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Metabolic differences between bronchial epithelium from healthy individuals and patients with asthma and the effect of bronchial thermoplasty

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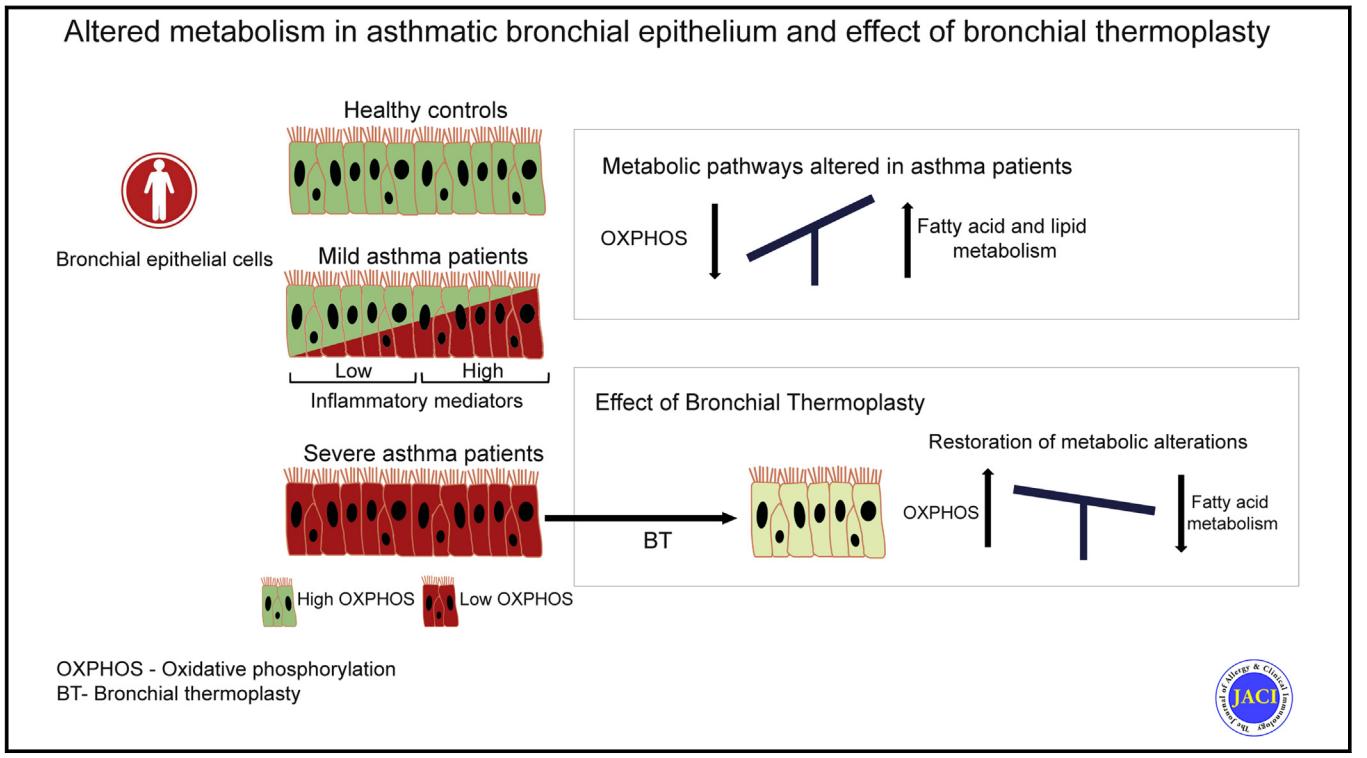
GRAPHICAL ABSTRACT



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Background: Asthma is a heterogeneous disease with differences in onset, severity, and inflammation. Bronchial epithelial cells (BECs) contribute to asthma pathophysiology.
Objective: We determined whether transcriptomes of BECs reflect heterogeneity in inflammation and severity in asthma, and whether this was affected in BECs from patients with severe asthma after their regeneration by bronchial thermoplasty.
Methods: RNA sequencing was performed on BECs obtained by bronchoscopy from healthy controls (n = 16), patients with mild asthma (n = 17), patients with moderate asthma (n = 5), and patients with severe asthma (n = 17), as well as on BECs from treated and untreated airways of the latter (also 6 months after bronchial thermoplasty) (n = 23). Lipidome and metabolome analyses were performed on cultured BECs from healthy controls (n = 7); patients with severe asthma (n = 9); and, for comparison, patients with chronic obstructive pulmonary disease (n = 7).
Results: Transcriptome analysis of BECs from patients showed a reduced expression of oxidative phosphorylation (OXPHOS) genes, most profoundly in patients with severe asthma but less profoundly and more heterogeneously in patients with mild asthma. Genes related to fatty acid metabolism were significantly upregulated in asthma. Lipidomics revealed enhanced levels of lipid species (phosphatidylcholines, lysophosphatidylcholines. and bis(monoacylglycerol)phosphate), whereas levels of OXPHOS metabolites were reduced in BECs from patients with severe asthma. BECs from patients with mild asthma characterized by hyperresponsive production of mediators implicated in neutrophilic inflammation had decreased expression of OXPHOS genes compared with that in BECs from patients with mild asthma with normoresponsive production. BECs obtained after thermoplasty had significantly increased expression of OXPHOS genes and decreased expression of fatty acid metabolism genes compared with BECs obtained from untreated airways.
Conclusion: BECs in patients with asthma are metabolically different from those in healthy individuals. These differences are linked with inflammation and asthma severity, and they can be reversed by bronchial thermoplasty. (J Allergy Clin Immunol 2021;148:1236-48.)

Key words: *Bronchial epithelium, metabolism, thermoplasty*

Abbreviations used

BEC:	Bronchial epithelial cell
BMP:	Bis(monoacylglycero)phosphate
BT:	Bronchial thermoplasty
COPD:	Chronic obstructive pulmonary disease
CXCL-8:	C-X-C motif chemokine ligand 8/IL-8
GSEA:	Gene set enrichment analysis
ICS:	Inhaled corticosteroid
IPA:	Ingenuity Pathway Analysis
LPC:	Lysophosphatidylcholine
OXPHOS:	Oxidative phosphorylation
PC:	Phosphatidylcholine
TASMA:	Unravelling Targets of Therapy in Bronchial Thermoplasty in Severe Asthma
TIA-1:	T-cell internal antigen-1
TiAR:	T-cell internal antigen-1 related protein

The airways of patients with obstructive airway diseases such as asthma and chronic obstructive pulmonary disease (COPD) are chronically inflamed.[1] Airway epithelial cells together with macrophages constitute the first line of defense to interact and respond to external stimuli, thus driving immune responses. This, together with their abundance, makes airway epithelial cells potential major contributors to the inflammation and pathogenesis of asthma[2] and COPD.[3] Recently, we identified a defect in translational control in bronchial epithelial cells from patients with asthma that correlated well with neutrophilic responses.[4] Several earlier reports have shown that both genetic and acquired defects[5] in airway epithelial cells[6] can lead to impaired mucociliary clearance[7] and barrier function.[8] Apart from genetic defects that may underlie such a defect, airway epithelial cells are continuously exposed to an inflammatory milieu, as a consequence of which epithelial cell biology may change. Even though asthma and COPD are different in etiology and pathophysiology, it is likely that the underlying innate immune mechanisms, especially those directed by bronchial epithelium, may at least in part be shared.

Previous studies of the differences between epithelial cells from patients with asthma in particular have focused on unbiased stratification of cohorts of patients with asthma by grouping into different clusters and on distinguishing asthma phenotypes by using transcriptomics[9] and proteomics.[10] Omics approaches comparing patients and healthy control subjects, however, are virtually lacking. The aim of this study was to compare the transcriptome of bronchial epithelial cells (BECs) from patients with mild to moderate to severe asthma with transcriptome of BECs from healthy controls, substantiate major differences between them, and relate these differences to the previously described translational defect. To put these findings into further perspective, we performed additional analysis in patients with asthma who underwent bronchial thermoplasty (BT). BT is a nonpharmacologic bronchoscopic treatment for severe asthma that delivers radiofrequency energy to heat the airway wall and thereby target airway smooth muscle cells. Furthermore, the bronchial epithelium is the most superficial airway wall layer that encounters radiofrequency heating energy, which has been shown to result in epithelial sloughing as detected by optical coherence tomography directly after BT.[11,12] Therefore, we postulated that BT may subsequently lead to restoration of bronchial epithelium, which would be reflected in the bronchial epithelial transcriptome.

METHODS

Subjects and design

This study comprised a cross-sectional design using bronchial epithelial brushes from 3 trials and 2 explorative studies. The RESOLVE trial (NCT1677)[13] involved patients with mild asthma and healthy controls. The MATERIAL trial (NTR01520051)[14] enrolled patients with mild asthma, and the RILCA (Role of Innate Lymphoid Cells in Asthma) study (NL48912.018.14) enrolled patients with moderate asthma and patients with severe asthma from the TASMA (Unravelling Targets of Therapy in Bronchial Thermoplasty in Severe Asthma) (NCT02225392) trial.[15] The patients with COPD were patients included from the RILCO (Role of Innate Lymphoid Cells in COPD) study (NL53354.018.15). All study protocols were reviewed and approved by the ethical review committee and were in accordance with the Declaration of Helsinki. All study participants provided written informed consent. The collection of BECs in the aforementioned trials and studies was

conducted by 1 center, namely, the Department of Respiratory Medicine of the Amsterdam University Medical Center, Amsterdam, The Netherlands. The TASMA trial was conducted at the Department of Pulmonology, University Medical Center Groningen, Groningen, The Netherlands, and Royal Brompton and Imperial College Hospital London, United Kingdom. The baseline characteristics of the patients with asthma and healthy controls who were involved in this study are provided in Table I.

The inclusion and exclusion criteria for patients with mild asthma (from the RESOLVE and MATERIAL trials) are described elaborately[16] in the Methods section of the Online Repository (available at www.jacionline.org). Patients with severe asthma (from the TASMA trial) fulfilling the World Health Organization or modified innovative medicines initiative criteria of severe refractory asthma were included.[17,18] The RILCA (Role of Innate Lymphoid Cells in Asthma) study and RILCO (Role of Innate Lymphoid Cells in COPD) were 2 explorative studies involving the impact of rhinovirus-16 challenge in patients with moderate asthma and patients with COPD, respectively, and exploring mechanisms driven by innate lymphoid cells. The inclusion and exclusion criteria are mentioned in detail in the Methods section of the Online Repository.

Corticosteriod use

The patients with mild asthma had not received inhaled or systemic corticosteroids or any other treatment other than inhaled short-acting β_2-agonists within 2 weeks before the start of the study. The patients with moderate asthma used a dose equivalent of no more than 500 μg of fluticasone propionate per day. The patients with severe asthma had been using an inhaled corticosteroid (ICS) at a dosage of at least 500 μg of fluticasone equivalent per day and a long-acting ß$_2$-agonist at a dosage of at least 100 μg of salmeterol per day or equivalent for the past 6 months, as well as a systemic corticosteroid (≤20 mg of prednisone equivalent per day). The patients with COPD were allowed specific medication, namely, long-acting ß$_2$-agonists and long-acting muscarinic agonists, but no ICSs.

Sampling and RNA isolation

Bronchoscopy was performed according to a standardized method.[14] Mucosal brushes collected by brushing the left lower lobe consisted predominantly (>95%) of bronchial epithelial cells. Two brushes were obtained from each participant and then pooled and pelleted by centrifugation at 1240 rpm (using a Rotanta 460S centrifuge) for 10 minutes at 4°C. The pellet was dissolved in 1 mL of TRIzol (Thermofischer Scientific, Paisley, United Kingdom) and stored at –80°C until the RNA was isolated. After all the samples had been obtained, they were thawed at room temperature, and after 200 μL of chloroform was added, they were shaken vigorously for 30 seconds. The samples were kept at room temperature for 10 minutes, after which the phases were separated by centrifugation at 16,000 *g* for 15 minutes at 4°C. The aqueous phase was concentrated with protocol 5.3 using the NucleoSpin system and an RNA XS extraction kit (Macherey-Nagel, Duren, Germany). The quality and concentration of the samples were assessed by using a fragment analyzer (Advanced Analytical Technologies, Inc, Ankeny, Iowa).

RNA sequencing, analysis, and heat maps

The NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (Ipswich, Mass) was used to process the samples. The sample preparation was performed according to the protocol for the NEBNextUltra Directional RNA Library Prep Kit for Illumina (NEB, catalog no. 7420S/L). Briefly, oligo-dT magnetic beads were used to isolate mRNA from total RNA. cDNA synthesis was performed after fragmentation of the mRNA. This was used for ligation with sequencing adapters followed by PCR amplification of the resulting product. The quality and yield after sample preparation were measured with the fragment analyzer. The size of the resulting products was consistent with the expected size distribution of between 300 and 500 bp. To evaluate the quality of the library preparation and kits used, the raw data were sampled and mapped to annotated genomic references. Mapping positions were classified as intragenic, exonic, intergenic, intronic, and rRNA. Clustering and DNA sequencing were performed according to the manufacture's protocol using the Illumina NextSeq 500. A concentration of 1.6 pM was used as the input. The reads were trimmed for adapter sequences by using Trimmomatic, version 0.30, before the alignment. Presumed adapter sequences were removed from the read when the bases matched a sequence in the adapter sequence set (TruSeq adapters) with 2 or fewer mismatches and an alignment score of at least 12. The reference Homo_sapiens.GRCh37.75 was used to align the reads. The mapping to the reference sequence was done by using a short reader aligner based on Burrows-Wheeler transform. The default of mismatch rate of 2% (3 mismatches in a read of 150 bases) was used. The frequency of the reads mapped on the transcript was determined in terms of counts, which were used as an input for the downstream analysis.

Additionally, reads per kilobase of exon per million reads mapped and fragments per kilobase of exon per million reads mapped values were calculated. Image analysis, base calling, and quality check were performed with Illumina data analysis pipeline RTA, version 2.4.11, and Bcl2fastq, version 17. The read counts were loaded into the DESeq2 software package, which is a statistical package within the R/BioConductor platform that is designed to determine the differentially expressed genes and in which an adjusted *P* value less than .05 was considered statistically significant. The DEseq2 method uses Benjamini-Hochberg correction as an adjustment for false discovery rates.

For the heat maps, $\log_2$ gene expression values were normalized with *z* scores calculated by using the equation $X - (\mu/\sigma)$, where X is the value of the individual sample, μ is the average of the row, and σ is the SD of the row. The clustering of genes represented in the heat maps was based on k-means clustering. The combination of *z* scores for all genes is represented below the heat maps in red and blue, with red indicating high expression and blue indicting low expression.

GSEA

The gene sets were identified by using the online platform accessed via the website www.gsea-msigdb.org. All differentially expressed genes with a *q* value less than 0.05 compared between 2 groups were used for gene set enrichment analysis (GSEA). The gene identifiers were uploaded in the molecular signature database of the website, and the overlapping gene sets were computed. The *P* values were derived on the basis of hypergeometric distribution for $k-1, K, N-K$, and n, where k is the number of genes in the intersection of the query set with a set from the Molecular Signature Database, K is the number of genes in the set from the Molecular Signature Database), N is the total number of all human genes, and n is the number of genes in the query set). The false discovery rate (q value) analog of the hypergeometric *P* value was determined by correction for multiple hypothesis testing according to Benjamini and Hochberg. The gene sets thus identified were plotted in heat maps to visualize gene expression of all individuals separately.

IPA

Ingenuity Pathway Analysis (IPA) is used to quickly visualize and understand complex omics data and perform insightful data analysis and interpretation. The IPA output represented in Fig 1, *E* is based on all genes differentially expressed in patients with severe asthma versus those expressed in healthy controls with a *q* value less than 0.05 (see Table E1 in this article's Online Repository at www.jacionline.org). These genes were uploaded in IPA, which displays the most significantly affected top canonic pathways. The output of graphical illustration shows all of the genes involved in the pathway, with the genes differentially expressed being highlighted.

Lipidomics and metabolome analysis

The BECs for lipidomics and metabolomics were obtained by brush during bronchoscopy (P0) from healthy individuals, patients with severe asthma, and

TABLE I. Baseline characteristics of patients with mild, moderate, and severe asthma and healthy controls for transcriptome analyses

Characteristic	Healthy controls	Patients with mild asthma	Patients with moderate asthma	Patients with severe asthma
Subjects (no.)	16	17	5	17
Age (y), mean (min-max)	22.42 (19-31)	22.82 (18-38)	37 (23-52)	44.82 (25-66)
Sex ratio (male:female), no./no.	4:12	6:11	3:2	5:12
FEV_1 before bronchodilator (L), mean (SD; min-max)	4.05 (0.72; 3.44-4.93)	3.94 (0.85; 2.56-5.16)	3.27 (0.88; 2.11-4.53)	2.79 (0.76; 1.7-4.37)
FEV_1 after bronchodilator (L), mean (SD; min-max)	4.12 (0.72; 3.52-5.05)	4.23 (0.84; 3.06-6.14)	3.41 (0.91; 2.21-4.64)	3.07 (0.76; 2.11-4.49)
FEV_1 before bronchodilator (% predicted), mean (SD; min-max)	105.5 (10.92; 90-121)	97.43 (6.64; 86-109)	93.5 (18.76; 93.5-122)	85.35 (26; 38-129)
FEV_1 after bronchodilator (% predicted), mean (SD; min-max)	107.5 (11.15; 98-124)	103.83 (8.63; 90-120)	97 (18.49; 73-125)	100.88 (20.91; 67-137)
FEV_1 reversibility (%),median (SD; min-max)	3 (2.86; –2 to 7)	7.5 (4.36; –4 to 12)	3 (2.87; 0-8)	10 (17.23; 1-64)
PC_{20} (mg/mL), median (SD; min-max)	>16.0	1.49 (0.03-7.35)	na	0.28 (0.0075-5.53)
F_{ENO} (ppb), mean (SD; min-max)	21.9 (11.4)	58.03 (37.14)	21 (10.04)	52 (63.97)

F_{ENO}, Fraction of exhaled nitric oxide represented in terms of ppb; *max*, maximum; *min*, minimum; *na*, not available; *PC_{20}*, histamine provocative concentration causing a 20% drop in FEV_1.

patients with COPD. The baseline characteristics for participants subjected to these analyses are provided in Table II.

The BECs were plated on 6-well plates precoated with PurCol (Advanced Biomatrix, Carlsbad, Calif) and grown until confluence in bronchial epithelial basal medium (Lonza, Basel, Switzerland) supplemented with growth factors (Lonza) and ciprofloxacin (Sigma, St Louis, Mo) at a concentration of 2 μg/mL. The cells were then passaged into T25 flasks (passage 1) until grown confluent. Subsequently, the cells were detached by using trypsin/EDTA (Lonza), and after the addition of trypsin-neutralizing solution, they were pelleted by centrifugation for 7 minutes at 1240 rpm (with a Rotanta 460S centrifuge) at 8°C. The pellet was washed twice with PBS and stored at –80°C until all samples were analyzed in parallel.

Lipidomics and metabolome analysis was performed on a high-performance liquid chromatography system (Ultimate 3000 binary high-performance liquid chromatography system, Thermo Scientific, Waltham, Mass), as described previously.[19] The extract of the cell pellet was injected onto a normal phase column (LiChrospher 2 × 250-mm silica-60 column) and a reverse-phase column (Acquity UPLC HSS T3, Milford, Mass). A Q Exactive plus Orbitrap (Thermo Scientific) mass spectrometer was used in the negative and positive electrospray ionization modes. In both ionization modes, mass spectra of the lipid species were obtained by continuous scanning from mass-to-charge ratio 150 to mass-to-charge ratio 2000 with a resolution of 280,000 full width at half maximum. Detailed analysis of the data is provided in the Methods section of the Online Repository.

BT

The TASMA trial was conducted by using a protocol approved by the Medical Ethics Committee (NL45394.018.13).[20] All subjects provided prior written, informed consent. The design of the TASMA trial has been elaborately described elsewhere.[12,20] A total of 23 patients[21] (6 patients with severe asthma were included in addition to those in the severe asthma group mentioned in Table I) were treated with BT by using the Alair System (Boston Scientific, Boston, Mass) according to the current standard[22] and sedated by using remifentanil/propofol[23] or general anaesthesia. Patients were treated with 50 mg of prednisolone 3 days before treatment, on the day of the procedure itself, and 1 day thereafter. During the first procedure, the right lower lobe was treated; during the second procedure, the left lower lobe was treated; and finally, both upper lobes were treated. The right middle lobe remained untreated; therefore, this region could be used to compare the treatment effect. Six months after BT, bronchoscopy was performed. During the bronchoscopy procedure, endobronchial brushes were obtained from the untreated middle lobe and treated left lower lobe airways. The baseline characteristics of the patients with severe asthma used to analyze the effects of BT on bronchial epithelium are provided in Table III; they are no different from those reported originally for all patients.[21]

RESULTS

Dysregulation of metabolic genes in bronchial epithelium from patients with severe asthma

Despite heterogeneity within each group, there were 472 genes differentially expressed in the BECs from patients with mild asthma (see Table E2 in this article's Online Repository at www.jacionline.org) and 640 genes in those BECs from patients with severe asthma (see Table E1) compared with the numbers in the BECs from healthy controls ($q < 0.05$). There were no genes differentially expressed between the BECs from patients with mild asthma and the BECs from patients with severe asthma, with the *q* value cutoff set at less than 0.05. Because of the small number of patients with moderate asthma used for analysis, there were was no comparison of differentially expressed genes in BECs between bronchial epithelium from patients with moderate asthma and bronchial epithelium from healthy controls. GSEA of differentially expressed genes in BECs from patients with mild asthma versus in BECs from healthy controls showed gene sets belonging to mitotic spindle, IL-2–signal transducer and activator of transcription 5 (STAT5) signaling, UV response, oxidative phosphorylation (OXPHOS), and inflammatory response (Fig 1, *A*). Similar comparisons for patients with severe asthma and healthy controls revealed gene sets belonging to OXPHOS, IFN-α response, mitotic spindle, apical junction, and late estrogen response (Fig 1, *B*).

Both the mitotic spindle and OXPHOS gene sets stood out in terms of BECs from patients with mild asthma and patients with severe asthma, with the mitotic spindle highest in the BECs of patients with mild asthma and OXPHOS highest in the BECs from patients with severe asthma. We further investigated the OXPHOS gene set. Heat maps of 15 OXPHOS genes were reduced (*shown in green*) (Fig 1, *C*) in BECs from the patients with severe and moderate asthma in comparison with those from the controls (shown in red) (Fig 1, *C*). In the patients with mild asthma there was a heterogeneous gene expression pattern, with 5 of 17 patients showing high expression (*red*) and the remainder showing low expression (*green*) of OXPHOS genes (Fig 1, *C*). Apart from these metabolic OXPHOS genes, fatty acid metabolism–related genes (fatty acid synthase [*FASN*], arachidonate 15-lipoxygenase [*ALOX15*], phospholipase C eta 1 [*PLCH1*], pyruvate dehydrogenase phosphatase regulatory subunit [*PDPR*], acyl-CoA oxidase 3 [*ACOX3*], and

A

Gene Set Name [# Genes (K)]	Description	# Genes in Overlap (k)	k/K	p-value	FDR q-value
HALLMARK_MITOTIC_SPINDLE [199]	Genes important for mitotic spindle assembly.	9		$8.62\ e^{-5}$	$2.24\ e^{-3}$
HALLMARK_IL2_STAT5_SIGNALING [200]	Genes up-regulated by STAT5 in response to IL2 stimulation.	9		$8.96\ e^{-5}$	$2.24\ e^{-3}$
HALLMARK_UV_RESPONSE_DN [144]	Genes down-regulated in response to ultraviolet (UV) radiation.	7		$3.39\ e^{-4}$	$5.65\ e^{-3}$
HALLMARK_OXIDATIVE_PHOSPHORYLATION [200]	Genes encoding proteins involved in oxidative phosphorylation.	8		$4.82\ e^{-4}$	$6.03\ e^{-3}$
HALLMARK_INFLAMMATORY_RESPONSE [200]	Genes defining inflammatory response.	7		$2.31\ e^{-3}$	$1.92\ e^{-2}$

B

Gene Set Name [# Genes (K)]	Description	# Genes in Overlap (k)	k/K	p-value	FDR q-value
HALLMARK_OXIDATIVE_PHOSPHORYLATION [200]	Genes encoding proteins involved in oxidative phosphorylation.	15		$7.65\ e^{-9}$	$2.69\ e^{-7}$
HALLMARK_INTERFERON_ALPHA_RESPONSE [97]	Genes up-regulated in response to alpha interferon proteins.	11		$1.08\ e^{-8}$	$2.69\ e^{-7}$
HALLMARK_MITOTIC_SPINDLE [200]	Genes important for mitotic spindle assembly.	14		$5.75\ e^{-8}$	$9.58\ e^{-7}$
HALLMARK_APICAL_JUNCTION [200]	Genes encoding components of apical junction complex.	13		$4\ e^{-7}$	$5.01\ e^{-6}$
HALLMARK_ESTROGEN_RESPONSE_LATE [200]	Genes defining late response to estrogen.	12		$2.58\ e^{-6}$	$2.58\ e^{-5}$

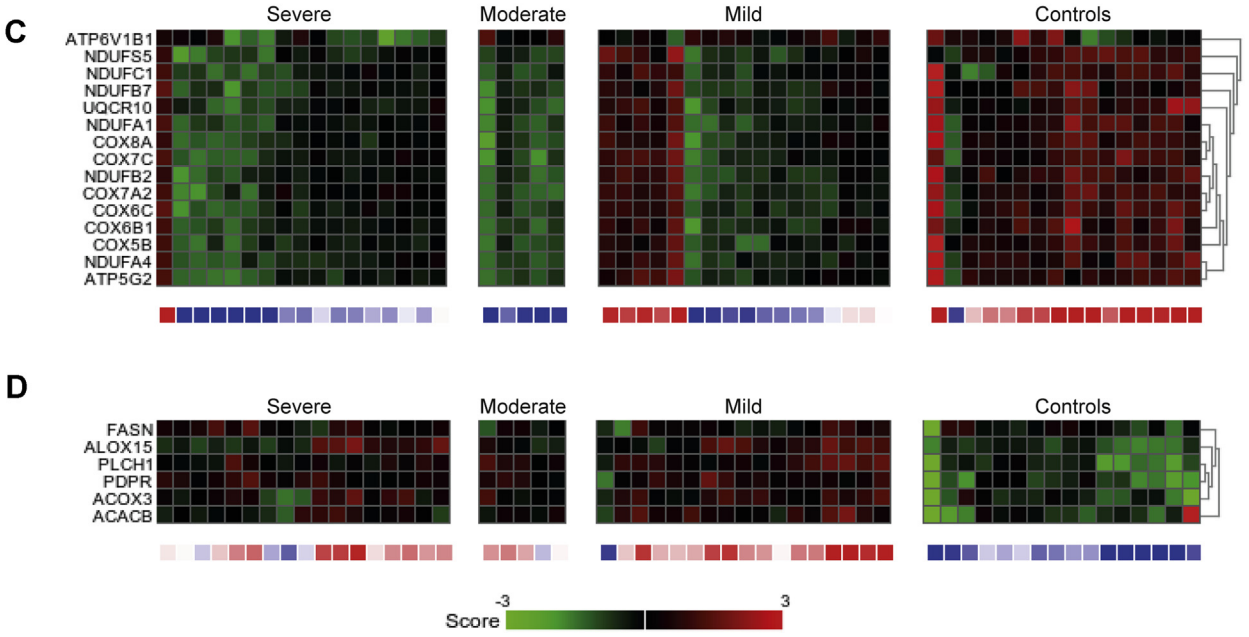


FIG 1. Metabolic genes in BECs from patients with asthma versus in BECs from healthy controls. GSEA showed differentially expressed gene sets in BECs from patients with mild (**A**) and severe (**B**) asthma, with green indicating highly significant and black indicating less significant q values (< 0.05). OXPHOS gene expression in BECs from patients with severe (n = 17), moderate (n = 5), and mild asthma (n = 17) versus in those from controls (n = 16) (**C**). **D,** As in (**C**), but for genes related to fatty acid metabolism (*in red and green, with red indicating high expression and green indicating low expression*). The combined *z* scores for the genes shown are represented below the heat maps (*in red and blue, with red indicating high expression and blue indicating low expression*). IPA of differentially expressed genes of the respiratory chain in BECs from patients with severe asthma versus in BECs from healthy controls (n = 17). **E,** Significantly down-regulated genes (adjusted *P* value of <.05) are shown in pink.

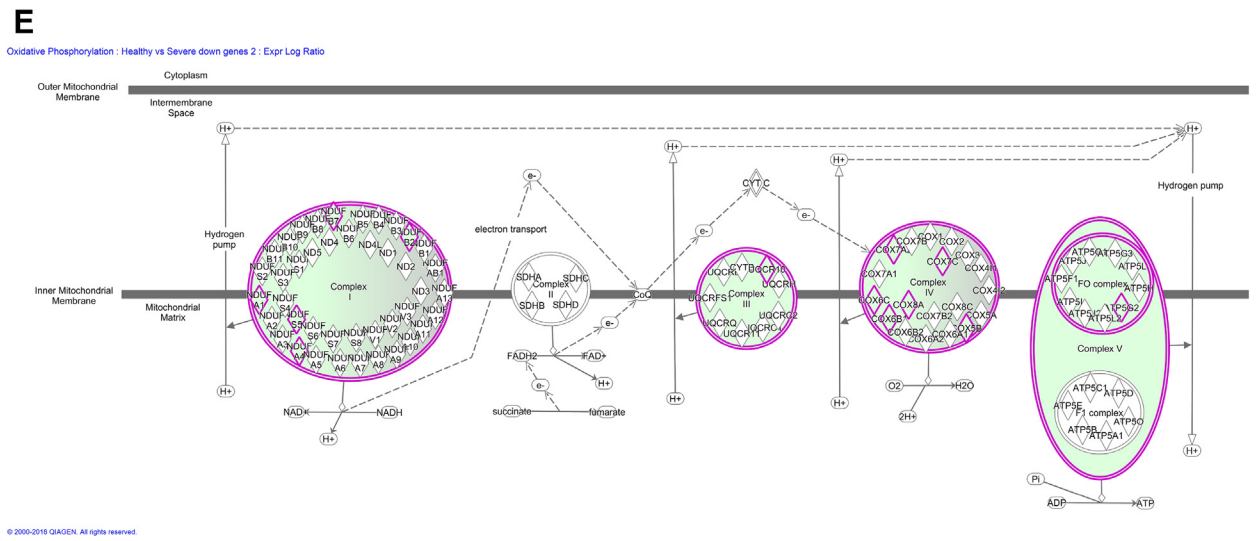


FIG 1. (Continued).

TABLE II. Baseline characteristics of patients with severe asthma, patients with COPD, and healthy controls for lipidomic and metabolomic analyses

Characteristic	Healthy controls	Patients with severe asthma	Patients with COPD
Subjects (no.)	7	9	6
Age (y), mean (min-max)	37 (24-56)	52.8 (40-63)	63.8 (52-74)
Sex ratio (male:female), no./no.	3:4	4:5	3:3
FEV_1 pre-bronchodilator (L), mean (SD; min-max)	3.94 (0.98; 2.6-5.09)	2.66 (0.33; 2.18-3.1)	2.228 (0.49; 1.49-2.99)
FEV_1 post-bronchodilator (L), mean (SD; min-max)	4.15 (1; 2.77-5.37)	2.61 (0.5; 1.58-3.14)	2.41 (0.48; 1.61-3.06)
FEV_1 pre-bronchodilator (% predicted), mean (SD; min-max)	105.75 (0.82; 105-107)	84.33 (23.13; 41-107)	70.8 (4.95; 65-77)
FEV_1 post-bronchodilator (% predicted), mean (SD; min-max)	111.5 (2.17; 108-114)	90.66 (21.15; 52-115)	77.2 (4.35; 72-85)
FEV_1 reversibility, median (SD; min-max)	6.5 (1.63; 3-7)	8 (4.4; 0-11)	6 (3; 2-11)

max, Maximum; *min*, minimum.

acetyl-CoA carboxylase-β [*ACACB*]) were also shown by heat maps. Interestingly, in the BECs from all patients with asthma (mild, moderate, or severe), these genes were upregulated (*red*) compared with those in the healthy controls (Fig 1, *D*). In addition to GSEA, IPA also showed that the OXPHOS pathway was the top dysregulated pathway in BECs from patients with severe asthma compared with in BECs from healthy subjects. The downregulated genes were from complexes I, III, IV, and V of the electron transport chain (Fig 1, *E*). Heat maps of 7 mitotic spindle genes displayed significantly enhanced expression, particularly in BECs from patients with mild and moderate asthma, but not in those from patients with severe asthma (see Fig E1 in the Online Repository at www.jacionline.org). The RNA sequencing data sets comparing bronchial epithelium from patients with mild, moderate, and severe asthma with that from healthy controls can be accessed by using the link https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE161245.

Elevated lipid profiles in BECs from patients with severe asthma versus in BECs from controls

To substantiate the findings for metabolic genes, lipid profiles and metabolome were analyzed after expansion of the bronchial epithelium *ex vivo*. As there was a clear and distinct reduction of OXPHOS gene expression in BECs from patients with severe asthma compared with in BECs from the controls, we compared these cohorts. Volcano plots showed all lipid species quantified in BECs and depicted the extent of differences in lipid profiles between the 2 groups. Phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs), lysophosphatidylethanolamines, and bis(-monoacylglycero)phosphates [BMPs]) (*shown in green*) (Fig 2, *A*) were significantly upregulated ($P < .05$ and $\log_2$ fold change > 1) in the BECs from patients with severe asthma compared with in the BECs from healthy controls. To visualize the 28 lipid mediators that were significantly upregulated in BECs from patients with severe asthma, we plotted these in heat maps. The levels of lipid species (PCs, LPCs, lysophosphatidylethanolamines, and BMPs) were increased in the BECs from the patients with severe asthma (*red*) compared with in those from the healthy controls (*blue*) (Fig 2, *B*). The metabolome analyses in BECs from patients with severe asthma and healthy controls did not show a clear distinction between these 2 groups (Fig 2, *C*). The metabolomic analysis measured a total of 75 metabolites, 16 of which are depicted in the heat maps in Fig 2, *C*. The remaining 59 metabolites that were not significantly different between patients with asthma and healthy controls are shown in Table E3 (in this article's Online Repository at www.jacionline.org).

Distinct lipid species in BECs from patients with COPD versus in BECs from healthy controls and patients with severe asthma

Previous reports showed altered metabolites and glycerophospholipids in PBMCs and airway smooth muscle cells[24] from

TABLE III. Baseline characteristics of patients with severe asthma who underwent the BT procedure

Characteristic	Value in patients with severe asthma
Subjects (no.)	23
Age (y), mean (min-max)	47.69 (25-64)
Sex ratio (male:female), no./no.	7:16
FEV_1 before bronchodilator (L), mean (SD; min-max)	2.67 (0.57; 1.59-3.66)
FEV_1 after bronchodilator (L), mean (SD; min-max)	3.03 (0.54; 2.11-4.26)
FEV_1 before bronchodilator (% predicted), mean (SD; min-max)	89.3 (19.89; 57-129)
FEV_1 after bronchodilator (% predicted), mean (SD; min-max)	101.13 (18.66; 67-137)
FEV_1 reversibility, mean (SD; min-max)	10 (11.65; 0-57)
PC_{20} (mg/mL), mean (SD; min-max)	0.41 (0.0075-32)
ACQ score before BT, mean (SD; min-max)	2.62 (0.57; 1.5-3.66)
AQLQ score before BT, mean (SD; min-max)	3.98 (0.81; 2.65-6)

ACQ, Asthma Control Questionnaire; *AQLQ*, Asthma Quality of Life Questionnaire; *max*, maximum; *min*, minimum; PC_{20}, histamine provocative concentration causing a 20% drop in FEV_1 value.

patients with COPD.[25] Hence, we analyzed whether BECs from patients with COPD also displayed a similar difference in metabolic status after *ex vivo* expansion. The heat maps of lipidomics analysis of BECs from patients with COPD compared with BECs from healthy controls showed a prominent upregulation of mainly PC lipid species. In addition, lipid species (triglycerides and LPCs) were also enhanced in BECs from patients with COPD (Fig 3, *A*). Strikingly, when BECs from COPD were compared with those from patients with severe asthma, there was also a significant increase in triglycerides in the patients with COPD (Fig 3, *B*). With respect to the metabolites analyzed, the levels of uridine diphosphate (UDP)-hexose, 2-dehydrogluconate-6P, deoxyadenosine monophosphate (dAMP), oxidized nicotinamide adenine dinucleotide (NAD^+), fructose 1,6 diphosphate, creatine-P, glutamine, α-ketoglutarate, nicotinamide adenine dinucleotide hydrogen (NADH), adenine, and creatinine were significantly lower than in the BECs from patients with COPD than in the BECs from the healthy controls. Heat maps representing these different metabolites in the 2 groups are provided in Fig 3, *C*. The other 64 metabolites with levels that were not significantly different between bronchial epithelium from patients with COPD and bronchial epithelium from healthy controls are shown in Table E4 (in this article's Online Repository at www.jacionline.org).

BT treatment–induced metabolic shift in BECs

BECs obtained from treated and, as controls, untreated airways (ie, the right middle lobe) of patients with severe asthma 6 months after initiation of the treatment were subjected to transcriptomics to analyze differentially expressed genes. Interestingly, the heat maps (Fig 4, *A*) and *z* scores (Fig 4, *B*) showed that the BECs obtained from the treated airways had significantly upregulated OXPHOS genes compared with the BECs from untreated airways. In addition, reduced glycolysis gene expression in the BECs from treated airways was observed in the heat maps (Fig 4, *C*) and *z* scores (Fig 4, *D*) when compared with the glycolysis expression in the BECs from untreated airways. Thus, thermoplasty induced a metabolic shift in bronchial epithelium toward that observed in healthy controls. The RNA sequencing data sets comparing bronchial epithelium from untreated and BT-treated regions can be accessed by using the link https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE161453.

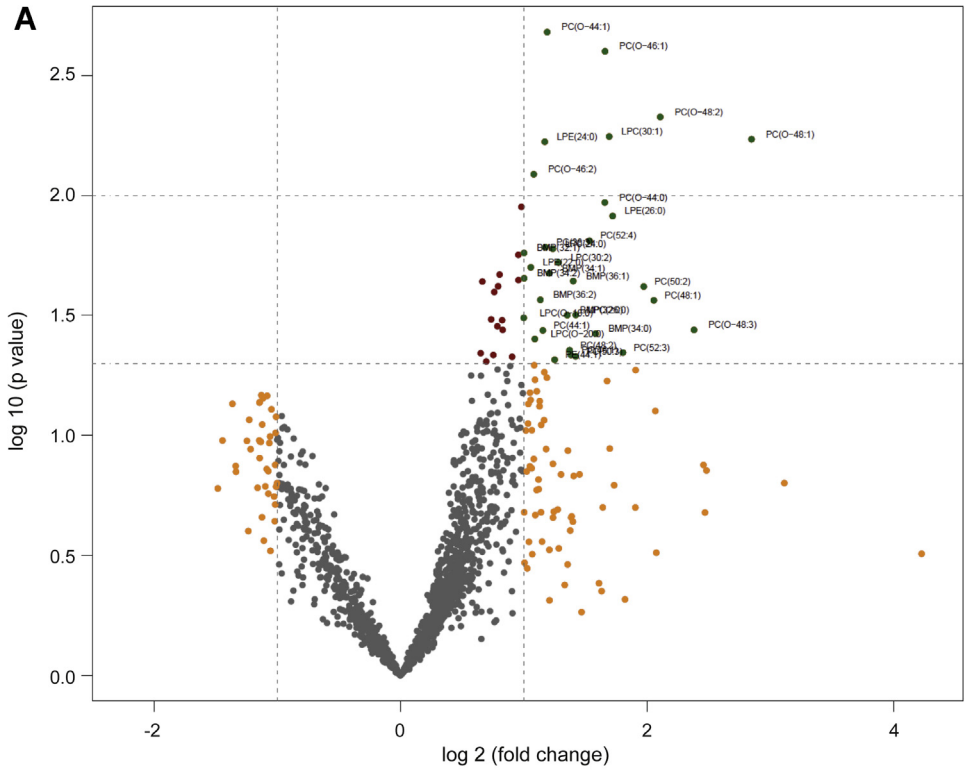
Hyperresponsive BECs have reduced expression of OXPHOS genes

Earlier, we identified BECs from patients with mild asthma with a defective translational control as hyperresponsive (exaggerated and corticosteroid-unresponsive production of C-X-C motif chemokine ligand 8/IL-8 [CXCL-8], and other cytokines) as opposed to normoresponsive (lower and corticosteroid-responsive production of CXCL-8 and other cytokines).[4] This epithelial hyperresponsiveness was correlated with neutrophilic inflammation. The transcriptome of hyperresponsive BECs from patients with mild asthma (subgroup from Table I and Fig 1) displayed a prominent reduced expression of OXPHOS genes compared with that in their normoresponsive counterparts (Fig 4, *E*).

DISCUSSION

Unbiased transcriptome analysis of BECs from patients with severe asthma compared with those from healthy controls showed a profound reduction in OXPHOS genes belonging to complexes I, III, IV, and V of the electron transport chain, whereas for BECs from patients with mild asthma, this reduction was heterogeneous. Genes related to fatty acid metabolism, however, were significantly upregulated in BECs from all patients with asthma, thus differentiating patients with asthma from healthy controls. This differential expression in bronchial epithelium was validated by lipidomics with enhanced levels of lipid species (PCs, LPCs, and BMPs). The reduction in metabolites in bronchial epithelium of patients with severe asthma was observed trendwise only, with no clear differences. Most interestingly, in BECs from patients with severe asthma who had received BT, a metabolic shift toward that in BECs from healthy individuals was observed. In BECs from patients with COPD also, there was a marked upregulation of lipid profiles compared with that in BECs from healthy controls, and the upregulation was even more pronounced than that in BECs from patients with severe asthma.

Together, these data indicate that bronchial epithelial cells are subject to metabolic adaptations in asthma. Studies have shown a metabolic shift from OXPHOS to glycolysis, particularly for macrophages[26] and dendritic cells,[27] which has been linked to inflammation. Another study showed that enhanced glycolysis in lung epithelium is required for IL-1α/β-induced proinflammatory responses.[28] There could be several reasons for this attenuated OXPHOS gene expression. For example, inflammatory



S.No	Lipid species	S.No	Lipid species	S.No	Lipid species	S.No	Lipid species
1	PC(O−44:1)	11	PG(38:3)	21	PC(48:1)	31	PC(46:1)
2	PC(O−46:1)	12	LPC(24:0)	22	LPC(26:0)	32	PC(50:3)
3	PC(O−48:2)	13	BMP(32:1)	23	BMP(32:0)	33	PE(44:1)
4	LPC(30:1)	14	LPC(30:2)	24	LPC(O−18:0)		
5	PC(O−48:1)	15	LPE(22:0)	25	PC(O−48:3)		
6	LPE(24:0)	16	BMP(34:1)	26	PC(44:1)		
7	PC(O−46:2)	17	BMP(34:2)	27	BMP(34:0)		
8	PC(O−44:0)	18	BMP(36:1)	28	LPC(O−20:0)		
9	LPE(26:0)	19	PC(50:2)	29	PC(48:2)		
10	PC(52:4)	20	BMP(36:2)	30	PC(52:3)		

FIG 2. Functional analysis of lipid profiles and metabolites in BECs cultured *ex vivo*. Volcano plots (significance [–log10(*P* value)] vs effect size [$\log_2$(fold change)]) on the y-axis and x-axis, respectively) of lipid profiles in BECs from healthy controls (n = 7) compared with in BECs from patients with severe asthma (n = 9) cultured *ex vivo*. **A,** The levels of lipids (*dots labeled in green, red, or yellow*) are significantly ($P < .05$) reduced in control subjects with a log fold change greater than 1 or with a fold change less than 1, or reduced with a fold change of greater than 1 but nonsignificant, respectively. **B,** Heat maps of lipid profiles in BECs cultured *ex vivo* from healthy controls and patients with severe asthma ($P < .05$ with a log fold change greater than 1). The asterisks in the heat maps indicate patients with severe asthma with low levels of lipid species. **C,** Heat maps of metabolome in BECs cultured *ex vivo* from healthy controls (n = 6) and patients with severe asthma (n = 8). ($P < .05$ and log fold change >1), with red indicating upregulation and blue indicating downregulation.

mediators such as TNF-α, can inhibit COX1 by tyrosine phosphorylation, thereby switching from aerobic metabolism to glycolysis.[29] Interestingly, here we have shown that the attenuated OXPHOS gene expression is observed in BECs that are hyperresponsive, which results in an increased production of proinflammatory mediators, prominently, the neutrophilic chemoattractant CXCL-8, owing to a defective translocation of the translational repressor T-cell internal antigen-1–related protein (TiAR).[8] Interestingly, TiAR and the closely related T-cell internal antigen-1 (TIA-1) have also been implicated in mitochondrial biogenesis.[30] In addition, TiAR and TIA-1 knockdown attenuates complex V (ATP synthase) of the OXPHOS pathway.[31] Therefore, a possible scenario is that a defective TiAR and/or TIA-1 in BECs from patients with asthma leads to the observed metabolic shift and the resulting lipid mediators may contribute to activation of the bronchial epithelium,[32] which can lead to chronic inflammation in asthma.[33] Alternatively, but not mutually exclusively, enhanced reactive oxygen species and mitochondrial damage in bronchial epithelium were found to parallel high levels of CXCL-8, IL-6, and IL-1β production.[34] Further support for a link between mitochondrial dysfunction and neutrophilic inflammation comes from clustering of genes in sputum from patients with asthma, in which

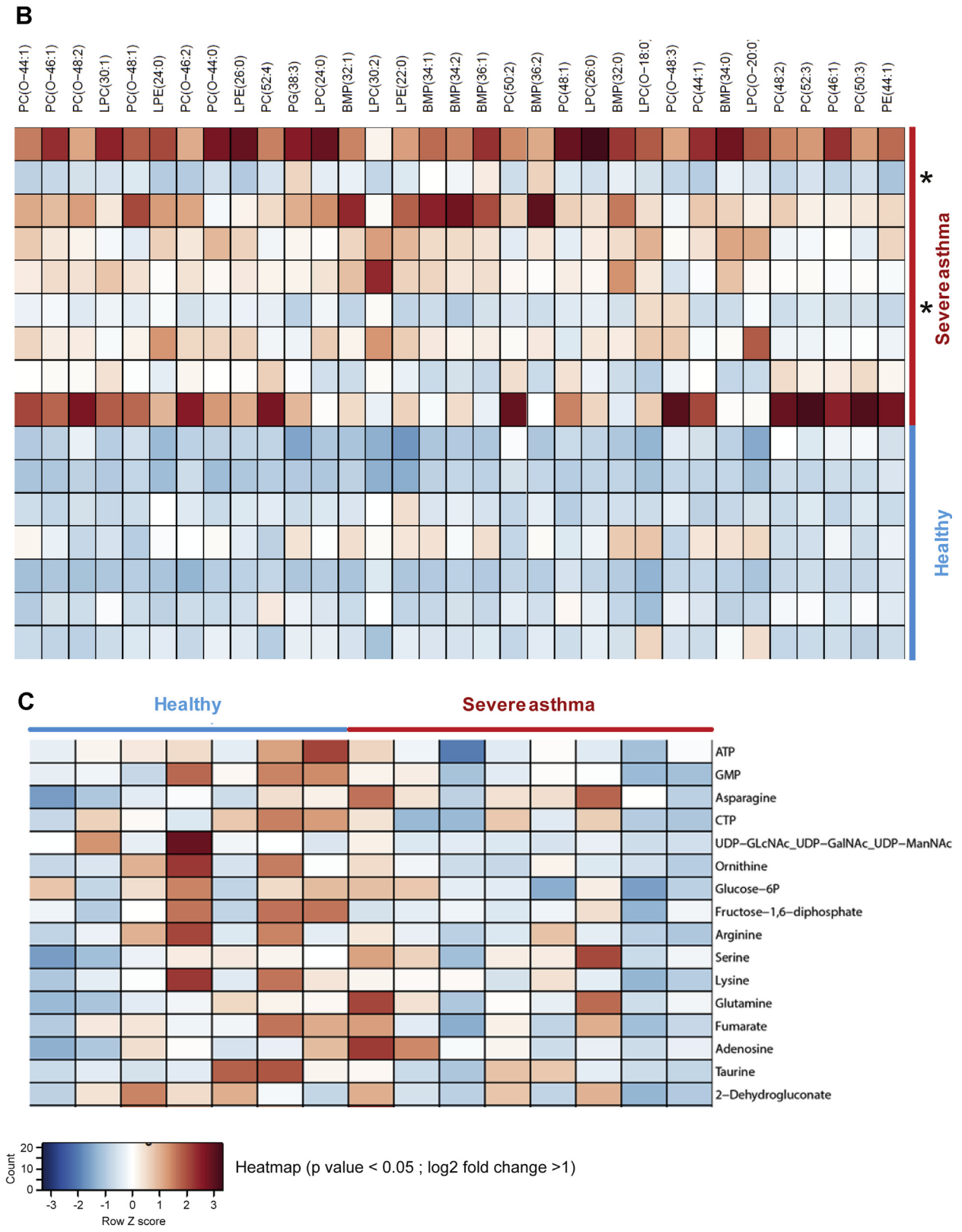


FIG 2. (Continued).

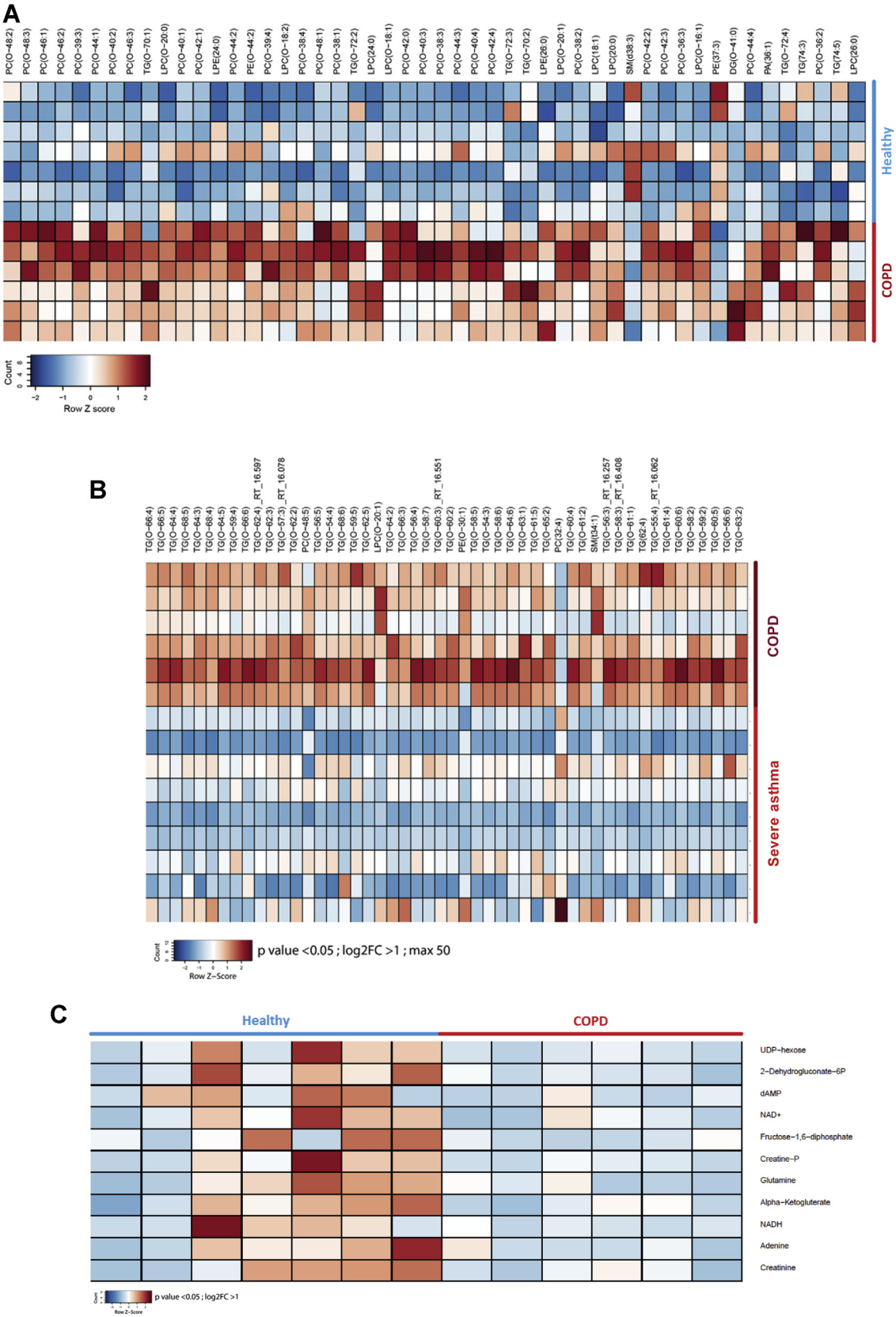


FIG 3. Lipidomics and metabolome analysis of expanded BECs from patients with COPD versus BECs from controls and patients with severe asthma. **A**, Heat maps of lipid profiles in *ex vivo*–cultured BECs from patients with COPD (n = 6) and healthy controls (n = 7) ($P < .05$ with log fold change >1). **B**, Heat maps of lipid profiles in *ex vivo*–cultured BECs from patients with COPD (n = 6) and patients with severe asthma (n = 9) ($P < .05$ with log fold change > 1). **C**, Heat maps of metabolome in *ex vivo*–cultured BECs from patients with COPD (n = 6) and healthy controls (n = 7) ($P < .05$ with log fold change > 1), with red indicating upregulation and blue indicating downregulation.

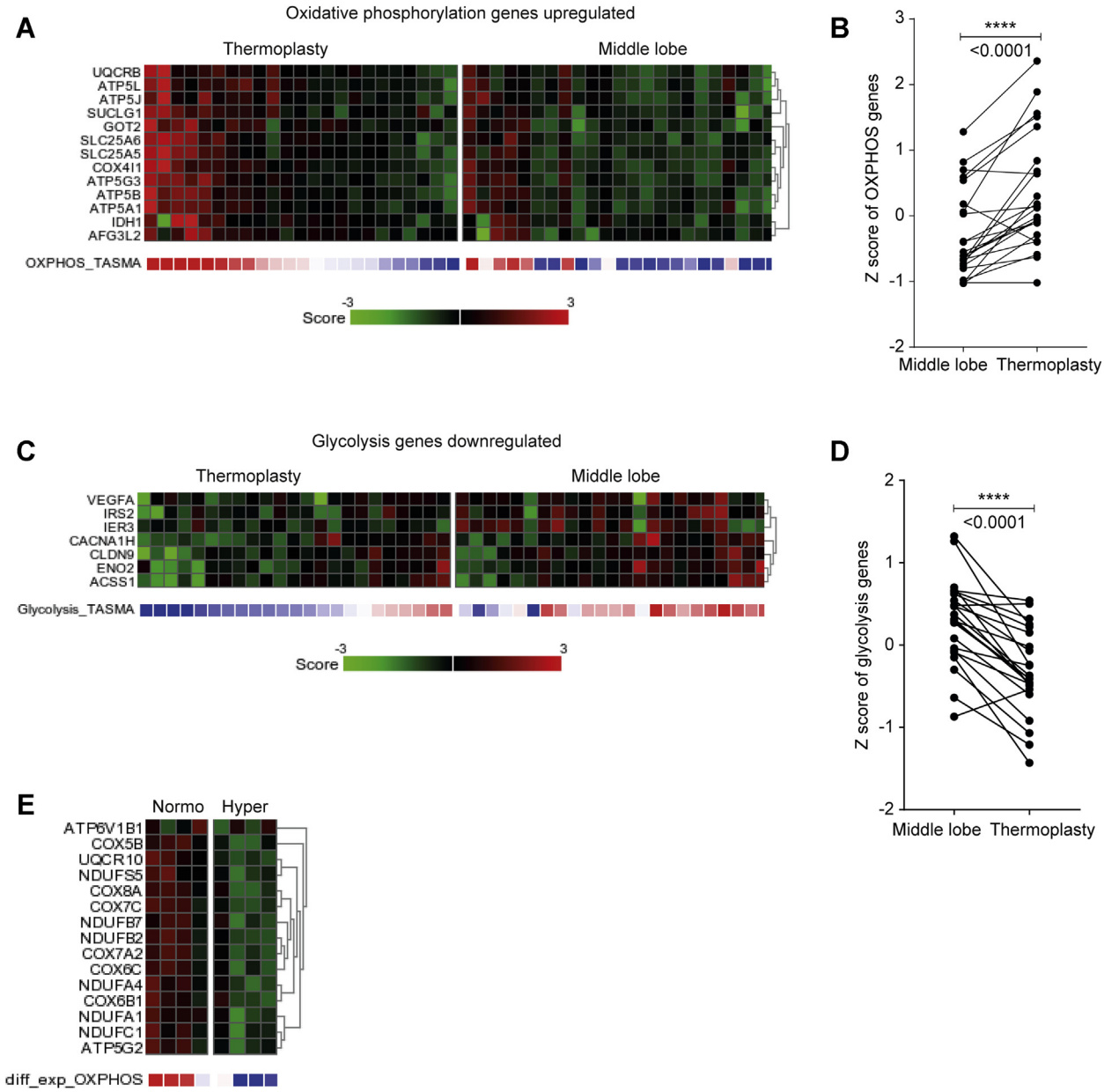


FIG 4. BT treatment alters metabolic gene expression in BECs from patients with severe asthma. **A** and **B,** Upregulation of OXPHOS genes shown in heat map (**A**) and line graph plotting *z* scores (**B**) in a pairwise comparison of BECs obtained 6 months after thermoplasty from the treated (left lower lobe) and untreated region (middle lobe). **C** and **D,** Also, similar pairwise analysis in heat maps (**C**) and plotted *z* scores (**D**) show reduced expression of glycolysis-related genes in BECs from treated versus untreated airways in patients with severe asthma (n = 23). **E,** Hyperresponsive (see Ravi et al[4]) BECs (n = 4) from patients with mild asthma displayed enhanced expression of OXPHOS genes (the same genes mentioned in Fig 1, *A*), as compared with that in normoresponsive BECs (n = 4).

case high neutrophilic inflammation was strongly associated with a significant reduction in OXPHOS genes in transcriptome-associated cluster 2 (TAC2),[35] and ether lipids from neutrophils[36] downregulate mitochondrial activity.[37] Finally, BECs from patients with COPD, which is associated with neutrophilic inflammation,[38] also display a very distinct increase in lipid species, even when compared with that from patients with asthma. Together, these findings provide strong evidence for a prominent reduction in mitochondrial activity in BECs that is linked to neutrophilic inflammation in asthma, and possibly in COPD.

The differences in metabolites between BECs from patients with severe asthma and those from healthy individuals appear to affect purine metabolism, amino acid biosynthesis, and glycolysis. In BECs from patients with COPD, the pathways that may be affected are creatine metabolism and the tricarboxylic acid cycle. This translation of metabolites to metabolic pathways, however, is based merely on the presence of 3 or more significantly different metabolites and should thus be considered with caution. The consequences of these different metabolic pathways on the functioning of BECs remain to be determined, but they likely

affect biosynthesis, energy housekeeping, and ultimately inflammatory responses and possibly lung function. In our analysis, 2 patients with severe asthma displayed markedly lower levels of lipid species and had lower FEV_1 % reversibility than other the patients did. Despite the low numbers, it would be interesting to explore whether higher lipid metabolism in bronchial epithelium results in higher FEV_1 % reversibility in patients with severe asthma.

Altered mitochondrial metabolism in airway epithelial cells may affect various epithelial functions, such as that of the mucociliary escalator. Epithelial cells in healthy lungs require energy for proper hydration of the mucus layer and mucociliary clearance by ciliary beat.[39] Consequently, mitochondrial damage in bronchial epithelium of patients with asthma leads to ciliary dysfunction, resulting in poor mucus clearance,[40] and in COPD this has been linked to exacerbations.[41] In the current study, the BECs from some patients with asthma from the steroid-naive cohort with mild asthma displayed reduced OXPHOS gene expression. Therefore, at least for this cohort, the reduced expression of OXPHOS genes is independent of corticosteroid use.

BT is a treatment option for patients with severe asthma who are uncontrolled despite optimal medical therapy, including a high dose of inhaled bronchodilators and inhaled or oral corticosteroids. The results from biopsy and imaging studies are conflicting as to whether the untreated parts of the airways are also altered by BT.[12,42] However, our results show a significantly increased expression of OXPHOS genes in BECs obtained from treated parts compared with in BECs from untreated parts (middle lobe). Interestingly, imatinib, a protein tyrosine kinase inhibitor, is shown to improve airway hyperresponsiveness in severe refractory asthma,[43] and it also inhibits the platelet-derived growth factor that induces airway smooth muscle cell proliferation.[44] In addition, imatinib induces an increase in OXPHOS gene expression in bronchial epithelium, specifically, in the responders to the treatment.[45] This suggests that reversing metabolic defects in bronchial epithelium from patients with asthma could be beneficial for patients with asthma.

For the first time, a persistent and altered metabolism in bronchial epithelium has been demonstrated *in vivo* in patients with mild asthma and, more prominently, in patients with severe asthma. The strengths of our study comprise the inclusion of patients with asthma who differ in severity of their asthma, healthy controls, and patients with COPD. For severe asthma, we have also shown that BT affects this altered metabolism in BECs. There was an earlier report comparing BECs obtained from sequential BT sessions,[46] but as the samples were collected on different days, those analyses may have been biased. In the BT trial we collected and analyzed BECs from treated versus nontreated airways in parallel. However, there are some potential limitations to the current study. First, the patients with severe asthma were taking both oral corticosteroids and ICSs, which may have influenced the alteration of metabolic genes in their bronchial epithelium. In some patients with mild steroid-naive asthma, however, there was a reduced expression of OXPHOS and enhanced fatty acid metabolism genes. Second, functional validation by lipidomics and metabolome analysis was done after culturing and expanding the bronchial epithelium. This *ex vivo* expansion of BECs may affect gene expression[47] and we cannot exclude the possibility that mitochondrial dysfunction in bronchial epithelium of patients with severe asthma is reversible by culturing BECs *ex vivo*. However, it also has been demonstrated that cultured bronchial epithelial cells maintain their phenotype *ex vivo*[4] and depict asthma severity.[48] This indicates that bronchial epithelium can retain intrinsic features, even after culturing *ex vivo*.[4] Third, because of the high variability of lipid mediators measured in lung epithelial lining fluids,[49] it is possible that more patients should have been included for functional validation by metabolome analysis. The transcriptome data, however, were substantiated by lipidomics. Fourth, OXPHOS is known to be attenuated by aging,[50] and both those patients with severe asthma and those with COPD were older than those in the healthy cohort. However, the reduced expression of OXPHOS genes was also observed in younger patients included in the cohort of patients with mild asthma, and thus, it is unlikely that aging underlies the observed differences between patients and healthy controls. In addition, BT apparently resets the metabolic changes in BECs, suggesting that the reduced expression of OXPHOS genes is acquired rather than due to aging. With respect to the latter, we cannot exclude the possibility that regional differences in the airways underlie the observed different bronchial epithelial transcriptomes after BT. Finally, for our study we used submerged cultures, which typically contain undifferentiated BECs as opposed to, for example, BECs grown at air-liquid interface cultures, which are considered more representative of airway epithelial cells. Submerged cultures were chosen because the cells could be analyzed after a shorter culture time span (2 weeks), as opposed to the 6 weeks required for air-liquid interface cultures, and were therefore less likely to lose their phenotype. As our control BECs were also cultured submerged, our findings are genuine, but the consequences of this for differentiated cells need to be established.

In summary, we have shown metabolic differences between BECs from patients with asthma and patients with COPD and BECs from healthy individuals, with the differences worsening with the severity of asthma. As these metabolic defects link to the earlier reported epithelial hyperresponsiveness,[4] these metabolic effects appear to underlie airway inflammation. In this context, it is of interest that BT partially normalizes the metabolic differences, thereby resetting the bronchial epithelium, which may contribute to the response to BT treatment.

Key messages

- **Bronchial epithelium from patients with severe asthma is metabolically altered.**
- **Levels of lipid species in bronchial epithelium from patients with asthma or COPD bronchial epithelium are enhanced.**
- **BT treatment reverses these metabolic alterations.**

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