). Moreover the specific *B. breve* M16-V has a well-evaluated safety profile for food use (69). The current study provides further preclinical support for good tolerability of *B. breve*

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TABLE 3 Overview of lung inflammation data measured in Broncho Alveolar Lavage Fluid.

Lung Inflammation analyzed in Broncho Alveolar Lavage Fluid								
	PBS–PBS		PBS–PRO		PBS-PRE		PBS-SYN	
Cells	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Total Cells	3.50	0.28	3.65	0.34	4.12	0.60	3.94	0.29
Treatment effect (Fractional change PBS-PBS)	-		0.04 $^{n.s}$		0.18 $^{n.s}$		0.13 $^{n.s}$	
Macrophages	3.04	0.25	2.90	0.35	3.13	0.34	3.57	0.28
Treatment effect (Fractional change PBS-PBS)	-		−0.05$^{n.s}$		0.03 $^{n.s}$		0.17 $^{n.s}$	
Neutrophils	0.30	0.11	0.46	0.20	0.65	0.39	0.16	0.029
Treatment effect (Fractional change PBS-PBS)	-		0.5 $^{n.s}$		1.2 $^{n.s}$		−0.5 $^{n.s}$	
Lymphocytes	0.14	0.05	0.26	0.17	0.28	0.11	0.13	0.03
Treatment effect (Fractional change PBS-PBS)	-		0.8 $^{n.s}$		1.0 $^{n.s}$		−0.1 $^{n.s}$	
Cytokines	Mean	SEM						
MIP	5.89	0.914	n.a.					
KC	2.77	0.269	n.a.					
	LPS–PBS		LPS – PRO		LPS-PRE		LPS-SYN	
Cells	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Total Cells	41.05	3.82	39.29	2.95	33.71	3.34	29.33	2.01
Challenge effect (fractional change PBS-PBS)	10.74****		-					
Treatment effect (% difference LPS-PBS)	-		−4.3 $^{n.s}$		−17.9 $^{n.s}$		−28.6**	
Macrophages	15.98	1.74	18.59	2.35	12.98	1.14	12.68	0.77
Challenge effect (Fractional change PBS-PBS)	4.25****		-					
Treatment effect (% difference LPS-PBS)	-		16.3 $^{n.s}$		−18.8 $^{n.s}$		−20.7 $^{n.s}$	
Neutrophils	23.61	2.73	19.57	2.58	19.32	2.81	14.01	1.78
Challenge effect (Fractional change PBS-PBS)	77.7****		-					
Treatment effect (% difference LPS-PBS)	-		−17.1 $^{n.s}$		−18.2 $^{n.s}$		−40.7**	
Lymphocytes	1.25	0.22	1.13	0.28	1.37	0.25	1.63	0.24
Challenge effect (Fractional change PBS-PBS)	7.8***		-					
Treatment effect (% difference LPS-PBS)	-		−9.4 $^{n.s}$		9.4 $^{n.s}$		30.6 $^{n.s}$	
NLR	24.52	3.95	27.23	6.01	24.39	6.67	10.94	2.22
Challenge effect (Fractional change PBS-PBS)	25.0****		-					
Treatment effect (% difference LPS-PBS)	-		11.0 $^{n.s}$		−0.5 $^{n.s}$		−55.3**	
Cytokines	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
MIP	9.15	1.42	5.98	1.32	7.59	1.60	6.19	0.89
Challenge effect (% change PBS-PBS)	55.3$^{\#}$		-					
Treatment effect (% difference LPS-PBS)	-		−34.7 $^{n.s}$		−17.1 $^{n.s}$		−32.3$^{\#}$	
KC	4.14	0.39	5.22	1.00	4.09	0.44	3.75	0.43
Challenge effect (% change PBS-PBS)	49.6$^{\#}$		-					
Treatment effect (% difference LPS-PBS)	-		26.1 $^{n.s}$		−1.2 $^{n.s}$		−9.3 $^{n.s}$	

ns $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$.

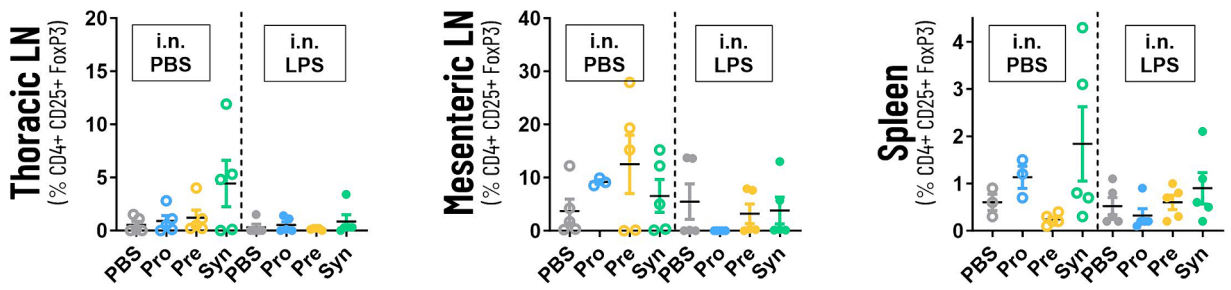

FIGURE 5
The graphs depicts the analysis of the numbers of systemic Tregs in thoracic lympnodes, mesenteric lympnodes and spleen tissue. The highest numbers of Tregs were counted in thoracic lympnodes and spleen of the mice that received the synbiotic formula of B *Breve* M16-V and GOS:FOS:lvPectin 9:1:2. Prebiotic supplementation with GOS:FOS:lvPectin 9:1:2 had a high count in mesenteric lymphnodes of unchallenged mice but not in challenged mice. This preliminary screening did not result in any significant results but a cautiously optimistic alignment with other data warrants further investigation.

M16-V in context of hyperactivation of a key innate immune pathway, also when applied in a synbiotic combination with GOS:FOS:lvPectin 9:1:2. Vital signs and body weight measurements were not negatively influenced by any of the tested prophylactic formula containing *B Breve* M16-V and/or GOS:FOS:lvPectin 9:1:2.

Although gut microbiota can boost the defensive capacity of neutrophils in context of infection (34, 66, 67), none of the here evaluated gut modulation formula promoted an increase in the numbers of pulmonary neutrophils. In unchallenged mice the intragastric pro-, pre-, and synbiotic supplements did not induce influx of pulmonary inflammatory cells and in the LPS challenged mice all groups that received an enteric supplement had lower neutrophil counts compared to the LPS-placebo group. Of the analyzed enteric supplements the synbiotic supplement containing the combination of *B. breve* M16-V and GOS:FOS:lvPectin 9:1:2 had the most favorable effect on the LPS induced pulmonary neutrophila, defined by a significant decrease in numbers of neutrophils and a lower pulmonary NLR score. The differential cell analysis was only in part reflected in the cytokine profile. Both MIP and KC were significantly upregulated in challenged compared to unchallenged mice but in contrast to the cellular response the synbiotic treatment effect was observed for the MIP marker, reflecting macrophage activity and not for KC reflecting neutrophil chemotaxis.

It would be interesting to extent analysis to systemic measurements of cytokines, neutrophils and NLR. Blood NLR is a relatively easy to measure marker for systemic inflammation and incorporating this marker in future gut-lung studies can shed further light on the effect of diets on progression of severe inflammatory disorders (86). Furthermore, it will be interesting to perform differential analysis of lung and systemic immune cells including dendritic cells and effector T cells such as Th1, Th2, and Th17. More specifically it will be relevant to further examine CD4(+)Foxp3(+) regulatory T cells (Tregs), since these can be upregulated by gut microbiota and have been identified as having immune-suppressive effects beyond the gut (87). The preliminary exploration of the numbers of systemic Tregs was performed using thoracic lympnodes and spleen tissue of a small subset of the mice (supplementary data). The highest numbers of Tregs were counted in thoracic lympnodes and spleen of the unchallenged and challenged mice that received the synbiotic formula of *B. breve* M16-V and GOS:FOS:lvPectin 9:1:2. Prebiotic supplementation with GOS:FOS:lvPectin 9:1:2 had a high count in mesenteric lymphnodes of unchallenged mice but not in challenged mice. The means were however very spread out and significant results were not obtained, but a cautiously optimistic pattern does warrant further investigations. A follow-up study will be needed to further unravel if Tregs play a role in any of the observed beneficial effects of dietary supplements on lung health.

The beneficial effect of synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin 9:1:2 in context of pulmonary neutrophilia was also reflected in the significantly improved functional parameter of lung resistance. It was interesting to see that in contrast to the individual data points, the linear regression analysis of the slope patterns also provided indications for a treatment effect of probiotics, $p < 0.0001$ and prebiotics $p = 0.0042$. Further analysis is however needed to see if this trend will result in a more profound effects of probiotics and prebiotics in a more stressful setting than the here used highest methacholine challenge.

In addition to lung inflammation and lung function, lung morphology was analyzed also. Of the LPS challenged mice the prebiotic and synbiotic group presented less, however not a significant decrease in the disruption of alveolar walls compared to the LPS-placebo group. A previous study did show that *B. breve* has the ability to inhibit alveolar damage but in that specific study the amount of neutrophils was not affected by *B. breve*, in contrast to the prebiotic GOS/FOS fiber mixtures that did attenuate both neutrophil influx and alveolar damage (76). Comparing these LPS studies is hampered by the differences in study design. In the current study 7 i.n doses of LPS were spread over a time frame of 15 days with the section 1 day after the final challenge whereas the previous study used 8 doses spread over 24 days with the section 4 days after the final LPS challenge (76). Compared to the former study, the shorter timelines of the current study were better suited for studying effects on overwhelming innate immune activation (e.g., pulmonary neutrophil infiltration). The more stretched time-period of the previous study however provided a better window for analyzing the dietary effect on changes in the lung architecture, with a consequence of having a limited window of studying the effect on innate immune activation.

Another novel insight from the current LPS model compared to the previous LPS model, was that i.n. delivered LPS caused an attenuation in the production of fecal SCFAs over time, compared to the control mice that received PBS (37.6% less increase of acetic acid and 64% less increase of propionic acid). Moreover enhanced levels of butyric acid, acetic acid and propionic acid, following supplementation with

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TABLE 4 Overview of FoxP3+ regulatory T cells in thoracic -, and mesenteric lymph nodes and spleen.

% CD4+ CD25+ FoxP3 cells								
	PBS–PBS		PBS–PRO		PBS-PRE		PBS-SYN	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TLN	0.544	0.316	0.902	0.512	1.2	0.719	4.422	2.179
Treatment effect (fractional change PBS-PBS)	-		0.66		1.21		7.13	
MLN	3.72	2.24	9.167	0.406	12.5	5.48	6.56	3.081
Treatment effect (fractional change PBS-PBS)	-		1.46		2.36		0.76	
Spleen	0.6	0.173	1.133	0.233	0.24	0.051	1.84	0.787
Treatment effect (fractional change PBS-PBS)	-		0.89		−0.60		2.07	
	LPS–PBS		LPS–PRO		LPS-PRE		LPS-SYN	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TLN	0.308	0.298	0.524	0.301	0.142	0.038	0.862	0.637
Challenge effect (fractional change PBS-PBS)	−0.43		-					
Treatment effect (% difference LPS-PBS)	-		70.1		−53.9		180	
MLN	5.48	3.335	-	-	3.2	1.859	3.82	2.536
Challenge effect (fractional change PBS-PBS)	0.47		-					
Treatment effect (% difference LPS-PBS)	-		n.a.		−41.6		−30.3	
Spleen	0.52	0.183	0.32	0.146	0.6	0.152	0.9	0.333
Challenge effect (fractional change PBS-PBS)	−0.13		-					
Treatment effect (% difference LPS-PBS)	-		−38.46		15.38		73.08	

synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin, associated with better lung health. This finding suggests bidirectional gut lung crosstalk involving microbial derived metabolites which confirms previous proposed links between microbes, metabolites, and gut-lung health (30, 88, 89). In other rodent models it has also been shown that common triggers for pulmonary inflammation, such as cigarette smoke and environmental particulate matter can cause intestinal inflammatory responses, alter the gut microbiota and trigger decreased SCFA production (90, 91). Moreover in an elastase induced model for emphysematous inflammation it was previously shown that increased cecal SCFA coexisted with improved lung health that was induced by a diet, more specifically a whey peptide-based enteral supplement (82).

The exact timelines of SCFA production and mechanisms of the lung driven effect on gut microbiota derived metabolites, are not yet elucidated. In the current study mice were relatively young, 6–8 weeks and we speculate that alongside their normal weight gain, the gut microbiota was still developing explaining the SCFA gain. Building on insights from other investigators one could speculate that LPS spilling over from the lungs could have a direct negative effect on the gut microbiota. Supportive of this idea is the finding that LPS that is cleared from systemic circulation causes intestinal inflammation driven by the different localization of LPS in intestinal epithelial cells and in more differentiated enterocytes (92). In contrast to systemic derived LPS, the presence of luminal LPS from commensal gut bacteria, is usually well tolerated by the mucosal immune system which can in part be explained by TLR4 subcellular distribution and ligand-specific dynamic regulation (40). An indirect mechanism of action could also be speculated upon, since other investigators showed that acute instillation of LPS in mouse lungs changes bacterial microbiota in the lungs, and causes an increase in number of bacteria in blood and gut (93).

More support for an indirect lung-gut crosstalk can be found in a study in mice showing that airways infected with an influenza virus can cause outgrowth of *E. coli* combined with abnormal inflammatory responses involving T-helper 17 cells and intestinal damage. These effects were explained via migrating lung derived CC-chemokine receptor 9 positive ($CCR9^+$)$CD4^+$ T cells (94). Intestinal injury was also observed secondary to pneumonia (95). The specific effect of neutrophilic lung diseases, such as COPD, on changes in gut microbiota has not been thoroughly studied (27). Investigators did report that smoking, which is a key risk factor for pulmonary neutrophilia, affects intestinal microbiota and smokers specifically express decreased abundance of *Bifidobacterium* spp. (27, 96, 97). Moreover, *in vitro* and *in vivo* studies showed that cigarette smoke has a negative effect on the production of SCFAs by *Bifidobacterium* (90, 98). In light of this data, the current study adds encouraging insight by showing that the synbiotic supplement containing *B. breve* M16-V and GOS:FOS:lvPectin 9:1:2 was capable of preventing the decreased SCFA production ánd improving lung health. Taken together the findings from the current study emphasize the potential benefit of bidirectional gut lung cross-talk in context of pulmonary neutrophilia.

5 Limitations and recommendations

Apart from fecal SCFA analysis the current study is limited in gut ecosystem analysis. In a follow up study it will be interesting to analyze intestinal inflammation and the composition of the intestinal microbiome before and after LPS treatment and nutritional supplementation. This would help to shed further light on the relation between lung inflammation and fecal SCFA production. Moreover, it is recommended to assess systemic-, and lung SCFA concentrations, in

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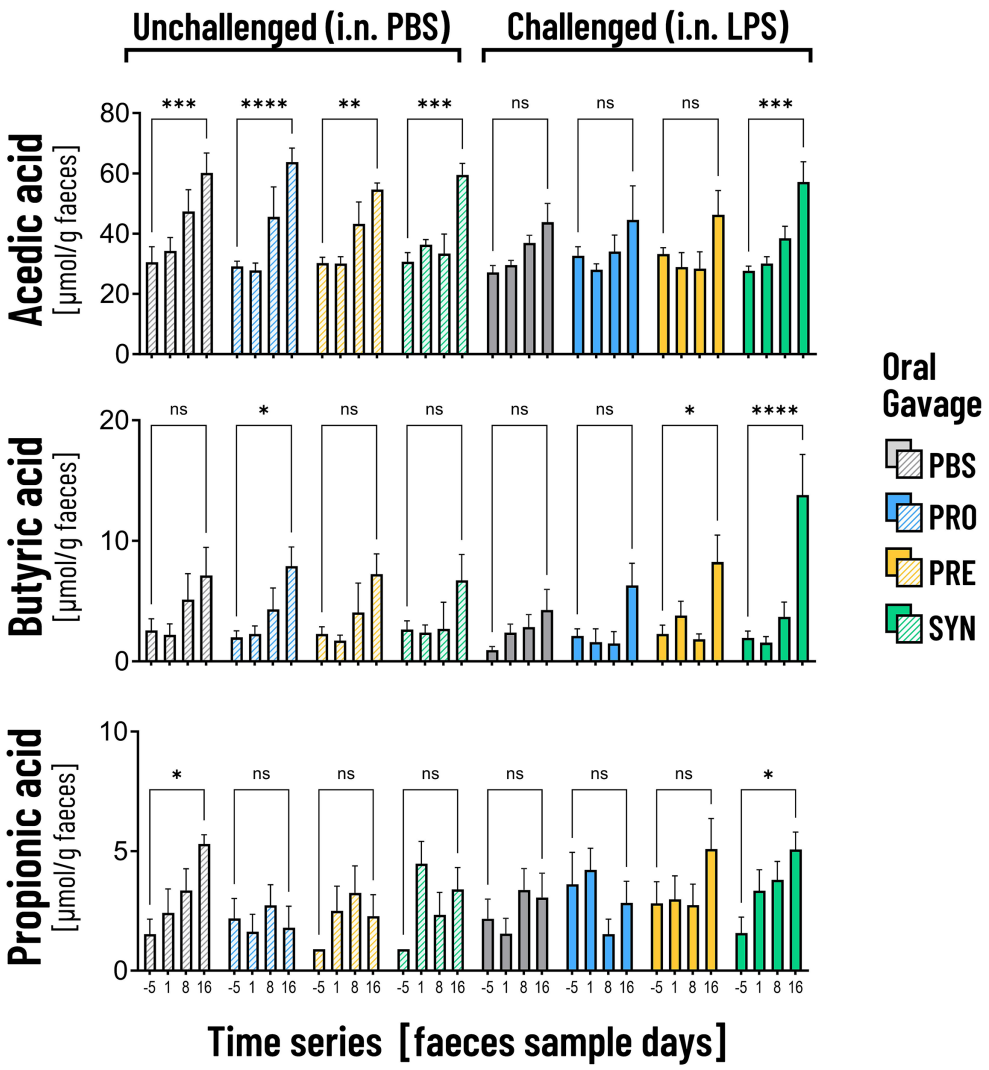

FIGURE 6
The graphs depict the analysis of acetic acid, butyric acid and propionic acid in fecal samples on 4 time points. Unchallenged mice (striped bars) showed a significant increase in the production of acetic acid over time (**$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). In the LPS challenged mice the increase was only visible in mice that received the synbiotic mixture of B. breve M16-V and GOS:FOS:lvPectin (***$p < 0.001$). Mice that received this synbiotic mixture also had a significant increase in levels of butyric acid and propionic acid on day 16. The probiotic B. breve M16-V on itself only induced a significant increase in butyric acid in unchallenged mice. The prebiotic supplement GOS:FOS:lvPectin upregulated butyric acid in challenged mice but not acetic acid nor propionic acid.

addition to fecal SCFA levels. In addition a recent study pointed toward the value of analyzing pulmonary TLR expression levels in explaining a beneficial effect of probiotics in context of lung disease (99). The specific study showed that mRNA expression levels of TLR2, TLR4 and TLR9 were all upregulated in lung tissue in response to cigarette smoke which was reduced significantly by oral feeding with *Lactobacillus rhamnosus*.

Although the LPS trigger in the current study was strategically chosen as a robust and reproducible stressor, activating an innate immune pathway that is broadly relevant for neutrophilic lung disorders including COPD, ARDS and persistent neutrophilic asthma (44–53), it does have limitations. It should be acknowledged that such persistent airway diseases can involve more complex mixtures of stressors (e.g., cigarette smoke, air pollutants and bacterial or viral infections) driving simultaneous activation of multiple inflammatory cascades. It must also be realized that although valuable insights can be obtained from rodent models (e.g., lung function and gut-lung interactions), there are limitations in cross-species translation. Significant differences exist between mouse and human lung architectures, their digestive and immune systems (100–102). Further research is thus warranted to investigate whether the here presented lung health improving effects of the synbiotic mixture of *B. breve* M16-V and GOS:FOS:lvPectin, can be reproduced in humans and in context of more complex mixtures of stressors, such as cigarette smoke, air pollutants and hyperinflammatory infectious disorders.

It is also important to realize that the here presented study describes the effect of prophylactic supplements which translates to prevention of hyperinflammation rather than management of existing chronic inflammatory disorders. To overcome this limitation, it is recommended to include the analysis of safety and effectiveness of microbiota manipulation after onset of dysregulated lung inflammation. For further

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TABLE 5 Overview of short chain fatty acid data measured in fecal samples.

Acetic acid	PBS–PBS		PBS–PRO		PBS-PRE		PBS-SYN	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Day −5	30.51	5.14	29.10	1.77	30.27	1.89	30.66	3.03
1	34.27	4.45	27.80	2.45	30.14	2.20	36.34	1.68
8	47.40	7.19	45.57	9.94	43.24	7.23	33.39	6.50
16	60.13	6.62	63.76	4.63	54.66	2.16	59.49	3.84
Fractional difference within group (−5 versus 16)	1.17***	0.31	1.24****	0.23	0.87**	0.17	1.05***	0.22
% difference between group (PBS-PBS day 16)	-		6.0		−9.1		−1.1	
	LPS–PBS		LPS–PRO		LPS-PRE		LPS-SYN	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Day −5	27.14	2.32	32.71	2.96	33.24	2.11	27.67	1.53
1	29.60	1.52	28.04	1.95	28.91	4.80	30.13	2.21
8	36.89	2.58	34.03	5.53	28.43	5.52	38.46	4.01
16	43.77	6.24	44.54	11.32	46.23	8.06	57.14	6.67
Fractional difference within group (−5 versus 16)	0.73 n.s.	0.33	0.32 n.s.	0.30	0.39 n.s.	0.24	1.04***	0.18
% difference between group (LBS-PBS day 16)	-		1.8		5.6		30.5	

Butyric acid	PBS–PBS		PBS–PRO		PBS-PRE		PBS-SYN	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Day −5	2.571	0.961	1.986	0.540	2.286	0.580	2.657	0.706
1	2.200	0.914	2.271	0.664	1.714	0.465	2.386	0.632
8	5.129	2.151	4.314	1.768	4.057	2.430	2.700	2.200
16	7.129	2.333	7.900	1.599	7.243	1.678	6.714	2.151
Fractional difference within group (−5 versus 16)	6.1 n.s.	3.2	9.7*	4.5 n.s.	7.3 n.s.	4.2	2.8 n.s.	1.9
% difference between group (PBS-PBS day 16)	-		10.8		1.6		−5.8	
	LPS–PBS		LPS–PRO		LPS-PRE		LPS-SYN	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Day −5	0.943	0.291	2.100	0.606	2.29	0.72	1.94	0.57
1	2.371	0.716	1.600	1.100	3.80	1.20	1.54	0.51
8	2.843	1.045	1.486	0.986	1.84	0.44	3.69	1.24
16	4.267	1.705	6.286	1.860	8.24	2.23	13.80	3.36
Fractional difference within group (−5 versus 16)	5.9 n.s.	3.7	7.2	3.7	4.3*	2.2	7.3****	1.4
% difference between group (LBS-PBS day 16)	-		47.3		93.2		223.4 #	

Propionic acid	PBS–PBS		PBS–PRO		PBS-PRE		PBS-SYN	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Day −5	1.529	0.629	2.186	0.830	0.900	0.000	0.900	0.000
1	2.414	1.002	1.629	0.729	2.500	1.036	4.471	0.938
8	3.357	0.905	2.729	0.866	3.257	1.122	2.329	0.948
16	5.300	0.389	1.800	0.900	2.286	0.896	3.400	0.911
Fractional difference within group (−5 versus 16)	4.11*	0.93	0.76 n.s	1.05	1.54 n.s	1.00	2.78 n.s	1.01
% difference between group (PBS-PBS day 16)	-		−66.04		−56.87		−35.85	
	LPS–PBS		LPS–PRO		LPS-PRE		LPS-SYN	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Day −5	2.171	0.821	3.614	1.332	2.814	0.903	1.571	0.671
1	1.543	0.643	4.214	0.907	2.986	0.985	3.343	0.876
8	3.371	0.896	1.529	0.629	2.743	0.874	3.800	0.767
16	3.057	1.021	2.829	0.910	5.086	1.279	5.071	0.723
Fractional difference within group (−5 versus 16)	1.48 n.s	1.06	0.47 n.s	0.73	2.37 n.s	1.22	3.98*	1.06
% difference between group (LBS-PBS day 16)	-		−7.46		66.37		65.88	

ns $p > 0.05$; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$.

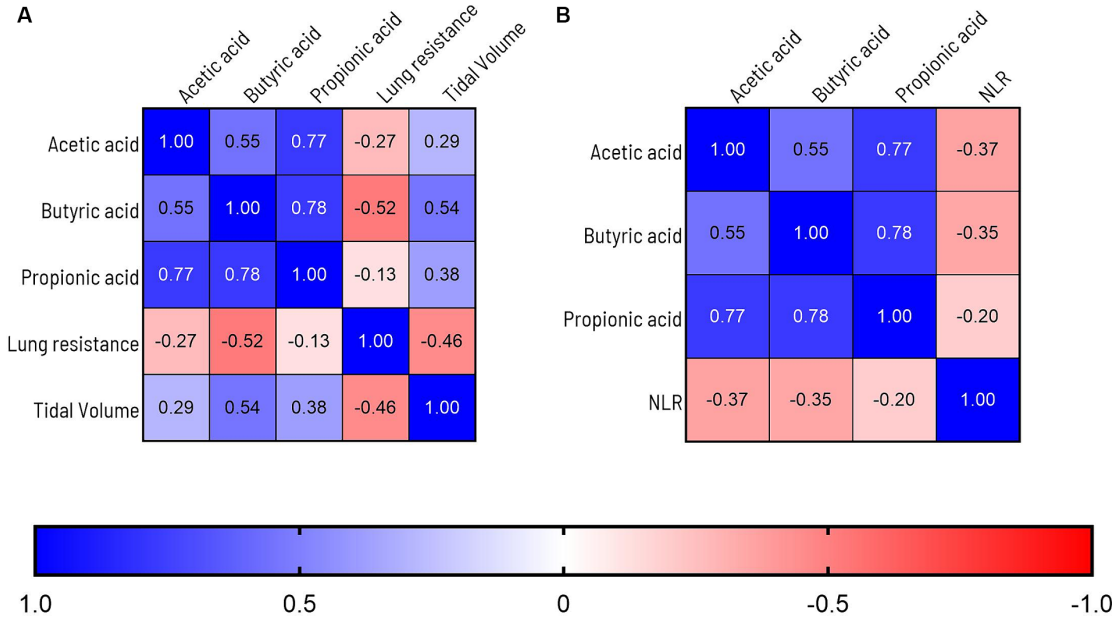

FIGURE 7
(A) Graph depicts Pearson correlations analysis of overlapping lung health and SCFA data points. A moderate correlation is observed between levels of butyric acid and improved lung function parameters. Levels of acetic acid were weakly correlated to improved lung resistance and tidal volume and propionic acid was moderately correlated to tidal volume and weakly to lung resistance. **(B)** Levels of acetic acid, butyric acid were moderately correlated to improvement of the BALF Neutrophil to Lymphocyte Ratio (NLR). To label the strength of the Pearson associations, the r values between, 0–0.3 (or −0.3) are considered as weak, 0.3–0.7 (or −0.7) as moderate, 0.7–1.0 (or −1) as a strong positive (or negative) correlations (85).

analysis of the effect and underlying mechanisms of gut modulation on emphysematous disease, it is also recommended to perform longer studies, allowing for more gradual alveolar wall breakdown that better represent the long timelines of disease progression in humans. Furthermore, it is relevant to elaborate investigations on the effect of lung disorder progression on the composition of gut microbiota and metabolites. The bidirectional effect of lung diseases on gut microbiota and metabolites composition are most ideally analyzed using human material since this will provide more ethical and relevant guidance of insights on safety and effectiveness of gut microbiota modulating therapies and its time-frames of delivery.

A final limitation of the current study that was identified is the absence of a reference drug. It is thus advised to compare the effects of gut microbiota manipulation on lung health and disease with reference medications and in combination with standard of care treatments.

6 Concluding remarks

Despite shortcomings, the here presented work provides relevant insight on bi-directional gut-lung crosstalk in context of pulmonary neutrophilia (Graphical abstract). Repeated pulmonary challenge with LPS induced pulmonary neutrophilia and negatively impacted fecal SCFA concentrations. The study provided proof of concept for tolerability and effectiveness of the synbiotic combination of *B. breve* M16-V and scGOS/lvFOS/lvPectin to prevent the LPS-induced decline in fecal SCFA levels and to dampen the development of LPS induced pulmonary neutrophilia, to improve pulmonary NLR and to dampen lung function decline. Targeting neutrophils has recognized potential in the management of severe acute and poorly controlled chronic inflammatory airway disorders (103, 104). The observed beneficial lung effects associated with enhanced SCFA production. Taken together, this experiment provided supportive data for the value of SCFA producing synbiotic nutritional concepts, more specifically the mixture of *B. breve* M16-V and scGOS/lvFOS/lvPectin, to prevent adverse functional consequences in context of neutrophilic lung disorders.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was approved by Utrecht Universities Committee on Animal Research. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

GB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. MD: Data curation, Investigation, Writing – review & editing. EM: Data curation, Investigation, Writing – review & editing. IA: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. JB: Writing – review & editing. AK: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. GF:

Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. JG: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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Conflict of interest

JB and JG are both in part affiliated with Danone Nutricia Research BV, a company having commercial interest in dietary products. GB was in part employed by Impact Station.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

ALI	Acute lung injury
ANOVA	Analysis of variance
APC	Allophycocyanine
ARDS	Acute respiratory distress syndrome
BAL	Broncho alveolar lavage
BALF	Broncho alveolar lavage fluid
B. breve	*Bifidobacterium breve*
BSA	Bovine serum albumin
BW	Body weight
CAMPs	Commensal associated molecular patterns
CBA	Cytometric bead array
COPD	Chronic obstructive pulmonary disease
FACS	Fluorescence activated cell sorting
FITC	Fluorescein isothiocyanate
GC	Gas chromatograph
GM-CSF	Granulocyte macrophage-colony stimulating factor
GOS:FOS:lvPectin	Short-chain galactooligosaccharides, long-chain fructo-oligosaccharides, and low-viscosity pectin
H&E	Hematoxylin/eosin
IgG	Immunoglobulin G
IL23 P19/P40	Mouse interleukin-23 (p19/p40)
i.n	Intra nasally
SCFA	Short chain fatty acids
scGOS/lcFOS	Short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides
KC	Keratinocyte-derived chemokine
Lm	Mean linear intercept
LPS	Lipopolysaccharides
MAMPs	Microbial associated molecular patterns
MFI	Mean fluorescence intensity
MIP1a	Macrophage inflammatory protein-1α
MLN	Mesenteric lymph nodes
NLR	Neutrophil to lymphocyte ratio
n.s.	Not significant
PAMPs	Pathogen associated molecular patterns
PBS	Phosphate-buffered saline
PE	Phycoerythrin
R_L	Lung resistance
SEM	Standard error of mean
Th	T helper cell
TLN	Thoracic lymp nodes
TLR	Toll like receptor
Tregs	CD4(+)Foxp3(+) regulatory T cells
V_t	Tidal volume

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Relationship between dietary protein, serum albumin, and mortality in asthmatic populations: a cohort study

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Background: Currently, there is limited research on the correlation between protein levels in the body and asthma. We used data from the NHANES to explore the relationship of dietary protein, serum albumin, with mortality in individuals with asthma to better understand their impact on asthma.

Method: This investigation involved 3005 individuals with asthma from the NHANES dataset. Studying potential links between dietary protein, serum albumin, and mortality in asthmatic populations utilized the Cox proportional hazards models, trend test, restricted cubic splines (RCS), and Kaplan-Meier survival analysis. Furthermore, subgroup analyses were carried out to explore these connections within specific populations.

Result: After considering all potential variables, multivariate Cox proportional hazard models proved that dietary protein intake did not have an independent connection with all-cause mortality, but serum albumin was inversely linked with all-cause mortality. Each unit rise in serum albumin (g/l) was linked to a 13% decrease in the likelihood of all-cause mortality. RCS confirmed a negative and linear connection of serum albumin with all-cause mortality. The Kaplan-Meier survival curve suggested that asthmatic adults with greater serum albumin levels had a decreased risk of mortality compared to those with lower levels.

Conclusion: The investigation proved a negative linear connection of serum albumin with all-cause mortality in asthma patients. However, there was no independent link discovered between dietary protein intake with mortality. This indicates that serum albumin could be a significant factor in predicting long-term outcomes for asthma patients.

KEYWORDS

protein intake, albumin, asthma, mortality, National Health and Nutrition Examination Survey (NHANES)

1 Introduction

One of the most prevalent chronic non-communicable illnesses worldwide, asthma is characterized by a variety of sporadic and variable symptoms related to bronchospasm and airway inflammation, including dyspnea, tightness in the chest, wheezing, coughing, and sputum (1). Over the past few decades, there has been a significant increase in the prevalence, morbidity, death, and economic burden of asthma worldwide, particularly in children. Globally, asthma claims the lives of about 180,000 individuals each year (2, 3). Some patients develop a partial or whole resistance to glucocorticoids during treatment, necessitating the use of comparatively large doses of glucocorticoids to control their symptoms. In other individuals, increasing loss of lung function is a contributing factor to death (4). More than 1% of all disability-adjusted life years lost globally are estimated to be attributable to asthma (5, 6).

In many industrialized nations, obesity has become an epidemic due to the widespread adoption of the Western diet. Saturated fats, refined carbs, and sodium intake rise when processed meals are consumed frequently and fruits, vegetables, and whole grains are consumed less frequently. Asthma is one of the chronic inflammatory disorders that are more likely to develop in those who follow this kind of poor diet (7). According to certain research, a high intake of protein and fat and a high intake of carbohydrates are inversely connected with lung function (8). For the majority of patients with chronic illnesses, inadequate food consumption that falls short of their needed calorie and protein intake leads to poor quality of life (9).

Albumin has a molecular weight of 66.3 kD (10, 11). Protein A, which is the most prevalent in plasma, is of critical importance in facilitating the movement of charged and electrically neutral molecules and ions and maintaining the colloid osmotic pressure of the blood (12). Vascular permeability increases are a common feature of inflammation. These increases make it easier for large amounts of albumin to pass through the interstitial and epithelial surfaces of many organs, including the lungs (13, 14). Significant elevations in tissue albumin levels might potentially confer advantageous consequences, including the provision of antioxidant defense for tissues (15). Human serum albumin is unique because it has fourteenteen disulfide bonds made up of thirty-four cysteine residues and a free thiol at position Cys-34 (11). This is the main pathway by which albumin scavenges reactive oxygen and nitrogen species (ROS and RNS) and thus participates in redox processes (12). Albumin is regarded as a significant extracellular antioxidant in plasma within this particular context.

Currently, there is insufficient study on the link between protein levels in the body with asthma. So we used NHANES data to investigate the relationship of dietary protein intake, serum albumin levels, and mortality in persons with asthma to better understand their impact on asthma.

2 Materials and methods

2.1 Study data and population

The data for that investigation were collected from the National Health and Nutrition Examination Survey (NHANES) public database, which was managed by the Centers for Disease Control and Prevention (CDC) in the USA. The NHANES database collected vital and health statistics for the country. The NHANES study was conducted using a complex multistage stratified sample design to identify a diverse sample of the US population that was not in institutions. Every participant underwent informed consent prior to the data collection procedures and thorough health examinations. The NHANES study protocol received approval from the National Center for Health Statistics (NCHS)' Research Ethics Review Board. Figure 1 illustrates that NHANES had a participation of 39156 people from 2011 to 2018. Adhering to specific inclusion and exclusion criteria, our study population excluded: (1) individuals without asthma or with missing data (n = 33402); (4) those with missing follow-up data (n = 2156); (3) those lacking serum albumin or dietary protein intake (n = 593). Finally, our investigation involved a sample of 3005 asthmatic people in the USA.

2.2 All variables

According to the NHANES introduction, participants' dietary protein and other nutrient intake were evaluated through a 24-hour dietary recall method. A professional technician inquired about the types and quantities of foods and medications consumed within a day, which were then documented in the NHANES computer-assisted dietary survey system. Estimates for the intake of each food component were calculated using the University of Texas Food Intake Analysis System and the USDA Survey Nutrient Database. The DcX800 method, a bichromatic digital endpoint technique, is utilized to evaluate serum albumin concentration. Bromcresol purple (BCP) reagent and albumin mix to create a complex during the process. The system maintains records of any variations in absorbance at 600 nm. The amount of albumin in the sample is directly proportional to the change in absorbance. The death index (NDI), as of December 31, 2018, was used to determine the follow-up status of our study populations. The NCHS offered further details regarding the matching approach. We utilized the 10th edition of the International Statistical Classification of Diseases (ICD-10) to establish mortality status. Our main outcome variable was all-cause mortality.

To mitigate the potential influence of various factors, we included numerous covariates in our study. The covariates considered in the analysis were sex (male and female), age, race, education, poverty to income ratios (PIR), marriage, body mass index (BMI), smoking status, intake of alcohol, energy, total sugar and total fat, hypertension history, diabetes history, cardiovascular disease (CVD) history, chronic obstructive pulmonary disease (COPD) history, cancer history, glucocorticoid drug (whether glucocorticoid drugs were used in the past month), serum creatinine, white blood cell number (WBC), blood neutrophils number (BNEU), and blood eosinophils number (BEOS), and serum total protein. Has a doctor or other healthcare provider ever diagnosed you with asthma? was one of the questions on standardized questionnaires given to participants during their visits. Participants who answered positively were classified as having a diagnosis of asthma. The NHANES website provides a comprehensive description of laboratory procedures.

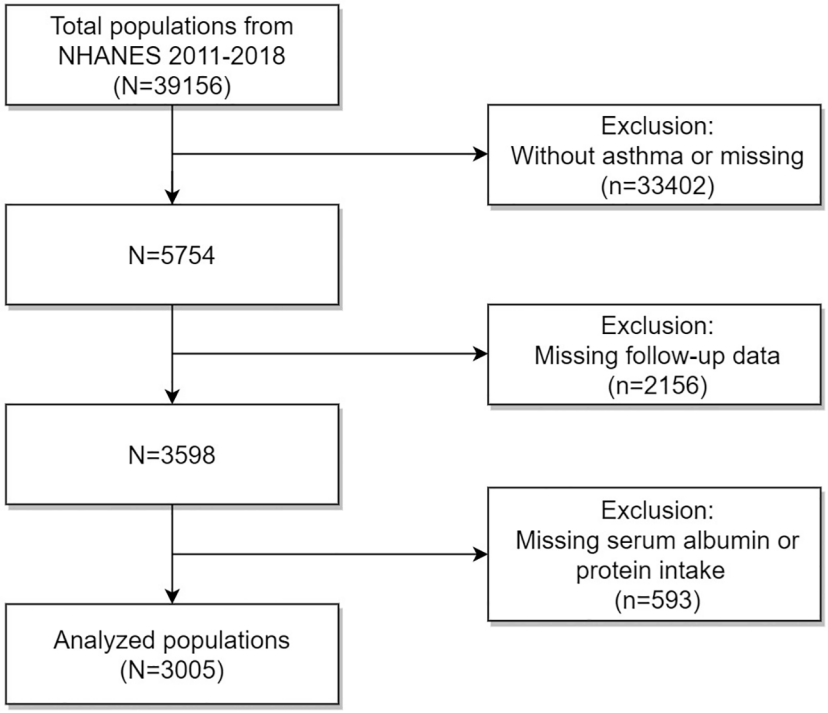

FIGURE 1
Flow diagram of selecting populations for analysis.

2.3 Statistical analysis

Statistical analyses were performed utilizing R software. $P < 0.05$ was regarded as statistical significance. Initially, we built three univariate and multivariate Cox proportional hazards regression models to assess the independent association between dietary protein, serum albumin, and all-cause mortality in individuals with asthma. Three constructed models were as follows: Model X (adjusted for none), Model Y (adjusted for age, race, sex, education, marriage, and PIR), and Model Z (adjusted for sex, age, race, education, marriage, PIR, BMI, smoking, intake of alcohol, energy, total sugar and total fat, hypertension history, diabetes history, CVD history, COPD history, cancer history, glucocorticoid drug, serum creatinine, WBC, BNEU, BEOS, and serum total protein). Subsequently, we utilized the trend test and restricted cubic spline (RCS) to examine whether the correlation was linear. Meanwhile, stratified analyses were carried out to explore the connection between serum albumin and all-cause mortality across diverse asthmatic populations. Lastly, we also carried out the Kaplan-Meier analysis to explore the prognostic impact of serum albumin on all-cause mortality in asthmatics. Multiple imputation was used to handle variables with missing values, and the total proportion of missing values for each covariate was less than 10%.

3 Results

3.1 Baseline characteristics

The investigation involved 3005 individuals with asthma (1269 men and 1736 women), and their characteristics were analyzed based on follow-up outcomes (Table 1). Our investigation found that the average age of participants was 45.66 years old, with the majority of the population being white populations. The average period of follow-up for populations was 57.96 months. Variations in the distributions of age, race, education, marriage, PIR, smoking, intake of energy and fat, hypertension, diabetes, CVD, COPD, cancer, glucocorticoid drugs, serum creatinine, WBC, BNEU, serum albumin, and serum total protein were found to be statistically significant among the follow-up outcome groups (Table 1). However, there were no significant variations in sex, BMI, alcohol consumption, sugar intake, protein intake, or BEOS ($p > 0.05$). In comparison to the survival populations, the death populations exhibited reduced serum albumin levels.

3.2 Association between dietary protein, serum albumin, and all-cause mortality

We applied univariate and multivariate Cox proportional hazard models to investigate the correlation of dietary protein intake, serum albumin, and all-cause mortality in asthmatics (Table 2). In the univariate Cox hazard model (Model X), without adjustment of covariates, dietary protein intake was inversely related to all-cause mortality. But in the multivariate Cox hazard models (Model Y and Z), with the adjustment of diverse covariates, dietary protein intake was not independently related to all-cause mortality. It was found that serum albumin was negatively linked to all-cause mortality in both univariate and multivariate Cox hazard models (Models X, Y, and Z), even after different covariates were

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TABLE 1 Baseline characteristics based on survival status.

	Survival	Death	P value
Sex (%)			0.073
Male	1186 (41.83%)	83 (48.82%)	
Female	1649 (58.17%)	87 (51.18%)	
Age (years)	44.46 ± 18.03	65.67 ± 15.53	<0.001
Race (%)			<0.001
Other race populations	948 (33.44%)	34 (20.00%)	
White populations	1161 (40.95%)	95 (55.88%)	
Black populations	726 (25.61%)	41 (24.12%)	
Education (%)			0.002
Less than high school	552 (19.47%)	51 (30.00%)	
High school	636 (22.43%)	40 (23.53%)	
More than high school	1647 (58.10%)	79 (46.47%)	
Marriage (%)			0.008
Married	1336 (47.13%)	62 (36.47%)	
Single	1271 (44.83%)	97 (57.06%)	
Living with a partner	228 (8.04%)	11 (6.47%)	
PIR	2.36 ± 1.84	1.89 ± 1.76	0.001
BMI (kg/m2)	30.82 ± 8.30	30.80 ± 8.95	0.979
Smoking status (%)			<0.001
Smoker	1257 (44.34%)	105 (61.76%)	
Non-smoker	1578 (55.66%)	65 (38.24%)	
Alcohol intake (gm)	9.94 ± 29.33	8.48 ± 26.48	0.524
Energy intake (kcal)	2146.42 ± 1072.36	1945.25 ± 1025.30	0.017
Sugar intake (gm)	114.35 ± 83.90	111.98 ± 90.41	0.722
Fat intake (gm)	84.09 ± 50.54	74.06 ± 43.37	0.011
Protein intake (gm)	80.87 ± 45.93	73.90 ± 48.12	0.055
Fat intake (gm)	78.06 ± 53.27	66.62 ± 46.78	0.004
Hypertension (%)			<0.001
No	1755 (61.90%)	43 (25.29%)	
Yes	1080 (38.10%)	127 (74.71%)	
Diabetes (%)			<0.001
No	2429 (85.68%)	110 (64.71%)	
Yes	406 (14.32%)	60 (35.29%)	
CVD history (%)			<0.001
No	2458 (86.70%)	95 (55.88%)	
Yes	377 (13.30%)	75 (44.12%)	
COPD history (%)			<0.001
No	2577 (90.90%)	115 (67.65%)	
Yes	258 (9.10%)	55 (32.35%)	
Cancer history (%)			<0.001
No	2562 (90.37%)	126 (74.12%)	
Yes	273 (9.63%)	44 (25.88%)	
Glucocorticoid drugs (%)			<0.001
No	2405 (84.83%)	108 (63.53%)	
Yes	430 (15.17%)	62 (36.47%)	
Serum creatinine (umol/l)	77.63 ± 33.29	104.39 ± 120.64	<0.001
WBC (1000 cells/uL)	7.47 ± 2.38	8.10 ± 3.76	0.001
BNEU (1000 cell/uL)	4.39 ± 1.83	5.09 ± 2.18	<0.001
BEOS (1000 cells/uL)	0.23 ± 0.17	0.25 ± 0.23	0.092
Serum albumin (g/L)	42.02 ± 3.66	39.97 ± 4.18	<0.001
Serum total protein (g/L)	71.17 ± 4.62	69.80 ± 6.17	<0.001

Continuous and categorical variables were displayed individually as mean ± SD or proportions. HR, hazard ratio; CI, confidence interval; PIR, poverty to income ratios; CVD, cardiovascular disease; COPD, chronicobstructive pulmonary disease; WBC, white blood cell; BNEU, blood neutrophils; BEOS, blood eosinophils.

taken into account. In Model Z, which controlled all covariates, the risk of all-cause death decreased by 13% for every additional unit of serum albumin (g/L). Also, the trend test of the correlation between serum albumin and all-cause mortality was statistically significant in all three models (p for trend < 0.05), which meant that serum albumin was linked to all-cause mortality in asthmatics in a way that was both linear and inverse.

3.3 Dose-response relationship

The restricted cubic spline (RCS) was especially sensitive to identifying linear or nonlinear correlation. Hence, we used RCS based on Model Z to ascertain whether the connection between serum albumin and all-cause mortality in asthmatics was linear or not (Figure 2). After adjustment for all covariates, we observed a linear and negative correlation between serum albumin and all-cause mortality in asthmatics (P for non-linearity > 0.05).

3.4 Subgroup analyses

Subgroup analyses were carried out to evaluate the correlation between serum albumin and mortality throughout various asthmatic populations. The outcomes, grouped by sex, age, race, history of hypertension, diabetes, CVD, COPD, cancer history, and usage of glucocorticoids, were displayed in Table 3. A linear and inverse correlation was observed among all asthmatic subgroup populations

TABLE 2 Association between dietary protein intake, serum albumin, and all-cause mortality in asthmatics.

	Model X	Model Y	Model Z
	HR (95% CI) P value	HR (95% CI) P value	HR (95% CI) P value
Protein intake	1.00 (0.99, 1.00) 0.0257	1.00 (1.00, 1.00) 0.8251	1.00 (1.00, 1.01) 0.1972
Protein intake quartile groups			
Q1	Reference	Reference	Reference
Q2	0.83 (0.56, 1.23) 0.3461	0.81 (0.54, 1.20) 0.2910	0.83 (0.54, 1.28) 0.4094
Q3	0.59 (0.39, 0.91) 0.0161	0.74 (0.47, 1.15) 0.1815	0.76 (0.45, 1.27) 0.2926
Q4	0.60 (0.39, 0.91) 0.0171	0.94 (0.59, 1.48) 0.7804	1.09 (0.56, 2.13) 0.7983
P for trend	0.0055	0.6017	0.8611
Serum albumin	0.84 (0.81, 0.87) <0.0001	0.86 (0.82, 0.90) <0.0001	0.87 (0.83, 0.92) <0.0001
Serum albumin quartile groups			
Q1	Reference	Reference	Reference
Q2	0.66 (0.45, 0.97) 0.0340	0.73 (0.49, 1.08) 0.1122	0.75 (0.50, 1.13) 0.1716
Q3	0.39 (0.25, 0.60) <0.0001	0.47 (0.30, 0.73) 0.0008	0.51 (0.32, 0.81) 0.0042
Q4	0.23 (0.15, 0.36) <0.0001	0.36 (0.23, 0.57) <0.0001	0.41 (0.25, 0.69) 0.0007
P for trend	<0.0001	<0.0001	0.0002

Model X adjusted for none. Model Y adjusted for age, race, sex, education, marriage, and PIR. Model Z adjusted for all covariates. HR, hazard ratio; CI, confidence interval.

except for white populations age<40, with a BMI of 25–30, and with a cancer history. And serum albumin and cancer history might have interaction effects with all-cause mortality (p for interaction < 0.05).

3.5 Kaplan–Meier survival curve

In Figure 3, we carried out a Kaplan-Meier analysis based on serum albumin quartile groups to examine the prognostic impact of serum albumin on mortality in asthmatics. We observed that the risk of mortality was lower for asthmatic adults with the highest serum albumin quartiles group (Q4), compared with asthmatics with the lowest serum albumin quartiles group (Q1).

4 Discussion

In this investigation, we selected 3005 NHANES participants with asthma based on designed exclusion criteria. In the follow-up outcome group, the difference in the distribution of serum albumin was statistically significant, whereas there was no significant difference in protein intake. Serum albumin levels were reduced in the death population compared with the survival population. We used univariate and multivariate Cox proportional hazard models to investigate the connection between dietary protein intake, serum albumin and all-cause mortality in patients with asthma. The results suggested that dietary protein intake was not independently related to all-cause mortality, whereas serum albumin was negatively related to all-cause mortality (P < 0.05). In model Z, which controlled for all covariates, each unit increase in serum albumin (g/l) was associated with a 13% reduction in the risk of all-cause mortality. RCS confirmed a linear negative connection of serum albumin with all-cause mortality in asthmatics. The results of subgroup analyses suggested a linear negative connection of serum albumin with all-cause mortality in all subgroups, except for white populations age<40, with a BMI of 25–30, and with a cancer history. And there was no interaction among all subgroups. Kaplan-Meier survival curves suggested asthmatic populations in the higher serum albumin quartile (Q4) had a lower risk of death compared with asthmatics in the lower groups (Q1).

In this study group, higher age, lower income, smoking, and co-morbidities were associated with a higher risk of death in asthma patients. This is consistent with the findings of many previous studies (16, 17). Several previous studies have found that lower albumin levels are associated with increased mortality in both the short and long term (18, 19). The negative association of serum albumin levels with mortality in long-term follow-up has also been found in elderly people (20, 21), post-surgical patients (19), patients after acute myocardial infarction (22), and patients with chronic kidney disease (23), which is consistent with our findings. However, our study considered a wider variety of potential factors, applied a more rigorous statistical approach and confirmed a linear association between serum albumin and mortality in asthmatics. In addition, we utilized stratified analyses to investigate the connection of serum albumin levels and all-cause mortality in various groups to avoid the impact of confounding variables.

Serum albumin levels are reduced in various conditions, such as inflammation, protein malnutrition, end-stage liver disease, or malignancy (24). Human serum albumin (HSA) is a cysteine-rich serum protein that is taken up by many cells through receptor-mediated liquid-phase endocytosis. This biological process has been documented in lung epithelial cells and may occur in other cell

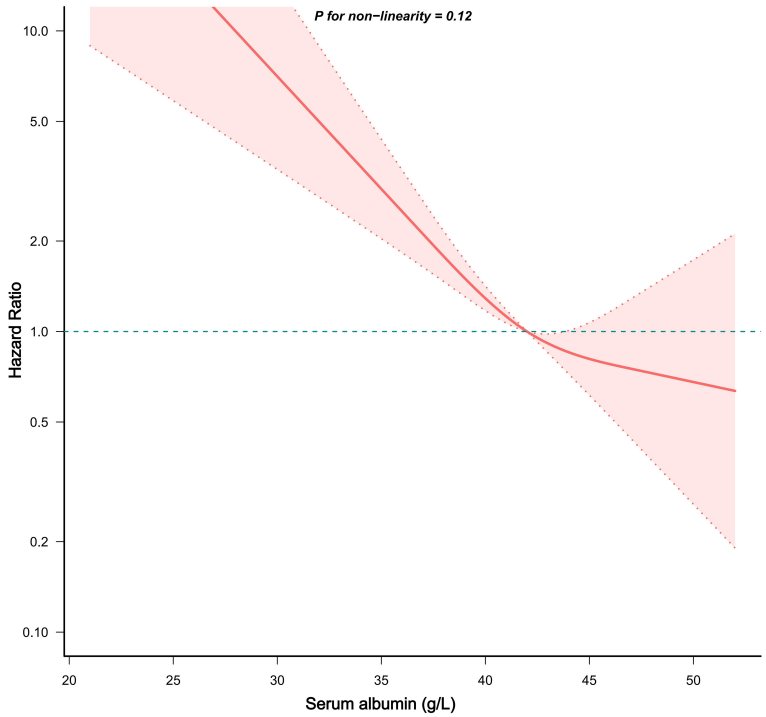

FIGURE 2
Dose-response correlation between serum albumin and mortality in asthmatics. The red solid line and red area represented the HRs and their accompanying 95%CIs, respectively.

types (25). It has been demonstrated that albumin can protect cells from oxidative damage and regulate nuclear factor kappa-B (NF-κB) activation by specifically regulating cellular glutathione (GSH) levels (26). In previous studies, abnormal CXCR4/CXCL12 signaling was involved in a variety of pathophysiological processes, such as cancer and organismal inflammation. The natural fragment of serum albumin, EPI-X4, has been identified as an endogenous peptide antagonist and inverse agonist of CXCR4, and novel CXCR4 antagonists developed on the basis of this feature can be used in the treatment of atopic dermatitis, asthma, and other CXCR4-related diseases (27).

Serum albumin is extensively involved in the development of various respiratory diseases. Capillary bronchiolitis is the leading cause of infant hospitalization in the USA and is accompanied by life-threatening apnea. It has been suggested that low serum albumin levels appear to be associated with an increased risk of apnea and are potentially a predictor of future apnea (28). A cross-sectional study that included 3286 individuals found a positive correlation between serum albumin levels and lung function, including forced vital capacity (FVC) and forced expiratory volume in one second (FEV 1) (29). Decreased blood albumin levels are a well-known clinical indication of malnutrition, resulting in weakened respiratory muscles and diminished lung function (29, 30). Moreover, chronic obstructive pulmonary disease (COPD) is a progressive disease characterized by chronic respiratory inflammation and parenchymal lung damage. Biological processes such as systemic inflammation and oxidative stress are involved in the pathogenesis of COPD. Serum albumin is a negative acute-phase protein with antioxidant properties. A systematic review and meta-analysis revealed that serum albumin concentrations were significantly depressed in patients with stable COPD compared to non-COPD controls. This result suggests that systemic anti-inflammatory and antioxidant defense mechanisms are defective in patients with COPD (31). A study on anti-oxidative stress in obstructive sleep apnea (OSA) patients found that their serum albumin levels were lower. This may make oxidative stress worse, which can lead to heart and metabolic diseases (32). Besides that, serum albumin is an independent predictor of the severity of lung necrosis in children with community-acquired necrotizing pneumonia (NP) (33).

Our investigation uniquely concentrated on exploring the connection of dietary proteins, serum albumin, and mortality in asthmatics, a topic that has not been previously addressed. Our study includes a substantial number of asthma patients that are nationally representative and considers a wide variety of potential factors that could affect the results. We showed a reverse linear connection of blood albumin levels and overall mortality in individuals with asthma using multivariate Cox hazard models and RCS. Our work provides fresh insights for future research on managing and treating asthma.

Still, we recognize that there are some constraints to our study. Our study has a nationwide sample size; however, most of the data was gathered from the population of the USA. Varying levels of economic development in countries result in diverse food patterns that significantly influence people's nutritional status and disease rates. Due to the limitations of the NHANES database, we included

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TABLE 3 Stratified associations between serum albumin and all-cause mortality in asthmatics.

Subgroup	N	HR (95% CI) P value	P for interaction
Sex			0.202
Male	1269	0.83 (0.77~0.89) <0.001	
Female	1736	0.89 (0.83~0.95) <0.001	
Age			0.541
<40	1274	1.01 (0.83~1.22) 0.959	
40-60	873	0.87 (0.78~0.97) 0.012	
≥60	858	0.85 (0.8~0.9) 0.001	
Race			0.093
White populations	1256	0.95 (0.88~1.03) 0.201	
Black populations	767	0.87 (0.79~0.95) 0.002	
Other race populations	982	0.77 (0.7~0.86) 0.001	
BMI			0. 843
<25	766	0.84 (0.76~0.92) <0.001	
25-30	823	0.94 (0.84~1.04) 0.234	
≥30	1416	0.87 (0.8~0.93) <0.001	
Hypertension			0.134
No	1798	0.84 (0.76~0.93) 0.001	
Yes	1207	0.88 (0.83~0.93) 0.001	
Diabetes			0.825
No	2539	0.88 (0.83~0.94) <0.001	
Yes	466	0.84 (0.76~0.92) <0.001	
CVD history			0.082
No	2553	0.91 (0.85~0.97) 0.006	
Yes	452	0.83 (0.77~0.89) <0.001	
COPD history			0.146
No	2692	0.85 (0.8~0.9) <0.001	
Yes	313	0.9 (0.83~0.99) 0.023	
Cancer history			0.001
No	2688	0.82 (0.78~0.87) <0.001	
Yes	317	1.05 (0.94~1.17) 0.416	
Glucocorticoid drugs			0.382
No	2513	0.85 (0.8~0.9) 0.001	
Yes	492	0.89 (0.81~0.97) 0.009	

All stratified analyses adjusted for all covariates except for the stratification variable. HR, hazard ratio; CI, confidence interval; CVD, cardiovascular disease; COPD, chronic obstructive pulmonary disease.

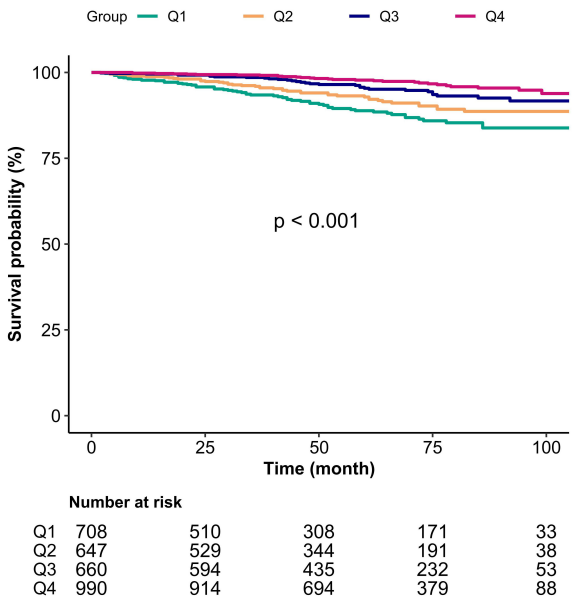

FIGURE 3
Kaplan-Meier analysis of mortality by groups of serum albumin quartile in asthmatics. Q1-Q4, Grouped by serum albumin quartiles.

asthmatic individuals based on questionnaire data as opposed to the lung function test. The 24-hour dietary recall approach records food intake over a 24-hour period, which might not accurately reflect patients' regular eating patterns and may be subject to memory bias. Serum albumin levels are reduced in various conditions, thus it is a non-specific and prognostically relevant marker of asthma. Cohort studies can establish a causal association between dietary protein, serum albumin, and mortality in asthma patients. However, there are unmeasured and unknown factors that may influence the study results.

5 Conclusion

This investigation proved a negative linear connection of serum albumin with all-cause mortality in asthma patients. However, there was no independent link discovered between dietary protein intake and mortality. This indicates that serum albumin could be a significant factor in predicting long-term outcomes for asthma patients.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: All data can be accessed via the NHANES official website (http://www.cdc.gov/nchs/nhanes/index.htm).

Ethics statement

The studies involving humans were approved by Prior to implementing the data collection methodologies and conducting comprehensive health assessments, all participants willingly completed informed consent. The NHANES study protocol received approval from the Research Ethics Review Board of the NCHS (Ethical approval number: Protocol #2011-17, Protocol #2018-01). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

RZ: Writing – original draft, Methodology, Investigation, Conceptualization. JL: Writing – original draft, Software, Methodology, Investigation. MG: Writing – review & editing, Software, Methodology, Data curation. JW: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. SG: Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Serum growth differentiation factor 15 as a biomarker for malnutrition in patients with acute exacerbation of chronic obstructive pulmonary disease

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Background: Chronic obstructive pulmonary disease (COPD) is a common respiratory disease that often coexists with malnutrition during acute exacerbation (AECOPD) and significantly affects the prognosis. Previous studies have shown that growth differentiation factor 15 (GDF15) levels promote appetite suppression, weight loss, and muscle weakness, and are markedly high in peripheral blood following inflammatory stimulation. However, it is still unknown whether serum GDF15 levels can be used to predict malnutrition in patients with AECOPD.

Methods: A total of 142 patients admitted to the Department of Respiratory Medicine at Anshun People's Hospital between December 2022 and August 2023 were selected for this study. The participants were divided into two groups: malnutrition group (n = 44) and non-malnutrition group (n = 98) based on a body mass index (BMI) < 18.5 kg/m^2, according to the Global Leadership Initiative on Malnutrition (GLIM) criteria. Serum GDF15 levels were measured using the enzyme-linked immunosorbent assay (ELISA) and compared between the two groups. Spearman correlation analysis was used to examine the association between serum GDF15 levels, baseline data, and clinical indicators. Binary logistic regression was used to identify the independent risk factors for AECOPD combined with malnutrition. The predictive value of serum GDF15, albumin (ALB), and a combination of these was evaluated to identify malnutrition in patients with AECOPD using a receiver operating characteristic (ROC) curve.

Results: Serum GDF15 levels in patients with malnutrition and AECOPD were significantly higher than those in patients without malnutrition, whereas the serum ALB levels were significantly lower than those in patients without malnutrition (p < 0.001). Moreover, serum GDF15 levels were negatively correlated with BMI (r = −0.562, p < 0.001), mid-arm circumference (r = −0.505, p < 0.001), calf circumference (r = −0.490, p < 0.001), total protein (r = −0.486, p < 0.001), ALB (r = −0.445, p < 0.001), and prognostic nutritional index (r = −0.276, p = 0.001), and positively correlated with C-reactive protein (r = 0.318, p < 0.001), COPD assessment test score (r = 0.286, p = 0.001), modified medical research council classification (r = 0.310, p < 0.001), and

global initiative for chronic obstructive pulmonary disease grade (r = 0.177, p = 0.035). Furthermore, serum GDF15 levels were an independent risk factor for malnutrition in patients with AECOPD (OR = 1.010, 95% CI, 1.003∼1.016). The optimal cut-off value of serum GDF15 level was 1,092.885 pg/mL, with a sensitivity of 65.90% and a specificity of 89.80%, while the serum ALB level was 36.15 g/L, with a sensitivity of 86.40% and a specificity of 65.00%, as well as a combined sensitivity of 84.10% and a specificity of 73.90%. Serum GDF15 and serum ALB levels had a good predictive ability (AUC = 0.856, AUC = 0.887), and the ROC revealed a greater combined prediction value for the two (AUC = 0.935).

Conclusion: Serum GDF15 levels could be used as a potential biomarker in the prediction of malnutrition in patients with AECOPD, offering a guidance for future clinical evaluation of malnutrition.

KEYWORDS

chronic obstructive pulmonary disease, acute exacerbation, growth differentiation factor 15, malnutrition, biomarker

Introduction

Chronic obstructive pulmonary disease (COPD) is a common respiratory disorder characterized by structural alterations in the airways and/or alveoli, caused by a prolonged exposure to harmful gases and particles, mostly from cigarette smoking, resulting in persistent respiratory symptoms and irreversible airflow limitation (1). The World Health Organization anticipates that COPD will become the third leading cause of global mortality by 2030 (2). Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) usually refers to the deterioration of clinical symptoms, including increased cough and sputum production, chest tightness, and dyspnea (3). Repeated episodes of acute exacerbation cause an intensification of inflammation and a significant reduction in lung function, often resulting in rapid disease progression and increased treatment challenges. AECOPD is frequently accompanied by comorbidities such as heart failure, respiratory failure, and malnutrition (4). The prevalence of malnutrition in COPD patients has been extensively documented in numerous studies, with estimates ranging from 30 to 60% (5). The rate of hospitalization and mortality is significantly increased when coupled with malnutrition (6). The risk of death has almost tripled, particularly during acute exacerbation, profoundly impacting patient prognosis and imposing a substantial economic burden (6, 7). Therefore, early identification and prediction of malnutrition in patients with AECOPD as well as timely implementation of nutritional management strategies are crucial for optimizing clinical prognosis and reducing disease burden.

Growing evidence revealed that dietary factors, nutrients, and gut-lung axis are closely linked to the occurrence and worsening of COPD (8, 9). High intake of red or processed meat, refined grains, saturated fats and diet rich in sweets are associated with an increased risk of the occurrence of COPD (10). However, a diet rich in fruits, vegetables, whole grains, and dietary fiber is inversely associated with the decrease in lung function and risk of COPD (10), being particularly effective in smokers and ex-smokers, suggesting that healthy dietary patterns protect against the deleterious effects of smoking on lung function (11). There is an imbalance of intestinal microbiota in patients with COPD, which may be caused by the colonization of intestinal microbiota by lung microbiota or the induction of anorexia, leading to chronic inflammation and immune disorders. This phenomenon is known as "gut-lung axis" (9, 12). Gut microbiota possess anti-inflammatory effects and improve the immune defense by producing a large amount of metabolites including short-chain fatty acids through dietary fiber fermentation. Nevertheless, when the intake of healthy food is reduced in COPD patients, especially dietary fibers, the lack of fermentable dietary fiber in the gut leads to gut microbiota imbalance and affects the immune response in the lungs, promoting inflammation and malnutrition (12). Moreover, low protein intake is associated with an increased risk of mild-to-moderate COPD exacerbation and with a low body mass index (BMI) (13). However, high-protein oral nutritional supplements significantly improve the nutritional status of COPD patients and reduce the risk of death (14). This evidence shows that dietary patterns and nutritional interventions have important implications malnutrition in patients with COPD.

Malnutrition is currently recognized and mainly diagnosed according to the Global Leadership Initiative on Malnutrition (GLIM), and the European Society of Clinical Nutrition and Metabolism (ESPEN) was used as standard (15, 16). The ESPEN consensus recommends using validated screening tools to identify patients at risk for malnutrition and then diagnosing malnutrition based on a BMI of less than 18.5 kg/m^2 or unintentional weight loss, low BMI, and low fat-free mass index (16). Similarly, the GLIM diagnostic criteria are also based on the assessment of malnutrition risk followed by a definitive diagnosis (at least one clinical presentation of unwanted weight loss, low BMI, reduced

muscle mass, and one etiologic presentation of reduced food intake, inflammation, or a disease state) (15). Previous studies in patients validated the Asian BMI cutoff value of 18.5 kg/m^2 for the diagnosis of malnutrition using the GLIM criteria (17, 18). However, BMI is often influenced by multiple factors. For example, AECOPD combined with heart failure and chronic kidney disease increases water load, resulting in body fluid overload and weight gain that does not accurately reflect nutritional status (19). The coexistence of AECOPD and heart failure activates various neurohumoral pathways in the body, including the sympathetic nervous system, renin-angiotensin-aldosterone system, and arginine vasopressor system (20). Cytokines such as arginine vasopressin, renin, and aldosterone secreted by these systems contribute to water and sodium retention in the body, leading to apparent "obesity and/or normal" presentation of patients (20). Additionally, when AECOPD is complicated by chronic kidney disease (CKD), structural or functional impairment of the kidneys results in decreased glomerular filtration rate and damage to the glomerular filtration barrier (21). This leads to a discharge of a large amount of proteins, including albumin, this phenomenon is known as the proteinuria. Since albumin maintains colloid osmotic pressure, lower serum albumin levels lead to interstitial edema (22). These conditions result in fluid overload in the body. Therefore, using BMI alone as an indicator of malnutrition for such patients may not be reliable, and additional ambulatory blood biomarkers may be beneficial. Hence, we need to identify a strategy that can be performed routinely in clinical practice, to complement the ones listed above.

Growth differentiation factor 15 (GDF15) is a member of the transforming growth factor-β superfamily with reduced expression in various human tissues, including the liver, epithelial cells, and macrophages (23). However, GDF15 expression rapidly upregulates in response to stress conditions such as inflammation or hypoxia (23). In addition to its involvement in several physiological processes including cell proliferation, differentiation, and repair, GDF15 may also play a role in various pathological processes. Elevated levels of GDF15 have been associated with cancer, cardiovascular diseases, and renal diseases, making it a potential disease biomarker (24–26). Several studies have suggested that elevated GDF15 levels in the serum are strongly associated with appetite suppression, weight loss, and muscle weakness (27, 28). Furthermore, the concentration of GDF15 in COPD patients is significantly higher than that in healthy individuals, up to 2.1 times higher (29). Moreover, GDF15 levels in AECOPD patients are higher than those in stable COPD patients and are strongly associated with reduced lung function, worsened clinical symptoms, increased frequency of acute exacerbation, and an elevated risk of mortality (29–31). Previous studies in COPD patients have associated elevated GDF15 serum levels with reduced muscle mass (32). However, it is still unknown whether serum GDF15 levels can be used to predict malnutrition in patients with AECOPD.

This study aimed to assess the predictive value of serum GDF15 for malnutrition in patients with AECOPD and to determine the optimal cut-off value for its use in clinical practice.

Materials and methods

Study population

A total of 142 AECOPD patients admitted to the Department of Respiratory Medicine of Anshun People's Hospital between December 2022 and August 2023 were included in this study. Patients were divided into malnutrition (n = 44) and non-malnutrition (n = 98) group, defined as BMI < 18.5 kg/m^2 according to the GLIM criteria. This study was approved by the Ethics Committee of the Anshun People's Hospital (Ethics number: 2023 Specialty 1), and informed consent was obtained from all participants.

The inclusion criteria were as follows: (1) diagnosis of AECOPD according to the Global Initiative for chronic obstructive lung disease (GOLD) criteria; (2) age ≥ 40 years.

The exclusion criteria were as follows: (1) presence of bronchial asthma, pulmonary tuberculosis, bronchiectasis, or other respiratory diseases; (2) severe liver and kidney diseases, heart failure, thyroid diseases, tumors, or other conditions significantly affecting the nutritional status; (3) individuals with mental illness who could not cooperate; and (4) patients who did not complete routine blood and biochemical tests or had incomplete data after admission.

Data collection

The following data were collected: gender, age, smoking status, BMI, mid-arm circumference (MAC), calf circumference (CC), comorbidities, nutrition risk screening 2002 (NRS2002) score, disease duration, number of acute exacerbations in the past year, COPD assessment test (CAT) score, and modified Medical Research Council (mMRC) classification. Pulmonary function tests were performed during hospitalization, including forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and FEV_1 as a percentage of the predicted value (FEV_1%/pre). Patients were divided into four groups based on the global initiative for chronic obstructive pulmonary disease (GOLD) grade: grade 1 (FEV_1%/pre ≥ 80%), GOLD grade 2 (50% ≤ FEV_1%/pre < 80%), GOLD grade 3 (30% ≤ FEV_1%/pre < 50%), and GOLD grade 4 (FEV_1%/pre < 30%). BMI measurement: on the second day of admission, patients were instructed to wake up in the morning, remove any clothing or accessories that may interfere with measurements, and record their height and weight. Two measurements were taken and averaged to determine the BMI using the formula: weight (kg)/height^2 (m^2). MAC measurement: the circumference from the acromion on the non-dominant side to the midpoint of the olecranon line was measured twice using a tape measure with subjects' upper limbs naturally hanging down. The average values were calculated. CC measurement: two measurements were taken using a tape measure around the broadest part of their non-dominant lower leg after allowing the subjects to stand naturally with their legs shoulder-width apart. The average values were calculated.

Fasting peripheral venous blood was collected from all subjects to detect serum GDF15 levels. The supernatant was separated by centrifugation at 2,000 RPM for 20 min after natural coagulation

at room temperature and stored in an ultra-low temperature refrigerator at −80 °C until further use. Serum GDF15 levels were measured using a GDF-15 Kit (Jiangsu Jingmei Biotechnology Co., Ltd., Jiangsu, China) following the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS 26.0 for Windows (IBM Corporation, Armonk, NY, United States). Normally distributed data were presented as mean ± SD, and the *t*-test was used for group comparisons. Non-normally distributed data, median, and interquartile range were used, and the Mann-Whitney U test was used for group comparisons. Count data are expressed as percentages (%), and the chi-square test was used for group comparisons.

A Spearman correlation analysis was performed to investigate the relationship between serum GDF15 levels, baseline data, and clinical indicators. The binary logistic regression analysis was used to identify independent risk factors for malnutrition in patients with AECOPD. A receiver operating characteristic (ROC) curve analysis evaluated the predictive value of serum GDF15, serum ALB, and their combination in identifying malnutrition among AECOPD patients by calculating the area under the curve (AUC). The significance level was set at $p < 0.05$.

Results

Baseline characteristics of subjects

This study included 142 patients and their baseline characteristics are shown in Table 1. Overall, the mean age of all patients with AECOPD was 67.67 ± 8.89 years and included 120 males and 22 females, with a malnutrition prevalence rate of approximately 30.99%. Next, we analyzed the differences between patients with and without malnutrition. MAC and CC were significantly lower in patients with malnutrition and AECOPD, as shown in Table 1. In terms of clinical symptoms, the CAT score and mMRC classification were higher in patients with malnutrition and AECOPD. In addition, the GOLD grade was significantly increased in patients with malnutrition and AECOPD.

Serum GDF15 levels and clinical indicators of subjects

Table 2 shows that patients with malnutrition had higher GDF15 levels in the serum and C-reactive protein (CRP) than patients without malnutrition, while total protein (TP) and ALB levels, as well as the prognostic nutritional index (PNI) were lower in patients with malnutrition. The differences were statistically significant. Patients with and without malnutrition showed no significant differences in hemoglobin, neutrophil/lymphocyte ratio (NLR), lymphocyte/monocyte ratio (LMR), and platelet/lymphocyte ratio (PLR).

Serum GDF15 levels and baseline characteristics and clinical indicators

Serum GDF15 levels were significantly negatively correlated with BMI ($r = -0.562$, $p < 0.001$), MAC ($r = -0.505$, $p < 0.001$), CC ($r = -0.490$, $p < 0.001$), TP ($r = -0.486$, $p < 0.001$), ALB ($r = -0.445$, $p < 0.001$), and PNI ($r = -0.276$, $p = 0.001$). This was confirmed by the positive correlation between serum GDF15 levels, CAT score ($r = 0.286$, $p = 0.001$), mMRC classification ($r = 0.310$, $p < 0.001$), and GOLD grade ($r = 0.177$, $p = 0.035$). The results are presented in Table 3.

Risk factors for malnutrition in AECOPD patients

After performing a binary logistic regression analysis, we used non-malnutrition and malnutrition as dependent variables (non-malnutrition = 0, malnutrition = 1), and the indicators with statistically significant differences in the text as independent variables. Table 4 illustrates that serum GDF15 levels (OR = 1.010, 95% CI, 1.003∼1.016) independently contributed to the risk of malnutrition in patients with AECOPD.

Predictive value of serum GDF15 levels, serum ALB levels and their combination in malnutrition patients with AECOPD

The optimal cut-off value of serum GDF15 levels and serum ALB levels for predictive malnutrition in AECOOPD patients was 1,092.885 pg/mL and 36.15 g/L, respectively, with a sensitivity of 65.90 and 86.40%, respectively, as well as specificity of 89.80 and 65.00%, respectively. The combination had a sensitivity of 84.10% and specificity of 73.90%. The AUC of serum GDF15 levels was 0.856 (95% CI, 0.791∼0.920) and the AUC of serum ALB levels was 0.887 (95% CI, 0.831∼0.943), and the combination AUC was 0.935 (95% CI, 0.895∼0.975). These results demonstrated that the combined detection had a stronger predictive ability for malnutrition in AECOPD patients. The results are presented in Table 5 and Figure 1.

Discussion

In this study, we examined the levels of serum GDF15 in AECOPD patients with and without malnutrition and the differences between the two groups were compared. We found a significant increase in serum GDF15 levels in AECOPD patients with malnutrition, which was negatively correlated with nutritional indicators such as BMI, MAC, CC, TP, ALB, and PNI, while demonstrating a positive correlation with the inflammatory marker CRP. Furthermore, serum GDF15 levels were positively correlated with CAT score, mMRC classification, and GOLD grade. Although our findings are mainly consistent with previous research reports, several new discoveries have emerged: (1) serum GDF15 is an independent risk factor for AECOPD patients with comorbid

TABLE 1 Baseline characteristics of the included subjects.

Variables	Malnutrition (n = 44)	Non-malnutrition (n = 98)	p-value
Gender			0.191
Male	39 (88.6)	78 (79.6)	
Female	5 (11.4)	20 (20.4)	
Mean age, years	68.27 ± 7.43	68.19 ± 8.37	0.957
BMI, kg/m^2	18.269 (17.56, 18.43)	22.63 (20.25, 25.80)	< 0.001
MAC, cm	24.80 (23.85, 27.58)	29.50 (27.25, 32.03)	< 0.001
CC, cm	30.31 ± 2.93	33.52 ± 3.18	< 0.001
Index of smoking, pack-years	49.00 (39.00, 60.00)	45.50 (0.00, 62.50)	0.240
Comorbidity			
Hypertension	12 (27.3)	25 (25.5)	0.825
Coronary heart disease	2 (4.5)	5 (5.1)	0.887
Diabetes mellitus	3 (6.8)	9 (9.2)	0.639
Respiratory failure	26 (59.1)	42 (42.9)	0.073
Number of acute exacerbations in the past year, times	1.5 (1.00, 2.00)	1.00 (0.00, 2.00)	0.082
Course of disease, years	8.00 (3.13, 14.50)	5.75 (3.00, 11.00)	0.212
NRS2002 score	6.00 (5.00, 7.00)	3.00 (2.00, 3.25)	< 0.001
CAT score	22.93 ± 4.73	16.76 ± 5.78	< 0.001
mMRC classification	4 (3, 4)	3 (2, 3)	< 0.001
GOLD grade	4 (3, 4)	3 (2, 4)	0.001

Data are expressed as mean ± SD, median (interquartile range), or frequency (%). BMI, body mass index; MAC, mid-arm circumference; CC, calf circumference; NRS2002 score, nutritional risk screening 2002; CAT score, COPD assessment test; mMRC classification, modified medical research council; GOLD grade, global initiative for chronic obstructive pulmonary disease.

TABLE 2 Serum GDF15 and clinical indicators of subjects.

Clinical indicators	Malnutrition (n = 44)	Non-malnutrition (n = 98)	p-value
GDF15, pg/mL	1,117.47 ± 93.00	951.61 ± 128.15	< 0.001
CRP, mg/L	22.13 (11.24, 55.89)	12.05 (3.52, 38.86)	0.002
TP, g/L	65.70 (64.50, 66.80)	68.80 (67.40, 69.83)	< 0.001
ALB, g/L	35.10 (34.25, 35.90)	37.00 (36.20, 38.60)	< 0.001
PNI	39.80 (38.14, 42.01)	42.65 (40.76, 45.99)	< 0.001
Hemoglobin, g/L	134.05 ± 22.09	141.28 ± 25.32	0.104
NLR, %	5.54 (3.43, 8.56)	4.69 (3.00, 7.36)	0.320
LMR, %	2.07 (1.48, 3.26)	2.39 (1.59, 3.26)	0.059
PLR, %	175.74 (112.9, 227.28)	142.90 (104.38, 216.48)	0.180

Data are expressed as mean ± SD, median (interquartile range). GDF15, growth differentiation factor 15; CRP, C-reactive protein; TP, total protein; ALB, albumin; PNI, prognostic nutritional index, albumin+5*lymphocyte count; NLR, neutrophil/lymphocyte ratio; LMR, lymphocyte t/monocyte ratio; PLR, platelet/lymphocyte ratio.

malnutrition; (2) serum GDF15 levels hold promise as a predictive biomarker for identifying malnutrition during AECOPD episodes, with the combination of serum ALB levels having a higher predictive ability for malnutrition in patients with AECOPD.

In clinical practice, BMI is commonly used as an initial assessment tool to evaluate the nutritional status of patients. However, it should be emphasized that BMI is influenced by various factors and may not accurately reflect an individual's actual nutritional status. Studies have demonstrated that even when COPD patients are within the normal BMI range, they often exhibit significant muscle quality decline and dysfunction, both being signs of malnutrition (32). These conditions can cause respiratory muscle weakness and exacerbate the progression of COPD. Furthermore, in patients with AECOPD who have comorbidities such as heart failure or chronic kidney disease, fluid overload can result in weight gain and falsely increased BMI levels, overestimating their actual nutritional status (19). Therefore, relying solely on BMI to diagnose malnutrition may result in an inaccurate assessment due to its susceptibility to diseases. To evaluate the nutritional status, especially among AECOPD patients, patients with severe liver, and kidney diseases as well as heart failure were excluded in our study, even though a BMI < 18.5 kg/m^2 was the criteria for a diagnosis of

TABLE 3 Spearman correlation analysis between serum GDF15 levels and baseline data and clinical indicators.

Variables	GDF15, pg/mL	
	r	*p*-value
BMI, kg/m^2	−0.562	< 0.001
MAC, cm	−0.505	< 0.001
CC, cm	−0.490	< 0.001
TP, g/L	−0.486	< 0.001
ALB, g/L	−0.445	< 0.001
CRP, mg/L	0.318	< 0.001
PNI	−0.276	0.001
CAT score	0.286	0.001
mMRC classification	0.310	< 0.001
GOLD grade	0.177	0.035

BMI, body mass index; MAC, mid-arm circumference; CC, calf circumference; TP, total protein; ALB, albumin; CRP, C-reactive protein; PNI, prognostic nutritional index, albumin+5*lymphocyte count; CAT score, COPD assessment test; mMRC classification, modified medical research council; GOLD grade, global initiative for chronic obstructive pulmonary disease.

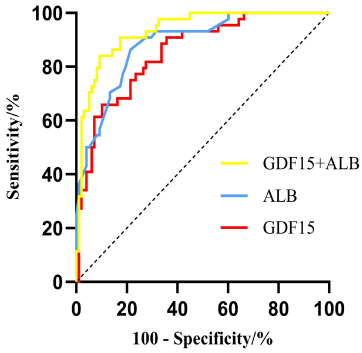

FIGURE 1
ROC curves of serum GDF15 levels and albumin levels, and their combination for predicting malnutrition in AECOPD patients.

malnutrition according to GLIM standards. The findings revealed a malnutrition prevalence rate of approximately 30.99% in our patient population, which was consistent with previous research results. Therefore, it is imperative to conduct comprehensive evaluations considering the potential coexisting medical conditions when using BMI alone for the clinical diagnosis of malnutrition.

ALB is a widely used clinical indicator for malnutrition assessment. This study revealed that AECOPD patients with malnutrition had lower serum ALB levels than those without malnutrition. Moreover, serum ALB levels show high predictive efficiency for malnutrition in AECOPD patients. Nevertheless, a binary logistic regression analysis showed that the serum albumin levels were not an independent risk factor for malnutrition in AECOPD patients. This finding might be related to the small sample size included in this work. Therefore, in the future, we will perform a large sample, multicenter prospective study to further validate the results. Moreover, the average half-life of albumin is approximately 20 days (33). Patients with AECOPD often experience reduced substance intake and increased catabolism, leading to potential lagging phenomena that reflect rapid changes in nutritional status. Serum GDF15 levels can rapidly increase under inflammatory conditions, with a half-life of approximately 3 h (34), and they are also linked to weight loss and weakness.

TABLE 4 Binary logistic regression analysis of risk factors in patients with malnutrition and AECOPD.

Variables	β	SE value	Wald value	*p*-value	OR	95% CI
MAC, cm	−0.573	0.298	3.683	0.055	0.564	(0.314∼1.012)
CC, cm	0.464	0.276	2.817	0.093	1.590	(0.925∼2.733)
TP, g/L	−0.189	0.317	0.357	0.550	0.827	(0.445∼1.540)
ALB, g/L	−0.901	0.543	2.751	0.097	0.406	(0.140∼1.178)
CRP, mg/L	−0.002	0.006	0.084	0.772	0.998	(0.986∼ 1.011)
PNI	0.105	0.132	0.631	0.427	1.111	(0.857∼1.440)
GDF15, pg/mL	0.010	0.003	9.174	0.002	1.010	(1.003∼1.016)
CAT score	0.111	0.094	1.404	0.236	1.118	(0.930, 1.343)
mMRC classification	0.198	0.842	0.055	0.814	1.219	(0.234, 6.346)
GOLD grade	0.083	0.551	0.022	0.881	1.086	(0.369, 3.199)

MAC, mid-arm circumference; CC, calf circumference; TP, total protein; ALB, albumin; CRP, C-reactive protein; PNI, prognostic nutritional index, albumin+5*lymphocyte count; GDF15, growth differentiation factor 15; CAT score, COPD assessment test; mMRC classification, modified medical research council; GOLD grade, global initiative for chronic obstructive pulmonary disease.

TABLE 5 Predictive value of serum GDF15 levels, serum ALB levels and their combination in malnutrition patients with AECOPD.

Indicator	AUC	95% CI	Cut-off value	Sensitivity/%	Specificity/%	Youden index	*p*-value
GDF15 (pg/mL)	0.856	(0.791∼0.920)	1,092.885	65.90	89.80	0.557	< 0.001
ALB (g/L)	0.887	(0.831∼0.943)	36.15	86.40	65.00	0.514	< 0.001
GDF15+ALB	0.935	(0.895∼0.975)	–	84.10	73.90	0.58	< 0.001

GDF15, growth differentiation factor 15; ALB, albumin; GDF15+ALB, growth differentiation factor 15+albumin.

These findings suggested that serum GDF15 levels might serve as a more effective indicator of nutritional status than serum ALB levels in critically ill patients in a short time. Furthermore, in AECOPD patients with comorbidities, such as heart failure or chronic renal failure, serum albumin might be lost through urinary excretion, resulting in hypoalbuminemia (33). One of the roles of serum albumin is to maintain plasma colloid osmotic pressure. Hypoalbuminemia may lead to fluid overload and promote weight gain, which is detrimental to the early detection of malnutrition (22, 33). Therefore, a comprehensive evaluation incorporating multiple factors is necessary to accurately assess the nutritional condition.

GDF15 belongs to the transforming growth factor-β superfamily and its expression is typically low in the human body under physiological conditions (23). However, its expression rapidly increases during inflammation and hypoxia and often serves as an inflammatory marker during the acute phase. Studies have revealed a significant increase of GDF15 levels among patients with COPD and AECOPD than those in good health, which is strongly associated with disease prognosis. Furthermore, high GDF15 levels have been observed in patients with old age and chronic kidney disease, and are closely linked to nutritional status (35, 36). Previous research has also demonstrated a strong correlation between GDF15 levels and muscle quality in patients with COPD. When muscle quality declines and dysfunction occurs in COPD patients, the respiratory muscles weaken, resulting in increased airway obstruction and more respiratory effort during static states. This results in significant energy expenditure and malnutrition in some individuals. Currently available methods for assessing muscle quality and function include magnetic resonance imaging (MRI), dual-energy X-ray absorptiometry (DEXA), and bioelectrical impedance analysis (BIA). However, it is difficult to perform procedures due to high costs, specific equipment, and specialized training. Clinicians commonly employ MAC and CC measurements as preliminary indicators of muscle quality evaluation (37). The results of this study also confirmed a significant negative correlation between serum GDF15 levels and TP, ALB, and PNI, particularly with BMI ($r = -0.562$, $p < 0.001$), MAC ($r = -0.505$, $p < 0.001$), and CC ($r = -0.490$, $p < 0.001$). This indicated that GDF-15 was involved in malnutrition in patients with AECOPD. Furthermore, serum GDF15 levels were significantly increased in AECOPD patients with malnutrition and were identified as an independent risk factor for AECOPD in patients with malnutrition. In this study, we separately analyzed the predictive efficacy of serum GDF-15 and albumin levels in patients with malnutrition and AECOPD. ROC analysis showed that serum GDF-15 levels had a lower sensitivity than serum albumin, but a higher specificity in predicting malnutrition in patients with AECOPD. Using this indicator alone with serum GDF15 levels to assess malnutrition might result in false negative results because serum GDF-15 levels might increase with age (38). Therefore, serum GDF15 levels combined with serum albumin levels were used to predict AECOPD malnutrition in patients, and the findings revealed a high sensitivity and specificity, with an AUC of 0.935. This indicated that the combined detection had a greater predictive ability for malnutrition in AECOPD patients.

The underlying mechanism by which GDF15 contributes to malnutrition in AECOPD remains unclear. It is possible that GDF15 may contribute to the development of malnutrition by activating signal transduction pathways and interacting with neurotrophic factors (39–41). GDF15 reduces body weight by inhibiting food intake, and its effect is mediated by its binding with its receptor glial cell derived neurotrophic factor receptor alpha like (GFRAL). GFRAL belongs to the family of glial cell-derived neurotrophic factor receptors, which are mainly present in neurons located in the nucleus tractus solitaris region of the posterior brain and the posterior region of the brainstem, which are the centers for the induction of vomiting and/or anorexia. About the main mechanism for anorexia response may be GDF15 combined with GFRAL specificity, leads to the activation and phosphorylation of the Ret signal transduction pathway, and activation of signal transduction molecules such as Akt, Erk and PLC (42). It has also been found that GFRAL is localized on cholecystokinin (CCK) positive neurons; GDF15 activates CCK neurons, and GDF15-induced anorexia is attenuated by CCK signaling blockade, suggesting that GDF15 is mediated by anorexigenic signaling in CCK neurons in the brainstem (43). GDF15 also reduces food intake by delaying gastric emptying through the vagus nerve, and this change is abolished after bilateral vagotomy (34). Borner (44) found a significant difference in the effect of delayed gastric emptying only after the treatment with high concentrations of GDF15, suggesting that this effect requires high levels of GDF15 to be effective. It may also be associated with the inflammatory response observed in AECOPD patients, as GDF15 levels are significantly elevated in these conditions. Furthermore, this study discovered a correlation between GDF15 and CRP levels. CRP is an acute-phase protein synthesized by the liver and is widely used as an early, sensitive indicator of inflammation (45). Elevated CRP levels hinder nutritional support efficacy also contributing to the early detection of malnutrition (45). Hypoxia exacerbates gastrointestinal congestion, resulting in reduced intake among patients, increased metabolism, and a negative nitrogen balance, all contributing to malnutrition (46). Therefore, when GDF15 levels are elevated in AECOPD patients with concurrent malnutrition, potential targeted anti-inflammatory interventions may improve malnutrition.

This study has certain limitations. Firstly, it is important to acknowledge that this study lacked a control group of individuals who were in good health and had stable COPD conditions. It should be also noted that this was an observational study with a relatively small sample size, and no additional research was performed on serum GDF15 levels and the prognosis of patients with AECOPD malnutrition, for example, hospitalization duration, hospitalization expenses, mortality, and acute exacerbation time should be considered the next time. To validate the reliability of serum GDF15, we need to conduct more prospective, multi-center investigations in the future. Furthermore, a recent study has shown that constructing the mesoporous PdPt-assisted LDI MS for biomarker analysis results in a more accurate early diagnosis of COPD and AECOPD (47). In the future, we may be able to use this platform to detect serum biomarkers, such as GDF15 and ALB to identify malnutrition in patients with AECOPD to provide early nutritional therapy.

Accordingly, we hypothesized that serum GDF15 might be used as a potential biomarker to detect malnutrition in patients with AECOPD.

Conclusion

Serum GDF15 levels could be used as a potential diagnostic biomarker for predicting malnutrition in patients with AECOPD, providing direction for future clinical evaluations of malnutrition.

Data availability statement

The original contributions presented in this study are included in this article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by the Medical Ethics Committee of Anshun People's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

GS: Data curation, Formal analysis, Investigation, Writing – original draft. LY: Data curation, Investigation, Writing – review & editing. ZT: Data curation, Writing – review & editing. YW: Data curation, Writing – review & editing. XH: Data curation, Formal analysis, Writing – review & editing. YT: Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Association between nutrition-related indicators with the risk of chronic obstructive pulmonary disease and all-cause mortality in the elderly population: evidence from NHANES

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Background: This study aims to use six nutrition-related indicators to assess the relationship between nutritional status and the risk of COPD as well as the all-cause mortality rate, and to determine the most reliable predictive indicators.

Methods: Data from the National Health and Nutrition Examination Survey (NHANES) spanning the years 2013 to 2018 were extracted. Nutritional status was evaluated using Controlling nutritional status (CONUT) score, Geriatric Nutritional Risk Index (GNRI), Advanced Lung Cancer Inflammation Index (ALI), Prognostic Nutritional Index (PNI), Triglycerides (TG) × Total Cholesterol (TC) × Body Weight (BW) Index (TCBI), and Albumin-to-Globulin Ratio (AGR) nutritional-related indicators. Multivariate weighted logistic and Cox regression models were employed to assess the correlation between the six nutritional-related indicators and the risk of COPD and as all-cause mortality. The restricted cubic spline tests were applied to explore potential nonlinear relationships, and ROC curves and C-index analyses were conducted to compare the predictive capabilities of different indicators. Stratified analysis and propensity score matching (PSM) to assess the robustness of the results.

Results: In this study, Lower ALI, lower GNRI, and higher CONUT scores were positively correlated with an increased risk of COPD (OR: 1.77, 95% CI: 1.10–2.84) (OR: 8.66, 95% CI: 2.95–25.5), and (OR: 5.11, 95% CI: 1.72–15.2), respectively. It was found that ALI and GNRI had a non-linear relationship with the risk of COPD. After propensity score matching (PSM), the associations between ALI, GNRI, CONUT scores, and COPD remained consistent. Lower ALI, PNI, and GNRI scores were positively associated with all-cause mortality in COPD patients (HR: 2.41, 95% CI: 1.10–5.27), (HR: 3.76, 95% CI: 1.89–7.48), and (HR: 4.55, 95% CI: 1.30–15.9), respectively, with GNRI displaying a non-linear relationship with all-cause mortality. ROC curve and C-index analyses indicated that ALI had the best predictive ability for both COPD risk and all-cause mortality.

Conclusion: ALI, GNRI, and CONUT scores are correlated with the risk of COPD, while ALI, PNI, and GNRI scores are associated with all-cause mortality in COPD patients. Compared to other nutritional scores, ALI may provide more effective predictive value for both risk and all-cause mortality.

KEYWORDS

advanced lung cancer inflammation index, chronic obstructive pulmonary disease, nutritional status, cross-sectional study, population-based study, NHANES

Introduction

Chronic obstructive pulmonary disease (COPD) is one of the most common respiratory system diseases and has become the third leading cause of death globally. It is characterized by persistent and usually progressive airflow limitation, which is caused by abnormalities in the airways and/or alveoli, leading to chronic respiratory symptoms such as difficulty breathing, coughing, and sputum production (1). There are reports indicating that COPD is more common in the elderly population, with the incidence rate in individuals aged 60 and above being nearly four times higher than that in individuals below 60 (2). Malnutrition is closely associated with the occurrence of COPD in the elderly population (3, 4), and it can increase the risk of exacerbations in COPD, affecting the prognosis of COPD patients, including poor exercise tolerance, increased risk of hospitalization, severe airflow obstruction, or even death (5, 6). Therefore, nutritional status assessment should be widely incorporated into the screening and management of COPD in the elderly.

The European Society for Clinical Nutrition and Metabolic Care (ESPEN) consensus statement in 2017 has long emphasized the use of easily accessible and simple nutritional screening tools in clinical settings to identify patients at risk of malnutrition (7). Several new laboratory-based nutritional indicators have emerged in recent years, including the Controlling Nutritional Status (CONUT) Score (8), Advanced Lung Cancer Inflammation Index (ALI) (9), Geriatric Nutritional Risk Index (GNRI) (10), Prognostic Nutritional Index (PNI) (11), Triglycerides (TG) × Total Cholesterol (TC) × Body Weight (BW) Index (TCBI) (12), and Albumin-to-Globulin Ratio (AGR) (13). CONUT has been used as an indicator for assessing the risk of mortality in patients with rheumatoid arthritis and type 2 diabetes (14, 15). ALI has been used to predict the prognosis of hypertension and heart failure (16, 17). GNRI, TCBI, PNI and AGR have also shown good characteristics in predicting disease risk and prognosis (18–21).

Previous evidence suggests that malnutrition is associated with the development of COPD (3, 22), but the inflammatory processes, oxidative stress, and immune function cannot be overlooked (23, 24). The regulation of nutritional status is based on inflammatory and oxidative stress processes, both of which are interconnected with the immune system (25). For instance, malnourished COPD patients are more susceptible to the effects of inflammation and oxidative stress (26), and weight loss commonly observed in patients with COPD may be related to inflammation. Certain pro-inflammatory cytokines interacting with glucagon-like peptide-1 (GLP-1) released from intestinal tissues may lead to unintended weight loss (27). Nutritional indicators such as the CONUT, composed of lymphocyte count, albumin, and TC, and the PNI, composed of lymphocyte count and albumin, not only assess nutritional status but also involve immune status. Lymphocytes primarily mediate adaptive immunity, playing a regulatory or protective role, and low lymphocyte count often indicates poor immune status, while albumin levels typically reflect nutritional status (28, 29). The ALI, consisting of body mass index (BMI), albumin, and neutrophil to lymphocyte ratio (NLR), addresses both nutritional and inflammatory conditions. Previous studies have shown that the NLR within ALI can serve as a systemic inflammatory marker for the risk of COPD (30). Additionally, the GNRI, related to albumin levels and weight, the TCBI combining triglycerides, total cholesterol, and body weight, and the AGR, composed of albumin and globulin, primarily assess nutritional status. Previous studies have often focused on single inflammation assessments in COPD (31, 32), and there is a lack of research focusing on the relationship between nutritional status assessment and the risk and prognosis of COPD.

This study, based on the National Health and Nutrition Examination Survey (NHANES) database, aims to assess the associations between six nutrition-related indicators and the risk of COPD as well as the all-cause mortality in the elderly population in the United States. Furthermore, we endeavor to identify the optimal predictive indicators in this context.

Materials and methods

Study population

NHANES, led by the Centers for Disease Control and Prevention, employs a complex, multistage probability sampling design. It is a nationally representative survey aimed at assessing the health and nutritional status of adults and children in the United States. The survey encompasses demographic, dietary, examination, laboratory, and questionnaire data. The NHANES research protocol has received approval from the National Center for Health Statistics Research Ethics Review Board, and written informed consent has been obtained from all participants.

For this cross-sectional analysis, we selected data from 2013 to 2018 as the basis for our analysis, as clear answers regarding the definition of COPD were only available during this period, covering a total of 29,400 individuals. Firstly, since we are focusing on the elderly population in the United States, we excluded individuals under the age of 65. Additionally, considering the complex sampling design and sample weights of NHANES, we removed missing values. (1) Individuals who did not clearly answer whether they had COPD or had missing self-data were excluded. (2) Individuals lacking data on variables such as albumin, globulin, TC, TG, BW, BMI, neutrophil count, or lymphocyte count, which are crucial for calculating nutritional indicators, were excluded. (3) Data missing from covariates were also excluded. In the end, a total of 3,180 individuals met the inclusion criteria for this study (Figure 1).

Exposure variable

The specific calculation methods for each indicator are detailed in Table 1. Levels of albumin, globulin, TC, and TG were

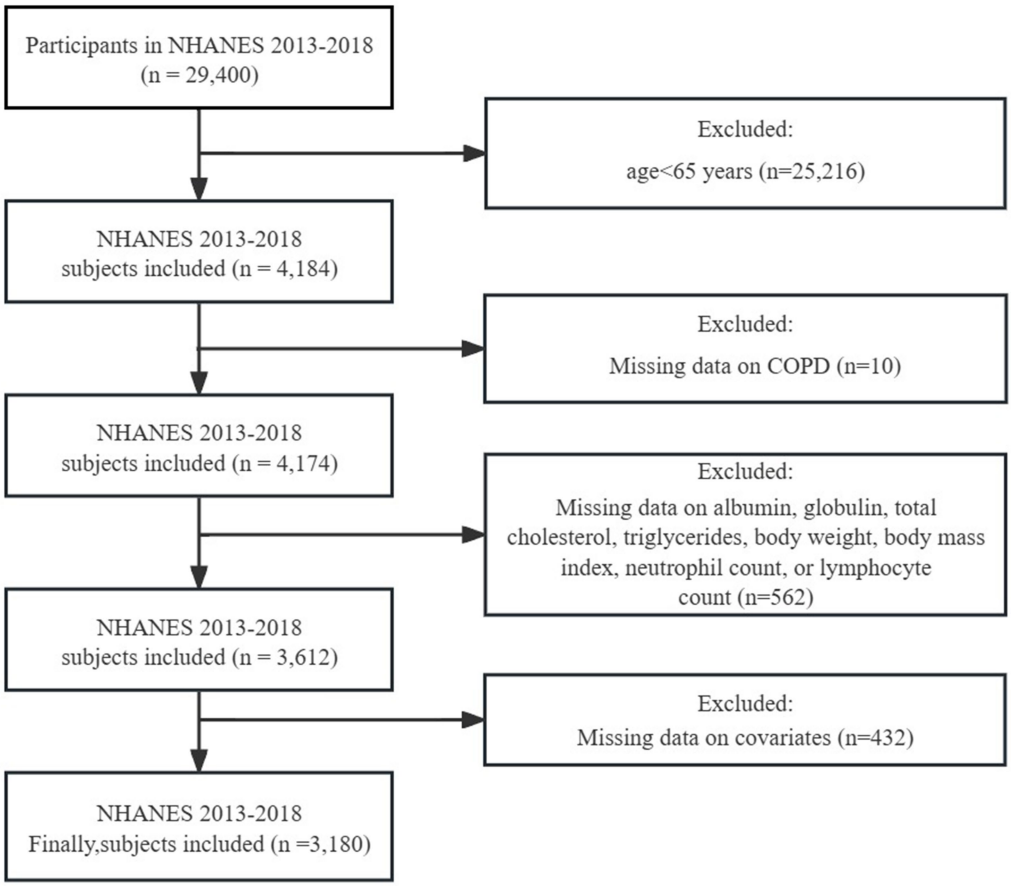

FIGURE 1
Flowchart for the selection of eligible participants.

TABLE 1 Details of the nutritional indices utilized in the study.

Nutrition-related indicators	Calculation formula	Reference
CONUT score	Albumin (g/dL) Score + Total lymphocyte (n/mm^3) Score + Total cholesterol (mg/dL) Score. Albumin Score: The respective scores for albumin levels of ≥3.5, 3.0–3.49, 2.5–2.99, and <2.5 g/dL are 0, 2, 4, and 6 points. Total lymphocyte Score: The respective scores for total cholesterol levels ≥1,600, 1,200-1,599, 800–1,199, and <800/mm^3 are 0, 1, 2, and 3 points. Total cholesterol Score: The respective scores for total cholesterol levels ≥180, 140–179, 100–139, and <100 mg/dL are 0, 1, 2, and 3 points.	8
ALI	Body mass index (kg/m^2) × albumin level (g/dL)/ neutrophil to lymphocyte ratio	9
GNRI	[1.489 × albumin (g/L)] + 41.7 × (present weight/ideal body weight) ideal body weight (male) = height(cm)-100- (height(cm) -150)/4 ideal body weight (female) = height(cm) -100- (height(cm) -150)/2.5	10
PNI	Albumin (g/L) + 5 × Total lymphocyte count (10^9/L)	11
TCBI	Triglycerides (mg/dL) × Total Cholesterol (mg/dL) × Body Weight (kg)/1,000	12
AGR	Albumin (g/L)/Globulin (g/L)	13

measured using the Roche Cobas 6,000 (c501 module) analyzer. Whole blood cell counts were conducted on blood specimens using an automated hematology analyzer (Coulter DxH 800 analyzer), providing blood cell distribution for all participants, including lymphocyte and neutrophil counts. Height and weight were measured during examinations at the Mobile Examination Center (MEC). Nutrition-related indicators ALI, PNI, TCBI, and AGR were categorized into three groups based on tertiles (T1, T2, and T3 corresponding to the first, second, and third tertiles). Based on CONUT scores, the severity of malnutrition was

defined in three groups: normal (0–1), mild to moderate (2–8), and severe (9–12) (8). Three levels of nutrition-related risk were defined based on GNRI levels: no risk (GNRI >98), low to moderate risk (GNRI: 82 to ≤98), and major risk (GNRI <82) (10).

Outcome variable

The definition of COPD is a positive response to the question: "Has a doctor or other health professional ever told you that you have COPD?" When participants answered "yes," we considered them to have COPD. Previous research utilizing NHANES data has indicated that self-reported COPD diagnosis is an effective method (33–35).

Identification of mortality

To assess the impact of nutrition-related indicators on overall mortality in COPD patients, we further conducted a cohort analysis. All-cause mortality was determined using death data recorded by the National Death Index (NDI) as of December 31, 2019. These records can be linked to NHANES data, and death files are available online at https://www.cdc.gov/nchs/data-linkage/mortality.htm. The definition of causes of death is based on the International Classification of Diseases, 10th Revision (ICD-10).

Covariates definition

Covariates in this study include age, gender, race, education level, marital status, BMI, poverty income ratio (PIR), smoking status, hypertension, diabetes, cardiovascular disease (CVD), and hyperlipidemia. BMI is categorized as Normal (<25 Kg/m^2), Overweight (≥25, <30 Kg/m^2), and Obese (≥30 Kg/m^2). PIR is divided into three groups: Low (≤1.39), Medium (>1.39, ≤3.49), and High (>3.49). Smoking status is categorized as current, former, or never smoking. Current smokers are individuals who have smoked over 100 cigarettes and currently smoke occasionally or continuously. Former smokers are individuals who have smoked over 100 cigarettes but are not currently smoking. Never smokers are individuals who have smoked fewer than 100 cigarettes in their lifetime. For CVD, a positive response to whether a doctor or other health professional has ever told you that you have congestive heart failure/coronary heart disease/angina/heart attack/stroke was defined as having CVD. Hyperlipidemia is defined as HDL ≤ 40 mg/dL in males and ≤50 mg/dL in females, or triglycerides ≥150 mg/dL, or total cholesterol ≥200 mg/dL, or low-density lipoprotein ≥130 mg/dL. Additionally, individuals reporting the use of cholesterol-lowering medication were also classified as having hyperlipidemia. We obtained the average blood pressure from three consecutive measurements taken at rest. Hypertension was defined as self-reported hypertension, or average systolic blood pressure ≥ 140 mmHg, or average diastolic blood pressure ≥ 90 mmHg, or the use of antihypertensive medication. Diabetes was defined as self-reported diagnosis of diabetes, or fasting blood glucose ≥7.0 mmol/L, or 2-h oral glucose tolerance level/random blood glucose ≥11.1 mmol/L, or glycated hemoglobin (HbA1c) ≥6.5 mmol/L, or the use of antidiabetic medication.

Statistical analyses

Participants' characteristics were reported as mean ± standard deviation (SD) for continuous variables and as percentages for categorical variables. Participants were divided into two groups based on whether they had COPD. The weighted t-test was used to assess differences in continuous variables between COPD and non-COPD participants, while the weighted chi-square test was used to evaluate differences in categorical variables.

In cross-sectional study analysis, weighted multivariable logistic regression analysis was conducted to assess the association between six nutrition-related indicators and COPD risk. The results were presented as odds ratios (ORs) with 95% confidence intervals (CIs). Logistic regression models were evaluated by gradually adjusting covariates: the crude model was unadjusted, model 1 adjusted for age, gender, and race, model 2 further adjusted for PIR, BMI, education level, and smoking status, and model 3 additionally adjusted for cardiovascular disease, hypertension, diabetes, and hyperlipidemia. To explore the potential nonlinear associations between nutrition-related indicators and the risk of COPD, restricted cubic splines (RCS) were further fitted. Three knots were set at the 10th, 50th, and 90th percentiles, with the 50th percentile as the reference.

In the cohort study analysis, we evaluated the relationship between six nutrition-related indicators and the overall survival of COPD patients using Kaplan–Meier (KM) curves. The analysis was conducted using a two-sided log-rank test. Additionally, weighted multivariable Cox regression analysis was performed to assess the correlation between the six nutrition-related indicators and the all-cause mortality of COPD patients. The results were presented as hazard ratios (HRs) and 95% confidence intervals (CIs). The same adjustments for covariates were applied to evaluate the Cox regression model and explore potential nonlinear associations between nutrition-related indicators and the risk of all-cause mortality in COPD patients.

Finally, the predictive value of nutrition-related indicators for assessing the risk of COPD and all-cause mortality was compared using the receiver operating characteristic (ROC) curve and the C-index. The best predictive indicators were determined. Stratified analysis was performed based on gender (male or female), smoking status (non-smoker or smoker), cardiovascular disease (yes or no), diabetes (yes or no), hypertension (yes or no), and hyperlipidemia (yes or no) to explore the interactions between these factors and the best predictive indicators. In addition, to further validate the association between nutrition-related indicators and the risk of COPD, a sensitivity analysis was conducted using a 1:2 nearest neighbor propensity score matching (PSM) method to balance the case and control groups, including age, gender, race, PIR, smoking status, education level, CVD, and diabetes as confounding factors for matching. Statistical analysis was performed using R Studio (version 4.2.2) and involved R packages such as "survey," "survival," "survminer," "rms," "ggplot2," "pROC," "MatchIt," and "jskm." The significance level was set at $p < 0.05$ (two-tailed).

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Results

General characteristics of the study population

The study involved a total of 3,180 participants (mean [SE] age of 72.6 [5.3] years), including 272 diagnosed with COPD (171 males [weighted proportion 56%], 101 females [weighted proportion 44%]). Compared to the non-COPD group, COPD patients had a higher proportion of non-Hispanic White individuals (196 [86%] vs. 1,454 [79%]), a higher percentage of smokers (74 [25%] vs. 240 [7.0%]), lower education levels (College Graduate or above) (34 [19%] vs. 673 [32%]), and were more likely to have comorbidities such as CVD (147 [53%] vs. 747 [24%]) and diabetes (115 [42%] vs. 1,050 [29%]). Additionally, there were statistically significant differences between the two groups in terms of poverty level and age (all *p*-values <0.05) (Table 2).

TABLE 2 Baseline characteristics of study participants by incident COPD.

Characteristic	Overall, *N* = 3,180 (100%)[1]	COPD, *N* = 272 (8.3%)[1]	Non-COPD, *N* = 2,908 (91.7%)[1]	*P*-value[2]
Age (years)	72.6 (5.3)	73.5 (5.3)	72.6 (5.3)	**0.027**
Gender				**0.002**
Female	1,605 (55%)	101 (44%)	1,504 (56%)	
Male	1,575 (45%)	171 (56%)	1,404 (44%)	
Race				**0.016**
Non-Hispanic White	1,650 (79.1%)	196 (86%)	1,454 (79%)	
Non-Hispanic Black	566 (7.4%)	39 (5.3%)	527 (7.6%)	
Other Race	624 (9.5%)	27 (7.2%)	597 (9.3%)	
Mexican American	340 (4.0%)	10 (1.5%)	330 (4.1%)	
PIR				**0.003**
Low (≤1.39)	1,010 (19%)	111 (27%)	899 (18%)	
Medium (>1.39, ≤3.49)	1,319 (40%)	118 (46%)	1,201 (40%)	
High (>3.49)	851 (41%)	43 (27%)	808 (42%)	
BMI (Kg/m^2)				>0.9
Normal (<25)	790 (24%)	69 (25%)	721 (24%)	
Overweight (≥25, <30)	1,183 (37%)	98 (36%)	1,085 (37%)	
Obese (≥30)	1,207 (39%)	105 (38%)	1,102 (39%)	
Smoking status				**<0.001**
Current smoker	314 (8.0%)	74 (25%)	240 (7.0%)	
Former smoker	1,280 (41%)	152 (55%)	1,128 (40%)	
Never smoker	1,586 (51%)	46 (20%)	1,540 (53%)	
Education attainment				**<0.001**
Less Than 9th Grade	446 (6.4%)	28 (6.4%)	418 (6.4%)	
9-11th Grade	384 (9.2%)	50 (16%)	334 (8.5%)	
High School Grad/GED	742 (23.2%)	84 (32%)	658 (23.1%)	
Some College or AA degree	901 (30%)	76 (26.6%)	825 (30%)	
College Graduate or above	707 (31.2%)	34 (19%)	673 (32%)	
Marital status				0.3
Married/cohabiting	1,768 (61%)	130 (56%)	1,638 (62%)	
Never married	122 (3.0%)	15 (3.0%)	107 (3.0%)	
Widowed/divorced/separated	1,290 (36%)	127 (41%)	1,163 (35%)	
CVD	894 (26%)	147 (53%)	747 (24%)	**<0.001**
Hypertension	2,359 (70%)	204 (73%)	2,155 (70%)	0.3
Diabetes	1,165 (31%)	115 (42%)	1,050 (29%)	**0.003**
Hyperlipidemia	2,653 (86%)	227 (86.7%)	2,426 (85.4%)	0.7

[1]Mean ± SD for continuous; n (%) for categorical. [2]t-test adapted to complex survey samples; chi-squared test with Rao & Scott's second-order correction. PIR, poverty income ratio; BMI, body mass index; CVD, cardiovascular disease.

Association between nutrition-related indicators with the risk of COPD

Based on a weighted logistic regression model, we assessed the relationship between six nutrition-related indicators and the risk of COPD. In the fully adjusted model (Model 3), the results revealed a significant association between an elevated CONUT score (9–13) (OR: 5.11, 95% CI: 1.72–15.2), lower GNRI score (<82) (OR: 8.66, 95% CI: 2.95–25.5), and lower ALI score (T1: ≤43.88) (OR: 1.77, 95% CI: 1.10–2.84) with an increased risk of COPD (Table 3).

Association between nutrition-related indicators and all-cause mortality in COPD patients

In the cohort study, the median follow-up time was 33 months. Among 271 COPD patients, there were 79 cases (29.2%) of all-cause mortality. The KM curve demonstrated the incidence of all-cause mortality in the COPD patient population (Figure 2), showing that COPD patients with lower PNI, GNRI, and ALI indices experienced poorer overall survival.

TABLE 3 Association of Nutrition-Related Indicators with the risk of COPD using weighted logistic analysis.

	Crude model	Model 1	Model 2	Model 3
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
CONUT				
0–1	Reference	Reference	Reference	Reference
2–8	1.93(1.34,2.79) ***	1.72(1.16,2.56) **	1.92(1.24,2.96) **	1.49(0.95,2.33)
9–13	5.43(1.71,17.2) **	4.73(1.42,15.7) *	6.91(2.47,19.3) ***	5.11(1.72,15.2) **
P for trend	$p = 0.07$	$p = 0.09$	$p < 0.05$	$p = 0.09$
GNRI				
<82	9.10(3.18,26.1) ***	7.91(2.77,22.6) ***	6.88(2.73,17.4) ***	8.66(2.95,25.5) ***
82–98	2.43(0.89,6.57)	2.12(0.75,5.98)	1.55(0.60,4.05)	1.39(0.55,3.51)
>98	Reference	Reference	Reference	Reference
P for trend	p < 0.05	$p < 0.05$	$p < 0.05$	$p < 0.05$
AGR				
T1(≤1.41)	1.12(0.69,1.81)	1.26(0.75,2.10)	1.09(0.63,1.87)	1.04(0.58,1.87)
T2(>1.41, ≤1.65)	0.83(0.50,1.38)	0.86(0.51,1.44)	0.78(0.44,1.38)	0.82(0.44,1.53)
T3(>1.65)	Reference	Reference	Reference	Reference
P for trend	$p = 0.63$	$p = 0.37$	$p = 0.66$	$p = 0.92$
ALI				
T1(≤43.88)	2.11(1.43,3.12) ***	1.85(1.21,2.83) **	1.81(1.14,2.89) *	1.77(1.10,2.84) *
T2(>43.88, ≤66.69)	1.05(0.58,1.89)	0.99(0.54,1.81)	0.97(0.52,1.79)	1.00(0.54,1.86)
T3(>66.69)	Reference	Reference	Reference	Reference
P for trend	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
PNI				
T1(≤49)	1.32(0.84,2.09)	1.25(0.80,1.97)	1.46(0.87,2.43)	1.37(0.82,2.29)
T2(>49, ≤53)	0.99(0.61,1.61)	0.99(0.61,1.59)	1.13(0.69,1.83)	1.18(0.72,1.94)
T3(>53)	Reference	Reference	Reference	Reference
P for trend	$p = 0.21$	$p = 0.31$	$p = 0.12$	P = 0.21
TCBI				
T1(≤1347.82)	1.34(0.89,2.02)	1.32(0.86,2.02)	1.61(0.93,2.80)	1.62(0.85,3.09)
T2(>1347.82, ≤2442.15)	0.86(0.58,1.26)	0.86(0.59,1.27)	0.99(0.62,1.57) *	1.07(0.67,1.71)
T3(>2442.15)	Reference	Reference	Reference	Reference
P for trend	$p = 0.16$	$p = 0.19$	P = 0.09	$p = 0.17$

Crude model: no covariates were adjusted. Modle 1: adjusted for age, sex, and race. Modle 2: adjusted for age, sex, race, PIR, BMI, education level, smoking status. Modle 3: adjusted for age, sex, race, PIR, BMI, education level, smoking status, cardiovascular disease, hypertension, diabetes and Hyperlipidemia.95% CI, 95% confidence interval; OR, odds ratio; CONUT score, Controlling Nutritional Status score; GNRI, Geriatric Nutritional Risk Index; AGR, Albumin-to-Globulin Ratio; ALI, Advanced Lung Cancer Inflammation Index; PNI, Prognostic Nutritional Index; TCBI, Triglycerides × Total Cholesterol × Body Weight Index. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $p < 0.05$ was considered statistically significant.

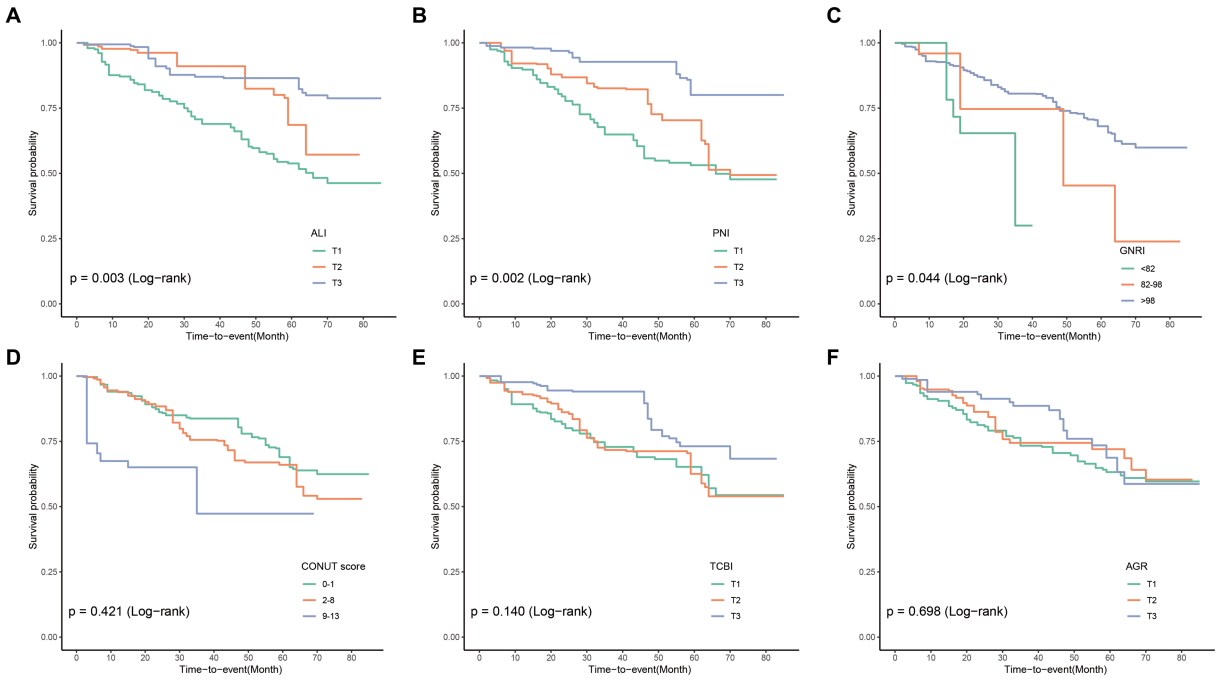

FIGURE 2
Compute Kaplan–Meier curves for all-cause mortality categorized by ALI **(A)**, PNI **(B)**, GNRI **(C)**, CONUT **(D)**, TCBI **(E)**, and AGR **(F)**. ALI, Advanced Lung Cancer Inflammation Index; PNI, Prognostic Nutritional Index; GNRI, Geriatric Nutritional Risk Index; CONUT score, Controlling Nutritional Status score; TCBI, Triglycerides × Total Cholesterol × Body Weight Index; AGR, Albumin-to-Globulin Ratio.

We used a weighted Cox regression model to evaluate the relationship between nutrition-related indicators and all-cause mortality in COPD patients. The results of Model 3 showed a significant association between PNI, GNRI, ALI, TCBI, and all-cause mortality in COPD patients. Specifically, compared to the highest quartile (T3), the lowest quartile (T1) of PNI and ALI was associated with increased risk of all-cause mortality in COPD patients (HR: 3.76, 95% CI: 1.89–7.48), (HR: 2.41, 95% CI: 1.10–5.27) (Table 4). On the other hand, compared to the high GNRI, the low GNRI was associated with increased risk of all-cause mortality in COPD patients (HR: 4.55, 95% CI: 1.30–15.9). Furthermore, compared to the highest quartile, the second quartile of TCBI was associated with increased risk of all-cause mortality in COPD patients (Table 4).

Nonlinear relationship assessment

To further explore the nonlinear associations between nutritional-related indicators (treated as continuous variables) and the risk of COPD and all-cause mortality, we employed RCS with weighting. Based on the multivariate regression model (Model 3), RCS analysis revealed a nonlinear association between ALI and GNRI with the risk of COPD (p for nonlinear <0.05), while a linear association was observed between CONUT and the risk of COPD (p for nonlinear = 0.51) (Figure 3A). For all-cause mortality rates, the results indicated a nonlinear association between GNRI and the all-cause mortality of COPD patients (*p* for nonlinear <0.05), while no such nonlinear association was observed for ALI and PNI (Figure 3B).

Comparison of nutritional-related indicators in predicting disease risk and all-cause mortality

We compared the ability of nutritional-related indicators to predict disease risk and all-cause mortality based on the ROC curves and C-index derived from the crude model. In assessing COPD incidence risk, ALI outperformed GNRI and CONUT, with the highest area under the ROC curve (AUC) value (ALI: 0.601 vs. CONUT: 0.582 vs. GNRI: 0.528) (Figure 4A) and the highest C-index (ALI: 0.601 vs. CONUT: 0.582 vs. GNRI: 0.529) (Supplementary Table S1). For all-cause mortality assessment, ALI exhibited the highest AUC value compared to PNI and GNRI (ALI: 0.587 vs. PNI: 0.559 vs. GNRI: 0.533) (Figure 4B), and a relatively higher C-index (ALI: 0.642 vs. PNI: 0.648 vs. GNRI: 0.522) (Supplementary Table S2). Similar results were observed across other models (Model 1 to Model 3). Therefore, we consider ALI to be the optimal indicator for predicting the risk of COPD incidence and all-cause mortality among COPD patients in this study.

Subgroup and sensitivity analysis

Overall, ALI is considered the optimal indicator for predicting the incidence risk and all-cause mortality of COPD. Subgroup analyses stratified by gender, smoking status, CVD, diabetes, hypertension, and hyperlipidemia revealed that, compared to the highest tertile (T3), the lowest tertile (T1) of ALI exhibited a stronger correlation with COPD risk and all-cause mortality in females,

TABLE 4 Association of Nutrition-Related Indicators with all-cause mortality in COPD patients was investigated using weighted Cox regression analysis.

	Crude model	Model 1	Model 2	Model 3
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
CONUT				
0–1	Reference	Reference	Reference	Reference
2–8	1.30(0.71,2.38)	1.21(0.68,2.15)	1.17(0.66,2.07)	1.32(0.79,2.20)
9–13	2.44(0.55,10.8)	3.06(0.87,10.8)	3.81(1.02,14.3) *	4.04(1.05,15.6) *
P for trend	$p = 0.24$	$p = 0.08$	$p < 0.05$	$p < 0.05$
GNRI				
<82	3.91(2.07,7.39) ***	3.78(1.24,11.5) *	4.66(1.44,15.1) *	4.55(1.30,15.9) *
82–98	1.68(0.60,4.66)	1.68(0.63,4.49)	1.38(0.43,4.44)	1.33(0.46,3.83)
>98	Reference	Reference	Reference	Reference
P for trend	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
AGR				
T1(<=1.41)	1.33(0.67,2.67)	1.74(0.82,3.70)	1.56(0.71,3.40)	1.71(0.81,3.62)
T2(>1.41, <=1.65)	1.16(0.50,2.71)	1.28(0.51,3.19)	1.42(0.51,4.01)	1.46(0.50,4.26)
T3(>1.65)	Reference	Reference	Reference	Reference
P for trend	$p = 0.42$	$p = 0.15$	$p = 0.27$	P = 0.16
ALI				
T1(<=43.88)	3.44(1.70,6.96) ***	3.40(1.49,7.78) **	3.38(1.75,6.54) ***	2.41(1.10,5.27) *
T2(>43.88, <=66.69)	1.53(0.56,4.17)	1.55(0.66,3.63)	2.06(0.93,4.57)	1.00(0.46,2.17)
T3(>66.69)	Reference	Reference	Reference	Reference
P for trend	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
PNI				
T1(<=49)	4.09(2.23,7.49) ***	3.60(1.78,7.27) ***	3.38(1.75,6.54) ***	3.76(1.89,7.48) ***
T2(>49, <=53)	2.77(1.40,5.46) **	2.68(1.27,5.66) *	2.06(0.93,4.57)	2.22(0.96,5.09)
T3(>53)	Reference	Reference	Reference	Reference
P for trend	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$
TCBI				
T1(<=1347.82)	2.04(0.79,5.30)	1.74(0.66,4.54)	1.70(0.63,4.57)	1.88(0.71,5.02)
T2(>1347.82, <=2442.15)	1.91(0.97,3.79)	2.23(1.07,4.63) *	2.67(1.28,5.58) **	2.91(1.35,6.27) **
T3(>2442.15)	Reference	Reference	Reference	Reference
P for trend	$p = 0.14$	$p = 0.26$	$p = 0.29$	P = 0.21

Crude model: no covariates were adjusted. Modle 1: adjusted for age, sex, and race. Modle 2: adjusted for age, sex, race, PIR, BMI, education level, smoking status. Modle 3: adjusted for age, sex, race, PIR, BMI, education level, smoking status, cardiovascular disease, hypertension, diabetes and Hyperlipidemia.95% CI, 95% confidence interval; OR, odds ratio; CONUT score, Controlling Nutritional Status score; GNRI, Geriatric Nutritional Risk Index; AGR, Albumin-to-Globulin Ratio; ALI, Advanced Lung Cancer Inflammation Index; PNI, Prognostic Nutritional Index; TCBI, Triglycerides × Total Cholesterol × Body Weight Index. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; $p < 0.05$ was considered statistically significant.

individuals with hyperlipidemia, and those without hypertension (Supplementary Tables S3, S4). Additionally, no significant interactions were observed between ALI levels and the stratified variables, with all interaction *p*-values exceeding 0.05.

After using the nearest neighbor PSM to establish a control group, the relationship between six nutrition-related indicators and the risk of COPD was analyzed again. After PSM, the dataset included 564 participants in the non-COPD group and 263 participants in the COPD group, with no statistically significant differences in covariates (Supplementary Table S5). Logistic regression analysis after matching showed that in the fully adjusted model, compared to high ALI, low ALI was significantly associated with an increased risk of COPD (OR: 1.77, 95% CI: 1.10–2.84), while CONUT score and GNRI score also remained consistent with the results of the logistic regression before matching (Supplementary Table S6).

Discussion

This study investigated the relationships between six nutrition-related indicators, including GNRI, ALI, TCBI, PNI, CONUT, and AGR, and the risk of COPD incidence and all-cause mortality in a nationally representative sample of the elderly population in the United States. We compared the performance of these indicators for

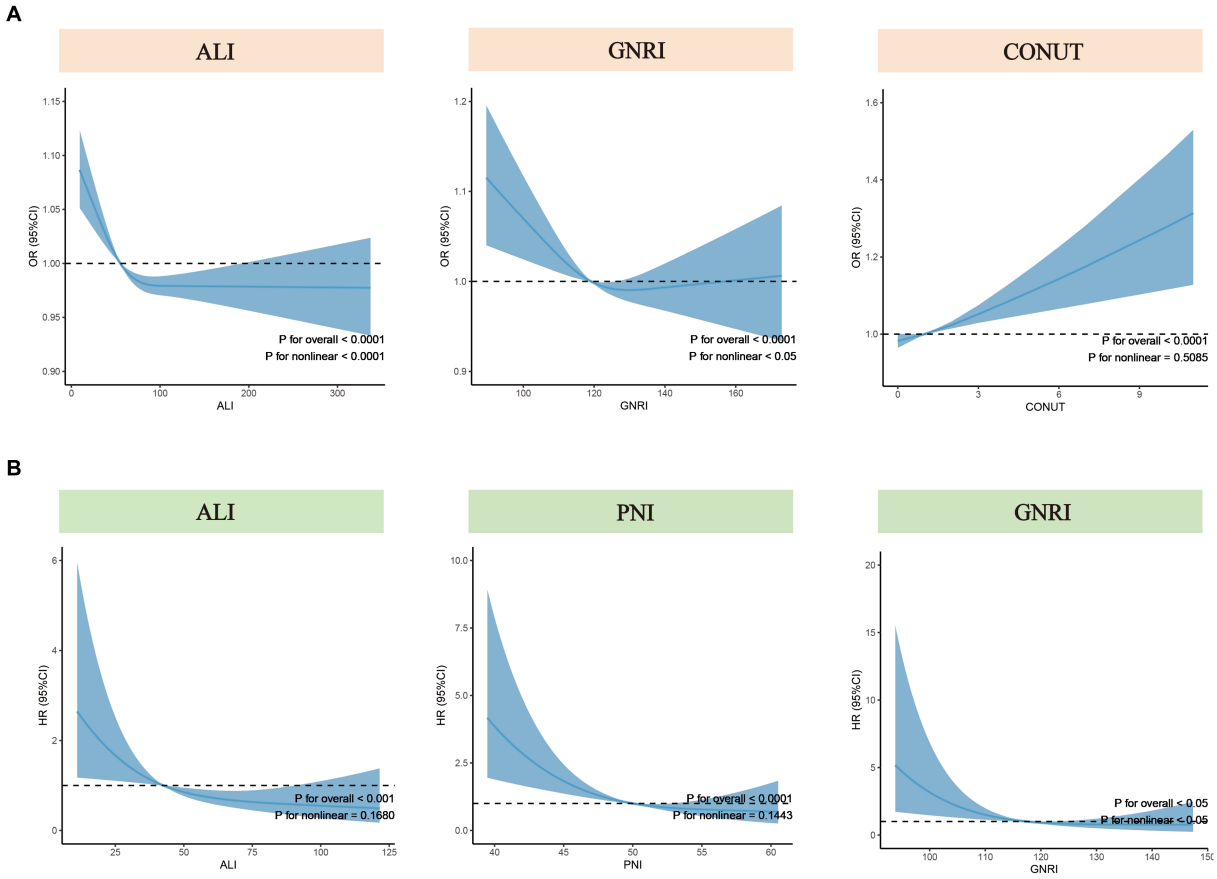

FIGURE 3
Nonlinear tests for nutritional-related indicators regarding COPD risk and all-cause mortality. **(A)** Nonlinear tests between ALI, GNRI, and CONUT (from left to right) and COPD risk. **(B)** Nonlinear tests between ALI, PNI, and GNRI (from left to right) and all-cause mortality among COPD patients. ALI, Advanced Lung Cancer Inflammation Index; PNI, Prognostic Nutritional Index; GNRI, Geriatric Nutritional Risk Index; CONUT score, Controlling Nutritional Status score; OR, odds ratio; HR, hazard ratio.

the first time in predicting COPD risk and all-cause mortality. We found that malnutrition in the elderly population was significantly associated with a higher risk of COPD incidence and all-cause mortality, with ALI, GNRI, and CONUT being correlated with COPD risk, and ALI, GNRI, and PNI being associated with all-cause mortality among COPD patients. Additionally, we observed nonlinear relationships between ALI, GNRI, and COPD risk, as well as between GNRI and all-cause mortality. Furthermore, compared to other nutrition-related indicators, ALI emerged as the top predictor for assessing both COPD risk and all-cause mortality in the elderly population.

From an overall perspective, poor nutritional status has an impact on the risk of developing COPD and all-cause mortality rate. Firstly, previous reports have clearly indicated that inflammation and oxidative stress are the core pathological processes of COPD (36). Malnutrition can potentially increase inflammation and oxidative stress (37), and it can also affect the immune system, as immune responses are strongly regulated by oxidative stress and inflammation (38). Consequently, this may further weaken the body's immune response (25) and increase the risk of developing COPD. Furthermore, studies on the role of nutrients and antioxidants have shown that a high intake of foods rich in antioxidants (such as fresh fruits and vegetables) and antioxidant nutrients (both vitamins and non-vitamins) can effectively enhance antioxidant and anti-inflammatory abilities (39, 40), thus playing a positive role in reducing the incidence of COPD (41, 42). Moreover, for patients with COPD, disease-related malnutrition is a common problem (43). Malnutrition can accelerate the decline in respiratory function, leading to loss of lung tissue and decreased quality and thickness of respiratory-related muscles, such as the diaphragm (44). Additionally, under conditions of malnutrition, respiratory muscles become weak and fatigue earlier, and there is poorer lung diffusion capacity and lower exercise tolerance (45). It is worth noting that malnutrition weakens immune defenses, significantly increasing the risk of lung infections and also being one of the reasons for increased risk of death (22). In summary, poor nutritional status can have a profound impact on the risk of developing COPD and overall health outcomes. Adequate intake of antioxidants and proper nutrition play an important role in maintaining lung health and reducing the risk of COPD.

Previous clinical studies have explored the associations of GNRI, ALI, TCBI, PNI, CONUT, and AGR with various disease outcomes, with fewer studies focusing on COPD. Chai X (46) investigated the association between GNRI and all-cause mortality in individuals aged 18 and above, suggesting a correlation between malnutrition and higher all-cause mortality in COPD. Suzuki E et al. (47) demonstrated that PNI could serve as a potential predictor for

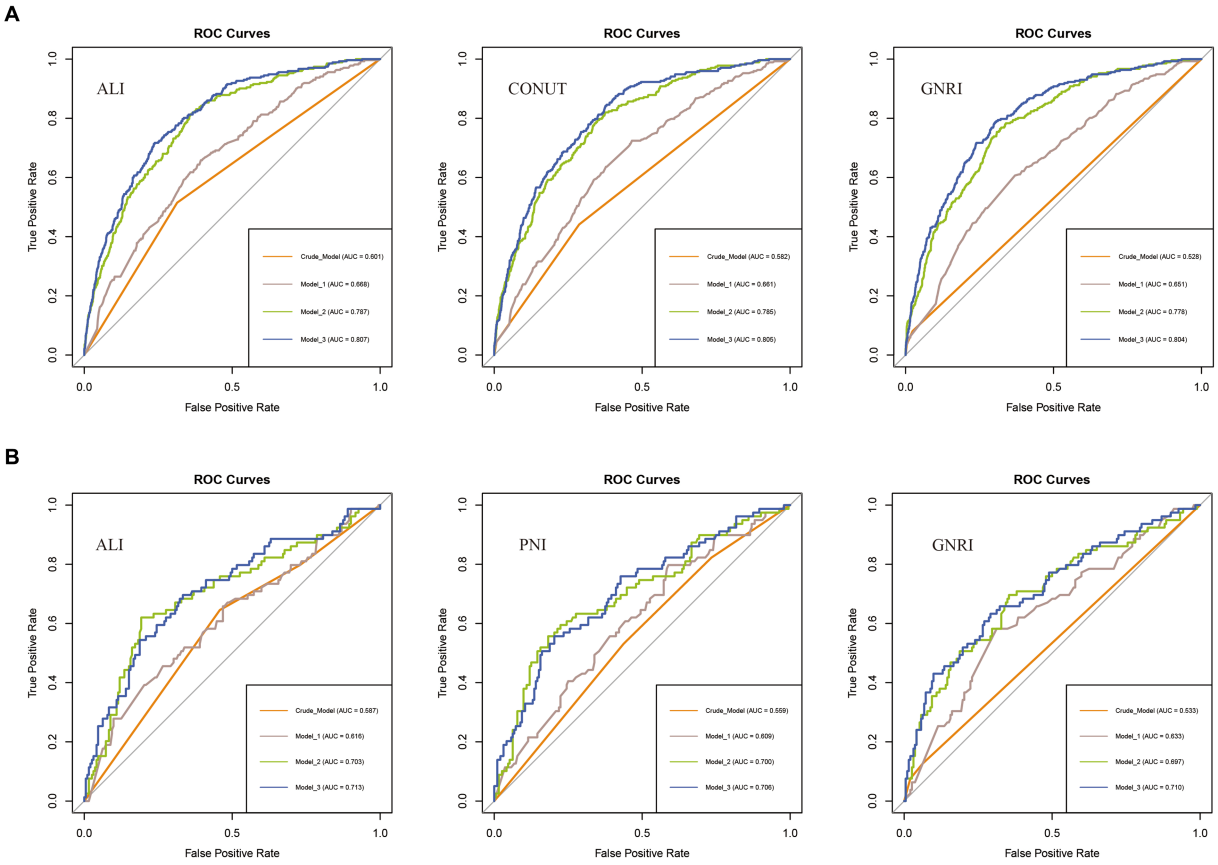

FIGURE 4
ROC curves for predicting COPD risk and all-cause mortality. **(A)** ROC curves for predicting COPD risk by ALI, CONUT, and GNRI (from left to right). **(B)** ROC curves for predicting all-cause mortality among COPD patients by ALI, PNI, and GNRI (from left to right). ALI, Advanced Lung Cancer Inflammation Index; PNI, Prognostic Nutritional Index; GNRI, Geriatric Nutritional Risk Index; CONUT score, Controlling Nutritional Status score.

exacerbation in elderly subjects with COPD. Additionally, studies have indicated that CONUT scores have prognostic value for frequent exacerbations in elderly COPD patients (48). Our findings align with previous research, but we also reveal that GNRI is not only associated with all-cause mortality in COPD but also with the risk of COPD incidence in the elderly population, with these relationships being nonlinear. A recent study in the general United States population suggested that, compared to other nutritional scores (TCBI, CONUT, GNRI), PNI might offer superior predictive value for all-cause mortality. However, our study among elderly population presents a different conclusion, highlighting ALI as a novel predictor for COPD risk and all-cause mortality. Through ROC curve and C-index analysis, we found that among the six nutrition-related indicators, ALI exhibited superior predictive efficacy for both COPD risk and all-cause mortality.

ALI, as the best predictive indicator, includes three factors: BMI, serum albumin levels, and the NLR. It is calculated by multiplying BMI by the ratio of serum albumin to the NLR. BMI and serum albumin levels reflect nutritional status (49, 50), while NLR reflects inflammatory status (51), making ALI a comprehensive assessment index based on nutrition and inflammation. BMI is a fundamental measure of body fat content, and the Global Leadership Initiative on Malnutrition (GLIM) diagnostic criteria for malnutrition utilize BMI (49). It is well known that obesity is often associated with increased rates of chronic diseases. However, research suggests that a BMI below 18.5 is a risk factor for COPD (52, 53), while overweight and obesity (BMI $\geq$ 25 Kg/m^2) may decrease the risk of COPD (53, 54), highlighting a paradox (55). According to our research, it appears that there exists an L-shaped non-linear relationship between ALI and the risk of COPD. A moderately higher BMI may correspond to elevated ALI values, suggesting a better nutritional status. Then, serum albumin is a multifunctional plasma protein, accounting for over 50% of total plasma proteins, and it possesses crucial antioxidant properties. In COPD, inflammation is central to its development, with oxidative stress amplifying inflammatory responses (56). Higher albumin levels contribute to improved antioxidant capacity and protect tissues from inflammatory damage (57). A meta-analysis indicates a significant decrease in serum albumin concentration in COPD patients compared to non-COPD controls (58), while a cross-sectional study of a British population suggests that elevated albumin levels help lower the risk of COPD (59). Thus, the level of albumin is closely associated with the occurrence and progression of COPD. Inflammation increases with the severity of COPD (51), and chronic, persistent inflammation often leads to changes in neutrophils, which further mediate the production and release of specific inflammatory mediators (60, 61), ultimately resulting in irreversible airway

damage. Due to the combination of different blood cell populations reflected by NLR expression, which can better indicate inflammatory status compared to individual blood cell markers, NLR has been studied in recent years as a systemic marker of inflammation. Multiple studies have also indicated associations between NLR and the risk of COPD, exacerbation severity, and mortality risk (30, 62), and adjusting nutritional status is also of significant importance for effectively reducing inflammation (37). In conclusion, there exists a complex interaction between malnutrition and inflammation. Therefore, these combined factors may be reasons for the potentially superior predictive value of ALI compared to other nutritional-related indicators.

Additionally, our research suggests a more significant relationship between ALI and COPD risk and all-cause mortality rates in females. Several studies in COPD genetics, such as the COPD Gene study, indicate gender-related genetic components in COPD incidence (63). Moreover, research suggests that females are more susceptible to smoking-related lung injuries (64, 65) and experience more severe inflammatory responses (66). Furthermore, studies propose that females exhibit more severe COPD symptoms (67) and have a greater risk of exacerbations (68, 69). However, the specific mechanisms still need further clarification.

Our study has several strengths. Firstly, it is based on a large sample with national representativeness. Secondly, it identifies ALI as associated with COPD risk for the first time and as an independent predictor of all-cause mortality. However, we acknowledge some limitations. Firstly, our study is cross-sectional, precluding causal inferences. Secondly, the population of COPD patients is defined through self-reporting, which may introduce recall bias. Nonetheless, these patients typically have medical histories, and their COPD diagnoses are typically confirmed by healthcare professionals, a method widely used in previous studies to define COPD (33–35). Thirdly, despite controlling for demographic indicators, behavioral risk factors, and various chronic diseases in the analysis, the results may still be influenced by unknown confounding factors. Fourthly, the study was conducted on a representative sample from the United States, and the generalization of the study results to other populations may not be direct.

Conclusion

In conclusion, this research, involving a nationally representative sample of the elderly population in the United States, indicates that nutritional-related indicators, including lower ALI, GNRI, and higher CONUT scores, are associated with the risk of COPD. Furthermore, lower ALI, GNRI, and PNI scores are linked to all-cause mortality. Importantly, compared to other indicators, ALI exhibits optimal performance in predicting both COPD risk and all-cause mortality among COPD patients. The assessment of ALI can enhance the identification of COPD and serve as a valuable prognostic marker in clinical practice.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

We used publicly available data that were obtained with ethical approval from their respective institutional review boards and informed consent from all participants. No administrative permissions were required for accessing the data.

Author contributions

YX: Conceptualization, Methodology, Software, Writing – original draft, Writing – review & editing. ZY: Conceptualization, Methodology, Software, Writing – original draft. KL: Conceptualization, Methodology, Writing – original draft. LL: Conceptualization, Validation, Writing – review & editing. LX: Data curation, Software, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1380791/full#supplementary-material

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Prospective study of protein intake and mortality among US adults with chronic obstructive pulmonary disease

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Background: Protein is crucial for the rehabilitation of patients with chronic obstructive pulmonary disease (COPD), and appropriate daily protein intake is essential for COPD patients. However, the specific role of protein intake in COPD and its impact on mortality remain uncertain. This study aims to ascertain the relationship between protein intake and mortality in COPD patients.

Methods: This investigation included 522 adult COPD patients from the National Health and Nutrition Examination Survey (NHANES) between 2013 and 2018, with a focus on evaluating protein intake. Multivariate Cox proportional hazard models were constructed to analyze the correlation between protein intake and the prognosis of COPD patients. Additionally, the restricted cubic spline (RCS) was employed to investigate the potential non-linear association between protein intake and mortality.

Results: A total of 522 patients with COPD were categorized into 4 groups based on the quartiles of protein intake: Q1 (< 25th percentile, 11.7–48.5 gm), Q2 (25–50th percentile, 48.5–67.7 gm), Q3 (50–75th percentile, 67.7–94.3 gm), and Q4 (≥ 75th percentile, 94.3–266.6 gm). Cox regression analysis revealed a significant trend in the p value of the Q3 group compared to the Q1 group when adjusting for other variables. The RCS-fitted Cox regression model indicated no non-linear relationship between protein intake levels and COPD mortality.

Conclusion: There is no evidence of a non-linear relationship between protein intake and all-cause mortality in COPD patients. Further investigation is warranted to comprehend the intricate relationship between protein intake and COPD outcomes.

KEYWORDS

COPD, protein intake, mortality, multivariate cox, NHANES

1 Introduction

Chronic obstructive pulmonary disease (COPD) represents a persistent and progressive inflammatory disorder affecting the airways, primarily characterized by irreversible airflow limitation and impaired ventilation (1, 2). As a leading contributor to global mortality and morbidity, COPD imposes a substantial economic burden on society (3). The influence of nutritional intake on the recovery of various diseases is well-established, and malnutrition exerts detrimental effects on disease outcomes.

Proteins, the principal constituents of cells, tissues, and organs, are vital for human health. Research has demonstrated that insufficient protein intake in patients with chronic respiratory diseases correlates with reduced physical activity and impaired lung function (4, 5). The effects of protein intake vary across different patient populations. In critically ill children admitted to pediatric intensive care units, adequate protein intake is associated with improved clinical outcomes and a decreased risk of mechanical ventilation (6). Previous studies have shown that in intensive care units, high protein intake is closely associated with the physical recovery of adult critically ill patients. Increasing protein intake not only significantly improves physical performance and muscle strength in these patients but also increases the recovery rate of independent walking (7, 8). These findings establish a crucial foundation for the management of clinical nutrition, stressing the importance of reasonable adjustment of protein intake during clinical diagnosis and treatment. In patients with prolonged mechanical ventilation, higher protein intake is associated with a shorter duration of ventilator use and successful weaning (9). However, in patients with diabetes, higher protein intake correlates with poorer glycemic control (10). The recommended protein intake for diabetic patients ranges from 10 to 20% of energy intake or 0.8–1.3 g/kg body weight, depending on age (11).

While some investigations have discovered that prioritizing energy and protein-rich foods may help improve the nutritional status and quality of life of COPD patients (12), others have reported insufficient protein intake may lead to an increased risk of exacerbating mild to moderate COPD (13). Nevertheless, certain research has identified a considerable correlation between pulmonary function and protein intake in COPD patients (14), with a notable association between protein intake extrapolated from food frequency questionnaires and forced vital capacity (FVC) and vital capacity (VC).

However, there is a paucity of literature on protein intake and all-cause mortality in COPD patients. This study sought to fill this gap by investigating the relationship between protein intake levels and COPD-related mortality using data from the National Health and Nutrition Examination Survey (NHANES). The analysis provides new insights into COPD treatment, laying the groundwork for further research.

2 Materials and methods

2.1 Study population

NHANES[1] (15) is a cross-sectional survey based on the population, assessing the nutritional status and health of the US household population. In each 2-year study period, it randomly includes around 10,000 individuals. Data were acquired through interviews, covering socioeconomic, health-related, and demographic information, coupled with examinations, including medical assessments, laboratory tests, and physiological measurements conducted by trained medical personnel (16). NHANES has received approval from the National Center for Health Statistics Institutional Review Board, with all participants providing written informed consent (17). The survey aims to determine disease prevalence, identify risk factors, and provide essential data supporting the development of nutrition and health policies.

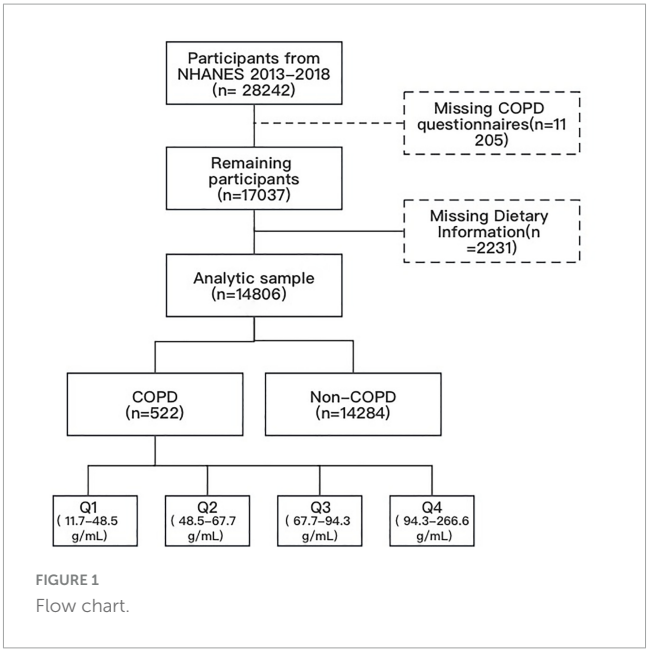

FIGURE 1
Flow chart.

This research amalgamated data from three NHANES cycles spanning 2013 to 2018. Our analysis encompassed all individuals aged 20 and over who participated in the COPD survey (responding affirmatively to the question "Has a doctor or other health professional ever told you that you had COPD?") and had measured protein intake. Participants with missing covariate data were excluded A total of 522 COPD patients were included for further analysis (Figure 1).

2.2 Determination of protein intake

Dietary intake data were utilized to assess the varieties and quantities of beverages and foods (inclusive of all types of water) consumed within 24 hours preceding the interview (midnight to midnight). These data included energy and nutrient intakes, and other food components. The US Department of Health and Human Services (DHHS) and the National Center for Health Statistics (NCHS) oversees the survey design and all data collection, whilst USDA's Food Surveys Research Group (FSRG) manages methods of data review and processing, databases maintenance, and dietary data collection. Protein intake was categorized into quartiles for statistical analysis: Q1 (< 25th percentile, 11.7–48.5 gm), Q2 (25–50th percentile, 48.5–67.7 gm), Q3 (50–75th percentile, 67.7–94.3 gm), and Q4 (≥ 75th percentile, 94.3–266.6 gm), with Q1 as the reference standard.

2.3 Mortality outcome

Death status and follow-up time information were obtained from the National Death Index in NHANES (as of

1 https://www.cdc.gov/nchs/nhanes/

May 2022). The cause of death was ascertained through the application of the International Classification of Diseases 10th Edition (ICD-10). The primary outcome of this study was all-cause mortality.

2.4 Covariates

Covariate factors encompassed age, gender (female, male), race (non-Hispanic whites and blacks, Mexican Americans, others), education level (less than 9th grade, 9–11th grade, high school graduate/GED or equivalent, some college or AA degree, college graduate or above), marital status (married or living with a partner, divorced or separated, widowed, and never married), family income to poverty ratio, smoking status (former, now, never), congestive heart failure (CHF, yes or no), alcohol (former, heavy, mild, moderate, never), diabetes (yes or no), hypertension (yes or no), BMI, coronary heart disease (CHD, yes or no), chronic kidney disease (CKD, yes or no), hyperlipidemia (yes or no), stroke (yes or no), atherosclerotic cardiovascular disease (yes, no), heart attack (yes or no), and study period 2013, 2014, 2015, 2016, 2017, 2018. Serum albumin (g/L), serum total protein (g/L) were considered as continuous variables.

2.5 Statistical analyses

Statistical analyses were performed using R version 4.2.1. COPD patients were stratified into four groups based on protein intake quartiles (Q1, Q2, Q3, Q4). Descriptive statistics were applied to analyze population characteristics, with appropriate sampling weights used. Continuous variables were compared by Kruskal-Wallis H test or analysis of variance, while chi-square tests were employed for categorical variables. A two-sided P-value below 0.05 was deemed statistically significant. Missing data were addressed through multiple imputations.

The relationship between protein intake and all-cause mortality was evaluated using Cox proportional hazard regression analysis. Hazard ratio (HR) and corresponding 95% confidence interval (CI) were calculated for three models: Model 1 (unadjusted), Model 2 (adjusted for age, gender, race, and education level, marital), and Model 3 (additionally adjusted for family poverty income ratio, smoking status, alcohol, diabetes, hypertension, BMI, CHD, CHF, CKD, hyperlipidemia, stroke, atherosclerotic cardiovascular disease, heart attack based on the factors adjusted in Model 2). Log-rank tests were used for statistical comparisons between groups according to protein intake.

Restricted cubic spline (RCS) models were established to explore the non-linear relationship between variations in protein intake and the risk of all-cause death in COPD patients, adjusting for the same factors as Model 3. If the non-linear relationship was identified, the Cox proportional hazard regression model was employed to identify an HR-related threshold point and validate the association between changes in protein intake both over and below the threshold and mortality.

3 Results

3.1 Baseline characteristics

A total of 522 participants with COPD were involved and categorized into four groups based on protein intake quartiles. Table 1 presents patient characteristics. Participants had an average age of 63 years, with 57% being females. The average protein intake among enrolled patients was 70gm. In comparison to the lowest quartile, participants exhibiting higher protein intake were inclined to be non-Hispanic White, male, and diabetic. No statistically significant variations were noted in biochemical indicators and other characteristics across the remaining groups ($P > 0.05$).

3.2 Multivariate cox proportional hazard regression analysis of dietary protein intake and its components in COPD

Out of the 522 patients, 100 deaths were recorded. The linear relationship between continuous protein intake and all-cause mortality in COPD patients was explored using three Cox regression models: Model 1, Model 2, and Model 3. No statistical significance was observed, with p values for Model 1, Model 2, and Model 3 at 0.8, 0.09, and 0.5, respectively (Table 2).

Subsequently, we adjusted for age, gender, race, education level, and marital status across the protein intake quartiles: Q1 (< 25th percentile, 11.7–48.5 gm), Q2 (25–50th percentile, 48.5–67.7 gm), Q3 (50–75th percentile, 67.7–94.3 gm), and Q4 (≥ 75th percentile, 94.3–266.6 gm). In Model 2, the all-cause mortality in the four groups was 1.00 (reference), 0.83 (95% CI: 0.41, 1.68; $P = 0.6$), 0.43 (95% CI: 0.21, 0.89; $P = 0.22$), 0.7 (95% CI: 0.32, 1.57; $P = 0.4$), respectively. In Model 3 further adjusted for family poverty income ratio, smoking status, alcohol, diabetes, hypertension, BMI, CHD, CHF, CKD, hyperlipidemia, stroke, atherosclerotic cardiovascular disease, and heart attack, the all-cause mortality in the four groups was 1.00 (reference), 0.93 (95% CI 0.49, 1.74; $P = 0.8$), 0.56 (95% CI 0.27, 1.16; $P = 0.12$), 0.69 (95% CI 0.32, 1.46; $P = 0.3$) (Table 3), separately.

Following adjustments for age, gender, race, education level, and marital status, no significant non-linear association was observed between protein intake and all-cause mortality, but a non-linear trend between the two was noted.

Therefore, we utilized RCS and smooth curve fitting to further examine the correlation. The adjusted smoothing map indicated no non-linear relationship between protein intake and all-cause mortality (Figure 2). In Figure 2B, the RCS curve analysis showed that after adjusting for gender, age, race, education level, and marital status, protein intake and all-cause mortality risk showed a U-shaped relationship. The lowest value was 90.3. It means that COPD patients have the lowest risk of all-cause mortality when the protein intake is 90.3gm. In Figure 2C, after further adjusting for BMI, smoking, drinking, household income-poverty ratio, comorbidities, and laboratory tests, the U-shaped relationship remained, but without statistical significance.

TABLE 1 Baseline characteristics according to the protein intake quartiles.

Characteristic	Overall	Q1 (11.7–48.5)	Q2 (48.5–67.7)	Q3 (67.7–94.3)	Q4 (94.3–266.6)	*p*-value
N (%)	522	131 (25.1)	130 (24.9)	130 (24.9)	131 (25.1)	
Age, years, mean (SD)	63 (54, 73)	61 (51, 73)	63 (56, 77)	63 (57, 72)	62 (48, 72)	0.2
Gender, *n* (%)						<0.001
Female	261 (57)	87 (67)	80 (74)	59 (53)	35 (35)	
Male	261 (43)	44 (33)	50 (26)	71 (47)	96 (65)	
Race, *n* (%)						0.016
Mexican American	34 (3.4)	6 (2.3)	11 (3.2)	5 (2.9)	12 (5.2)	
Non-Hispanic Black	85 (6.7)	21 (7.4)	25 (11)	18 (4.2)	21 (4.9)	
Non-Hispanic White	305 (78)	72 (77)	76 (76)	82 (77)	75 (81)	
Other Hispanic	44 (3.2)	20 (5.3)	9 (5.0)	4 (0.4)	11 (2.7)	
Other Race—Including Multi-Racial	54 (9.1)	12 (7.8)	9 (5.1)	21 (16)	12 (5.9)	
Education, *n* (%)						0.5
Less than 9th grade	52 (5.5)	19 (9.2)	15 (5.5)	7 (3.0)	11 (4.3)	
9-11th grade (Includes 12th grade with no diploma)	95 (14)	26 (16)	22 (13)	28 (18)	19 (7.6)	
High school graduate/GED or equivalent	147 (29)	38 (30)	37 (29)	30 (27)	42 (28)	
Some college or AA degree	164 (35)	38 (29)	39 (41)	46 (30)	41 (41)	
College graduate or above	64 (18)	10 (15)	17 (12)	19 (23)	18 (19)	
Marital, *n* (%)						0.4
Divorced	94 (13)	20 (12)	22 (13)	27 (16)	25 (11)	
Living with partner	30 (4.7)	7 (5.3)	10 (7.6)	6 (2.5)	7 (4.2)	
Married	236 (55)	55 (52)	52 (46)	57 (54)	72 (68)	
Never married	45 (6.2)	12 (5.4)	8 (3.9)	12 (8.7)	13 (6.0)	
Separated	26 (3.9)	8 (4.2)	7 (4.2)	6 (4.2)	5 (2.8)	
Widowed	91 (17)	29 (21)	31 (25)	22 (15)	9 (8.2)	
Family poverty income ratio, Mean (SD)	2.04 (1.05, 3.81)	1.93 (1.08, 3.75)	1.88 (1.02, 3.13)	2.10 (1.01, 3.67)	2.22 (1.21, 4.49)	0.4
Smoking status, *n* (%)						0.2
Former	83 (17)	17 (9.8)	25 (23)	23 (21)	18 (15)	
Never	255 (50)	59 (47)	63 (44)	66 (51)	67 (57)	
Now	184 (33)	55 (43)	42 (33)	41 (28)	46 (28)	
Alcohol, *n* (%)						0.3
Former	120 (21)	34 (25)	26 (14)	25 (14)	35 (30)	
Heavy	79 (17)	15 (14)	18 (16)	22 (17)	24 (22)	
Mild	193 (38)	40 (38)	51 (37)	52 (44)	50 (30)	
Moderate	81 (18)	24 (15)	25 (26)	18 (15)	14 (16)	
Never	49 (6.8)	18 (7.3)	10 (7.3)	13 (9.3)	8 (2.7)	
Diabetes, *n* (%)	240 (43)	63 (48)	45 (29)	69 (59)	63 (34)	<0.001
Hypertension, *n* (%)	367 (64)	93 (67)	87 (64)	90 (62)	97 (64)	>0.9
BMI, mean (SD)	30 (26, 34)	30 (26, 33)	30 (25, 34)	28 (25, 34)	30 (28, 38)	0.11
Protein, mean (SD)	70 (48, 93)	37 (29, 43)	58 (53, 62)	78 (73, 83)	118 (106, 151)	<0.001
Coronary heart disease, *n* (%)	82 (15)	29 (25)	15 (8.3)	25 (16)	13 (10)	0.042
Congestive heart failure, *n* (%)	87 (14)	28 (15)	19 (12)	19 (13)	21 (14)	>0.9
Chronic kidney disease, *n* (%)	186 (28)	47 (31)	45 (26)	43 (24)	51 (34)	0.5

(Continued)

TABLE 1 (Continued)

Characteristic	Overall	Q1 (11.7–48.5)	Q2 (48.5–67.7)	Q3 (67.7–94.3)	Q4 (94.3–266.6)	*p*-value
Hyperlipidemia, *n* (%)	413 (83)	108 (81)	103 (85)	103 (82)	99 (82)	>0.9
Stroke, *n* (%)	65 (14)	15 (12)	14 (12)	17 (14)	19 (19)	0.7
Atherosclerotic cardiovascular disease, *n* (%)	172 (33)	51 (43)	40 (25)	40 (29)	41 (33)	0.2
Heart attack, *n* (%)	81 (14)	26 (19)	19 (12)	13 (6.7)	23 (18)	0.039
Serum albumin, Mean (SD)	41.0 (39.0, 43.0)	41.0 (39.0, 43.0)	41.0 (39.0, 42.6)	41.0 (38.0, 43.0)	42.0 (39.0, 43.0)	0.7
Serum total protein, Mean (SD)	69.0 (66.0, 72.0)	69.0 (66.5, 73.0)	68.4 (65.0, 72.0)	69.0 (66.0, 72.0)	68.0 (64.1, 71.0)	0.2

SD, standard deviation.

TABLE 2 HR with 95% CI for mortality based on the protein intake as a continuous variable.

	HR	95% CI	*p*-value
All-cause mortality			
Model 1	1.00	0.99, 1.01	0.8
Model 2	1.00	0.99, 1.01	0.9
Model 3	1.00	0.99, 1.00	0.5

Non-adjusted (Model 1). Adjusted for race, gender, age, education level, and marital (Model 2). Further adjusted for family poverty income ratio, smoking status, alcohol, diabetes, hypertension, BMI, coronary heart disease, chronic kidney disease, congestive heart failure, hyperlipidemia, stroke, atherosclerotic cardiovascular disease, heart attack based on the factors adjusted in Model 2 (Model 3); HR, hazard ratio; CI, confidence interval.

4 Discussion

This study represents the first prospective exploration of the relationship between protein intake and COPD mortality by analyzing COPD patients in the NHANES 2013–2018 cycle. The findings revealed no significant non-linear association between protein intake levels and mortality. Hence, further investigation is required to elucidate the intricate interplay between protein intake and mortality in COPD patients.

COPD stands as a significant global health concern that is anticipated to rank fifth in disease burden worldwide by 2020. It is estimated to ascend to the fourth most common cause of death by 2030 and the seventh in disability-adjusted life years (DALYs) loss globally (18). COPD has garnered growing attention from the medical community in recent years (19). Nutritional dietary protein intake has been a longstanding focal point of research in COPD, but the clinical understanding of its impact on the incidence, progression, and outcomes of COPD remains inadequately understood. Nowadays, there is a lack of studies examining the association between nutritional status and disease recovery or mortality in COPD patients. Nevertheless, research has identified a notable correlation between dietary protein intake and forced vital capacity (FVC) as well as vital capacity (VC) in patients with COPD (4). In general, patients with chronic respiratory diseases may undergo weight loss and accelerated muscle wasting during acute exacerbations, owing to malnutrition, reduced activity, hypoxia, systemic inflammation, and/or the combined effects of systemic corticosteroids (20). Thus, understanding the role of dietary protein intake in the management and prognosis of COPD becomes paramount.

Higher protein intake in COPD patients may be linked to the observed increased protein degradation rate. Evidence suggests elevated muscle protein degradation rates in COPD patients (21), characterized by heightened components of the ubiquitin 26S proteasome system (22) and an augmented role of autophagy (23). However, the potential repercussions of protein synthesis signal transduction remain unclear, specifically in response to catabolic triggers (24).

TABLE 3 log (HR) (95% CI) for mortality according to the protein intake.

	Q1 (11.7–48.5)	Q2 (48.5–67.7)	Q3 (67.7–94.3)	Q4 (94.3–266.6)
All-cause mortality				
Number of patients	101	103	106	112
Model 1 HR(95% CI) *p*-value	1	1.08 (0.50, 2.32) 0.9	0.62 (0.28, 1.34) 0.2	0.82 (0.36, 1.85) 0.6
Model 2 HR(95% CI) *p*-value	1	0.83 (0.41, 1.68) 0.6	0.43 (0.21, 0.89) 0.022	0.70 (0.32, 1.57) 0.4
Model 3 HR(95% CI) *p*-value	1	0.93 (0.49, 1.74) 0.8	0.56 (0.27, 1.16) 0.12	0.69 (0.32, 1.46) 0.3

Non-adjusted (Model 1). Adjusted for age, gender, race, and education level, marital (Model 2). Further adjusted for family poverty income ratio, smoking status, alcohol, diabetes, hypertension, BMI, coronary heart disease, congestive heart failure, chronic kidney disease, hyperlipidemia, stroke, atherosclerotic cardiovascular disease, heart attack based on the factors adjusted in Model 2 (Model 3); HR, hazard ratio; CI, confidence interval.

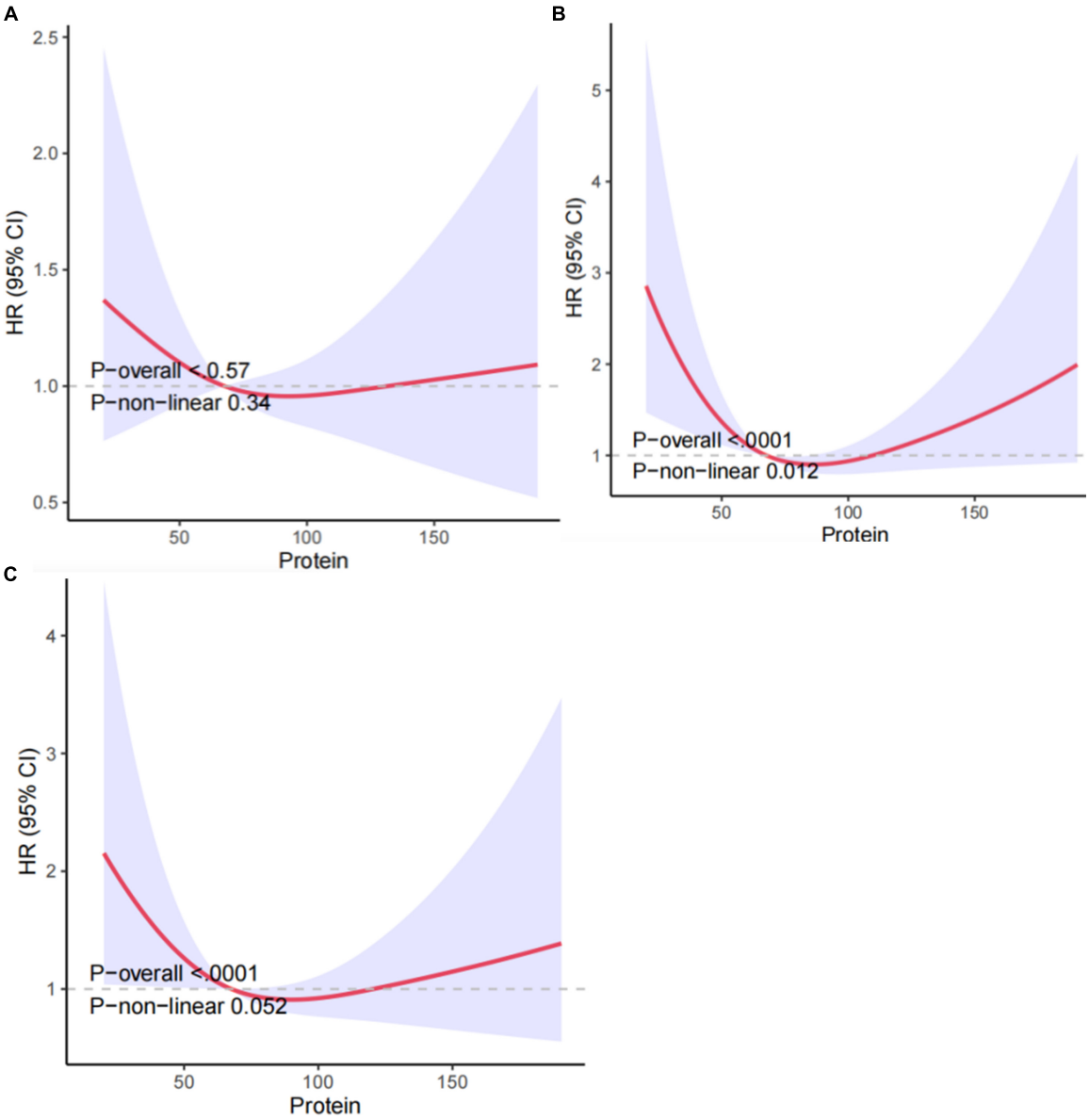

FIGURE 2
Association between protein intake and all-cause mortality in COPD patients: **(A)** non-adjusted Model 1, **(B)** Model 2 adjusted for gender, age, race, education level, and marital, **(C)** Model 3 further adjusted for age, gender, race, BMI, smoking, drinking, education level, hypertension, household income-poverty ratio, comorbidities and laboratory tests. HRs were calculated with the protein intake level of 62.8 as the reference. The solid line and the red region depict the estimated value and its corresponding 95% CI, respectively.

Protein intake in COPD patients has been reported to have varied effects. A study identifies insufficient protein intake in COPD patients referred for pulmonary rehabilitation (PR) (25), while others find no additional benefits in terms of muscle strength, physical function, and quality of life in non-sarcopenic COPD patients undergoing rehabilitation with the addition of protein supplementation (26). Another study discovers that energy and protein intake are below the calculated demand for all groups of COPD patients, revealing a notable correlation between protein intake and pulmonary function (27). Additionally, a study comparing the effects of a semi-solid snack and a liquid oral nutritional supplement (ONS) on energy, postprandial glucose, and protein intake reveals that postprandial glucose is elevated after consuming ONS compared to the snack after a meal (28). Overall, the role of protein intake in COPD patients is still under investigation, and further research is necessary to determine its optimal use in improving outcomes for these patients.

Nutritional interventions designed to stimulate protein synthesis and counteract increased protein degradation may help maintain muscle mass (29). Supplying adequate amino acids through nutritional interventions to support protein synthesis signaling could potentially induce a compensatory response to increased protein breakdown cues (30). Studies have indicated that the level of oxidative stress in the skeletal muscle of COPD patients is consistently elevated. In signaling pathways sensitive to oxidative stress and involved in the regulation of muscle mass, muscle biopsy

analysis has demonstrated the activation of Forkhead box O (FoxO) (31), myogenin-activated protein kinase (MAPK) (32), and NF-κB (33). MAPK and NF-κB signaling is also induced by increased inflammation and inflammatory cell infiltration. A previous study has confirmed the expression of pro-inflammatory cytokines (34). Therefore, these catabolic pathways (or upstream triggers, such as oxidative stress and inflammation) may serve as potential targets for nutritional regulation (22).

The important implication of nutritional status for the prognosis of COPD patients has been widely recognized. Studies have shown that good nutritional status can significantly improve the quality of life, reduce acute exacerbations, and may prolong survival in COPD patients (35, 36). Muscle consumption and atrophy are closely related to the severity of COPD and affect the quality of life and survival rate of COPD patients. Muscle dysfunction is a prevalent issue among COPD patients, affecting about 20% to 35% of patients, especially those with severe symptoms or long-term bed rest. Muscle atrophy is related to the imbalance of muscle protein synthesis and decomposition, primarily attributed to factors such as inflammation, oxidative stress, hypoxia, and hypercapnia (37, 38).

Amino acid metabolism is also disrupted in the muscle tissue of COPD patients. One notable change is the decrease in the concentration of branched-chain amino acids (such as leucine), which are essential for providing energy to the muscle tissue. Leucine can not only provide energy for muscle tissue but also aids in the repair process after muscle injury. Therefore, disruptions in amino acid metabolism may exacerbate muscle damage and functional decline (39).

COPD primarily manifests as emphysema and chronic bronchitis, both of which have a significant impact on patient prognosis. Disease exacerbation is typically closely related to reduced quality of life, heightened hospitalization rate, and shortened life expectancy. Compared with mild or moderate COPD patients, patients with severe or extremely severe emphysema have a significantly elevated risk of all-cause mortality and cardiovascular mortality (40, 41).

Many factors affect protein intake, and previous research has underscored the importance of the proportion of protein in total energy intake. When considering total energy intake, an appropriate adjustment in protein intake could significantly impact health and longevity (42). Various research has indicated the significance of total energy intake on protein consumption. A thorough understanding of the fluctuations in energy requirements at different life stages is essential for devising appropriate strategies for proteins and other macronutrient intake to optimize health and function (43).

Although our RCS model and Cox regression analysis demonstrate no non-linear relationship between protein intake levels and mortality in COPD patients, this study has certain limitations. Initially, the absence of repeated protein intake measurements prevents the assessment of dynamic protein intake levels and their association with mortality. Second, the single-day recall method may not accurately reflect the regular food intake of the participants. Then, participants who self-reported 'yes' to the item "Ever told you had COPD?" may introduce some bias into the results, as we could not further validate the applicable diagnostic criteria. Additionally, despite adjusting for certain covariates in this study, other unadjusted covariates such as muscle wasting, sarcopenia, dynapenia, emphysema, and bronchitis might influence the mortality or protein intake levels. Consequently, a comprehensive prospective cohort study evaluating dynamic protein intake levels is crucial to corroborate the correlation between protein intake levels and COPD mortality.

5 Conclusion

In summary, this study, utilizing NHANES data, underscores the correlation between protein intake levels and all-cause mortality in COPD patients. No substantial non-linear correlation is observed between protein intake levels and all-cause mortality. Our results demonstrate that high protein intake does not have a protective effect in COPD patients. These results provide a theoretical basis for subsequent experimental verification and potential interventions for appropriate protein intake, offering new insights to improve the survival rate of this patient population.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/.

Author contributions

HL: Conceptualization, Data curation, Methodology, Software, Visualization, Writing—original draft. QZ: Conceptualization, Investigation, Methodology, Software, Writing—original draft, Writing—review and editing. JL: Conceptualization, Project administration, Supervision, Writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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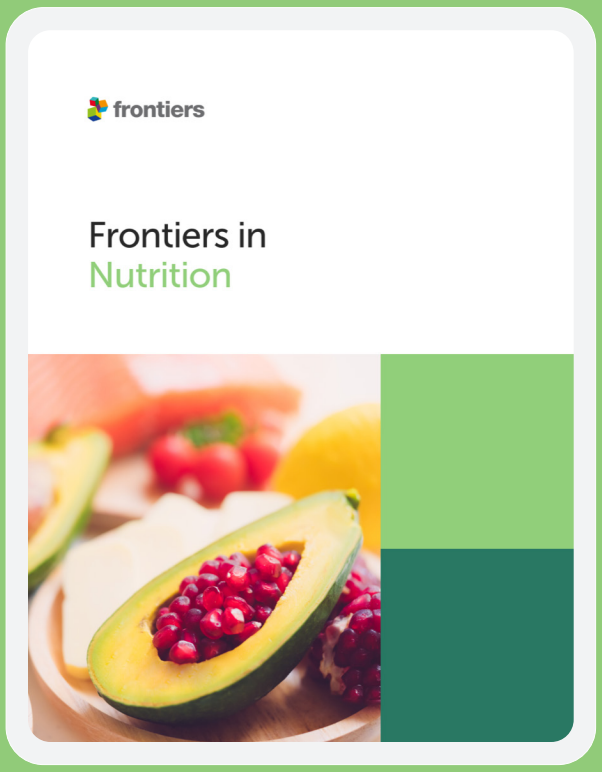

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