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ORIGINAL RESEARCH

Urinary Epinephrine Sulfate Can Predict Cardiovascular Risk in Moderate-to-Severe OSA: A Metabolomics-Based Study

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Purpose: There are currently no ideal indicators for predicting the cardiovascular risk of obstructive sleep apnea (OSA). This study aimed to employ urinary metabolomics to detect early cardiovascular risk in patients with moderate-to-severe OSA.

Patients and Methods: Male participants who underwent polysomnography from November 2020 to May 2021 were screened. Clinical data, polysomnography data and urine samples were collected. Untargeted metabolomics analyses of urine were performed. Multivariate analyses and receiver operating characteristic (ROC) curve analyses were subsequently performed to identify potential biomarkers. Associations between metabolites and clinical indicators and cardiovascular risk were examined through linear regression analyses with interaction and mediation analyses.

Results: Thirty-six male participants were included in the study, comprising 22 males with moderate-to-severe OSA and 14 age-matched controls, with an average age of 39.6 ± 9.2 years. We identified 65 metabolites in the study, involving pathways including pyrimidine, androgen, estrogen, vitamin B6 and sulfate/sulfite metabolism. Among them, epinephrine sulfate was the most significantly altered metabolite. ROC analyses highlighted that epinephrine sulfate had the highest area under the curve (AUC=0.883) for detecting moderate-to-severe OSA. Epinephrine sulfate was statistically correlated with OSA severity, hypoxia-related indicators (apnea-hypopnea index: r=0.685; oxygen desaturation index: r=0.743, p<0.0001), arterial stiffness (arterial augmentation index: r=0.361, p=0.031) and long-term cardiovascular risk (Framingham cardiovascular risk: r=0.375, p=0.024). Linear regression analysis revealed that epinephrine sulfate was significantly associated with an increased in the Framingham risk (β = 0.004, 95% CI = 0.000–0.009, p = 0.049), with the effect partly mediated by systolic blood pressure (27.6%) and not moderated by other factors. Additionally, it also significantly associated with the increased in the arterial augmentation index (β = 0.019, 95% CI = 0.000–0.037, p = 0.046), with the effect fully mediated by blood pressure and not moderated by other indices statistically.

Conclusion: There are significant metabolic pathway alterations in moderate-to-severe OSA patients. Urinary epinephrine sulfate markedly predicts early cardiovascular risk in OSA patients.

Keywords: metabolomics, Framingham cardiovascular risk score, epinephrine sulfate, obstructive sleep apnea

Introduction

Obstructive sleep apnea (OSA) is characterized by recurrent partial or complete obstructions of the upper airway during sleep, resulting in a reduction or complete absence of airflow. Individuals with OSA often experience poor sleep quality, morning headaches, and excessive daytime sleepiness, all of which adversely affect occupational performance, reduce productivity, and heighten accident risks both at work and while driving.[1,2] Additionally, OSA imposes substantial

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socioeconomic burdens.[2] Previous research has shown associations between OSA and various health conditions, including refractory hypertension, atrial fibrillation, diabetes, and stroke.[3–6]

With the increasing awareness of OSA and its multiple complications, the search for effective biomarkers has been a critical research focus. These biomarkers are essential for assessing treatment response, prognostication, and cardiovascular outcome prediction in patients with OSA.[7] Biomarkers reflecting inflammatory states (such as IL-6 and TNF-α) and metabolic status (such as glycated hemoglobin) have been explored. However, due to inconsistent conclusions across various studies, these are not ideal choices for clinical application.[7–12] For example, even in randomized controlled trials, changes in inflammatory markers such as TNF-α after OSA treatment vary across different studies.[9,10] Recent advancements in multiomics fields such as metabolomics, lipidomics, proteomics, and microbiomics have broadened the scope of biomarker discovery for OSA. Investigations indicate that alterations in metabolites associated with fatty acid, carbohydrate, and amino acid metabolism, as well as proteins involved in cytokine production and coagulation processes, are related to OSA development.[13,14] Furthermore, variations in the human microbiome composition in OSA patients have been associated with cardiovascular risks.[14] Despite the array of potential markers, clinical practice is still limited.

Metabolomics, the comprehensive analysis of metabolites in body fluids, cells, and tissues, has emerged as a potent tool in biomarker discovery. These metabolites are essential for cellular functions, reflect diverse disease-related changes and offer critical insights for phenotype characterization.[15,16] Our study, advancing the identification of meaningful biomarkers for OSA, employed urinary untargeted metabolomics to contrast metabolites in male patients with moderate-to-severe OSA against those with no or mild OSA. We aimed not only to pinpoint markers uniquely prevalent in moderate-to-severe OSA but also to analyze the association between differential metabolites and key clinical indicators, such as the Framingham cardiovascular risk score and vascular stiffness, to recognize early predictors of potential cardiovascular complications.

Materials and Methods

Study Population

Our study was conducted at the Sleep Center of Peking Union Medical College Hospital from November 2020 to May 2021. OSA was diagnosed per the International Classification of Sleep Disorders, Third Edition,[17] defining moderate-to-severe OSA as an apnea-hypopnea index (AHI) exceeding 15 events/hour, with predominantly obstructive respiratory events. Considering the male predilection and sex-specific physiological differences of OSA,[18] only adult male patients with suspected OSA who underwent polysomnography (PSG) were prospectively and consecutively included. The exclusion criteria for individuals were as follows: (1) had current use of any medication; (2) were active smokers; (3) had chronic respiratory or heart diseases, such as asthma, chronic obstructive pulmonary diseases, coronary heart disease or heart failure; (4) had liver/kidney diseases, nervous system diseases or mental diseases; (5) previously received treatment for sleep apnea with positive airway pressure therapy; (6) had acute infections during the preceding week; and (7) had a total sleep duration less than four hours. The study protocol was similar to that of a previous study.[19] Participants were categorized into two groups based on the AHI: the experimental group with moderate-to-severe OSA (AHI $\geq$ 15) and the control group (AHI $<$ 15). The study was approved by the Ethics Committee of Peking Union Medical College Hospital (PUMCH) and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Clinical Data Collection

The clinical information of each participant, including age, height, weight, BMI, and neck circumference, was collected on the night of PSG. Participants were assessed with the Epworth Sleepiness Scale (ESS) and the Pittsburgh Sleep Quality Index (PSQI). Blood pressure measurements for each participant were taken using an automated device, the Omron M2 HEM-7143-E. Measurements were calculated as the average of two consecutive readings taken at intervals of 5 to 10 minutes. Blood pressure recorded before bedtime on the evening of the PSG monitoring was designated as "blood pressure before sleep", while measurements taken the following morning upon awakening were referred to as "blood pressure after sleep". We calculated the average systolic and diastolic blood pressures (SBP and DBP) at two time points

as indicators to assess the participants' blood pressure levels in our subsequent statistical analyses.[20] Fasting venous blood samples were collected and sent to the clinical laboratory of PUMCH for lipid profiling the following morning.

Cardiovascular Assessment

Each participant's cardiovascular disease (CVD) risk was evaluated using the 2008 Framingham Heart Study Cox regression model, which predicts a ten-year risk of coronary heart disease, cerebrovascular disease, peripheral arterial disease, and heart failure.[21] To calculate this risk, the following data are required: age, SBP, total cholesterol (TC), HDL cholesterol (HDL-C), hypertension medication status, smoking status, and diabetes history.

The augmentation index (AI) is an indicator of arterial stiffness. It quantifies the extent to which central aortic pressure is increased due to reflected pulse waves.[22] Higher values of the augmentation index indicate greater arterial stiffness. Currently, this index can be calculated using the peripheral arterial tonometry device along with its built-in computer algorithms. Since the index is affected by heart rate, it can be normalized using data standardized at a heart rate of 75 bpm (AI@75). In our study, we utilized the EndoPAT device (Itamar Medical Ltd., Israel) to conduct our measurements. This assessment was performed in the morning following PSG, with the patient in a supine position for at least 20 minutes and maintaining silence throughout the procedure, which was performed per the manufacturer's guidelines. The standard procedure included three consecutive five-minute phases: (1) baseline measurement; (2) occlusion measurement during which brachial artery occlusion was performed on the nondominant upper arm with an occlusion pressure at least 60 mmHg above the systolic blood pressure (minimally 200 mmHg and maximally 300 mmHg); and (3) recording after cuff deflation.[22]

Polysomnography

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.[23] Apnea was defined as airflow cessation (a reduction in airflow of ≥90%) for ≥10 seconds. Obstructive apnea events were characterized by persistent respiratory effort throughout the entire period of airflow cessation, while central events were identified by the absence of respiratory effort during these episodes. Hypopnea was defined as a reduction in airflow of >30% for at least 10 seconds accompanied by a ≥3% decrease in oxygen saturation. The AHI was defined as the number of apnea and hypopnea events per hour of sleep. The diagnosis of OSA required that more than 50% of the respiratory events observed in a sleep study be obstructive rather than central. The oxygen desaturation index (ODI) refers to the number of times per hour of sleep that blood oxygen levels fall by more than 3% from the baseline value. T90% was the percentage of the total monitoring time during which the SpO_2 was <90%. Additionally, the average ($MeanSpO_2$) and lowest ($LowestSpO_2$) oxygen saturation levels throughout the night were recorded.

Urine Sample Collection and LC Separation for Metabolomic Profiling

Morning urine samples were collected in 50 mL centrifuge tubes after PSG monitoring, ensuring that the patients had not consumed any food or water before collection. The supernatant was stored at −80°C in 100-μL aliquots after centrifugation. The metabolomics test was performed at Peking Union Medical College Hospital's central laboratory. For metabolomic profiling, a 100-μL aliquot of urine was centrifuged at 4°C and 13000 rpm for 20 minutes to eliminate impurities. Following dilution with an equal volume of water, an 80-μL sample was transferred to sample vials, and 20-μL aliquots were used to prepare quality control (QC) samples for LC–MS optimization and normalization. Chromatographic separation was performed using a Waters ACQUITY UPLC I-Class system on an ACQUITY UPLC BEH C18 column (Waters Corporation, USA). The mobile phases for gradient elution (column temperature: 40°C; sample temperature: 10°C) were (A) water with 0.1% formic acid (FA) and (B) acetonitrile with 0.1% FA. The total analysis time was 16 minutes at a flow rate of 0.30 mL/min: 0–1.5 minutes, 5%–20% B; 1.5–12 minutes, 20%–90% B; 12–13 minutes, 90%–100% B; and 13–16 minutes, maintaining at 5% B for re-equilibration.

LC–MS Data Acquisition

In the positive-ion mode, primary and secondary mass spectrometry data were collected using Masslynx software based on MSE functionality. The settings employed included a capillary voltage of 0.5 kV, a cone voltage of 40 V, an ion source temperature of 100°C, a desolvation gas flow of 1000 L/h, and a cone gas flow of 50 L/h. Ions within the m/z range of 100–1200 Da were scanned over 16 minutes at 0.2 sec/cycle. In negative-ion mode, similar parameters were used with a capillary voltage of 0.8 kV. The raw mass spectrometry data were processed for peak detection using Progenesis QI software (Waters Corporation, USA).

Statistical Analysis

The metabolite identification utilized the KEGG (http://www.genome.jp/kegg/) and HMDB (http://www.hmdb.ca) databases. In our statistical analysis, metabolites were first filtered based on a coefficient of variation (CV) of less than 30% in the QC samples to ensure reproducibility and reliability. We then applied unsupervised Principal Component Analysis (PCA) to preliminarily assess the relationships between samples and the explanatory power of metabolites in sample grouping. Subsequently, orthogonal partial least squares discriminant analysis (OPLS-DA) was utilized to further identify differential metabolites between moderate-to-severe OSA and control groups. In addition, permutation tests in the OPLS-DA analysis quantified the explained variance in group separation (R^2Y) and the model's predictive ability for new data (Q^2), with values above 50% considered acceptable. Variable importance in projection (VIP) scores for each metabolite were calculated based on the OPLS-DA model; a two-tailed Student's *t*-test was also conducted between groups to determine the significance of these metabolites. The false discovery rate was estimated using q values to address the issue of multiple comparisons.[24] In our study, VIP >1, and a p value <0.05 were considered differentially abundant metabolites.[25] Heatmaps were used to visualize the differential metabolites between groups. Receiver operating characteristic (ROC) analysis was employed to evaluate the capability of each differential metabolite to distinguish between moderate-to-severe OSA, with a higher area under the ROC curve (AUC) indicating stronger discriminative power. KEGG enrichment analysis and pathway analysis were then conducted to determine which metabolic pathways were significantly altered in moderate-to-severe OSA, aiding in understanding the disease mechanisms and their impact. All metabolomic data analyses mentioned above were performed using MetaboAnalyst 5.0 software (http://www.metaboanalyst.ca/).

Comparative analyses of clinical characteristics between groups were performed using IBM SPSS Statistics 26.0 software (IBM Corporation, Armonk, N.Y., USA). Categorical variables are presented as frequencies and percentages and were analyzed using the chi-square test. Continuous variables are presented as the mean and standard deviation (for normally distributed data) or median with interquartile range (for nonnormally distributed data) and were analyzed with Student's *t*-test or the Mann–Whitney test, respectively. Linear regression was used to explore the relationships between OSA-related indicators, metabolites, and cardiovascular risk measured by Framingham cardiovascular risk and arterial stiffness. Interaction analyses based on regression were conducted to assess if there are interactions between variables, which were also performed with SPSS Statistics 26.0 software. The mediation analysis was performed using the PROCESS v4.1 plugin in SPSS to evaluate how the effect of a variable on the cardiovascular outcome is mediated by another variable. To minimize the impact of confounders, regression analyses included OSA-related factors such as age and BMI, and further incorporated Framingham risk assessment indicators as confounders in three adjusted models: Model 1 (adjusted for age and BMI), Model 2 (adjusted for age, BMI, and TC), and Model 3 (adjusted for age, BMI, and SBP). Relationships between targeted differentially abundant metabolites and clinical indicators were examined using Spearman correlation and partial correlation analyses, which included age and BMI as confounding factors, with analyses and correlation bar graphs constructed using R Version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). A two-tailed p value of <0.05 was set as the threshold for statistical significance.

Results

General Characteristics of the Participants

A total of 135 males were screened, and only 36 males were enrolled in our study. As illustrated in Supplementary Figure S1, 22 individuals were in the moderate-to-severe OSA group, and 14 age-matched males were in the control group. The

Table 1 Baseline Characteristics of the Participants

	All Participants (N=36)	Moderate-to-Severe OSA (N=22)	Controls (N=14)	*P* value
Age, years	39.6±9.2	39.8±9.2	39.2±9.6	0.851
BMI, kg/m^2	26.3±3.1	27.2±2.9	24.7±2.8	0.015*
Neck circumference, cm	39.4±2.7	40.2±2.7	38.2±2.4	0.033*
SBP before sleep, mmHg	130.4±13.4	132.4±13.0	127.4±13.8	0.286
DBP before sleep, mmHg	88.7±9.3	90.5±8.1	85.9±10.6	0.150
SBP after sleep, mmHg	128.2±12.4	130.1±12.3	125.2±12.5	0.261
DBP after sleep, mmHg	86.2±10.8	88.6±10.4	82.6±10.8	0.107
AI@75, %	−0.64±14.6	1.32±15.0	−3.71±14.0	0.321
TC, mmol/L	5.1±1.0	5.1±1.1	5.2±0.7	0.719
Framingham cardiovascular risk, %	4.76 [2.33, 8.39]	5.30 [2.59, 8.68]	3.68 [2.02, 6.63]	0.256
ESS	12.5±5.7	12.9±5.3	12.0±6.4	0.663
PSQI	7.5±3.3	6.8±3.0	8.8±3.5	0.074
TST, min	401.0±56.8	409.7±60.7	387.3±49.0	0.255
AHI, events/h	41.7 [6.4, 63.7]	58.6 [47.5, 69.3]	4.4 [0.7, 11.1]	<0.001*
AI, events/h	35.1 [3.5, 58.5]	53.0 [35.8, 64.6]	1.5 [0.7, 3.9]	<0.001*
HI, events/h	5.9 [0.8, 9.7]	6.1 [0.8, 13.3]	2.3 [0.6, 8.3]	0.160
OAI, events/h	27.1 [1.3, 55.2]	47.4 [33.1, 60.4]	1.0 [0.3, 2.2]	<0.001*
ODI, events/h	29.7 [5.1, 63.8]	54.1 [38.3, 69.9]	2.7 [0.8, 9.3]	<0.001*
MeanSpO_2, %	95.6 [92.7, 97.8]	94.3 [92.1, 96.2]	97.7 [95.6, 98.1]	0.001*
LowestSpO_2, %	80 0.0 [71.5, 88.8]	73.5 [68.8, 80.0]	91.0 [87.0, 92.5]	<0.001*
T90%	0.6 [0.0, 7.4]	3.7 [1.0, 13.9]	0.0 [0.0, 0.03]	<0.001*

Notes: *indicates $p < 0.05$.

Abbreviations: AHI, apnea-hypopnea index; AI@75, augmentation index normalized to a heart rate of 75 bpm; BMI, body mass index; DBP, diastolic blood pressure; ESS, Epworth sleepiness scale; OAI, obstructive apnea index; ODI, oxygen desaturation index; OSA, obstructive sleep apnea; PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressure; SpO_2, peripheral blood oxygen saturation; TC, total serum cholesterol; TST, total sleep time; T90%, percentage of cumulative time with oxygen saturation less than 90%.

participants' clinical information is provided in Table 1. The mean age of all the participants was 39.6±9.2 years old, and their mean BMI was 26.3±3.1 kg/m^2. Obstructive respiratory events constituted 97.9% [92.5%, 100.0%] of all respiratory events. Individuals with moderate-to-severe OSA exhibited significantly greater BMI and neck circumference values. There was no significant difference in age between the two groups. Cardiovascular assessments revealed no significant differences between the two groups; however, a trend toward higher blood pressure, higher 10-year cardiovascular risk, and reduced vessel wall elasticity was observed in the moderate-to-severe OSA group. In terms of sleep assessment, daily sleepiness and subjective sleep quality did not significantly differ between the two groups. The AHI, ODI, and nocturnal hypoxia indices were markedly elevated in the moderate-to-severe OSA group.

Metabolome Profiling and Multivariate Analysis

Untargeted metabolomics analyses of urine samples were also conducted. A total of 14,517 and 12,278 features were detected in the negative-ion and positive-ion modes, respectively. The base peak chromatograms (BPCs) for the QC samples are illustrated in Supplementary Figure S2A and B, with the peak intensity on the vertical axis and the retention time on the horizontal axis. These BPCs exhibited numerous, finely resolved peaks, indicative of efficient chromatographic separation.

The unsupervised PCA was used to assess the intergroup distribution of metabolites and the quality of the metabolomic data. In the negative-ion and positive-ion modes, the QC samples demonstrated tight clustering, indicating a stable analytical process with good reproducibility (Supplementary Figure S3A and B). In the negative-ion mode, the first principal component accounted for 27.4% of the variance in the original data, while the second principal component explained 8.6%. In the positive-ion mode, the first principal component explained 28.2% of the variance in the original data, and the second principal component accounted for 11%.

To identify differentially expressed metabolites between moderate-to-severe OSA patients and their controls, substances detected in both ionization modes were compiled and analyzed. In cases of duplication, the substance with the higher quantification value was selected. OPLS-DA was applied to minimize confounding factors unrelated to the classification (Figure 1A). The model exhibited an R^2Y of 66.6% and a Q^2 of 59.3%, indicating a robust fit and predictive accuracy. Permutation tests confirmed the robustness and effectiveness of the model (Figure 1B). VIP scores were determined for metabolites using the OPLS-DA model, and p values were derived from two-tailed Student's *t*-tests. Metabolites with VIP > 1 and $p < 0.05$ were considered to be differentially expressed. These metabolites were subsequently identified using the HMDB and KEGG databases. Ultimately, we identified 65 different metabolites in our study (Supplementary Table S1). A heatmap was generated to visualize the expression patterns of these metabolites and compare them between the two groups (Figure 1C), revealing a notable increase in epinephrine sulfate and pyridoxine phosphate in the OSA group. In contrast, the concentration of 3-methyl-3-butenyl apiosyl-(1->6)-glucoside was markedly lower in patients with OSA (Figure 1D-1F). After correction for multiple testing, the q value for epinephrine sulfate remained below 0.05 (Supplementary Table S1). ROC analyses were also performed, and the results are detailed in Supplementary Table S2. Epinephrine sulfate also exhibited the strongest discriminatory ability for moderate-to-severe OSA of all biomarkers, with an area under the curve (AUC) of 0.883.

Enrichment Analysis and Pathway Analysis Revealed Metabolic Dysregulation

To further investigate the abnormal metabolic profiles of patients with moderate-to-severe OSA, we conducted enrichment analyses and pathway analyses within the KEGG database. The bubble chart shows the enrichment analysis of the top 25 metabolites (Figure 2A), with the size of each bubble corresponding to the enrichment ratio and the color gradient reflecting the p value. Metabolic pathways such as pyrimidine metabolism, androgen and estrogen metabolism, vitamin B6 metabolism, and sulfate/sulfite metabolism are prominently featured. The scatter plot shows the results of the pathway analysis (Figure 2B). Points on this plot are sized proportionally to their pathway impact values. The color intensity denotes the p value. The plot highlights many metabolic pathways, with pyrimidine metabolism and sulfur metabolism showing the most substantial pathway impacts and lowest p values, indicating that these pathways are highly relevant to the disease state.

Metabolite-Based Cardiovascular Risk Factors for OSA

Epinephrine sulfate was the metabolite with the most pronounced differential expression in our moderate-to-severe OSA patients. Using Spearman correlation analysis, we investigated the associations between epinephrine sulfate concentrations and various clinical parameters in the study population (Figure 3A) and found that epinephrine sulfate concentrations exhibited strong correlations with hypoxia-related parameters (ODI, lowest SpO_2, mean SpO_2, and T90%) and the AHI (Figure 3B). Additionally, risk factors for OSA (such as the degree of obesity and neck circumference) were also correlated with the epinephrine sulfate concentration. Notably, the concentration of epinephrine sulfate also exhibited a moderate correlation with blood pressure. Additionally, both the AI@75 and Framingham risk model showed correlations with epinephrine sulfate ($r=0.361$, $p=0.031$; $r=0.375$, $p=0.024$), suggesting its utility in indicating an increased risk of elevated blood pressure, relatively poor vascular elasticity, and a greater risk of long-term cardiovascular complications in such patients (Figure 3C and D).To minimize the impact of potential confounders, we conducted Spearman's partial correlation analysis, adjusting for variables, namely age and BMI. Even after adjusting for confounders, these correlations remained significant (Figure 3E).

The relationship between epinephrine sulfate concentration and the Framingham 10-year cardiovascular risk score was further evaluated. Table 2 presents the results of linear regression analyses. According to the univariate linear regression, the epinephrine sulfate concentration was significantly positively correlated with the Framingham cardiovascular risk score (β 0.004, 95% CI=0.000–0.009, p=0.049). To validate the robustness of these associations, we incorporated the general characteristics of the patients (Model 1: age and BMI) and other variables from the Framingham cardiovascular risk model (Model 2: age, BMI, and TC; Model 3: age, BMI, and SBP). Epinephrine sulfate concentration was significantly positively correlated with cardiovascular risk according to the adjusted models. Other traditional OSA assessment metrics such as the AHI and ODI were significantly associated with the Framingham cardiovascular risk in Model 1 and 2. However,

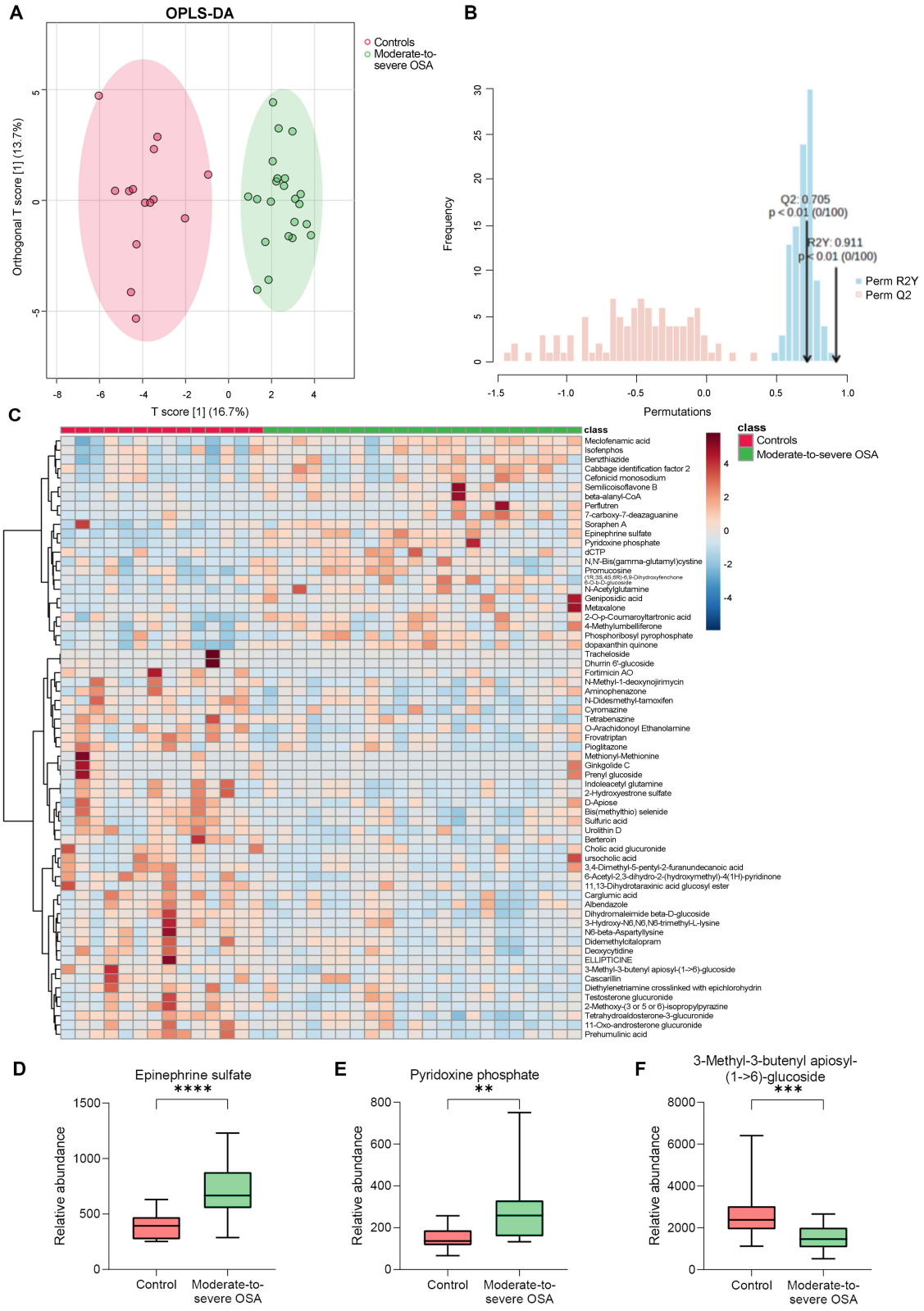

Figure 1 Metabolic changes associated with moderate-to-severe OSA. Orthogonal partial least square discriminant analysis (OPLS-DA) plots (**A**) and permutation analysis (**B**) for the metabolic profiles between the moderate-to-severe OSA group and controls. (**C**) Heatmap showing the differential expression patterns of the metabolites between the two groups. (**D-F**) The box plot illustrates the three metabolites with the most significant differences between moderate-to-severe OSA patients and the control group. **, ***, and **** indicate $p < 0.01$, 0.001, and 0.0001, respectively.

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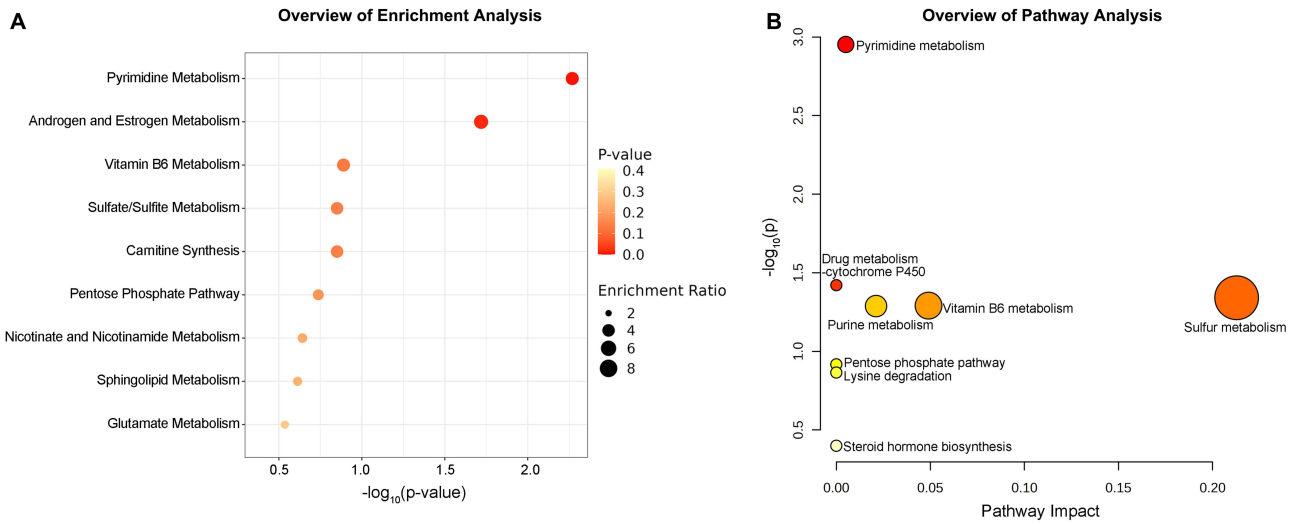


Figure 2 Abnormalities in metabolic pathways in patients with moderate-to-severe OSA. The results of the (**A**) enrichment analysis and (**B**) pathway analysis for the top 25 differential metabolites. (**A**) A bubble chart displays the results of the enrichment analysis. Each bubble's size represents the enrichment ratio, and the color gradient and the horizontal axis both reflect the *p* values.(**B**) A scatter plot illustrates the results of the pathway analysis. The size and horizontal positioning of each point indicate the pathway impact values, whereas the color and vertical axis represent the *p* values.

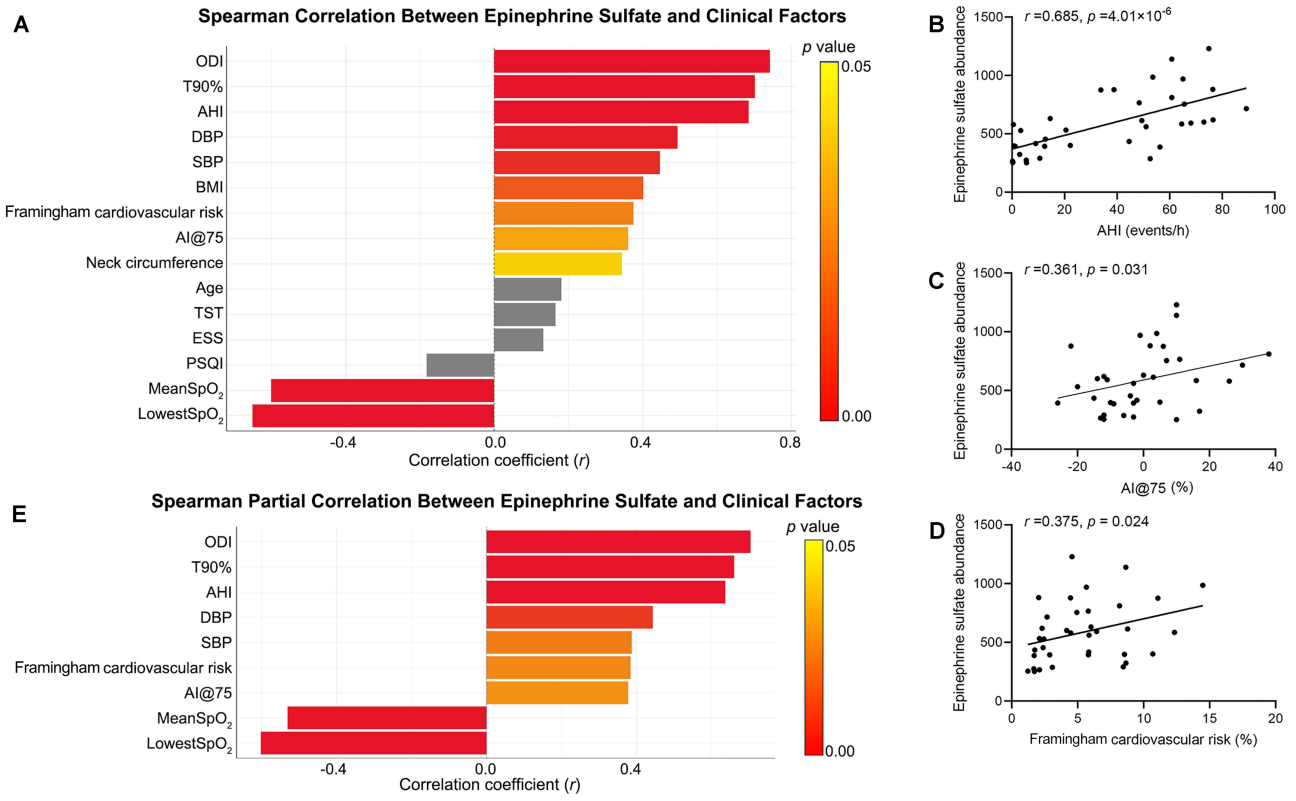


Figure 3 Spearman correlation analyses between epinephrine sulfate concentration and clinical indicators. (**A**) Results of Spearman correlation analyses. Each bar represents a different clinical indicator, with the length and direction of the bar indicating the strength and direction of its correlation with epinephrine sulfate. Indicators with p values <0.05 are color-coded based on the color gradient, while indicators with p values ≥0.05 are shown in gray. (**B–D**) Epinephrine sulfate concentrations are positively correlated with the AHI, AI@75, and the Framingham cardiovascular risk. (**E**) Results of Spearman partial correlation analyses. Age and BMI were included as confounding factors.

Abbreviations: AHI, apnea-hypopnea index; AI@75, augmentation index normalized to a heart rate of 75 bpm; BMI, body mass index; DBP, diastolic blood pressure; ESS, Epworth sleepiness scale; ODI, oxygen desaturation index; OSA, obstructive sleep apnea; PSQI, Pittsburgh Sleep Quality Index; SBP, systolic blood pressure; SpO_2, peripheral blood oxygen saturation; TST, total sleep time; T90%, percentage of cumulative time with oxygen saturation less than 90%.

Table 2 Relationships Between OSA-Related Indicators and Framingham Cardiovascular Risk

Framingham Cardiovascular Risk	β(95% CI) of Clinical Factors About OSA			
	Crude Model	Model 1	Model 2	Model 3
Epinephrine Sulfate	0.004 (0.000, 0.009)*	0.003 (0.001, 0.005)**	0.003 (0.001, 0.005)**	0.002 (0.000, 0.004)*
AHI	0.012 (−0.029, 0.053)	0.021 (0.001, 0.04)*	0.023 (0.004, 0.041)*	0.016 (−0.002, 0.034)
ODI	0.012 (−0.029, 0.054)	0.023 (0.003, 0.042)*	0.025 (0.007, 0.044)**	0.018 (−0.001, 0.036)
MeanSpO_2	−0.041 (−0.397, 0.315)	−0.105 (−0.27, 0.06)	−0.126 (−0.285, 0.034)	−0.089 (−0.237, 0.06)
LowestSpO_2	−0.052 (−0.162, 0.057)	−0.046 (−0.095, 0.004)	−0.045 (−0.092, 0.003)	−0.040 (−0.084, 0.004)
T90%	0.035 (−0.045, 0.115)	0.032 (−0.003, 0.066)	0.031 (−0.003, 0.064)	0.023 (−0.010, 0.055)

Notes: *and **indicate $p<0.05$ and $p<0.01$, respectively. Model 1: adjusted for age and BMI; Model 2: adjusted for age, BMI and TC; Model 3: adjusted for age, BMI and SBP.
Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; CI, confidence interval; ODI, oxygen desaturation index; OSA, obstructive sleep apnea; SBP, systolic blood pressure; SpO_2, peripheral blood oxygen saturation; T90%, percentage of cumulative time with oxygen saturation below 90%; TC, total serum cholesterol.

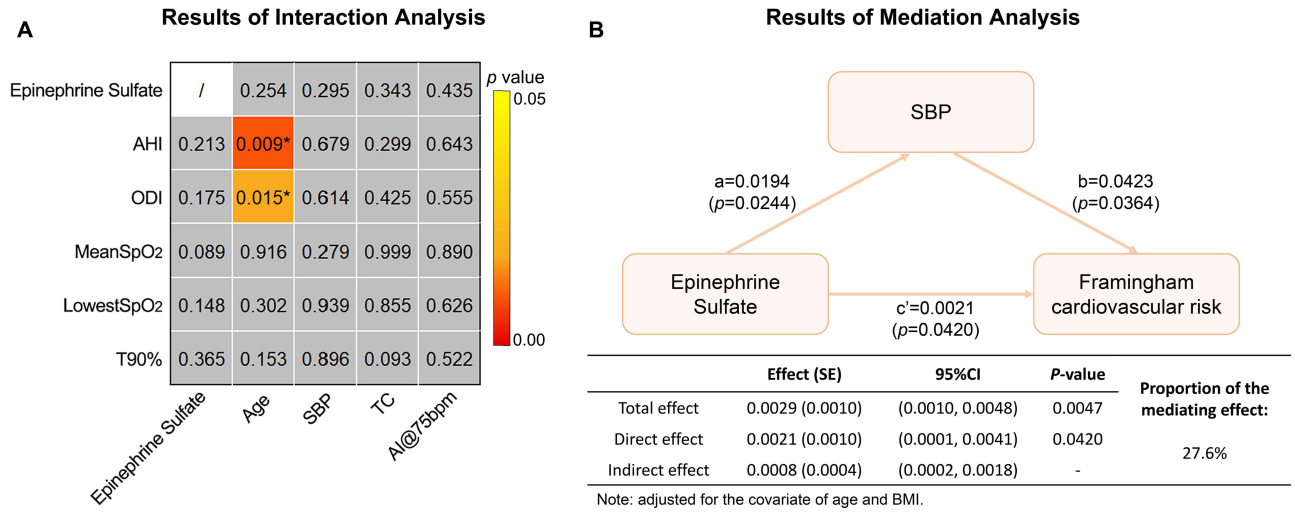
Table 3 Relationships Between OSA-Related Indicators and AI@75

AI@75	β(95% CI) of Clinical Factors About OSA			
	Crude model	Model 1	Model 2	Model 3
Epinephrine Sulfate	0.019 (0.000, 0.037)*	0.022 (0.003, 0.041)*	0.023 (0.004, 0.042)*	0.011 (−0.007, 0.028)
AHI	0.134 (−0.037, 0.304)	0.229 (0.052, 0.406)*	0.226 (0.045, 0.406)*	0.181 (0.034, 0.328)*
ODI	0.149 (−0.022, 0.319)	0.257 (0.080, 0.433)**	0.253 (0.073, 0.434)**	0.197 (0.049, 0.346)*
MeanSpO_2	−0.617 (−2.127, 0.893)	−1.056 (−2.595, 0.484)	−1.012 (−2.588, 0.563)	−0.863 (−2.118, 0.392)
LowestSpO_2	−0.253 (−0.718, 0.212)	−0.338 (−0.808, 0.132)	−0.341 (−0.816, 0.135)	−0.273 (−0.657, 0.111)
T90%	0.264 (−0.068, 0.597)	0.295 (−0.030, 0.621)	0.299 (−0.030, 0.627)	0.188 (−0.088, 0.464)

Notes: *and **indicate $p<0.05$ and $p<0.01$, respectively. Model 1: adjusted for age and BMI; Model 2: adjusted for age, BMI and TC; Model 3: adjusted for age, BMI and SBP.
Abbreviations: AHI, apnea-hypopnea index; AI@75, augmentation index normalized to a heart rate of 75 bpm; BMI, body mass index; CI, confidence interval; ODI, oxygen desaturation index; OSA, obstructive sleep apnea; SBP, systolic blood pressure; SpO_2, peripheral blood oxygen saturation; T90%, percentage of cumulative time with oxygen saturation below 90%; TC, total serum cholesterol.

these indices did not show a consistent relationship with the Framingham cardiovascular risk in the Crude Model and Model 3. Additionally, other hypoxia indices did not exhibit any significant correlations with the Framingham cardiovascular risk across all evaluated models. Furthermore, since AI@75 also reflects the current arterial stiffness of patients and indicates their cardiovascular health, we also conducted linear regression analysis with AI@75 as the outcome variable (Table 3). The results show that epinephrine sulfate is positively correlated with AI@75. This relationship was still significant after adjusting for age and BMI, and further adjusting for TC. However, the correlation loses statistical significance after adjusting for SBP. For the AHI and ODI, the relationship with AI@75 was not significant in the crude model. However, after adjusting for age, BMI, TC, and SBP, these indices demonstrated a significant association with AI@75. Other hypoxia-related indices continued to show no significant relationship with AI@75.

After obtaining the Spearman correlation coefficient of epinephrine sulfate concentration with various OSA indicators, we conducted an interaction analysis and mediation analysis to further assess the role of this correlation in predicting CVD risk (Figure 4). Regarding the impact on Framingham cardiovascular risk, the results showed no significant interaction between epinephrine sulfate and the other indicators ($p > 0.05$) (Figure 4A). Furthermore, in our patients, both the AHI and ODI interacted with age (Figure 4A), which might be the reason for the significant association between the AHI and ODI and Framingham risk after we adjusted for age in the linear regression model. In terms of



	Effect (SE)	95%CI	*P*-value	Proportion of the mediating effect:
Total effect	0.0029 (0.0010)	(0.0010, 0.0048)	0.0047	27.6%
Direct effect	0.0021 (0.0010)	(0.0001, 0.0041)	0.0420	
Indirect effect	0.0008 (0.0004)	(0.0002, 0.0018)	-	

Note: adjusted for the covariate of age and BMI.

Figure 4 Results of interaction and mediation analyses. (**A**) The heatmap displays the p values for the interaction effects between each metric on the y-axis and each metric on the x-axis in the linear regression for Framingham cardiovascular risk. Gray indicates no significant interaction, and a gradient from yellow to red signifies progressively lower p values, indicating increasingly significant interactions. * indicate $p < 0.05$. (**B**) The path diagram illustrates the mediating role of SBP in the effect of epinephrine sulfate on Framingham cardiovascular risk.

Abbreviations: AHI, apnea-hypopnea index; AI@75, augmentation index normalized to a heart rate of 75 bpm; ODI, oxygen desaturation index; SBP, systolic blood pressure; SpO_2, peripheral blood oxygen saturation; T90%, percentage of cumulative time with oxygen saturation less than 90%; TC, total serum cholesterol.

influencing the AI@75, there were no interaction effects observed among epinephrine, AHI, ODI, hypoxia indicators, age, SBP and TC (p>0.05). Subsequently, we conducted a mediation analysis. After adjusting for age and BMI, we conducted analyses using AHI, ODI, lowest and mean SpO2, T90%, AI@75, and TC as mediating variables for the risk of epinephrine sulfate on the Framingham cardiovascular risk (Supplementary Table S3). The mediation analyses indicated that SBP partially mediated the impact of epinephrine sulfate on cardiovascular risk. Specifically, 27.6% of the effect was attributable to an elevation in systolic blood pressure, with the remaining 72.4% being a direct effect of epinephrine sulfate in this model (Figure 4B). The mediation analysis regarding the effect of epinephrine sulfate on AI@75 revealed that the influence of epinephrine sulfate is entirely mediated by blood pressure (p for direct effect of epinephrine sulfate on AI@75 > 0.05, details in Supplementary Table S4). This can explain why the effect of epinephrine sulfate on AI@75 no longer reached statistical significance in the linear regression after controlling for blood pressure.

Discussion

In this study, we utilized nontargeted metabolomic analysis of urine samples and identified 65 metabolites, covering a range of metabolic pathways, including pyrimidine metabolism, hormone metabolism, vitamin B6 metabolism, and sulfate metabolism by enrichment and pathway analyses. Notably, epinephrine sulfate showed the most significant difference in patients with moderate-to-severe OSA compared with the controls. Additionally, correlation and regression analyses further indicated an association between higher epinephrine sulfate levels and the risk of adverse cardiovascular events.

Previous research has underscored the role of urine metabolomics in OSA studies. Given its noninvasive and convenient collection, urine is an ideal sample for metabolic analyses. Adam et al compared the effectiveness of urine, serum, and exhaled breath condensate metabolites in differentiating OSA from COPD and found that urine metabolites exhibit superior discriminatory power.[26] Xu et al also reported disparities in purine metabolites and amino acids between patients with OSA and simple snorers.[25] Similarly, Mohit et al identified distinct metabolites, including adrenaline and various amino acids, in severe OSA patients, which were also detected in our study.[27] In addition to urine metabolomics analysis, Laura's team, through a comparative analysis of overnight pre-sleep and post-sleep sweat metabolomics in patients with severe OSA, discovered distinct differences in lactose, succinate, urea, and oxoproline in sweat.[28] These variations were correlated with OSA severity and nocturnal hypoxia, highlighting the impact of OSA on energy metabolism, nitrogen metabolism, and oxidative stress. Consequently, OSA disrupts multiple metabolic

pathways within the body. Our study also revealed significant alterations in pathways related to nucleotide metabolism, energy metabolism, vitamin and cofactor metabolism, carbohydrate metabolism, and amino acid metabolism in patients with moderate-to-severe OSA.

In OSA, metabolic alterations reflect the multifaceted nature of the disease, which is influenced by hypoxia, inflammation, repeated arousal, and autonomic nervous system changes. Our research notably highlights alterations in the pyrimidine metabolic pathway, which plays a pivotal role in the synthesis of DNA, RNA, lipids, and carbohydrates, and also in the synthesis, degradation, recycling, transformation, and transport of these substances by various enzymes.[29] The synthesis of dihydronicotinamide riboside in the respiratory chain-dependent dihydrolactate dehydrogenase phase represents a key regulatory point impacted by hypoxic conditions, affecting cellular metabolic activity and exemplifying the effects of intermittent hypoxia in OSA.[30] Vitamin B6 plays a multifaceted role in cellular metabolism, including amino acid metabolism and neurotransmitter synthesis. Hypoxic conditions can induce the downregulation of pyridoxal kinase as a protective cellular response.[31] Furthermore, the levels of vitamin B6 are correlated with inflammatory markers such as C-reactive protein, suggesting a potential role in inflammatory response regulation.[32,33] The pentose phosphate pathway (PPP) reflects the organism's response to hypoxia and inflammation. Some studies associate this pathway with chronic neuroinflammation and dopaminergic neurodegenerative changes and high-altitude pulmonary circulation hypoxemia.[34,35] The carnitine synthesis pathway is critical for the body's autoregulation response to hypoxia and inflammatory stimuli. An increase in the serum and muscle carnitine levels can enhance muscle tissue blood flow and oxygen supply by improving endothelial function, thereby reducing cellular and biochemical disorders caused by hypoxia.[36] L-carnitine supplementation may aid in ameliorating inflammatory conditions and decreasing the levels of inflammatory cytokines.[37] In hypoxic conditions, the expression of purinosomes, key components of purine metabolism, increases. The purinosome is a dynamic metabolic complex composed of enzymes responsible for de novo purine synthesis, indicating that purine metabolism is an essential biological pathway under hypoxic conditions.[38] Additionally, alterations in sex hormone-related pathways are associated with the epidemiological characteristic of increased OSA incidence in males and the therapeutic effects of estrogen treatment in postmenopausal women.[39] This difference may be related to the increased activity of sulfiredoxin and Nrf-2, p38 MAP kinase activation, vagal C fiber inhibition, and HIF-1α attenuation.[40]

OSA is associated with cardiovascular risks, including hypertension, stroke, myocardial infarction, heart failure, arrhythmias, sudden cardiac death, and increased mortality.[41] Untreated OSA leads to intermittent hypoxemia and hypercapnia, frequent respiratory arousals, intrathoracic pressure swings, and alterations in autonomic nervous system functioning. These changes disrupt normal blood pressure regulation, impair endothelial function, provoke systemic inflammation, induce oxidative stress, and destabilize metabolism, elevating cardiovascular disease risk.[41] The Framingham 10-year cardiovascular risk score provides a comprehensive prediction of a patient's risk of developing cardiovascular events over the next decade. Research as early as published in 2000 utilized the initial version of the Framingham risk score to assess cardiovascular risk in OSA patients.[42] Subsequent studies also found higher AHI scores in populations with moderate to high Framingham cardiovascular risk.[43,44] In our study, compared to controls, patients with moderate-to-severe OSA had higher, yet not statistically different, Framingham cardiovascular scores. Some previous studies were similar with this study.[45] The possible reasons were as follows: a) the small sample size, which only showed relative size relationships without statistical differences; b) AHI could not fully reflect cardiovascular risk.[46] However, generally, moderate-to-severe OSA tends to increase the risk of future cardiovascular events. The AI is an indicator of arterial stiffness and previous researches in OSA patients has found that more severe OSA correlates with higher AI,[47–49] independent of peripheral blood pressure increase.[48] In our study, the AI@75 was higher in moderate-to-severe patients, although the difference was not statistically significant, likely due to the small sample size. Given the close association between OSA and cardiovascular health, our study further evaluated the relationship between cardiovascular risk and urinary epinephrine sulfate, a differentially abundant metabolite identified between moderate-to-severe OSA and control groups.

Through our analysis, we have determined that epinephrine sulfate effectively assesses cardiovascular risk in patients with OSA. Regression analysis revealed that, compared to the AHI and hypoxia markers, epinephrine sulfate provides the most robust regression outcomes for future cardiovascular risk in this subset of OSA patients. Additionally, interaction

analyses demonstrate that the assessment capabilities of epinephrine sulfate on cardiovascular risk are not significantly moderated by other factors; that is, the impact of epinephrine sulfate on cardiovascular risk does not vary significantly across age, systolic blood pressure, and other indicators. Furthermore, mediation analysis has uncovered that a portion of the impact of epinephrine sulfate on future cardiovascular risk is mediated through its effects on SBP. Additionally, epinephrine sulfate can also effectively indicate the current level of arterial stiffness in patients. Therefore, overall, epinephrine sulfate is suitable for assessing cardiovascular risk in the OSA population.

Epinephrine sulfate, which emerged as a key metabolite in our study, exhibited strong correlations with the AHI, ODI, meanSpO_2, and lowestSpO_2. Urinary epinephrine sulfate, a metabolite of epinephrine, is linked to abnormalities in epinephrine levels associated with sleep disorders, anxiety, and cardiovascular diseases.[50] A meta-analysis focusing on pediatric OSA patients revealed elevated levels of urinary norepinephrine and epinephrine in OSA patients, which correlated with disease severity (SMD 1.45, 95% CI 0.91–2.00, I^2=75%, p<0.001; SMD 1.84, 95% CI 0.00–3.67, I^2=97%, p=0.05).[51] Autonomic dysfunction is recognized as a key mechanism contributing to cardiovascular damage in OSA patients.[52] Our findings are in line with the results of a previous study showing that patients with moderate-to-severe OSA had increased urinary catecholamines, indicating autonomic dysfunction in these patients.[53]

Epinephrine sulfate also offers potential for cardiovascular risk assessment in patients with OSA. Currently, there is no clear consensus on predictive markers for cardiovascular risk in patients with OSA. Potential assessment markers include combinations of PSG metrics, such as the multilevel interval coded scoring by Margot,[54] biomarkers such as CRP and IL-6 for inflammation, amino acids, including homocysteine, exhaled nitric oxide tests, and microRNAs.[55] Metabolomics has revealed several potential cardiovascular risk factors across different populations.[56] However, these markers either lack specificity for OSA or are not yet practical for clinical application, and there is a paucity of direct analyses of metabolomics and cardiovascular risk in OSA patients. Our study showed that epinephrine sulfate is associated with various cardiovascular indices, such as vascular elasticity. The adjusted augmentation index, which is derived from pulse-wave analyses, is a measure of systemic arterial stiffness that is linked to cardiac risk factors and coronary artery disease (CAD) and potentially serves as a useful indicator for overall CAD risk assessment.[57] Moreover, in our study, epinephrine sulfate was an independent factor for increased Framingham 10-year cardiovascular risk, independent of other cardiovascular risk indicators, such as age, blood lipids, and obesity level. Its effect was partially mediated by increased blood pressure, further exacerbating cardiovascular risk. Existing researches indicated that urinary catecholamine metabolites were associated with SBP and cardiovascular disease.[58,59] Another study involving 34 hypertensive OSA patients also reported that approximately two-thirds of them exhibited elevated urinary normetanephrine level.[60] However, there are limited studies utilizing urinary catecholamine metabolites as biomarkers for cardiovascular risk in adult OSA population. Additionally, from a mechanistic standpoint, adrenergic substances themselves can directly influence the cardiovascular system and participate in its pathogenesis,[61] which supports our research findings. We present urinary epinephrine sulfate as a potential key biomarker for cardiovascular risk assessment in patients with OSA.

Our study is the first to use metabolomic approaches to identify biomarkers for arterial stiffness and long-term cardiovascular risk in male patients with moderate-to-severe OSA. Previously, metabolomics has been predominantly applied to assess the severity of OSA and aid in its diagnosis,[25–28,62] but few studies have evaluated the long-term cardiovascular risk in OSA patients. Our study bridges this research gap. Additionally, while there has been considerable research on the association between adrenergic substances and cardiovascular diseases, studies in adult OSA patients are insufficient. The identification of epinephrine sulfate, a metabolite of adrenergic metabolism, reemphasizes the importance of related physiological processes in the cardiovascular complications of OSA. Our research, while insightful, is subject to certain limitations. Primarily, to mitigate the impact of sex-specific metabolic differences, we included only male patients, which restricts the broader applicability of our findings. Indeed, physiological differences in sleep and sleep-related disorders exist between males and females, potentially related to differences in sex hormones.[63] In the context of OSA, the prevalence is somewhat lower in females, who are more likely to exhibit symptoms such as insomnia and anxiety, rather than the more typical daytime sleepiness and fatigue seen in OSA. Additionally, metabolomic differences between genders are evident, with studies indicating that over half of the metabolites differ between sexes.[64] Consequently, our study results cannot be directly generalized to the entire population. However, since males

remain the primary demographic affected by OSA and cardiovascular diseases, our research still holds clinical value. Consequently, further research involving female patients is essential to gain a more holistic understanding of metabolic alterations in OSA patients. Additionally, urinary metabolites can be influenced by various factors. In our study, urine samples were collected in the morning following PSG monitoring. However, it was difficult to strictly control the patients' lifestyle habits in the days preceding the PSG, which may have affected the metabolomic outcomes. Furthermore, the relatively small sample size in our study may have limited the statistical power of our analyses. Given the absence of prior studies examining the relationship between metabolites and long-term cardiovascular risk in adults with severe OSA, determining an optimal sample size or achieving sufficient statistical power was particularly challenging. We employed comprehensive statistical methods to minimize the impact of confounding variables and strengthen the reliability of our findings. We anticipate that future research involving larger cohorts will further validate and enhance the generalizability of our conclusions. Besides, our present study is a cross-sectional analysis employing indirect cardiovascular outcomes, which includes the collection of current clinical data, blood pressure measurements, and arterial stiffness assessments. We utilize the Framingham 10-year cardiovascular risk assessment to predict patients' cardiovascular risk. While this design increases the feasibility of the experiment and minimizes the impact of metabolomic variability over time,[65] it inherently restricts our conclusions to correlations rather than causal determinations. Furthermore, the Framingham cardiovascular risk score does not completely represent actual long-term cardiovascular events, such as myocardial infarction, stroke, or cardiovascular death. Building upon the foundation established by our initial findings, we anticipate that future research involving larger cohorts and longer follow-up periods, which employ direct cardiovascular events as endpoints, will both validate and expand upon our results. Such studies are likely to offer more conclusive evidence to aid in elucidating the intricate metabolic interactions associated with OSA.

Conclusion

In our metabolomics study, we focused on analyzing the urinary metabolic profiles of male patients with moderate-to-severe OSA. According to our findings, moderate-to-severe OSA significantly influences multiple metabolic pathways. Notably, epinephrine sulfate was the most differentially expressed substance between the study groups, positioning itself as a potential biomarker for cardiovascular risk assessment in OSA patients. This discovery highlights the value of combining metabolic biomarkers with clinical assessment tools for the improved and comprehensive evaluation of cardiovascular risk in patients with OSA.

Data Sharing Statement

The corresponding author, Jinmei Luo, will supply the relevant data in response to reasonable requests.

Ethics Approval and Informed Consent

The study protocol was approved by the ethics committees of PUMCH (JS-2627) and was conducted following the Declaration of Helsinki. Written informed consent was obtained from all participants. We ensure that all patients' personal information and data have been kept confidential, without involving any patient privacy issues.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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