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# Correlation between gut microbiota and metabolomics under intermittent hypoxic conditions

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## Abstract

**Objective** This study investigates the role of gut microbiota and metabolites as biomarkers in the diagnosis and assessment of Obstructive Sleep Apnea (OSA) by analyzing the correlation between microbiota and metabolomics in the host's gut under conditions of chronic intermittent hypoxia (CIH).

**Methods** We analyzed the composition and function of gut microorganisms and metabolites in OSA and CIH mouse models using 16S rRNA sequencing combined with targeted metabolomics to evaluate the association between clinical indicators and severity of OSA and gut flora and metabolites.

**Results** Compared with normal controls, we found: (1) The apnea hypoventilation index (AHI) and fasting blood glucose were higher in the moderate and severe OSA groups, the eosinophilia score (EP) and neutrophil percentage were significantly higher in the severe OSA group, and the percentage of lymphocytes in the blood count and platelet pressures were lower in the moderate group ( $P < 0.05$ ). (2) The OSA and CIH groups showed an increase in the abundance of Firmicutes and *Tenotrophomonas*, and a decrease in the abundance of Bacteroidota and *Ligilactobacillus* ( $P < 0.05$ ). (3) The OSA and CIH groups had elevated Nervonic acid and Erucic acid and decreased Arachidonic acid ( $P < 0.05$ ). In addition, correlation analysis showed that: There was a negative correlation between *Coprobacillus* and percentage of lymphocytes, and Bacteroidota and EP and AHI; and a positive correlation between *Lactococcus* and Neutrophils, Arachidonic acid and width of erythrocyte distribution, and Erucic and Neuronic acids and AHI ( $P < 0.05$ ).

**Conclusion** The CIH condition can lead to microbial and metabolite alterations, which correlate with disease severity and clinical indicators, and is expected to be a new biomarker for the diagnosis and assessment of severity of OSA.

**Keywords** Chronic Intermittent Hypoxia, Gut microbiota, Targeted metabolomics, Biomarkers, Disease Evaluation

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## Introduction

Obstructive sleep apnea (OSA) is closely related to stroke, Alzheimer's disease, coronary heart disease, hypertension, renal disease, metabolic dysfunction, etc., and is a primary disease [1–3]. The pathogenesis of OSA involves a variety of factors including anatomical and structural abnormalities, decreased muscle tone, and neurological control disorders. Chronic intermittent hypoxia (CIH) is a pathophysiological alteration specific to OSA, which can lead to oxidative stress, inflammation, sympathetic hypersensitivity and endothelial dysfunction [4]. According to statistics, there are currently 936 million adults aged 30–69 with OSA worldwide [5].

The diagnosis of OSA was mainly based on polysomnography (PSG), and the severity grading was mainly based on the Apnea Hypopnea Index (AHI) on the monitor, more and more studies have combined microbiomics and metabolomics to assess the severity of OSA at the level of species, genes, and metabolites altogether. The gut microbiota of patients with OSA differed from that of the normal group, particularly in the abundance of certain metabolites. The CIH model mice with elevated systemic inflammation have significantly altered intestinal flora, especially an increase in Bacteroidota and a decrease in *Bifidobacterium* [6]. The metabolic pathway predictions indicate that changes in the CIH gut microbiota are primarily associated with bile acid and fatty acid metabolism [7]. CIH modulates the abundance of intestinal microorganisms (e.g. *Akkermansia muciniphila*, *Clostridium Prazmowski*, *Lactococcus*, and *Bifidobacterium*) and functional metabolites (e.g. tryptophan, free fatty acids, branched-chain amino acids, and bile acids) and affects adipose tissue and liver lipid metabolism, as well as the level of lipid deposition in tissues and peripheral blood [7]. Johanna Roche et al. [4] explored the enrichment of Arachidonic acid, Docosahexaenoic acid, and 11-Z-eicosenoic acid as well as the reduction of Stearic acid, 5-hydroxyindoleacetic acid, Gluconic acid, and  $\alpha$ -scopoletin Deoxycholic acid in the feces of patients with OSA, using non-targeted gas chromatography-time-of-flight mass spectrometry. Moreover, the metabolism of rats was induced by CIH and significantly enhanced the body's inflammatory response, activating metabolic pathways involving microorganisms, including the biosynthesis of tropane, piperidine, and pyridine alkaloids, the proteasome, and the biosynthesis of type II polyketide products [8].

In this study, we analyzed the changes in intestinal microbiota and metabolomics of patients with OSA and CIH model mice, using Gas Chromatography-Mass Spectrometry (GC-MS), and performed correlation analysis with clinical indicators, to explore the value of new biomarkers for disease diagnosis and assessment of disease severity under CIH conditions.

## Materials and methods

### Study subjects

We randomly selected 60 patients, aged 18–80 years ( $44.83 \pm 15.53$ ), who were examined by PSG in outpatient clinics and hospitalization in Guizhou Provincial People's Hospital from December 2022 to December 2023. The patients completed the Epworth Sleepiness Scale (ESS) within 24 h of admission, which is distributed by the physician and collected on the spot. The ESS was completed prior to the PSG. The ESS is an 8-item questionnaire that measures daytime sleepiness. Each item is scored on a 4-point scale (response range, 0–3) to examine the likelihood of falling asleep under 8 different circumstances. The higher the score, the greater the degree of daytime sleepiness [9]. PSG examinations were conducted for 7 h per night, and total bedtime (TIB), total sleep time (TST), sleep staging, AHI, and oxygen saturation below 90% (T90) were recorded. According to the standards of the American Academy of Sleep Medicine,  $AHI < 5$  was defined as normal, and  $AHI \geq 5$  was defined as OSA, in which  $5 \text{ times/h} \leq AHI < 15 \text{ times/h}$  was considered as mild OSA,  $15 \text{ times/h} \leq AHI < 30 \text{ times/h}$  was considered as moderate OSA, and  $30 \text{ times/h} \leq AHI$  was considered as severe OSA [10]. Overnight PSG was conducted for each participant between 10 PM and 6 AM using the Embla N7000 system (Natus Medical Incorporated, Orlando, FL, USA). The process and manual scoring were performed by skilled technicians supervised by experienced sleep physicians per the American Academy of Sleep Medicine (AASM) manual 2.5 for the scoring of sleep and associated events in 2018 [11]. Apnea was defined as airflow cessation (a reduction in airflow of  $\geq 90\%$ ) for  $\geq 10$  s. Hypopnea was defined as a reduction in airflow of  $> 30\%$  for at least 10 s accompanied by a  $\geq 3\%$  decrease in oxygen saturation. The exclusion criteria included having an acute infection within the past week; chronic gastrointestinal disease and having received antibiotics or probiotics within the last 8 weeks; having chronic respiratory disease such as asthma, COPD, heart failure; oncology patients; hepatic/kidney disease, neurologic disease, or psychiatric disorders; previous treatment with positive airway pressure ventilation for sleep apnea; and suffering from severe metabolic diseases, such as diabetic ketoacidosis, hypothyroidism, hyperthyroidism. The study protocol was approved by the Ethics Committee of Guizhou Provincial People's Hospital [2024–123], and all subjects signed an informed consent form.

### Study materials

Twenty healthy SPF-grade C57BL/6 mice (male, 6–7 weeks old, 19–20 g) were purchased from Changsha Tianqin Biotechnology Laboratory Animal Co. Ltd (Hunan, China, License No.: SCXK (Xiang)2024–0021), and were subjected to a 12-h light/12-h dark cycle at

22–24 °C, and allowed to have free access to water and food for acclimatization feeding for 1 week. After a week, the mice were randomly divided into a normal control group (NC group) and a CIH group, with 10 mice in each group. Mice in the CIH group were placed in a hypoxic chamber, and nitrogen and oxygen were given to the closed chamber apparatus by cycling to ensure that the oxygen concentration was maintained at 8% for 3 min each time, for 30 s; the oxygen concentration was restored to 21% for 120 s afterward, and then maintained at 8% for 30 s; this cycle was repeated every day from 8:00 am to 16:00, for 8 h of modeling over 28 days. The NC group was fed in air, and all animal experiments complied with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guideline. Mice were euthanized by intraperitoneal injection of an overdose of sodium pentobarbital (dose: 280 mg/kg). After injection, death was verified by observing pupil dilation, loss of corneal reflexes and cardiac arrest (confirmed by thoracic palpation). All experimental manipulations followed the protocol approved by the Animal Ethics Committee of Guizhou Medical University (Approval No: No2403650) and were in accordance with the Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011). Flow chart of the experiment (Fig. 1).

### 16S rRNA gene sequencing

Feces were collected from all clinical investigators in sterile containers early in the morning (7:00–8:00 a.m.) on the day following PSG monitoring, and fecal samples were taken from C57BL mice undergoing CIH modeling on the 29th day of fasting for 12 h, and stored at −80 °C. Genomic DNA was extracted from samples using the CTAB method, followed by quality assessment (concentration and purity) via 1% agarose gel electrophoresis. DNA was diluted to 1 ng/μL for downstream amplification. The hypervariable V3–V4 regions of bacterial 16S rRNA were amplified using barcoded primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGGTATCTAAT-3') with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). PCR conditions included initial denaturation at 98 °C for 1 min, 30 cycles of denaturation (98 °C, 10 s), annealing (50 °C, 30 s), extension (72 °C, 30 s), and final elongation at 72 °C for 5 min. Amplicons were verified by 2% agarose gel electrophoresis, pooled in equimolar ratios, and purified using the Universal DNA Purification Kit (TianGen). Sequencing libraries were prepared with the NEB Next® Ultra DNA Library Prep Kit (Illumina), quantified using the Agilent 5400 system, and sequenced on an Illumina platform to generate 250-bp paired-end reads.

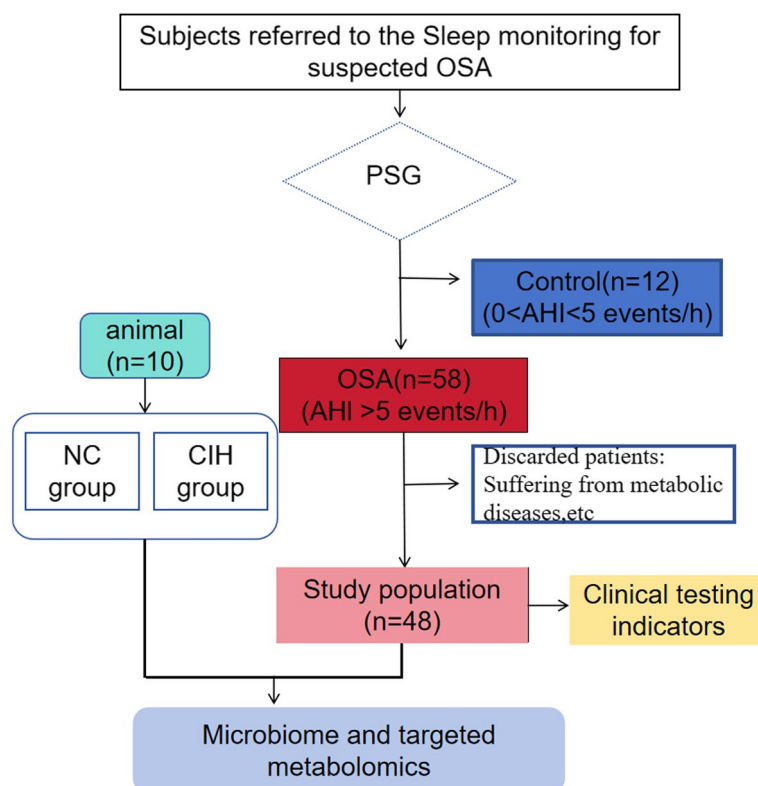


**Fig. 1** Flowchart of the study

### Targeted metabolomics analysis

This study advanced the identification of meaningful biomarkers for OSA by using fecal targeted metabolomics to compare metabolites from patients with OSA to metabolites from patients without OSA, and from CIH mice to NC mice. At the end of PSG monitoring and modeling, early morning fecal samples were collected in sterile containers to ensure that no food or water had been consumed prior to collection. Micromeritics Biocloud Bioassay for Targeted Metabolites. An appropriate amount of biological samples was taken and added to chloroform solution for vortex extraction, after stratification by 0.9% NaCl solution, the chloroform layer was obtained by centrifugation at 3500 rpm for 15 min, and the combined multiple dichloromethane extracts were concentrated by nitrogen blowing. The methyl esterification derivatization was completed by adding 5% sulfuric acid in methanol solution at 80 °C in a water bath for 2 h. After extraction with hexane, the extract was purified by centrifugation at 2000 rpm, and finally dissolved in isooctane by nitrogen blowing. The mass spectrometry was performed on an Agilent 7820 gas chromatography system coupled with a 5977 quadrupole mass spectrometer on a CP-Sil 88 column (100 m × 0.25 mm × 0.25 µm) with a helium carrier gas at a flow rate of 1.0 mL/min, and the programmed temperature increase was from 100 °C (5 min) to 240 °C (15 min) at 4 °C/min. The mass spectrum was performed using an electron bombardment ion source (EI) with a scanning range of  $m/z$  30–550 and single-ion monitoring (SIM) mode detection. Quantification was performed by external standard method to establish a standard curve, quality control (QC) samples were inserted into the analytical sequence and the RSD (< 30%) was calculated, and the data were subjected to peak identification, retention time correction and concentration calculation by Quant-My-Way software, and finally, the content of the solid samples was calculated by the formula  $(C \times V \times F)/M$  (µg/mg) [12].

An Agilent 7820 gas chromatography coupled to a 5977 quadrupole mass spectrometry system was used, operating in EI mode, with an inlet temperature of 260 °C, a quadrupole temperature of 150 °C, and a scanning mode of SIM, with a mass scanning range of  $m/z$  30–550. The chromatographic separation was performed using a CP-Sil 88 capillary column (100 m × 0.25 mm × 0.25 µm), the carrier gas was high-purity helium (flow rate 1.0 mL/min), the injection volume was 1 µL, the split ratio was 10:1, and the column temperature program was 100 °C initial temperature maintained for 5 min → increased to 240 °C at 4 °C/min → maintained for 15 min. The data were collected by Agilent MassHunter workstation, and the raw files were analyzed by Quant-My-Way software for peak identification, retention time calibration and

quantitative analysis, and the stability of the system was verified by inserting QC samples (RSD < 30%) [13, 14].

### Blood sample collection and biochemical analysis

On the night of PSG, clinical data including age, height, weight, body mass index (BMI), and neck circumference were collected from all participants. Fasting venous blood samples were drawn via standardized venipuncture between 7:00 AM and 8:00 AM the following morning and immediately aliquoted into three specialized vacuum tubes: EDTA-anticoagulated tubes (BD Vacutainer®) for complete blood count analysis, serum separation tubes (BD Vacutainer®) for lipid profiling, and gel separator tubes (BD Vacutainer®) for glucose measurement. All specimens were transported at 4 °C to the Clinical Laboratory of Guizhou Provincial People's Hospital and processed within 2 h. Complete blood counts were analyzed using a Sysmex XN-9000 automated hematology system. For lipid profiling, serum samples were centrifuged at  $3,000 \times g$  for 10 min (Eppendorf 5424R centrifuge) and analyzed on an Abbott C16000 biochemical analyzer using homogeneous assay kits (Cat. 9P02.29) for HDL-C, direct surfactant removal method kits (Cat. 9P02.30) for LDL-C, enzymatic reagent kits (Cat. 9P02.26) for total cholesterol, glycerol phosphate oxidase kits (Cat. 9P02.27) for triglycerides, and uricase method kits (Cat. 6S51.37) for uric acid. Plasma glucose levels were determined via hexokinase method (Cat. 3L82-42) on the same analyzer. All procedures strictly complied with Chinese national standards GB/T20468-2006 (Guidelines for Internal Quality Control of Clinical Laboratory Quantitative Measurements) and WS/T641-2018 (Internal Quality Control for Clinical Quantitative Testing), with daily calibration using Bio-Rad Liquichek™ controls and participation in the CNAS CL02:2023 external quality assessment program, maintaining intra-assay coefficients of variation (CV) below 3% for all parameters.

### Joint analysis of microbiomics and metabolomics

Microbiomics and metabolomics in the gut of patients with OSA and healthy individuals were correlated using heatmaps and network analysis in the BioinCloud tool (<https://www.bioincloud.tech/>).

### Statistical analysis

The statistical data were expressed as mean ± standard deviation. GraphPad Prism 10 and SPSS 22.0 (IBM, Armonk, NY, USA) one-way analysis of variance (ANOVA) and two-way ANOVA were used to assess the differences in clinical indicators among the four experimental groups. Group comparisons were performed by Student t-test and  $P < 0.05$  was used for statistical significance. In sequencing data, the Wilcoxon rank sum test was used to assess the microbial diversity index

between groups in fecal samples ( $P < 0.05$ ). The False Discovery Rate (FDR) method was used to correct  $p$ -values for multiple hypothesis tests to assess whether there were significant differences in species diversity between groups. The Wilcoxon rank sum test was used to compare bacterial abundance at the phylum and genus level between the two groups, where  $P < 0.05$  was used as the threshold for significant differences. Pearson correlation analysis was used to calculate Pearson correlation coefficients between species. A nonparametric Kruskal–Wallis rank sum test was used to screen for species with significantly different relative abundances between taxa. Finally, Linear Discriminant Analysis (LDA) was used to assess significantly different species ( $|LDA| > 2$ ,  $P < 0.05$ ). \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ ; ns, not significant.

## Results

### Clinical characterization of participants in the study

The 60 participants were divided into 12 in the control group, 8 in the mild OSA group, 10 in the moderate OSA group, and 30 in the severe OSA group according to the PSG monitoring results. There were no statistically significant differences between the groups in terms of age, gender, and BMI. Compared with the NC group, there was a significant difference in AHI in the moderate and severe OSA groups ( $P < 0.05$ ); EP was significantly higher in the severe OSA group ( $P < 0.05$ ); fasting blood glucose (FBG) was significantly higher in the moderate and severe OSA groups ( $P < 0.05$ ); the percentage of lymphocytes (LY) was decreased in the severe OSA group, and neutrophils (NE) were elevated ( $P < 0.05$ ); the moderate group had decreased platelet corpuscle pressure (PCT) ( $P < 0.05$ ) (Table 1).

### Microbial community composition

#### At the phylum and genus level, the composition of gut microorganisms varied significantly

Based on the results of species annotation, the top 20 species in terms of maximum abundance at the phylum and genus levels in both groups were selected to generate relative abundance bar charts. Compared to the NC group, at the phylum level, the relative abundance of gut microorganisms such as Firmicutes and Verrucomicrobiota was increased, whereas the relative abundance of Bacteroidota and Actinobacteriota was reduced in patients with OSA (Fig. 2A), and at the genus level, the relative abundance of gut microorganisms such as *Escherichia\_710834*, *Blautia*, and *Tenotrophomonas\_A\_615274* in patients with OSA was also all increased, while that of *Coprobaclaceae*, *Bacteroides* and *Lacticaeibacillus casei* decreased significantly (Fig. 2B).

Similarly, in CIH model mice, the relative abundance of Firmicutes and Verrucomicrobiota was increased

compared to the NC group, whereas that of Bacteroidota and Actinobacteriota was decreased (Fig. 2E). At the genus level, *Escherichia\_710834*, *Blautia*, and *Tenotrophomonas\_A\_615274* showed increased abundance, while *Coprobaclaceae*, *Bacteroides*, and *Lacticaeibacillus casei* exhibited significant decreases (Fig. 2F). These results demonstrate that the gut microbial composition of patients with OSA differs significantly from the NC group at both phylum and genus levels, with a general increase in Firmicutes abundance and decrease in Bacteroidota abundance ( $P < 0.05$ ), consistent with previous findings [15].

#### CIH may lead to imbalances in the intestinal flora, affecting host metabolism, immune function and overall health

The results of the species evolutionary tree with heat map suggested that seven phyla had low relative abundance in patients with OSA, with the Firmicutes\_A having a high percentage of human gut microorganisms, totaling 28, and being a dominant group. This was followed by the Bacteroidota, with a total of 8; and the Firmicutes, with 7 (Fig. 2C). The results of the Venn diagram suggested that there were 661 co-expressed OTUs in patients with OSA and NC, with 2,169 specifically expressed OTUs in patients with OSA and only 365 specifically expressed OTUs in NC group (Fig. 2D and E). This suggests the presence of more microbial changes or specific gene expression in the OSA state.

The species evolutionary tree with heatmap shows 10 phyla in the mouse gut microbes, of which the Firmicutes\_A and the Bacteroidota have a high percentage of mouse gut microbes, both with 17 each, which belong to the dominant group. The Firmicutes\_A has a high relative abundance in the CIH model mice, and the Bacteroidota has a relatively high abundance of UBA7173 in the NC group (Fig. 2G). The Venn diagrams show that the CIH group specifically expresses 962 OTUs, 532 OTUs were specifically expressed in the NC group, and 251 OTUs were coexpressed in the two groups (Fig. 2H). The CIH model mice had a higher number of microbiota or changes in gene expression.

#### Kruskal Wallis/ANOVA/LEfSe/DESeq2 analysis

The results of the DESeq2 volcano plot showed that 3 microorganisms were down-regulated in patients with OSA compared to the NC group, namely, *Chrysobacterium\_796* patients with OSA 614, *Bifidobacterium\_388775*, and *Clostridium\_P*; and 23 species were up-regulated, which mainly contained *Tenotrophomonas*, *Faecimonas*, *Clostridium\_T*, and so on (Fig. 2I). The results of the LDA histogram of the LEfS analysis and the cladogram results showed that the abundance of *Coprobaclaceae*, *Blautia\_A*, etc. was higher in patients with

**Table 1** Analysis of the clinical characteristics of study participants**Demographic and Clinical Characteristics of the Study Participants**

| Item                         | Normal Control | Mild OSA        | Moderate OSA   | Severe OSA      |
|------------------------------|----------------|-----------------|----------------|-----------------|
| N                            | 12             | 8               | 10             | 30              |
| Age (y)                      | 39.17 ± 17.20  | 37.38 ± 16.01   | 48.40 ± 11.80  | 47.90 ± 15.18   |
| BMI (kg/m <sup>2</sup> )     | 24.79 ± 3.32   | 26.23 ± 2.77    | 24.45 ± 8.35   | 23.61 ± 10.26   |
| Female sex,n                 | 7              | 4               | 3              | 7               |
| AHI (events/h)               | 3.26 ± 1.25    | 7.79 ± 3.24     | 21.37 ± 4.2*   | 70.49 ± 16.46*  |
| EP                           | 6.17 ± 1.53    | 9.13 ± 3.04     | 9.1 ± 2.47     | 10.47 ± 3.58*   |
| FBG                          | 2.29 ± 2.66    | 5.2 ± 0.58      | 6.22 ± 2.17*   | 6.57 ± 2.95*    |
| LDL-c                        | 3.66 ± 0.96    | 2.76 ± 1.59     | 2.53 ± 1.18    | 2.61 ± 1        |
| HDL-c                        | 1.09 ± 0.2     | 1.08 ± 0.3      | 0.94 ± 0.21    | 1.11 ± 0.39     |
| TG                           | 1.92 ± 0.86    | 1.5 ± 0.65      | 2.85 ± 2.13    | 2.15 ± 1.47     |
| TC                           | 5.36 ± 0.87    | 4.5 ± 1.95      | 4.74 ± 1.87    | 4.64 ± 1.38     |
| globulin                     |                | 27.33 ± 1.68    | 29.93 ± 0.67   | 29.36 ± 4.7     |
| DBIL                         | 4.73 ± 1.43    | 10.54 ± 9.1     | 5.8 ± 1.95     | 4.6 ± 2.76      |
| WBC                          | 6.55 ± 2.21    | 5.74 ± 0.95     | 5.8 ± 0.61     | 7.29 ± 2.05     |
| P-LCR                        | 30.99 ± 7.84   | 30.53 ± 11.28   | 29.58 ± 9.36   | 28.46 ± 7.37    |
| MO%                          | 31.5 ± 9.97    | 8.29 ± 1.14     | 7.28 ± 2.65    | 7.27 ± 1.53     |
| MO#                          | 0.72 ± 0.13    | 0.55 ± 0.15     | 0.42 ± 0.17    | 0.53 ± 0.18     |
| RBC                          | 5.19 ± 0.49    | 5.11 ± 0.47     | 4.69 ± 0.83    | 4.8 ± 0.71      |
| HCT                          | 45.99 ± 3.94   | 46.33 ± 3.26    | 43.64 ± 5.9    | 42.84 ± 5.58    |
| MCH                          | 29.7 ± 1.97    | 30.39 ± 0.92    | 32.32 ± 2.65   | 29.66 ± 2.33    |
| MCHC                         | 334.46 ± 9.08  | 293.56 ± 115.35 | 344.42 ± 10.72 | 330.8 ± 12.95   |
| RDW                          | 41.84 ± 3.26   | 43.81 ± 3.74    | 44.62 ± 5.36   | 43.88 ± 5.57    |
| LY                           | 36.66 ± 6.21   | 30.57 ± 8.98    | 30.2 ± 10.63   | 25.4 ± 10.16*   |
| A/G                          |                | 1.62 ± 0.05     | 1.51 ± 0.1     | 1.4 ± 0.26      |
| STB                          | 13.46 ± 5.07   | 25.02 ± 16.05   | 16 ± 3.95      | 11.82 ± 8.35    |
| ALB                          | 2.36 ± 0.76    | 1.72 ± 0.49     | 1.75 ± 0.56    | 1.77 ± 0.79     |
| MCV                          | 88.8 ± 5.49    | 90.87 ± 3.71    | 93.8 ± 7.01    | 89.63 ± 6.01    |
| MPV                          | 11.03 ± 1.14   | 10.64 ± 1.43    | 10.58 ± 1.11   | 10.43 ± 0.9     |
| BAS                          | 0.04 ± 0.01    | 0.04 ± 0.03     | 0.03 ± 0.02    | 0.03 ± 0.02     |
| BA                           | 0.71 ± 0.28    | 0.67 ± 0.5      | 0.46 ± 0.32    | 0.45 ± 0.26     |
| EO#                          | 0.23 ± 0.17    | 0.14 ± 0.1      | 0.09 ± 0.09    | 0.15 ± 0.19     |
| EO%                          | 2.9 ± 2.66     | 2.33 ± 1.41     | 1.56 ± 1.38    | 2.22 ± 2.6      |
| HB                           | 153.86 ± 14.21 | 155.14 ± 12.03  | 150.4 ± 21.36  | 142 ± 21.23     |
| PLT                          | 255.57 ± 58.27 | 256.17 ± 80.85  | 197.2 ± 37.22  | 241.17 ± 65.07  |
| PDW                          | 13.59 ± 2.77   | 13.14 ± 3.45    | 12.48 ± 2.49   | 12.4 ± 2.05     |
| ALT                          | 33.86 ± 25.76  | 51.6 ± 49.28    | 31 ± 14.11     | 35.22 ± 34.55   |
| TP                           |                | 71.7 ± 5.45     | 75.07 ± 1.23   | 69.7 ± 10.49    |
| PCT                          | 0.31 ± 0.03    | 0.29 ± 0.08     | 0.21 ± 0.05*   | 0.25 ± 0.07     |
| NE                           | 50.71 ± 8.48   | 58.14 ± 9.56    | 59.9 ± 10.76   | 64.67 ± 11.45*  |
| ANC                          | 5.05 ± 1.16    | 3.49 ± 0.9      | 3.5 ± 0.86     | 4.81 ± 1.83     |
| UA                           | 338.57 ± 89.34 | 348.4 ± 152.82  | 341.5 ± 95.13  | 305.75 ± 108.09 |
| UREA                         | 5.54 ± 1.36    | 4.22 ± 1.42     | 5.6 ± 0.98     | 5.16 ± 1.98     |
| Cr                           | 89.86 ± 21.51  | 85.4 ± 25.68    | 80.8 ± 19.49   | 81.13 ± 25.69   |
| U/C                          | 16.28 ± 3.26   | 13 ± 5.54       | 17.46 ± 2.32   | 16.46 ± 7.03    |
| GFR                          | 99.14 ± 22.97  | 93.2 ± 25.57    | 94.2 ± 20.02   | 87.13 ± 24.95   |
| Cysteine protein inhibitor C |                | 1.51 ± 0.6      | 75.11 ± 164.85 | 1.45 ± 0.65     |
| albumin                      |                | 44.37 ± 3.82    | 45.13 ± 1.86   | 40.34 ± 7.65    |
| IBIL                         | 8.73 ± 3.83    | 14.48 ± 7.92    | 10.2 ± 2.25    | 7.56 ± 5.56     |

**Abbreviations:** BMI body mass index, AHI apnea hypoventilation index, EP eosinophilia score, NE neutrophil, UREA urea, FBG fasting blood glucose, Cr creatinine, HCT hematocrit, IBIL indirect bilirubin, DBIL direct bilirubin, ALT alanine aminotransferase, MCH mean erythrocyte hemoglobin content, BX12 vitamin B12, MO% Monocyte Percentage, MO# Monocyte Absolute Count, LY lymphocyte, PDW platelet distribution width, RDW red blood cell distribution width, MPV mean platelet volume, P-LCR platelet large cell ratio, PCT platelet specific volume, PLT platelet count, WBC white blood cell count, MCV mean corpuscular volume, UA uric acid, LDLc low-density lipoprotein cholesterol, TC total cholesterol, GFR glomerular filtration rate, ALB albumin, EO# Eosinophil Absolute Count, EO% Eosinophil Percentage, RBC Red Blood Cell, BA basophil, HB hemoglobin, MCHC mean erythrocyte hemoglobin concentration

\*P < 0.05, vs. Normal Control



(See figure on previous page.)

**Fig. 2** **A, B** Microbial genus and phylum levels in the gut of the population. **E, F** Relative abundance differences of the top 20 species at the level of microbial genera and phylum in the gut of the mouse. **C, D** The NC group vs. patients with OSA—Phylogenetic evolutionary tree and between-groups abundance distribution heat and Venn diagrams. **G, H** The CIH mice vs. NC group—Phylogenetic evolutionary tree and between-groups abundance distribution heat and Venn diagram. **I** The NC group vs. patients with OSA—DESeq2 volcano diagram. **K, J** The NC group vs. patients with OSA—LDA histogram and cladogram diagram. **L** The CIH mice vs. NC group—DESeq2 volcano diagram. **N, M** The CIH mice vs. NC group—LDA value distribution histogram and cladogram graph. High-resolution versions of all subfigures are provided in Supplementary Files (Fig. 2)

OSA; while *Clostridium\_A*, *Alphaproteobacteria-α*, etc. were more abundant in the NC group (Fig. 2J and K).

As well, there were 17 microorganisms down-regulated in the intestines of CIH model mice compared to the NC group, mainly containing *Muribaculum*, *Faecimonas*, *Clostridium\_T*, and so on. Eleven species were up-regulated, such as *Anaerovibrio*, *Enterocloster*, *Dysosmibacter*, etc. (Fig. 2L). The results of the LEfS analysis of the LDA histogram and cladogram showed that compared with the NC group, 26 species of *Bacteroides*, *Clostridia\_258483*, *Firmicutes\_A*, and so on were more abundant in the intestinal tracts of the mice in the CIH model, while 20 species of *Muribaculum*, *Helicobacteraceae*, and 20 other species were more abundant in the NC group (Fig. 2N and M).

The results of the two-dimensional principal coordinate analysis (PCoA) plot based on the Bray–Curtis distance showed that there was some separation between the microbiomes of the patients with OSA and the NC group, but there were also individual sample points that crossed or overlapped (Fig. 3C), which indicated that there was a certain degree of difference in the structure of their microbiomes. The  $\alpha$ -diversity analysis as shown in the figure showed that there was no significant difference in the results of  $\alpha$ -diversity analysis of gut microbes in patients with OSA compared with the NC group; there was a significant difference in the gut microbiota of the CIH group ( $p=0.0472$ ) (Fig. 3A). The abundance of microbes in the CIH group (CHO1 index) was significantly higher than that in the NC group, and there was a greater intra-group variability in the CIH group (Fig. 3B). The microbiome structure of the CIH group was significantly different from that of the NC group. The distributions of the two groups in the figure did not overlap, indicating that the microbiome structure of the CIH group was significantly different from that of the NC group at the Bray–Curtis distance (Fig. 3D). The pathway abundance map at the KEGG L3 level showed that there were significant differences in apoptosis pathways between gut microorganisms in humans and animals, in addition to the metabolic pathways such as type II polyketide biosynthesis, citric acid cycle, glycolysis/glucose isomerization, methanogenesis, pyruvate metabolism, and steroid degradation in the gut microorganisms of the CIH group and the NC group (Fig. 3E and F). This study suggests that the microbiome structure of the CIH group was significantly different from that of the NC

group, exhibiting greater within-group variability. The diversity or composition of the microbiome structure of the CIH group may have changed significantly compared to the NC group, which may be closely related to the long-term intermittent hypoxic conditions.

#### Targeted metabolomics based on GC–MS

The top 20 metabolites in terms of content are shown in Figs. 4A, B and 5A, B. The rest of the metabolites are included in Others. Fatty acid metabolism was significantly altered in patients with OSA and the CIH group compared to the NC group, e.g. Neuronic acid, Erucic acid, and Linoleic acid were significantly increased in patients with OSA and the CIH group; on the contrary, Arachidonic acid and Lignoceric acid were significantly decreased in patients with OSA and the CIH group. Based on the R language MetaboAnalystR package [16], targeted metabolomics analysis identified different fecal metabolite profiles. Twenty-one metabolites in medium- and long-chain fatty acids were identified as shifted between patients with OSA and the NC group, and the CIH and NC group.

The Random Forest model observed changes in the levels of the 15 most important fatty acids in the fecal samples of patients with OSA and the CIH group, with 10 metabolites decreasing and 5 metabolites increasing. Ten metabolites, such as Arachidonic acid and Lignoceric acid, decreased; five metabolites, such as Erucic acid, Neuronic acid, and Linoleic acid, increased (Figs. 4C, D and 5C, D). The metabolite changes shown in the results further support the association of OSA with metabolic diseases, which may provide clinical biomarkers for predicting or monitoring the progression of OSA and metabolic diseases.

By applying Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) combined with the permutation test, we were able to effectively identify metabolites that were significantly different between groups. Before analysis, the data were standardized to ensure the fairness of comparison. A VIP threshold of no less than 1 and a  $p$ -value of no more than 0.05 were set, and 200 random permutations were performed to accurately calculate the  $p$ -value of the model. In addition, False Discovery Rate (FDR) correction was used to screen metabolites to enhance the reliability of the results. Scatterplot analyses of OPLS-DA and PLS-DA revealed a clear differentiation between the NC group and patients with OSA based on

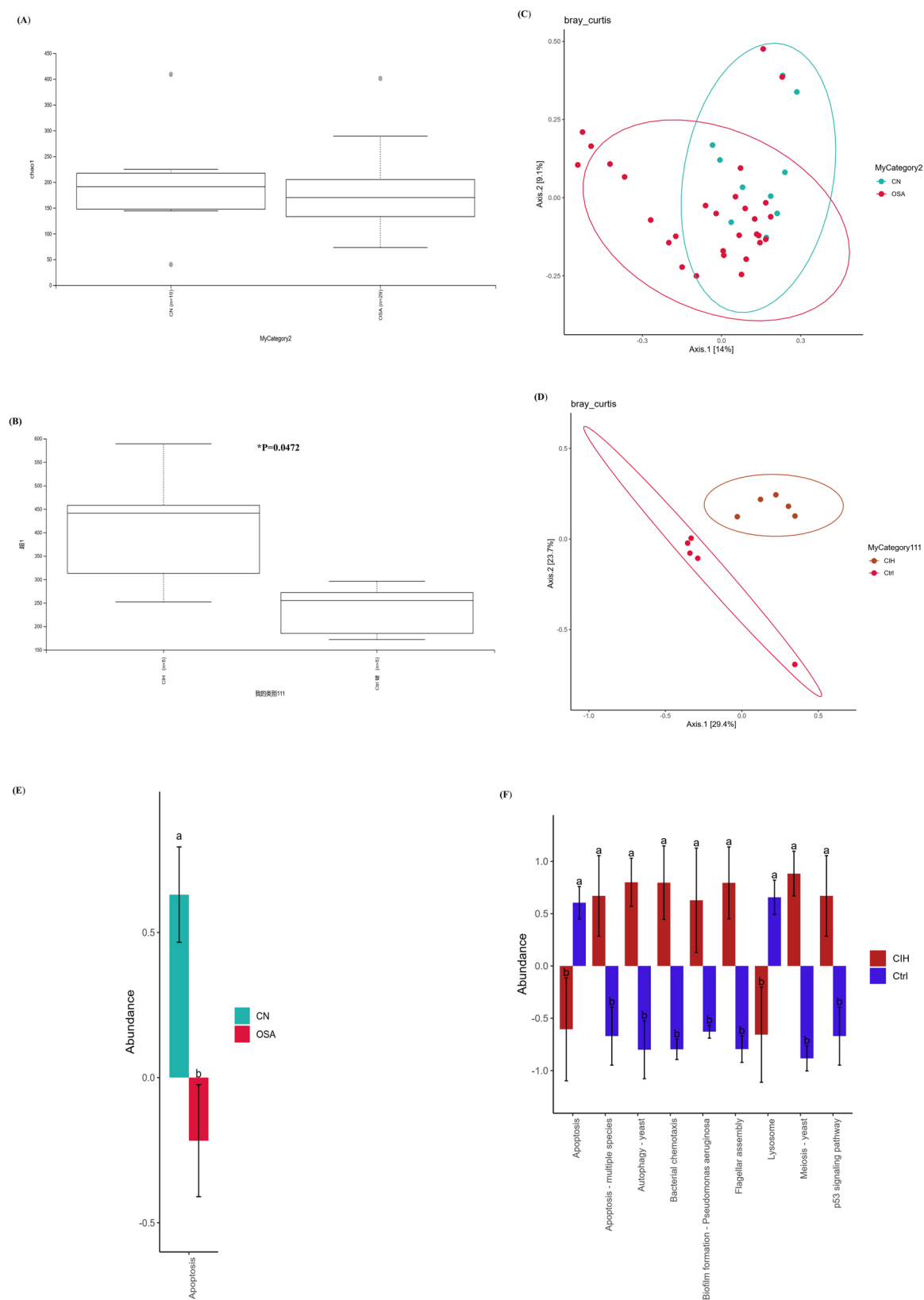


**Fig. 3** **A** and **C** Alpha diversity analysis and Principal Coordinate Analysis (PCoA) comparing the NC group vs. patients with OSA. **B** and **D** Alpha diversity analysis and PCoA comparing the CIH mice vs. NC group. **E** KEGG Level 3 pathway abundance profiles: the NC group vs. patients with OSA. **F** KEGG Level 3 pathway abundance profiles: the CIH mice vs. NC group. High-resolution versions are available in Supplementary Files (Fig. 3)

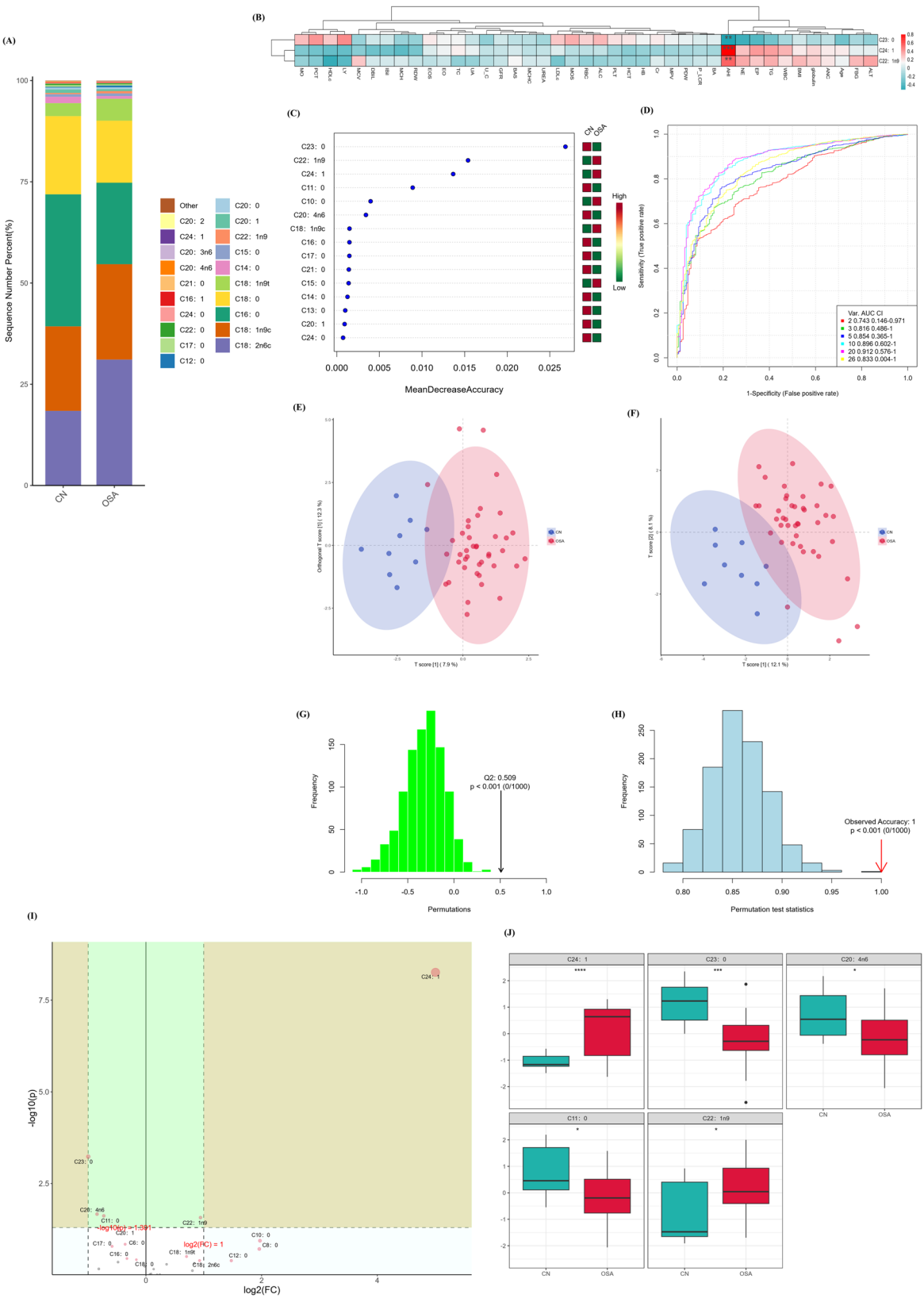


**Fig. 4** (See legend on next page.)

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**Fig. 4** **A** Stacked bar chart showing the top 20 metabolites in the NC group vs. patients with OSA. **B** Heatmap of metabolite profiles. **C** and **D** Top 15 metabolites ranked by importance in Random Forest analysis and results from Support Vector Machine (SVM) analysis. **E** OPLS-DA score plot. **G** OPLS-DA permutation test results showing the distribution of the test statistic and  $p$ -value. **F** PLS-DA score plot. **H** PLS-DA permutation test results showing the distribution of the test statistic and  $p$ -value. **I** Volcano plot based on T-test results. **J** ANOVA boxplot. High-resolution versions are available in Supplementary Files (Fig. 4). SVM: Support Vector Machine. PLS-DA: Partial Least Squares Discriminant Analysis. OPLS-DA: Orthogonal Partial Least Squares Discriminant Analysis. ANOVA: Analysis of Variance

fecal metabolic profiles. Although the NC group was not completely differentiated from the OSA group, with some sample points showing crossover or overlap, the results still demonstrated the effectiveness of the method (Figs. 4E, F, G, H, and 5E, F). The combined assessment of VIP values,  $p$ -values, and FDR-corrected  $p$ -values verified the significance and validity of the OPLS-DA model in distinguishing significantly different metabolites between groups (Fig. 4G and H).

The obtained quantitative peak area results of the 21 differential metabolites were analyzed. Their intergroup fold changes (FCs) were calculated and statistically analyzed as described above to obtain their  $p$ -values, which were then plotted as volcano plots. A multiplicity of difference greater than 2 with a  $P$  value less than 0.05 was observed in patients with OSA compared to the NC group ( $p < 0.05$ ,  $VIP > 1$ ). ANOVA box plots identified five differentially significant metabolites, among which Neuronic acid and Erucic acid were significantly up-regulated ( $p < 0.05$ ), and Arachidonic acid was significantly down-regulated ( $p < 0.05$ ) in patients with OSA compared to the NC group (Figs. 4I and J). Compared with the NC group, Neuraminic acid was significantly up-regulated ( $p < 0.05$ ), and Heptadecanoic acid was significantly down-regulated ( $p < 0.05$ ) in the CIH model (Fig. 5G and H).

### Correlation analysis

#### Association of human gut microorganisms, metabolites and clinical indicators

In this study, the heat map of the correlation between the gut microbiota of the population, the substances of interest at the portal level, and some of their clinical indicators is shown in Fig. 6A and B. There were significant correlations between certain flora and some of the clinical indicators. Among them, there were significant positive correlations between *Corynebacterium* and neutrophils; *Lactococcus* and neutrophils; *Leuconostoc\_B* and fasting blood glucose; *Howardella* and platelet pressure; and Firmicutes\_D and EP, among others. Significant negative correlations were found between *Corynebacterium*, *Lactococcus*, *Coprobacillus*, *Blautia\_A*, *Patescibacteria*, etc. and the percentage of lymphocytes; and between Bacteroidota and EP, AHI, etc. The significant associations of 26 metabolites in the intestines of the population with some of the clinical indicators detected by a targeted metabolomics approach in this study are demonstrated in Fig. 6C. The results showed significant positive

correlations ( $p < 0.05$ ) between Palmitic acid and LY; Erucic acid and AHI; and Neuronic acid and EP and AHI. Significant negative correlations ( $p < 0.05$ ) were observed between Palmitic acid and NE; Stearic acid and NE; and Eicosatrienoic acid and AHI.

#### Correlation analysis between microbiota and metabolites

Correlation analysis between gut microbiota and metabolites showed significant correlations. In this study, Pearson's correlation analysis was used to assess the relationship between metabolites and clinical indicators and the association between metabolites and microorganisms in the human gut, setting  $|r| > 0.4$  as the threshold, meaning that a significant correlation between the two variables was considered to exist only if the absolute value of the correlation coefficient was greater than 0.4. After correcting the  $p$ -value using FDR, it was found that the targeted metabolite eicosatrienoic acid in the human gut had a significant negative correlation with AHI, and that Nervonic acid and Erucic acid had a significant positive correlation with AHI (Fig. 6A). In addition, network diagrams and correlation heatmaps revealed significant positive correlations between *Barnesiella* and *Butyrivibrio* with Tridecanoic acid, Pentadecanoic acid, and Heptadecanoic acid; *Alistipes\_A\_871400* with Pentadecanoic acid; and significant negative correlations between CAG\_217, *Ruminiclostridium* and Palmitoleic acid; and JC017 with Tridecanoic acid and Pentadecanoic acid (Fig. 6B). The figure revealed complex correlations between specific intestinal flora and fatty acid metabolism, suggesting that these flora have a regulatory role in the synthesis and metabolism of specific fatty acids. This provides important clues to investigate the role of intestinal flora in the dysregulation of fatty acid metabolism in patients with OSA.

### Discussion

#### Intermittent hypoxia imbalances microbes and metabolites in the host gut

With gut microbes and metabolites playing an important role in the pathogenesis and development of patients with OSA, an imbalance of the gut microbiota may lead to an increased inflammatory response, which in turn affects the body's metabolic state and thus the pathophysiological process of OSA [17]. Collado et al. reported a higher abundance of *Aspergillus*, *Enterobacteriaceae*, and *Dantozoa*, and a higher proportion of Thick-walled

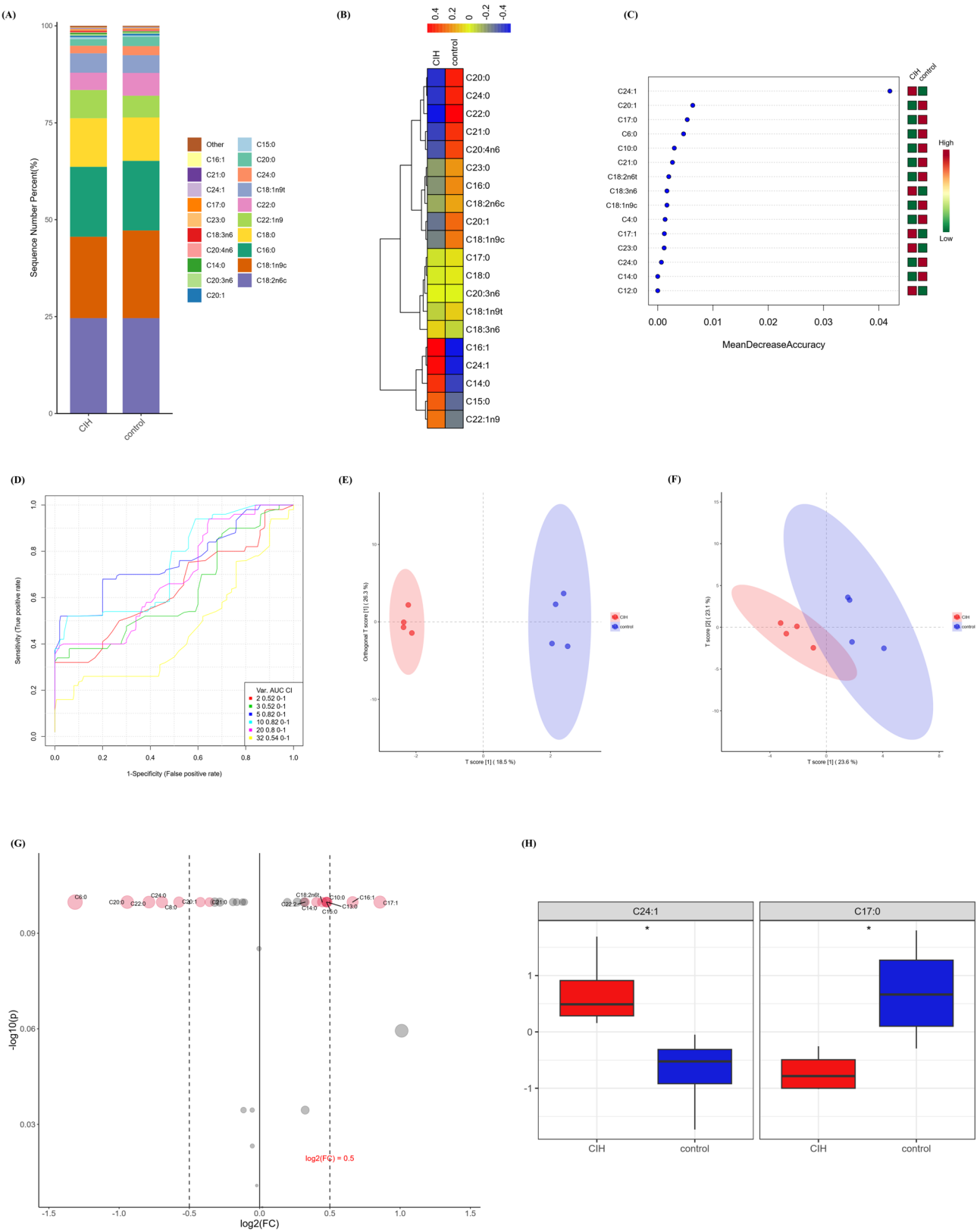


**Fig. 5** (See legend on next page.)

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**Fig. 5** **A** Stacked bar chart of the top 20 metabolites in the CIH group vs. NC group. **B** Heatmap of metabolite profiles. **C** Top 15 metabolites ranked by importance in Random Forest analysis. **D** SVM classification results. **E** OPLS-DA score plot. **F** PLS-DA score plot. **G** Volcano plot of statistical significance. **H** ANOVA boxplot comparing metabolite abundance across groups. High-resolution versions are available in Supplementary Files (Fig. 5). SVM: Support Vector Machine. PLS-DA: Partial Least Squares Discriminant Analysis. OPLS-DA: Orthogonal Partial Least Squares Discriminant Analysis. ANOVA: Analysis of Variance

versus *Anaplasma* were detected in snoring children compared to controls. In addition, microbial diversity and abundance were significantly lower in snoring children than in non-snorers [18]. In this study, we found that the increase in the abundance of Firmicutes, Verrucomicrobiota, *Blautia\_A*, and certain metabolites such as Nervonic acid and Erucic acid, along with the significant down-regulation of Arachidonic acid and *Actinobacteria* in the intestines of patients with OSA, suggests a potential link between metabolites derived from the intestinal microbiota and the metabolic derangements observed in OSA, filling in some of the gaps for previous research. Previous studies have revealed structural alterations in the gut microbiota of patients with OSA. Notably, Andras et al. [15] identified a significant decrease in Actinobacteria abundance and an increase in Proteobacteria through taxonomic analyses at both phylum and genus levels. At the genus level, they reported enrichment of *Lacticaseibacillus casei* and *Roseburia* in patients with OSA. These findings suggest that microbial dysbiosis in specific phyla may contribute to OSA pathophysiology. Our study corroborates this phenomenon, demonstrating a consistent trend of decreased Actinobacteria and increased Firmicutes abundance at the phylum level, aligning with existing literature. However, novel genus-level observations emerged: while we confirmed decreased *Ligilactobacillus* abundance, we specifically identified a marked reduction in *Limosiella mucosae* alongside a selective elevation of particular Firmicutes species.

Compared with the NC group, *Lacticaseibacillus casei*, *Bacteroides*, and *Tenotrophomonas* were increased in the CIH group, while *Muribaculum* and *Paramuribaculum* were significantly decreased in the intestinal tract of the CIH group. Based on these results, we hypothesize that the increased relative abundance of Firmicutes and Verrucomicrobiota may be related to OSA-associated metabolic changes or inflammatory responses. Meanwhile, the increase in *Escherichia coli*, *Blautia\_A*, and *Ruminococcus\_E* may be related to intestinal health or specific metabolic processes.

The ratio between the Firmicutes and Bacteroidota in the intestine (F/B ratio) is considered an important indicator of intestinal health [19], obesity [20], diabetes, metabolic syndrome, and other related diseases [21]. In this study, we found a significant correlation between the intestinal microbiota and long-chain fatty acid

metabolites in patients with OSA, which is considered to be related to processes such as the elevation of *Lacticaseibacillus casei* within the Firmicutes at the onset of OSA. This elevation helps prevent toxins and pathogens from penetrating the intestinal wall and repairs the damaged intestinal barrier through the production of short-chain fatty acids, such as Butyric acid [22–24].

The present study found an increase in *Tenotrophomonas* in the feces of patients with OSA, which may be related to various factors, including intestinal flora dysbiosis and a low-oxygen environment [8]. In this study, we found that *Lacticaseibacillus casei* decreased significantly in the CIH group compared to the NC group. *Lacticaseibacillus casei* can inhibit the growth of harmful bacteria, such as *Escherichia coli*, *Salmonella*, and *Helicobacter pylori*, and it occupies the surface of the intestinal epithelium to form a beneficial microbial barrier through a competitive adherence mechanism [25]. Hypoxia and oxidative stress in patients with OSA can change the microenvironment of the intestinal tract, thereby inhibiting its growth [26].

In this study, we found that the expression of Nervonic acid and Erucic acid was elevated in patients with OSA, while Arachidonic acid was down-regulated in patients with OSA. Nervonic acid and Erucic acid are important components of fatty acid metabolism. The increased abundance of Nervonic acid, which is associated with cell membrane integrity and neuronal cell function, is closely related to inflammation-related metabolic changes. Meanwhile, changes in Erucic acid metabolism are associated with dysregulation of lipid metabolism, inflammatory responses, or other metabolic syndromes [27]. Arachidonic acid is a precursor of several inflammation-regulating molecules, such as prostaglandins and leukotrienes, which modulate the inflammatory response and are essential for inflammatory regulation and maintenance of intestinal homeostasis [28]. Down-regulation of Arachidonic acid decreases the production of inflammatory mediators such as prostaglandins and leukotrienes, resulting in a weakened intestinal response to inflammation. This may lead to an increased risk of intestinal diseases or infections.

Based on the results of this study, it is hypothesized that CIH conditions contribute to a decrease in inflammatory modulators or Bacteriostatic organisms in the gut, leading to inflammatory infections as well as a range of exacerbated intestinal diseases.

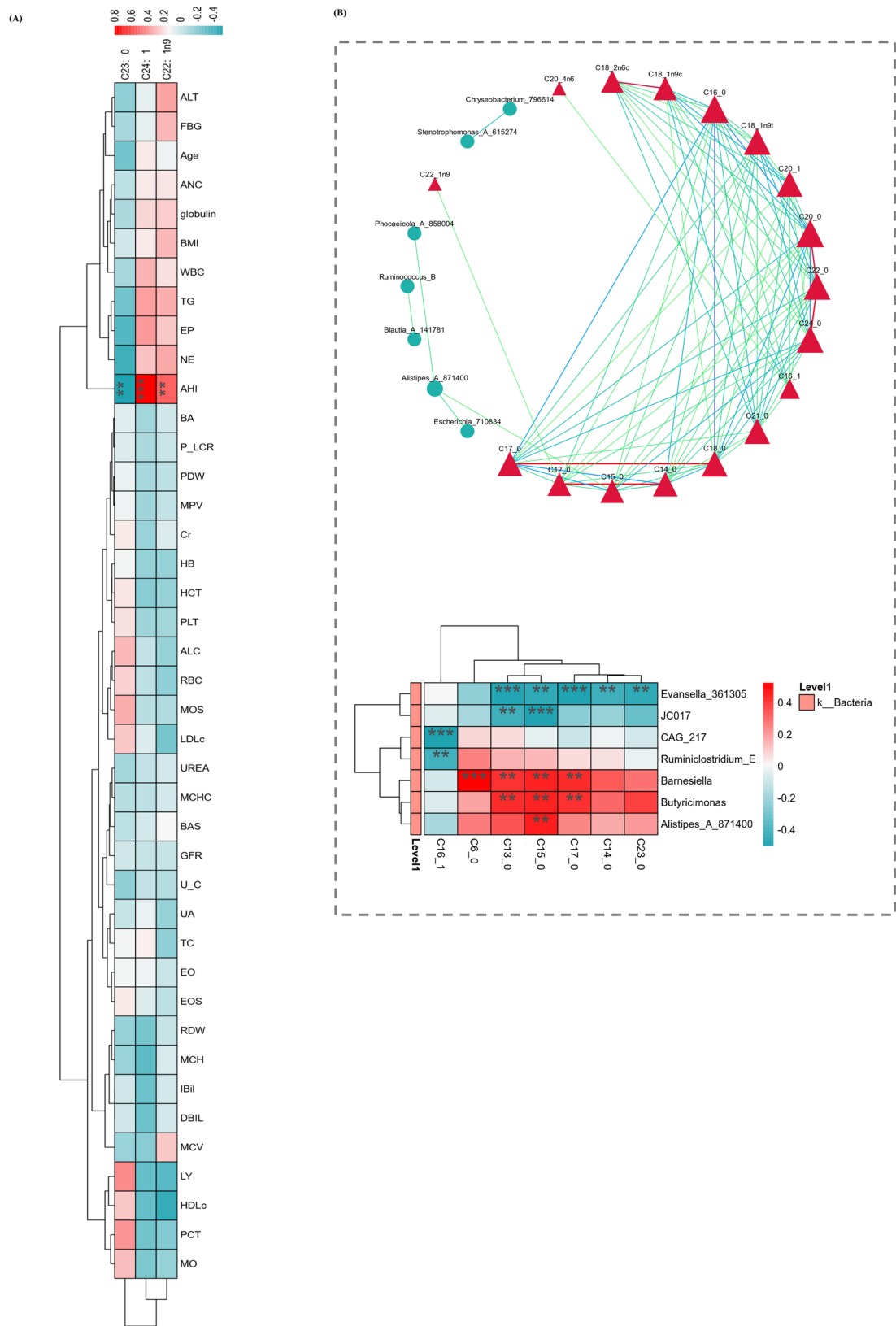


**Fig. 6** **A** Heatmap of correlations between targeted metabolites identified in the human gut and clinical parameters. **B** Microbial-metabolite correlation network diagram and associated heatmap. High-resolution versions are available in Supplementary Files (Fig. 6)

### Imbalances in OSA gut microbes and metabolites exacerbate apnea

In this study, we revealed a complex network of associations between gut flora and its metabolites and the pathological features of OSA under CIH conditions by integrating gut microbiota and metabolomics data. Neuronic acid, a long-chain monounsaturated fatty acid, is an important component of Neuromyelin. Elevated levels of Neuronic acid may affect the central nervous system (CNS) regulation of respiratory rhythms by interfering with myelin stability. Studies have shown that CIH in patients with OSA induces oxidative stress, leading to oligodendrocyte dysfunction and myelin damage [29]. Abnormalities in Erucic acid metabolism may exacerbate this process and further worsen AHI values by reducing the efficiency of nerve signaling in the respiratory centers [30]. Elevated levels of Erucic acid, a monounsaturated fatty acid, are closely associated with disorders of hepatic lipid metabolism. Animal experiments have shown that CIH can promote hepatic lipid synthesis through activation of the sterol regulatory element-binding protein (SREBP-1c) pathway, leading to fatty liver and elevated ALT [31]. The positive correlation between Erucic acid and AHI in the present study may reflect the mechanism of synergistic deterioration of hepatic lipid accumulation and OSA severity under CIH conditions. In addition, Erucic acid may exacerbate hepatic oxidative stress by inhibiting mitochondrial  $\beta$ -oxidation [31, 32], which further promotes the release of inflammatory factors (e.g. IL-6) and indirectly exacerbates apneic events.

In contrast, Eicosatrienoic acid is an odd-chain saturated fatty acid, and its negative association with AHI may alleviate the pathological process of OSA through immunomodulation. Studies have shown that odd-chain fatty acids can inhibit the NF- $\kappa$ B pathway and reduce the production of pro-inflammatory factors (e.g. TNF- $\alpha$ , IL-1 $\beta$ ) by activating peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) [33–35]. The negative correlation between Eicosatrienoic acid and AHI in the present study further supports the notion that it enhances anti-inflammatory responses by regulating the Th1/Th2 balance, thereby attenuating CIH induced airway inflammation and respiratory impairment [36].

*Barnesiella* and *Butyricimonas* are known producers of short-chain fatty acids (SCFAs), but their positive correlation with medium-chain fatty acids (e.g. Pentadecanoic acid) suggests that they may generate medium-chain fatty acids through the degradation of complex lipids by lipid hydrolyzing enzymes (e.g. esterases, lipases) [37]. Medium-chain fatty acids can enter the liver directly through the portal system and inhibit hepatic lipid synthesis by activating the AMPK pathway [38, 39], a mechanism that may partially explain their negative association with AHI. In addition, medium-chain fatty acids

may reduce systemic inflammation by decreasing endotoxin translocation through enhanced intestinal barrier function [40], which is consistent with the low-grade inflammatory remission observed in patients with OSA. Reduced abundance of *Ruminococcus*, a genus of cellulose-degrading bacteria, may lead to reduced dietary fiber utilization, which in turn inhibits intestinal Palmitoleic acid (a monounsaturated fatty acid) synthesis. Palmitoleic acid may inhibit the TLR4/NF- $\kappa$ B signaling pathway and attenuate intestinal inflammation through activation of the G protein-coupled receptor (GPR120) [41]. The negative correlation of *Ruminococcus* with Palmitoleic acid in the present study suggests that dysbiosis under CIH conditions may weaken this anti-inflammatory pathway, promoting increased intestinal permeability and systemic inflammation, ultimately exacerbating AHI. The negative correlation of JC017 with Pentadecanoic acid may reflect its inhibition of these fatty acids by competing for substrates. Odd-chain fatty acids (e.g. Pentadecanoic acid) can activate the farnesol X receptor (FXR) by regulating bile acid metabolism, which in turn inhibits hepatic lipid synthesis [42]. Thus, enrichment of JC017 may impair FXR signaling by reducing odd-chain fatty acids, promoting lipid accumulation and OSA progression.

The present study revealed the protective role of Eicosatrienoic acid in OSA, which may be related to PPAR $\gamma$ -mediated immunomodulation. This finding is similar to that of Chen et al. [43] in COPD, suggesting a pervasive function of odd-chain fatty acids in respiratory diseases.

### The animal experimental model reflect on OSA pathology in humans

Animal experimental models have played an important role in exploring the pathological mechanisms of OSA. By subjecting rats to repeated episodes of hypoxia and reoxygenation, researchers have found that CIH triggers a systemic oxidative stress response, leading to elevated levels of reactive oxygen species and inflammatory factors. Oxidative stress is indeed another pathological feature of OSA [44]. Conotte et al. [45] suggested that hypoxia-induced oxidative stress may promote metabolic changes in OSA. These changes are highly similar to abnormalities observed in inflammatory markers in the blood of patients with OSA in clinical settings [46, 47]. Further studies have shown that CIH impairs vascular endothelial function and promotes atherosclerotic plaque formation through activation of the HIF-1 $\alpha$  and NF- $\kappa$ B signaling pathways. This provides an experimental rationale for explaining the high prevalence of cardiovascular disease in patients with OSA [48, 49]. Xu et al. [50] compared metabolite levels in simple snorers, patients with OSA, and healthy subjects. A total of 21 and 31 metabolites in simple snorers and patients with OSA, respectively, showed differences from NC group. In a model of

airway obstruction in adult dogs, prolonged increases in upper airway resistance stimulate compensatory hypertrophy of the lingual muscles, as well as enhancement of hypoglossal nerve efferent activity. This phenomenon corroborates the mechanism of apnea due to upper airway muscle relaxation during sleep in human patients with OSA [51, 52].

A complex microbial community exists in mammals, dominated mainly by specialized anaerobic bacteria (Firmicutes and Bacteroidota). The dynamic equilibrium between the Firmicutes and Bacteroidota is a defining feature of the human gut [53]. When an animal is subjected to CIH, characteristic of OSA, the partial pressure of oxygen (PaO<sub>2</sub>) within the intestinal environment fluctuates, and its average level decreases. During CIH exposure, significant changes in the intestinal microbial community occur, mainly characterized by an increase in *Prevotella* and *Desulfovibrio* as well as a decrease in *Lactobacillus* [54].

However, animal models have significant limitations in simulating the complex pathological features of human OSA. Rodents have simpler upper airway structures than humans, such as the lack of an elongated soft palate prone to collapse and a retrognathic mandible, anatomical features that are important risk factors in patients with OSA [55]. Overall, relatively few studies have investigated the microbiome and metabolome of patients with OSA and animal models, and the unique features of OSA associated with these aspects need to be further elucidated. There are several other limitations to microbiome studies in OSA. First, sample sizes are usually small, making results less reliable. Second, few studies have investigated whether changes in gut flora occur with disease progression; therefore, longitudinal metabolomic studies and multi-omics studies using advanced methods are needed to increase the identification of microbiome biomarkers. In addition, the dominant genera and relative abundance of gut flora differ substantially between mice and humans; approximately 85% of bacterial genera in mice are absent in humans [56]. Thus, although mouse models are a valuable way to explore metabolic and microbial changes, there are limitations in translating these results to humans, especially regarding the microbiome. Differences in microbiome and metabolomic profiles observed across studies are further difficult to interpret because studies employ different gut microbiome sequencing tools and metabolomics methods. Although recent technological advances have made it possible to analyze metabolomes and microbiomes quickly and efficiently, the use of different platforms and software has resulted in the lack of a gold standard methodology for studying metabolomes and microbiomes.

### Limitations of the study

In this study, we revealed the association characteristics of gut microorganisms and metabolites under CIH conditions using a microbiomics co-targeted metabolomics approach. The cross-species comparisons were designed to validate the reproducibility of clinical observations in experimental models and to provide a basis for mechanistic studies. However, there are still many limitations:

Previous studies have used the CIH mouse model to mimic the pathological features of OSA. Although this model has been widely used in the study of OSA-related mechanisms [57], differences between it and the natural course of the disease in humans still need to be noted. First, the hypoxia pattern in experimental animals is induced by forced periodicity, which is different from the triggering mechanism of spontaneous apnea due to upper airway collapse in humans. Second, human OSA is often accompanied by comorbidities such as obesity and metabolic syndrome, whereas it is difficult for animal models to fully reproduce such multifactorial interactions. Additionally, the experimental period (usually 4–6 weeks) is significantly different from the chronic course of human OSA (several years or even decades). Furthermore, there are inherent compositional differences between rodent and human gut flora [58]. For example, the Bacteroides is predominant in mice, whereas the Firmicutes is predominant in humans [58]; *Lactocaseibacillus casei* are significantly more abundant in mice (10%–20%) [59–61] than in humans (~1%) [53]. These factors may influence the dynamic process of gut microbiota and metabolite changes.

Although we revealed a series of microbial-metabolite-clinical index associations, the fact that the human study was partly cross-sectional and the sample size was small (60 clinical samples, 10 animal samples) may have reduced statistical validity and increased the risk of type I/II errors. The cross-sectional design of this study revealed an association between OSA and intestinal flora but did not allow for a clear causal relationship. Future longitudinal cohorts or interventional studies (e.g., continuous positive airway pressure (CPAP) therapy) are needed to validate the dynamic effects of CIH on flora. OSA is a chronic disease with multi-system interactions, and intestinal flora dysbiosis may be both a causative factor and a secondary outcome. It is difficult to distinguish the contribution of direct effects of hypoxia from secondary metabolic disorders in the current data, which needs to be further resolved by time series analysis or conditional knockout modeling.

### Conclusion

In this study, we analyzed the differences and associations of gut microbiota and metabolites in patients with OSA and CIH model mice using microbiomics and

targeted metabolomics approaches. The results showed that the alteration of intestinal microbiota in CIH conditions, specifically the changes in the Firmicutes and the Bacteroidetes, may lead to alterations in the targeted differential metabolites Erucic acid and Arachidonic acid. These changes correlate with the severity of the disease and clinical indices, and thus may be promising as new biomarkers for the diagnosis of OSA and the assessment of disease severity.

Furthermore, this study also calls for more researchers to focus on gut microbiota in the context of OSA, and to jointly explore diagnostic and therapeutic strategies for controlling gut dysbiosis, thereby providing new ideas and directions for the precise diagnosis and treatment of OSA.

## Glossary

|       |                                     |
|-------|-------------------------------------|
| OSA   | Obstructive sleep apnea             |
| CIH   | Chronic intermittent hypoxia        |
| BMI   | Body mass index                     |
| AHI   | Apnea hypoventilation index         |
| NE    | Neutrophil                          |
| UREA  | Urea                                |
| FBG   | Fasting blood glucose               |
| Cr    | Creatinine                          |
| HCT   | Hematocrit                          |
| IBIL  | Indirect bilirubin                  |
| DBIL  | Direct bilirubin                    |
| ALT   | Alanine aminotransferase            |
| MCH   | Mean erythrocyte hemoglobin content |
| BX12  | Vitamin B12                         |
| MO%   | Monocyte Percentage                 |
| MO#   | Monocyte Absolute Count             |
| LY    | Lymphocyte                          |
| PDW   | Platelet distribution width         |
| RDW   | Red blood cell distribution width   |
| MPV   | Mean platelet volume                |
| P-LCR | Platelet large cell ratio           |
| PCT   | Platelet specific volume            |
| PLT   | Platelet count                      |
| WBC   | White blood cell count              |
| MCV   | Mean corpuscular volume             |
| UA    | Uric acid                           |
| LDLc  | Low-density lipoprotein cholesterol |
| TC    | Total cholesterol                   |
| GFR   | Glomerular filtration rate          |
| ALB   | Albumin                             |
| EO#   | Eosinophil Absolute Count           |
| EO%   | Eosinophil Percentage               |
| RBC   | Red Blood Cell                      |
| BA    | Basophil                            |

|          |                                           |
|----------|-------------------------------------------|
| HB       | Hemoglobin                                |
| MCHC     | Mean erythrocyte hemoglobin concentration |
| C24:1    | Neuronic acid                             |
| C20:2    | Eicosadienoic acid                        |
| C21:0    | Heneicosanoic acid                        |
| C20:3n6  | Eicosatrienoic acid                       |
| C16:1    | Palmitoleic acid                          |
| C12:0    | Lauric acid                               |
| C24:0    | Lignoceric acid                           |
| C22:1n9  | Erucic acid                               |
| C15:0    | Pentadecanoic acid                        |
| C18:1n9t | Trans-oleic acid                          |
| C18:2n6c | Linoleic acid                             |
| C18:1n9c | Oleic acid                                |
| C20:4n6  | Arachidonic acid                          |
| C20:0    | Arachidic acid                            |
| C17:0    | Heptadecanoic acid                        |
| C22:0    | Docosanoic acid                           |
| C20:1    | Eicosenoic acid                           |
| C14:0    | Myristic acid                             |
| C18:0    | Stearic acid                              |
| C16:0    | Palmitic acid                             |

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-025-04220-z>.

Supplementary Material 1.

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## Authors' contributions

All authors contributed significantly to the work of the report. Z.T.Y.:methodology and Writing—original draft; C.J.Q.:animal husbandry and gene sequencing; P.Y. and L.J.X.:resource and gene sequencing; L.W. and L.Y.Q.:clinical data collection; W.M. and N.C.:software and data calibration;Z.D.: conceptualization, study design, experimental guidance, and thesis revisions, and for the generous volunteer subjects recruited in this study.

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## Data availability

The raw 16S rRNA amplicon sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession numbers PRJNA1230830 and PRJNA1235339.

## Declarations

### Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Guizhou Provincial People's Hospital [2024–123] and the Experimental Animal Ethics Committee of Guizhou Medical University [2403650], and all subjects signed an informed consent form. This study is in line with the provisions of the Declaration of Helsinki. The authors declare that this study was conducted without any commercial or financial relationships that could be perceived as a potential conflict of interest.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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