Original Research *Feature article*

Metabolomic profiling of anaerobic and aerobic energy metabolic pathways in chronic obstructive pulmonary disease

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Impact statement

While there is no cure for chronic obstructive pulmonary disease (COPD), its progressive nature and the formidable challenge to manage its symptoms warrant more extensively mechanistic study on its pathogenesis. A new emphasis on COPD study is the change of energy metabolism. This is the first study using metabolomics to profile energy metabolism pathways and energy metabolism of COPD patients. Through comprehensive evaluation, we highlighted the significance of anaerobic and low-efficiency energy supply pathways in lung injury and linked it to the energy-inflammation-lung ventilatory function and the motion limitation mechanism in COPD patients. Targeting at these mechanisms in COPD may promote the development of effective treatment for this devastating disease.

Abstract

While there is no cure for chronic obstructive pulmonary disease (COPD), its progressive nature and the formidable challenge to manage its symptoms warrant a more extensive study of the pathogenesis and related mechanisms. A new emphasis on COPD study is the change of energy metabolism. For the first time, this study investigated the anaerobic and aerobic energy metabolic pathways in COPD using the metabolomic approach. Metabolomic analysis was used to investigate energy metabolites in 140 COPD patients. The significance of energy metabolism in COPD was comprehensively explored by the Global Initiative for Chronic Obstructive Lung Disease–GOLD grading, acute exacerbation vs. stable phase (either clinical stability or four-week stable phase), age group, smoking index, lung function, and COPD Assessment Test (CAT) score. Through comprehensive evaluation, we found that COPD patients have a significant imbalance in the aerobic and anaerobic energy metabolisms in resting state, and a high tendency of anaerobic energy supply mechanism that correlates positively with disease progression. This study highlighted the significance of anaerobic and low-efficiency energy supply pathways in lung injury and linked it to the energy-inflammation-lung ventilatory function and the motion limitation mechanism in COPD patients, which implies a novel therapeutic direction for this devastating disease.

Keywords: Chronic obstructive pulmonary disease, metabolomics, energy metabolism cycle, tricarboxylic acid cycle, anaerobic glycolysis

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Introduction

Chronic obstructive pulmonary disease (COPD) is a highly heterogeneous chronic respiratory disease that is characterized by an airway blockage and inflammation which is usually associated with smoking, chronic dust exposure, and environmental pollution.[1] The pathogenic condition can continuously exist without predisposing factors and appear as an acute aggravation under infection.[1]

Meanwhile, severe COPD in patients may also be accompanied by cardiovascular disease, skeletal muscle atrophy, chronic hypoxemia, and abnormal metabolic environmental homeostasis of the body as a consequence of long-term inflammatory stimulation.[2,3] In the later stages of the disease, severe airway remodeling and blockage of secretions lead to laborious breathing and chronic dyspnea, resulting in respiratory muscle fatigue and increased energy consumption.[4,5] While there is no cure for COPD, its progressive nature and the formidable challenge to manage its symptoms warrant a more extensive study of the pathogenesis and related mechanisms. A new emphasis in COPD study is the change of energy metabolism of COPD, which may turn out to be promising for therapeutic targets using metabolic drugs.[6] In the meantime, thanks to the rapid technological progress of metabolomics, the critical roles of energy metabolism in many chronic diseases have been highlighted by recent studies,[7,8] with promising therapeutic targets, e.g. mitochondria,[9] or peroxisome proliferator-activated receptors (PPARs).[10]

As the common hub of lipid and protein metabolism pathways, the tricarboxylic acid cycle (TCA) cycle mainly takes place in the mitochondria, where it plays a core role in the body metabolic system of the body through its activity in sugar oxidation under aerobic conditions, and without pyruvate accumulation.[5] Pyruvate is the main fuel for energy generation in the TCA cycle. However, under hypoxia, pyruvate is irreversibly converted to lactic acid through anaerobic glycolysis that results in low efficiency production.[11] This process is mainly located in the mature erythrocyte that lacks mitochondria and in the skeletal muscles during the normal body working period.[5] However, under pathological conditions, such as diabetes, cancer, COPD, or pyruvate kinase isoenzyme deficiency, the overall energy metabolic homeostasis is significantly affected.[5,12–14] The formation of lactic acid can limit the efficiency of the TCA cycle productivity, especially in COPD patients, resulting in a decrease in mitochondrial content and pyruvate transporters, which leads an intracellular shortage of adenosine triphosphate (ATP) supply.[15,16] Although the liver gluconeogenesis of lactic acid may become the body energy reserve, when the body is under an oxygen-deficient environment, it is unable to make efficient use of it.[14,17] However, a decreased productivity does not mean a decreased ATP at all levels.[18] Studies have found that the ATP content in the tissue interspace and the alveolar lavage fluid increase significantly, which accelerates the induction of neutrophilic granulocyte extravasation and causes macrophages to secrete a large amount of inflammatory mediators.[18] Under the influence of long-term smoking, the autophagy degradation and the mitochondrial fission separation in patients' tissue are unbalanced, resulting in decreased ATP production and increased reactive oxygen species (ROS) levels, which accelerates histiocytes' aging and death. Meanwhile, aging is also an important factor for the process progression.[19] Therefore, we hypothesized that COPD patients may be more prone to uneven expression of energy metabolism.

Due to the long-term decline in effective energy utilization and nutrient intake, COPD patients tend to show a poor nutrition status, especially skeletal muscle atrophy as one of the main clinical manifestations, and this phenotype is more obvious in patients with more severe disease.[20–22] Respiratory muscles are a specific group of skeletal muscles, that are responsible for pulmonary ventilation volume exchange, which can be affected by chronic inflammation, hypoxia, and protein deficiency. In addition, COPD patients' compensatory pulmonary hyperventilation (reduced range of motion) and polypnea exacerbate the burden of respiratory muscles. Functional muscle weakness may also occur if the patient is in the acute exacerbation phase.[21]

Metabolomics is the state-of-art molecular biology tool for the measurement of the energy metabolic cycle by dynamically observing small molecule changes as a consequence of the mechanisms of the body physiopathological changes that occur in COPD.[23] For the first time, we assessed the consequences of the chronic inflammation level of COPD patients on metabolomics' profiling, which has been shown to correlate with the body inflammation and nutrition levels in patients with COPD.[24,25] On this basis, this study further explores the mechanisms of disease progression in COPD patients from the perspective of energy metabolism.

Materials and methods

Grouping criteria

The diagnostic COPD criteria were established according to the latest 2020 Global Initiative for Chronic Obstructive Lung Disease report.[26] In this study, the subjects were from the First Affiliated Hospital of Guangzhou Medical University, and included 140 COPD patients and 20 healthy individuals. All the participants in the study signed informed consent.

Clinical stable criteria

Eighty COPD patients (20 in each category according to the GOLD 1–4 criteria) collected in our research met the standard of clinical stability (the clinical stability standard in keeping with Reis *et al.*,[27] and Nguyen *et al.*:[28] No coughing aggravation, no increase in sputum volume, no fever or new dyspnea, no contractible movement in the chest or abdomen, and no new patchy exudative lesions based on lung imaging), with an age range between 50 and 75 years. Among them, we collected clinical information from 18 COPD patients on the acute exacerbation phase on admission during the same hospitalization.

Four-week stable criteria

Marco *et al.*[29] and GOLD [26] indicated a set of COPD stable criteria that included absence of acute exacerbation within four weeks, no coughing symptoms, no significant aggravation of sputum and shortness of breath, no fever or other symptoms of infection, clinical symptoms not affecting normal activity level, no acute drug application, and no

significant decline in short-term lung function, including FeNO. Although the assessment criteria are roughly the same as the clinical stable standard; however, the longitudinal time of assessment was different. Therefore, we enrolled 30 stable patients who met the criteria for a period of more than four weeks, and defined them as four-week stable in this study to distinguish from the clinical stability standard above.

A total of 20 healthy subjects, matched for gender and age, were included for comparison. To compare the metabolite levels in COPD patients at different ages, we additionally included 30 COPD patients aged 40–50 years who qualified as GOLD 3–4, who were compared with the 50–75 years old COPD patients with GOLD 3–4 who were already in the study as described above. To avoid the influence of patients' age on metabolites, we conducted a comparative analysis on patients aged 50–75 years. Moreover, we also performed an exclusive comparative analysis on COPD patients aged 40–50 years (The information of 40-50 COPD patients was used in basic information-Table 1 and COPD age group comparison-Table 3), who were not involved in analyses of the 50–70 years old.

Before sample collection, all subjects were kept in a resting state (quiet and relaxed state) for at least 30 min before sample collection.

Preparation and preservation of serum samples

The blood sample was collected from the participants at the resting and fasting (>6 h) state and it was then separated within 2 h at room temperature (1500 r/min or 1000 *g*, 10–15 min), and finally stored at −80°C for testing.

Serum sample preprocessing

The morning fasting venous blood from each subject was collected and centrifugated (3500 r/min, 3–5 min, normal temperature) to separate the serum. A total of 50 μL serum was collected from each sample to test for metabolites using methanol (chromatography) as the extraction agent. The whole extraction process was repeated three times. Then started the derivatization (5 μL in sequence to join 1-Hydroxybenzotriazole-HOBt, 5-(diisopropylamino)-Amylamine-DIAAA 5 μL, 2-(7-Azabenzotriazol-1-yl)-N, N, N′, N′-tetramethyluronium hexafluorophosphate-HATU 5 μL, finally used the capacity to 50 μL the acetonitrile). Upon completion, 45 μL were extracted into the internal cannula and sealed.

UHPLC-Q-TOF/MS (ultra-high performance liquid phase series quadrupole flight-time secondary mass spectrometry) analysis

Agilent 1290 Infinity LC system (UHPLC, Santa Clara, CA) and Agilent Eclipse XBD-C18 column (2.1 × 100 mm, 1.8 mol/L) were used for liquid chromatography separation. Mobile phase A and mobile phase B were water (containing 0.1% formic acid) and acetonitrile (containing 0.1% formic acid), respectively. The gradient was set as follows: 0–1 min, 15–23%B; 1–8 min, 23–33%B; 8–8.5 min, 33–35%B; 8.5–15.5 min, 35–47%B; 15.5–16 min, 47–50%B; 16–23 min, 50–85% B; 23–25 min, 85–95%B; and 25–28.9 min, 95%B.

Agilent 6550 UHD accurate-mass Q-TOF/MS (UHPLC, Santa Clara, CA) was used for mass spectrometry. Parameters: Dry gas temperature 250°C, dry gas flow 15 L/min, sheath gas temperature 300°C, sheath gas flow 11 L/min, sprayer pressure 20psig, capillary voltage 5000 V, and nozzle voltage 500 V.

Chromatographic peak analysis

The targeted positioning method was adopted in the study. According to the known metabolite M/Z value, the metabolite standard was first detected to confirm the retention time, and then the target chromatographic peak area was confirmed by testing the sample against the retention time. The peak separation degree was highest at 30–35 min. Qualitative Analysis of MassHunter Acquisition B 05.00 (UHPLC, Santa Clara, CA) was used for spectrum analysis.

Data analysis

The categorical variable was expressed as frequency, and Fisher exact test was used to test for statistical differences. Continuous variables were expressed as median, upper, and lower ranges (IQR). The statistical differences between groups were analyzed using a non-parametric test (Mann–Whitney test). Spearman correlation coefficient was used to assess correlation. We considered $P< 0.05$ to be statistically significant for any unrelated variables. The order of magnitude of chromatographic peak area was significantly different in some metabolites. In order to make a clearer comparison, we standardized the data ($\frac{X-\mu}{\sigma}$, where X is the peak metabolite variable, μ is the mean value of the variable, and σ is the standard of deviation). In the comparison process of homologous metabolites, we used the chromatographic peak area for direct comparison. Data analysis and charting were performed using R (Bell Laboratories Version 4.0.0), GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, US) and IBM SPSS (Windows Statistical Version 22.0, IBM Corp, Chicago, IL, USA).

Results

Characteristics of participants

The number of white blood cells and the CRP indicator of inflammation were significantly increased in the COPD patients when compared with the healthy controls, with no differences in the BMI between the two groups. COPD patients had a higher smoking index and were more likely to smoke (Table 1).

Mechanism of energy metabolism and overall comparison of metabolites

We collated the metabolic pathways involved (Figure 1) and compared the metabolites that are most closely related to the energy cycle in the stable period (clinical stability and four weeks stable period) and the healthy control. The results demonstrate that the increasing trend of anaerobic glycolysis in COPD patients is more prominent when both

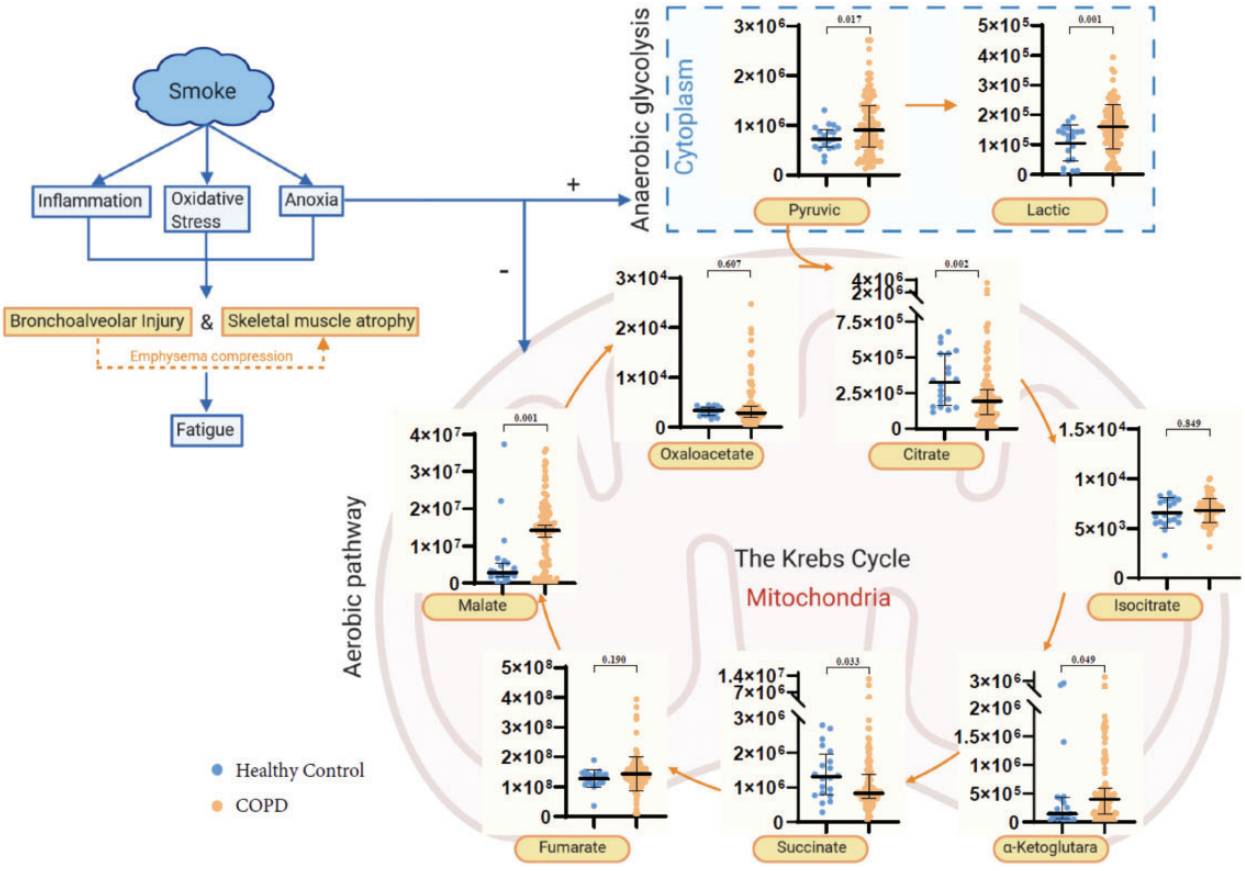

Figure 1. Analysis of the mechanism of energy metabolism cycle in COPD patients. The metabolite content value of COPD ($n = 110$) and healthy control group ($n = 20$) is shown in the figure. For comparing of same metabolites in the two groups, the metabolite content was measured using chromatographic integral peak area data instead of peak ratio data. Horizontal comparisons between different metabolites cannot be made directly due to the different orders of magnitude. Nonparametric analysis (Mann–Whitney) was used for statistical analysis. The pyruvic-lactic pathway is carried out in non-mitochondrial anaerobic conditions, which is defined in studies as the anaerobic pathway or anaerobic glycolysis. In the study, the TCA cycle pathway is in the mitochondria, and is defined as the aerobic pathway. (A color version of this figure is available in the online journal.)

groups of patients are in the resting state. Due to long-term smoking of COPD patients, the CO contained in the smoke significantly reduces the oxygen-binding capacity of hemoglobin, in keeping with previous reports.[30] In light of results demonstrating that oxidative stress and chronic inflammatory response damage the lung parenchyma, and result in respiratory and motor muscles' atrophy to different degrees, further reducing oxygen uptake capacity,[31,32] we hypothesized that this is one of the reasons leading to the non-mitochondrial production of metabolic energy by the anaerobic pathway.

As shown in Figure 1, when starting with citric acid, COPD patients have an altered TCA cycle compared with the healthy control group, demonstrating preference for anaerobic metabolism. To compare the difference between aerobic (TCA cycle) and anaerobic glycolysis, we made a general comparison after the standardization of all metabolites in the two pathways. The comparison of aerobic vs. anaerobic standardized mean parameters in the healthy control group were −0.056 vs. −0.034 ($P > 0.05$), indicating that there are no differences between the aerobic and anaerobic pathways in the resting state of healthy controls. The comparison of aerobic vs. anaerobic standardized mean parameters in COPD patients was −0.159 vs. −0.006 ($P < 0.05$). Thus, unlike controls, the energy cycle tended to be anaerobic, and in comparison to healthy subjects, the overall level of the COPD patients' aerobic pathway was lower, and that the aerobic vs. anaerobic comparison was −0.056 vs. −0.159 ($P < 0.05$). For anaerobic glycolysis, the values in COPD patients were significantly higher than in healthy people −0.006 vs. −0.034 ($P < 0.01$).

Comparative analysis of the COPD patient population

Changes in the metabolic activity level of COPD patients were compared according to the GOLD grading.

Figure 2 shows that pyruvate and lactic acid levels are highest in the GOLD 4 COPD patients. For the TCA cycle, the metabolites do not show reverse cycling differences with the increase of hypoxia when compared with healthy subjects. However, as shown in Table 2, the energy cycle metabolites demonstrate significant differences between the GOLD 1–4 categories (all $P < 0.01$), indicating that the energy metabolism level of COPD patients correlates with disease severity.

Comparison of GOLD acute onset and stable stage. For further comparison, we analyzed the information of the 18 patients with COPD acute exacerbation (further clinical

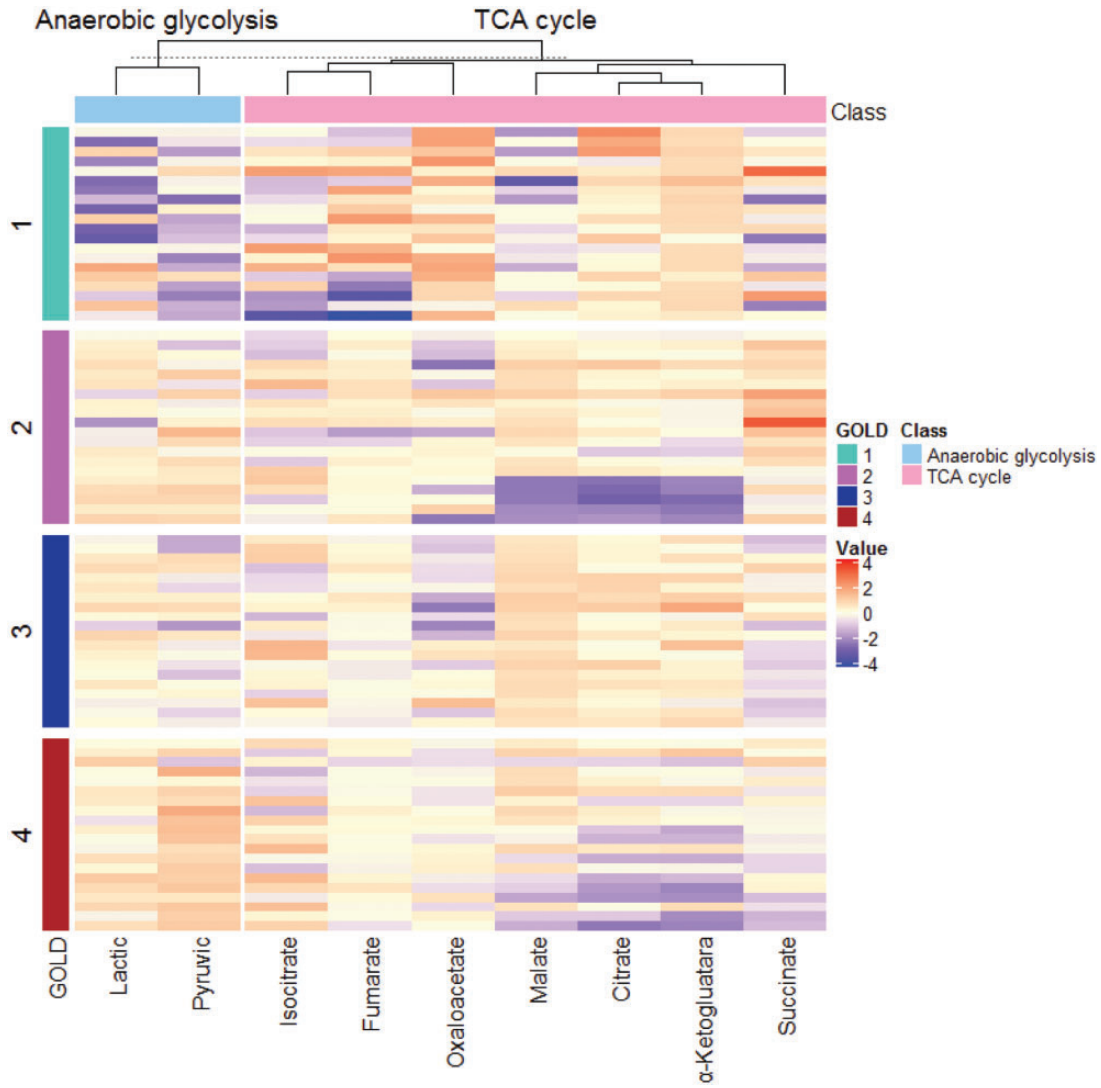

Figure 2. Heatmap of metabolite levels in COPD patients (Clinical stable period, $n = 80$) according to GOLD grading ($n = 20$ for each level). There was a significant difference in anaerobic glycolysis (Lactic and Pyruvic) and TCA cycle (Citrate, α-Ketogluatara, Oxaloacetate, Succinate, and Malate). The statistical method was the Kruskal–Wallis test. Because of the metabolite different order of magnitude in values, the data are standardized in the heat map using the $\frac{X-\mu}{\sigma}$ method (X is the peak metabolite variable, μ for the mean value of the variable, and σ is the standard of deviation). (A color version of this figure is available in the online journal.)

Table 1. Basic information of the participants of this study.

	Healthy control	COPD group	*P*
n	20	140	——
Sex, male/female	13/7	113/27	0.141
Age, years	52 (50.00, 64.75)	60.00 (53.00, 69.00)	0.056
BMI, kg/m^2	21.40 (18.50, 22.50)	19.40 (18.00, 22.05)	0.188
Smoke index	0	550.00 (225.00, 1050.00)	0.001
CRP, mg/dL	0.29 (0.13, 0.63)	9.26 (4.92, 14.28)	0.001
WBC, 10^9/L	5.49 (4.42, 6.21)	7.07 (5.65, 8.67)	0.001
Lymphcyte, 10^9/L	1.41 (1.30, 1.60)	0.75 (0.40, 1.25)	0.001
Neutrophile, 10^9/L	2.98 (2.37, 3.76)	4.37 (3.29, 5.86)	0.001
Monocyte, 10^9/L	0.25 (0.20, 0.30)	0.41 (0.26, 0.52)	0.009
Eosinophilic, 10^9/L	0.00 (0.00, 0.10)	0.01 (0.00, 0.18)	0.154
FeNO, ppb	–	22.00 (16.00, 33.75)	——
FEV1, L	——	1.35 (1.06, 1.94)	——
FEV1 pred, %	——	45.50 (37.28, 64.00)	——
FVC, L	——	2.89 (1.93, 3.50)	——
FVC pred, %	——	58.00 (48.00, 80.00)	——
FEV1/FVC	——	0.57 (0.46, 0.66)	——

Note: It should be noted that the data are presented with a median, therefore the smoking index showing 0 in the normal group, does not mean that no one smokes. The average was 9.
CRP: C-reactive protein; FEV1: forced expiratory volume in one second; FVC: forced vital capacity; Pred: prediction.

Table 2. Relative quantitative values of the metabolites.

	Citrate	Isocitrate	α-Ketogluatara	Succinate	Fumarate
GOLD 1	280,963 (207,466, 526,922)	6,016 (5,113, 7,267)	470,029 (436,684, 493,603)	797,968 (525,751, 1,502,068)	163,609,333 (79,253,087, 266,996,514)
GOLD 2	183,679 (24,824, 253,761)	6,505 (5,448, 7,572)	118,613 (17,983, 22,1351)	1,723,236 (958,589, 2,352,665)	148,593,599 (135,982,656, 161,390,248)
GOLD 3	262,118 (204,941, 420,180)	6,774 (5,811, 7,821)	304,838 (178,677, 492,505)	679,126 (483,345, 1,002,766)	127,104,517 (115,282,924, 142,195,930)
GOLD 4	102,591 (29,761, 266,119)	6,933 (5,601, 7,969)	84,242 (19,067, 176,249)	801,744 (559,955, 1,191,911)	133,445,197 (123,669,854, 144,972,015)
Statistic	17.88	2.54	33.84	20.22	6.03
P	0.001	0.468	0.001	0.001	0.11

	Malate	Oxaloacetate	Pyruvic	Lactic
GOLD 1	2,687,658 (589,079, 5,276,120)	9,142 (4,014, 15,023)	341,844 (250,720, 667,237)	97,024 (23,308, 215,680)
GOLD 2	1,262,9001 (1,502,792, 190,037,124)	2,437 (8,41, 3,496)	1,090,395 (713,105, 1,426,819)	158,513 (120,824, 177,039)
GOLD 3	19,723,638 (16,548,573, 25,057,067)	1,368 (9,20, 3,131)	634,869 (461,232, 1,028,257)	145,163 (1,24,575, 173,223)
GOLD 4	10,644,293 (1,451,906, 18,990,206)	2,367 (1,552, 3,343)	1,607,740 (1,302,137, 1,888,451)	159,121 (120,371, 192,866)
Statistic	23.91	29.21	38.41	17.63
P	0.001	0.001	0.001	0.001

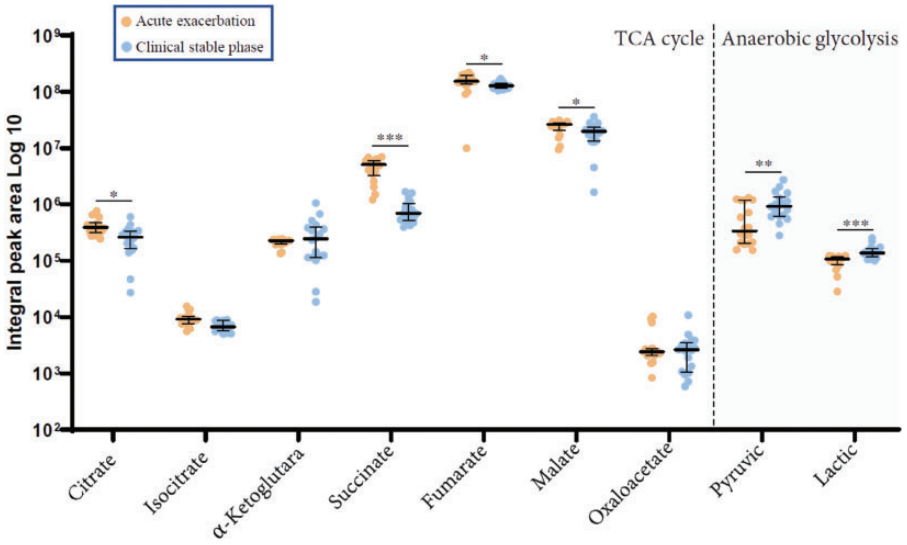

Figure 3. Comparative analysis of COPD patients ($n = 18$) during acute exacerbation vs. those who demonstrated clinical stability following COPD therapy. The statistical test adopted the Mann–Whitney test and the difference was presented as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. (A color version of this figure is available in the online journal.)

information in Supplementary Table 2). As shown in Figure 3, this group of COPD patients (GOLD 3–4) had corresponding changes in the TCA aerobic metabolism cycle that tracked with their exacerbation severity and was significantly higher than that of patients with clinical stability following hospital-based COPD therapy. However, their anaerobic metabolism level was significantly lower than levels in patients with clinical stability following COPD therapy (all $P < 0.05$). In the 18 patients, there was no significant difference in the aerobic vs. anaerobic pathway (whole course acute exacerbation + clinical stability) −0.055: −0.038 ($P > 0.05$) (Figure 4). However, there were significant differences in aerobic vs. anaerobic during acute exacerbation: −0.008 vs. −0.035 ($P < 0.05$), and during clinical stability: −0.047 vs. −0.003 ($P < 0.05$).

In addition, the difference in energy metabolism between the time point of clinical stability versus four-week stable period demonstrated differences in aerobic vs. anaerobic measures, which amounted to −0.093 vs. −0.056 ($P < 0.05$). Similarly, the parameters of anaerobic glycolysis (clinical stability vs. four-week stable period) amounted to −0.003 vs. −0.056 ($P < 0.05$). However, no correlation was observed between the two aerobic pathways.

Comparison of COPD in different age groups. In the absence of significant BMI differences, we compared the

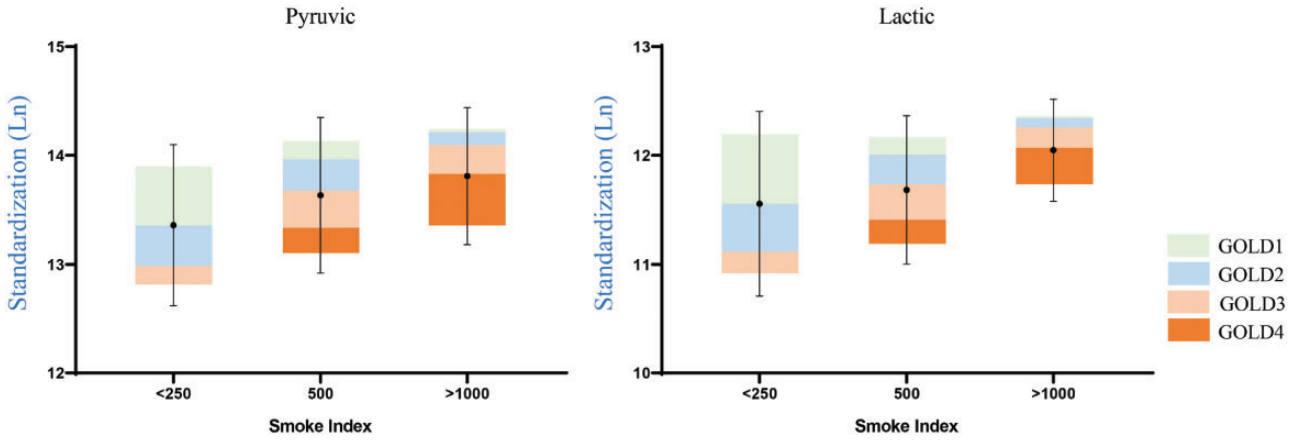

Figure 4. Association between smoking and the anaerobic pathway. The data of smoke index are collected from 140 COPD patients of this study that include clinical stable group, four-week stable group, and 40–50 COPD patient groups. The Mann–Whitney statistical method is adopted and shows a significant difference in <250 and >1000 smoke index of pyruvic and lactic group. $^{**}P < 0.01$, $^{***}P < 0.001$. Meanwhile, the proportion of patients with increasing smoking index was higher with the increase of GOLD grade. (A color version of this figure is available in the online journal.)

Table 3. Differential analysis of the differences in metabolic levels between the different COPD age groups.

Age	35–50	51–65	>65	*P*
Citrate	375,972 (333,375, 439,330)	238,925 (63,399, 334,124)	200,031 (151,142, 299,003)	0.001
Isocitrate	6,692 (4,152, 7,305)	6,489 (5,538, 7,899)	6,918 (5,460, 7,835)	0.198
α-Ketogluatara	439,659 (363,912, 550,148)	251,542 (61,623, 482,630)	167,429 (113,489, 394,310)	0.001
Succinate	696,406 (348,606, 829,340)	842,446 (669,952, 1,191,911)	966,372 (570,933, 1,896,617)	0.004
Fumarate	119,400,080 (103,157,208, 139,314,371)	140,545,866 (124,718,582, 158,899,600)	135,234,770 (120,553,859, 160,767,270)	0.017
Malate	20,079,419 (15,998,803, 24,086,870)	5,370,983 (1,412,507, 17,981,631)	16,654,705 (4760488, 20,786,000)	0.001
Oxaloacetate	2,017 (1,789, 2,274)	2,757 (1,636, 5,926)	2,391 (1,246, 3,831)	0.076
Pyruvic	727,170 (414,746, 775,189)	963985 (437,313, 1,494,262)	981,796 (556,114, 1,412,934)	0.017
Lactic	128,465 (92,982, 135,324)	155537 (94,247, 187,931)	150,542 (108,452, 183,044)	0.069

metabolic levels of the different age groups of COPD patients at clinical stability of GOLD 3–4 vs. resting condition (Table 3). There were significant differences in pyruvate, showing an upward trend, but no significant differences in lactic acid. The standardized mean parameters of aerobic in the 35–50 vs. 51–65 vs. >65) age groups amounted to −0.017 vs. 0.035 vs. 0.019, respectively ($P > 0.05$); and for anaerobic, they were −0.033 vs. 0.037 vs. 0.035, in the same age groups, respectively ($P > 0.05$).

Correlation analysis

The CO contained in cigarette smoke significantly affects patient oxygen intake, and therefore, is closely related to the anaerobic pathway. We also focused on the trend of pyruvate and lactic acid (Figure 4), revealing that the higher the smoke index, the higher the pyruvate and lactic acid levels, and the higher the GOLD grade, reflecting the severity of the disease.

Analysis of the correlation between aerobic and anaerobic metabolic pathways with pulmonary ventilation function and COPD patient symptoms demonstrated that the levels of pyruvate and lactic acid that represent the anaerobic metabolism pathways were significantly and negatively correlated with pulmonary ventilation function in COPD patients. Their levels were also closely correlated with the CAT comprehensive score, reflective of the patients' clinical manifestations (Figure 5 and Supplementary Table 1).

Discussion

Using the targeted metabolomics detection method for the analysis of aerobic vs. anaerobic energy metabolic pathways in patients with COPD, we uncovered that COPD patients have a significant increase in anaerobic metabolism at rest. Moreover, as some patients were given oxygen therapy to improve their pulmonary ventilation function, which, to a certain extent, affects the results of the study, we hypothesized that this difference would be even greater in patients who did not receive oxygen therapy. The higher the GOLD level, the more likely that the energy supply was originated through a mitochondria-independent anaerobic glycolysis. Patients admitted with acute exacerbation were mainly treated by therapies that promote the aerobic pathway, and patients who were clinically stable following hospital-based therapy were mainly treated by therapies that enhance the anaerobic pathway. This study did not find significant differences among different age groups. In addition, the correlation with the smoking index showed that the anaerobic pathway was associated with the hypoxia mechanism that is caused by smoking. The anaerobic pathway was also significantly associated with BMI and the CAT score.

Energy metabolism in COPD

The TCA and pyruvate-lactic pathways are the most important pathways of energy metabolism, and their

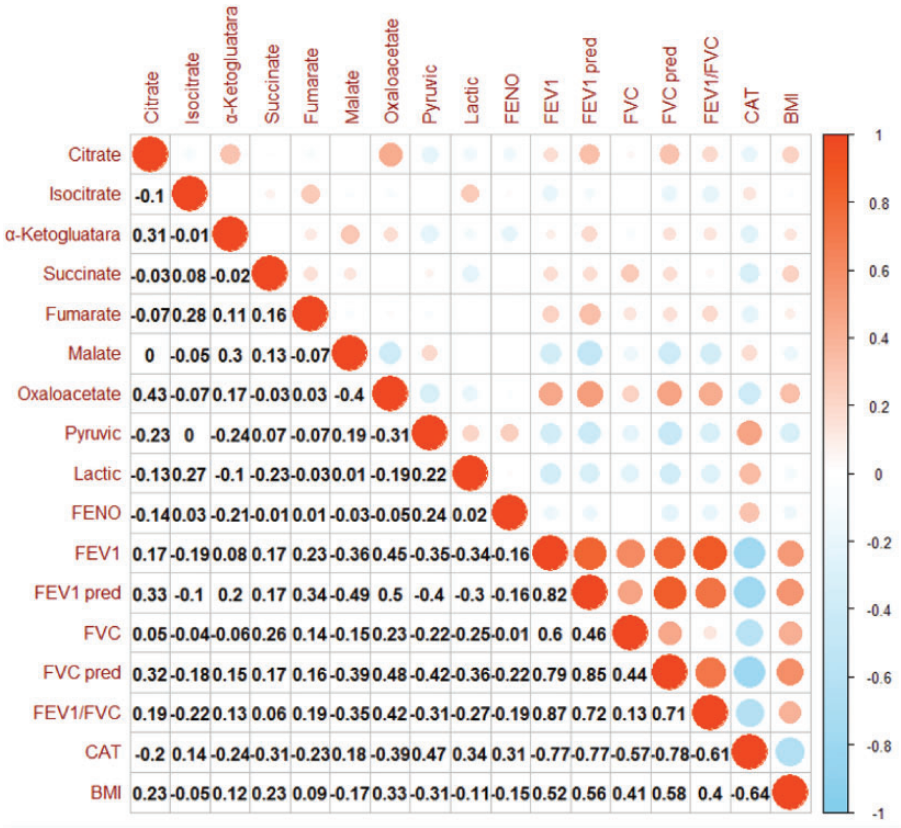

Figure 5. Comprehensive correlative analysis of energy metabolism in COPD patients, using the Spearman correlation method. The correlation heatmap shows significant correlation in lung function (FEV1 and FEV1/FVC), CAT, and BMI. Both lactic acid and pyruvate in the anoxylytic pathway, which represents anoxic degree, have also been shown to correlate with the above indicators (P all < 0.05), demonstrating a synchronous change in anoxic-inflammator-anoxic symptoms-nutritional status. (A color version of this figure is available in the online journal.)

corresponding aerobic and anaerobic mechanisms have been well characterized.[33,34] In this study, we used, for the first time, metabolomics to explore the energy metabolic mechanism in COPD patients with chronic hypoxia. Our results showed that the levels of pyruvate and lactic acid, and metabolites of the anaerobic pathway, were significantly increased in the COPD patient group compared with healthy subjects, which is likely related to the chronic hypoxia caused by smoking, lung parenchyma destruction, respiratory muscle injury, decline of exercise ability, and reduction of aerobic exercise. In addition, Hara *et al.*[19] proposed that oxidative stress activates the mechanism of mitochondrial autophagy degradation and inhibits the TCA cycle. Although there are several ways to utilize pyruvate, aerobic circulation is the most efficient way to consume pyruvate. When pyruvate consumption decreases, its accumulation leads to an increase in lactic acid level, which becomes the main source of biological energy and replaces energy that is produced through the normal pathway of oxidative phosphorylation. This process may also be one of the causes of the enhanced oxidative stress observed in COPD.[35,36] In reference to the TCA cycle metabolism, COPD patients show a distinct pattern compared to normal subjects, which may result from the accumulation of the TCA cycle intermediates, malic and fumaric, that are unable to enter the next circulation consumption that is used by the body. This could be exploited to promote the TCA cycle efficiency in COPD patients by regulating the activity of the intermediate enzymes fumarase and malic dehydrogenase.

The GOLD grading is an important standard for assessing COPD patient severity.[26] In this study, we found that the aerobic and anaerobic energy metabolic pathways show significant differences with the aggravation of the disease. The GOLD grading is mainly defined according to the pulmonary ventilation function that is reflected by FEV1, with oxygen intake being the most critical factor affecting the energy circulation efficiency.[37] Therefore, we believe that the higher the anaerobic/aerobic ratio, the lower is the body's productivity, with the patients more likely to develop symptoms, such as fatigue, dyspnea, and polypnea.

Under the dual effects of long-term low efficiency of energy supply (high anaerobic/aerobic relative ratio) and emphysema compression, the patients' decreased respiratory muscle motor function directly affects the pulmonary ventilation function.[38,39] Jaitovich *et al.*[40] showed that decreased ATP production is one of the causes of skeletal muscle atrophy in COPD patients. In addition, metabolic dysfunction, nutritional intake, and sensitivity to Ca2+ intake affect normal muscle strength and endurance, with lower limb disorders being more obvious than those in the upper limbs. CAT scores included not only symptoms, but also the patient's assessment of walking, climbing and physical activity.[41] To compare the effects of decreased

respiratory muscle strength on pulmonary ventilation, we also compared pulmonary function parameters while evaluating CAT. We found that anaerobic metabolites positively correlate with CAT score, and with the pulmonary function parameters, FEV1 pred, and FEV1/FVC ratio. This can be partly explained by the reduced energy efficiency of the body that causes the decline in respiratory muscle strength and affects the pulmonary ventilation function. However, this process is comprehensive, and the pressure of excessive lung inflation will also lead to the compression and atrophy of respiratory muscles. Using an *in vivo* model of smoking-induced emphysema, Mortaz *et al*.[18] found that the level of ATP in the alveolar lavage fluid of smokers was higher than that of non-smokers, which meant that the high level of extracellular free ATP provides free energy to neutrophils and encourages them to extravasate, accumulate, and secrete inflammatory factors and matrix metalloproteinases that trigger lung tissue destruction. The unbalanced energy fuel distribution could further reduce the effective energy supply of respiratory and skeletal muscles. Suggested by this study, inefficient energy supply and increased inflammatory energy consumption affect the homeostatic circulation. In addition, patients with decreased appetite and reduced nutrient intake have lower BMI, and worse energy circulation.

Energy metabolism in acute exacerbation

Some COPD patients develop acute exacerbations due to viral or bacterial infections, specific environment exposures, or other predisposing factors.[42] In the short-term, they have symptoms, such as an aggravated cough, increased sputum, aggravated dyspnea, and a significant decrease in the pulmonary ventilation function.[42] During the acute exacerbation period, the patients' aerobic metabolism was found to be higher than that of the anaerobic metabolism, while the anaerobic metabolism was higher than that of the aerobic metabolism in clinically stable patients following hospital-based treatment. However, this does not mean that energy consumption increases in patients with acute pulmonary exacerbations. Celli *et al*.[37] proposed that an acute myocardial ischemic injury that is caused by an acute kidney injury would lead to the inhibition of oxidative phosphorylation and a decrease in energy level, which clearly contradicts the results of this study. Musazzi *et al*.[43] proposed in an animal study that acute state stimulation would cause a rapid increase in metabolic level and induce rapid state changes in the body. Hall *et al*.[44] proposed that the energy intake and consumption of the human body are in a dynamic energy balance, which can be broken by external factors, such as diet, exercise, drugs, and diseases. Therefore, we speculate that the persistent inflammation, the BMI level progressive drop,[24] and the body's increased energy consumption in COPD patients result in energy imbalance, especially, for patients with acute exacerbation chronic obstructive pulmonary disease (AECOPD) GOLD3–4, who have an activation of their stress state. In the acute phase, this is characterized by symptoms such as wheezing, sweating, or fever, the energy consumption may significantly increase, and the TCA aerobic cycle also increases compared to anaerobic glycolysis, causing an acceleration of the energy imbalance. After treatment, the proportion of the anaerobic pathway exceeded that of the aerobic after reaching a clinically stable state. Compared with COPD patients who have been stable for a four-week duration or more, the lactic acid level was higher. We also observed an increase in the anaerobic metabolic pathway of patients in the acute phase that resulted in the accumulation of lactic acid and pyruvate in this short-term and high-energy stage. The increase in the anaerobic and aerobic metabolic pathway maintains the acute phase of the body's high energy consumption state. Unfortunately, we did not track the data in the clinically stable patients following hospital-based treatment, nor in the patients who had been stable for at least four weeks and, therefore, we were unable to do a longitudinal level analysis.

Aging and smoking

Different age groups have different energy metabolism profiles,[45] and therefore, we compared the metabolic levels of patients with grade GOLD 3–4 COPD at different ages. In the study, differences in individual metabolites, such as citric acid, succinic acid, and pyruvate were found, indicating that the productivity and the utilization efficiency of pyruvate in elderly patients were lower than that in middle-aged patients. However, the overall analysis of the anaerobic vs. aerobic metabolic pathways did not show significant differences, which may be due to the limited sample sizes of the study, or the FEV1 pred $< 70\%$ of the COPD patients, as the impact of chronic hypoxia on energy metabolism of the body is higher than that of the age factor.

Smoking is the main cause of COPD, due to the presence of CO in the smoke that affects the hemoglobin oxygen exchange capacity. Moreover, a long-term smoke exposure causes direct and indirect damage to cells and organelles, including mitochondria, the site of aerobic metabolism.[19,46] In this study, we show that a higher smoking index has higher pyruvate and lactic acid levels, and that the proportion of patients with grade GOLD 3–4 is also higher. Under smoke stimulation, mitochondrial DNA damage, mutation, and deletion can affect its normal function or activate autophagy.[19] Prakash *et al*.[47] proposed that mitochondria play an important role in energy production that is required for a range of physiological responses, such as calcium regulation, airway contraction, gene and protein regulation, and oxidative stress response. Therefore, mitochondrial dysfunction has undoubtedly a huge impact on COPD disease progression. Different cell types have distinct requirements for mitochondrial regulatory function, especially for type II alveolar epithelial cells with stem-like function, whose differentiation and repair of lung epithelial cells mainly depend on mitochondrial oxidative phosphorylation for energy supply.[48] In conclusion, our analysis of mitochondrial dysfunction showed that COPD patient metabolism is unable to properly use pyruvate aerobic metabolism for energy consumption, which can lead to an imbalance in lung homeostasis resulting in parenchymal

lung injury with impaired repair, emphysema, persistent airflow limitation, chronic wheezing and shortness of breath that further aggravate the fatigue and energy consumption of the gradually atrophic respiratory muscles.

Conclusions

For the first time, we used metabolomic profiling to investigate the aerobic and anaerobic energy metabolism of COPD patients. This study shows that their metabolism is associated with anaerobic glycolysis when compared with that of normal subjects. Patients with higher GOLD classification levels had more severe conditions, higher pyruvate and lactic acid levels, and a reduced efficiency energy supply. For AECOPD patients, their metabolism may use the aerobic pathway for a short period of time, due to secondary onset of the acute state, but the anaerobic pathway may also become simultaneously hyperactive, resulting in the accumulation of pyruvate and lactic acid. After treatment, the level of anaerobic metabolites is still higher than that of the aerobic pathway. Limited by the study sample size, no difference in energy pathways between different age groups was found. In conclusion, through comprehensive evaluation, we highlighted the significance of high-propensity anaerobic and low-efficiency energy supply pathways in lung injury and linked it to the energy-inflammation-lung ventilatory function and the motion limitation mechanism in COPD patients. Targeting these pathways in COPD may promote the development of effective treatment for this devastating disease.

AUTHORS' CONTRIBUTIONS

Conception and design of the research: BS and LZ; Drafting the manuscript: MX and YZe; Samples collection and detection: RL and YZe; Acquisition of data: ZH, YL, and YZh; Statistical analysis: HC, XZ and TZ; Analysis and interpretation of data: HHa, HQ, HHu and PZ.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

This study was approved by the Ethics Committee of Guangzhou Medical University, Ethics No.2017–92. All the participants signed the informed consent form.

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DATA AVAILABILITY

The metabolomics data has been deposited to the EMBL-EBI MetaboLights database (MTBLS2502, www.ebi.ac.uk/metabolights/MTBLS2502).

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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