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Article

Breath and Blood Metabolomics: A Comparative Study Using SESI-HRMS/MS and UHPLC-ESI-HRMS/MS

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Cite This: *Environ. Health* 2025, 3, 596−604

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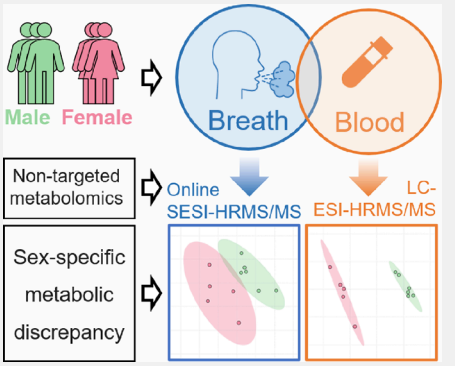
ABSTRACT: Breath metabolomics enables noninvasive and rapid acquisition of metabolic information by detecting volatile organic compounds (VOCs) in exhaled breath. Secondary electrospray ionization high-resolution tandem mass spectrometry (SESI-HRMS/MS) offers the highest coverage for detecting breath metabolites among current real-time breath analysis techniques. Although it has been generally recognized that metabolites in breath originate from the blood, a molecular-level understanding of the characteristics of metabolites in both breath and blood remains insufficient. In this study, nontargeted analyses of breath and blood samples from 11 healthy volunteers were performed using SESI-HRMS/MS and ultrahigh performance liquid chromatography electrospray ionization high-resolution tandem mass spectrometry (UHPLC-ESI-HRMS/MS), respectively. Tandem mass spectrometry was employed for metabolite annotation. Twenty-six breath-unique metabolites and 73 blood-unique metabolites were identified. Besides, seven metabolites were found in both breath and blood, including 7-oxabicyclo [2.2.1] heptane, levulinic acid, indole, pyroglutamic acid, malic acid, glutamic acid, and histidine. Intriguingly, the correlation of these metabolites between breath and blood was low ($r < 0.4$ or $p > 0.05$). Among all the confirmed metabolites, breath metabolites exhibit higher volatility according to their water−gas partition coefficient (log $P_{w/g}$) compared to blood metabolites. In addition, gender-derived differences in breath were significantly smaller than blood. In summary, this study indicates that breath metabolites are likely to offer complementary information on blood metabolites. When combined with blood metabolomics, this would be advantageous for the appropriate application of breath metabolomics in life sciences, such as in biomarker discovery.



KEYWORDS: *breath metabolomics, blood metabolomics, SESI-HRMS/MS, UHPLC-ESI-HRMS/MS, metabolite identification, metabolomics comparison*

1. INTRODUCTION

Metabolomics is regarded as the ultimate expression of genomics, transcriptomics and proteomics.[1] Metabolomics utilizing high-throughput technologies can cover endogenous and exogenous small-molecule metabolites (<1500 Da) such as amino acids, lipids, carbohydrates and organic acids in biological samples including blood, urine, tissues and exhaled breath.[2−5] This broad analytical approach allows for a comprehensive understanding of metabolic processes, thereby revealing key insights into the relevant mechanisms. Among the various branches of metabolomics, breath metabolomics has developed rapidly in recent years due to its merits of noninvasiveness and convenience. Studies have highlighted the potential of breath metabolomics in biomarker discovery, which is of great importance for the monitoring of disease occurrence and treatment, pharmacokinetics, exercise and exposure monitoring.[6−13]

The important hypothesis for using exhaled breath as a sample in metabolomics research is that it contains numerous trace volatile metabolites, the majority of which are derived from blood, subsequently traverse the blood-gas barrier into the alveoli and ultimately are exhaled from the body.[14] However, to the best of our knowledge, the understanding of the correlation of compounds between breath and blood is confined to drugs and a few of endogenous metabolites.[8,9,15−17] Recently, Ahmed et al. have applied breath and plasma metabolomics to assess inflammation in acute stroke.[18] Forty-six volatile organic compounds (VOCs) were analyzed in breath samples exclusively from patients with ischemic stroke by using sorbent tube followed by thermal desorption-gas chromatography-time-of-flight-mass spectrometry (TD-GC-ToF-MS). Correlations between 816 plasma metabolite features and 46 breath VOCs were preliminary explored. It

Received: November 24, 2024
Revised: February 14, 2025
Accepted: February 25, 2025
Published: March 5, 2025

https://doi.org/10.1021/envhealth.4c00248
Environ. Health 2025, 3, 596−604

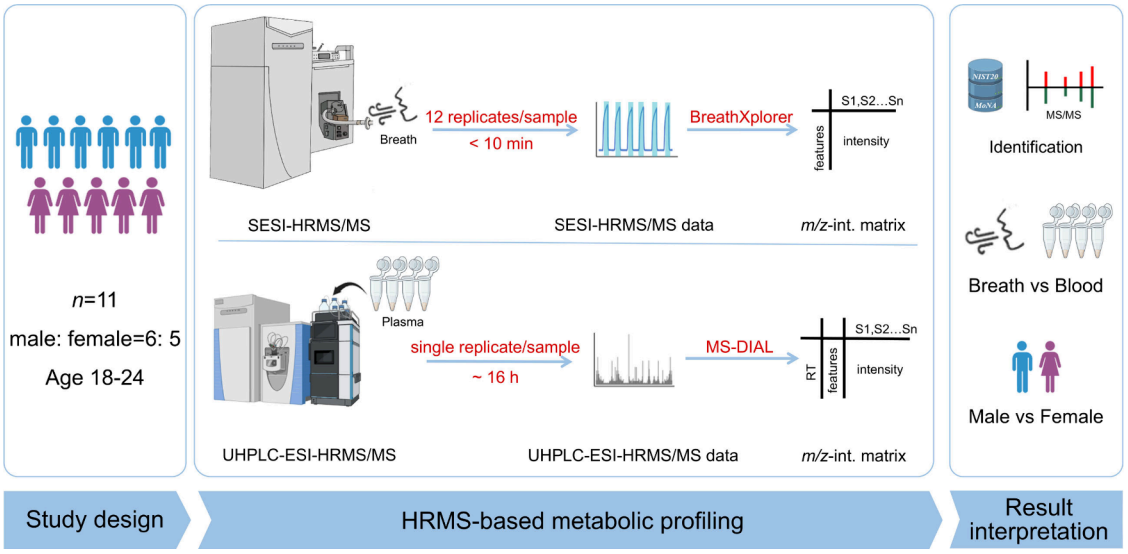


Figure 1. Breath (top) and blood (bottom) metabolomics study flow. Breath and blood samples were collected from 11 subjects (6 males and 5 females). Breath samples were analyzed in real-time using SESI-HRMS/MS, which was completed within 10 min for each sample. The *m*/*z*-int matrix was obtained via BreathXplorer. Blood samples underwent pretreatment and were detected by UHPLC-ESI-HRMS/MS. The entire process took approximately 16 h. The *m*/*z*-int matrix was extracted using MS-DIAL. Metabolites in both breath and blood were identified based on MS/MS matched to the NIST20 and MoNA databases. The identified metabolites were then utilized to investigate the similarities and differences between breath and blood metabolomics. Finally, an analysis of differences in metabolome between males and females was conducted.

was discovered that VOC functional groups are highly correlated with plasma features, for example with alkanes and terpenes, suggesting that some blood plasma metabolites are directly related to breath VOCs. However, the coverage of breath metabolites reported in this paper was rather restricted, especially since most of the detected compounds are nonpolar volatile ones. An in-depth investigation of the relationship between breath and blood metabolites has yet to be undertaken. The inability to fully understand the similarities and differences of metabolites between breath and blood has greatly hindered the application of breath analysis.

A systematic comparison of metabolomics in breath and blood requires the adoption of comparable detection technologies. Currently, breath metabolomics research predominantly depends on offline techniques such as gas chromatography−mass spectrometry (GC-MS). In recent years, methods utilizing online mass spectrometry have attracted increasing attention as these methods enable real-time detection of exhaled breath, as well as minimize sample loss during collection and pretreatment which commonly occurs in GC-MS analysis. These methods primarily include proton transfer reaction mass spectrometry (PTR-MS) and secondary electrospray ionization mass spectrometry (SESI-MS).[19,20] Compared with PTR-MS, SESI can be coupled with high-resolution mass spectrometry (HRMS), a significant greater number of features have been detected in breath.[20] This enhanced capability facilitates more precise assessments of physiological status through high-sensitivity detection of changes in breath metabolites. While SESI-HRMS has been widely applied in breath analysis,[11,21−25] it mainly identifies compounds based on the exact mass of the features,[10,26] which limits the reliability of breath biomarkers.[27] Ultrahigh performance liquid chromatography electrospray ionization high-resolution tandem mass spectrometry (UHPLC-ESI-HRMS/MS) is the most commonly used analytical tool for blood metabolomics,[28−31] offering broad metabolite coverage.[32,33] SESI, as an ionization technique derived from electrospray ionization (ESI), can ionize a wide range of species with a broad polarity range similar to that in ESI. Besides, similar relative peak intensities have been observed for the same compounds in both SESI and ESI.[21]

Building on SESI-HRMS, this study further developed an SESI-HRMS/MS analytical method. Nontargeted analysis of breath and blood was conducted using SESI-HRMS/MS and UHPLC-ESI-HRMS/MS, respectively (Figure 1). Metabolites in breath and blood were identified employing high-resolution MS/MS to provide relatively higher identification accuracy. A comprehensive analysis of the similarities and differences between breath and blood was conducted according to the identification results. This analysis will contribute to a better understanding of the application of breath metabolomics acquired by SESI-HRMS/MS.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

HPLC-grade acetonitrile, methanol, ammonium hydroxide, 0.1% (v/v) formic acid solution in ultrapure water and MS-grade ammonium acetate were purchased from Merck (Germany). MS-grade formic acid was purchased from Thermo (USA).

2.2. Subjects

The experiments were conducted between March 11 and 17, 2023. Breath and blood samples were collected from 11 healthy volunteers (6 males and 5 females, age: 18−24 years) with no smoking history (Table S1). The subjects were instructed to fast for 3 h prior to sampling, avoid intense exercise for 1 h before the test and refrain from consuming beverages such as alcohol, tea or coffee. They were also advised not to apply makeup, lipstick, lip balm or perfume before the test to minimize the impact of nonstudy factors on the samples.[10,26] This study was approved by the Medical Ethics Committee of Jinan University (No. JNUKY-2023−0059), and all subjects provided informed consent.

2.3. Breath Sample Collection and Analysis

Breath samples were analyzed in real-time using a SESI source (Super SESI, Fossil Ion Technology, Spain) coupled with a high-resolution

https://doi.org/10.1021/envhealth.4c00248
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mass spectrometer (Q Exactive, Thermo Fisher Scientific, USA) (Figure 1). Prior to sample collection, subjects rinsed their mouths for three times with clean water. Breath samples were exhaled through a mouthpiece (MicroGard, Vyaire Medical, USA) connected to the Super SESI. Each subject provided 6 exhalations in positive ion mode and 6 in negative ion mode. A flow sensor (Exhalion, Fossil Ion Technology, Spain) regulated the exhalation flow rate at 7 L min^{-1}. Before the experiment, the mass calibration of the Q Exactive was conducted using the commercial calibration solution Pierce LTQ Velos ESI (Thermo Fisher Scientific, USA). The instrument was calibrated using a 100 ppbv standard gas of α-terpinene (Dalian Special Gases, China) to ensure that the signal intensity was above 10^9.[26,34] Detailed parameter settings for Super SESI and Q Exactive can be found in Text S1.

2.4. Blood Sample Collection and Analysis

After collecting breath samples, blood samples were immediately collected through a disposable needle (Jiangxi Hongda Group, China) and a sodium heparin tube (Hebei Kangweishi Medical, China). Blood samples were remained at room temperature for less than 30 min, then centrifuged at 3000 rpm for 5 min at room temperature to obtain plasma, and finally stored at −80 °C until further analysis. After pretreatment (Text S2), nontargeted metabolomics analysis was performed using UHPLC (Vanquish, Thermo Fisher Scientific, USA) coupled with a high-resolution mass spectrometer (Q Exactive Plus, Thermo Fisher Scientific, USA) (Figure 1), with an injection volume of 5 μL. In positive ion mode, an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 3 μm, Waters, USA) was used, operating at 30 °C with a flow rate of 0.5 mL min^{-1}. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile). In negative ion mode, a HILIC column (2.1 × 150 mm, 5 μm, HILICON, Sweden) was used, operating at 30 °C with a flow rate of 0.2 mL min^{-1}. The mobile phase consisted of solvent A (20 mmol L^{-1} ammonium acetate, 10 mmol L^{-1} ammonium hydroxide, 5% acetonitrile, 95% water) and solvent B (acetonitrile). Detailed gradient elution procedures and instrument parameter settings can be found in Text S3 and Text S4.

2.5. Data Analysis

Raw files collected by SESI-HRMS/MS were converted to mzML format using MSConvert. Topological algorithm in BreathXplorer was used to extract the average peak heights of all features and MS/MS spectra detected in breath samples.[35] The UHPLC-ESI-HRMS/MS data of blood samples were processed utilizing MS-DIAL (version 4.9.22) to extract peak area and MS/MS spectra. Mass tolerance was 0.001 and 0.05 Da for MS1 and MS2, respectively. Minimum peak height was 10,000, and retention time tolerance was 0.1 min. Features with a sample average less than three times the blank average were excluded. Compound identification in breath and blood was based on MS/MS spectra matched against the NIST20 and MoNA databases using a reverse dot product spectral similarity algorithm with a 0.05 Da mass tolerance. A similarity score above 0.8 was used as the identification threshold. According to standard classification criteria, all identifications fall under level 2.[14]

Absolv module in ACD/Percepta was used to predict Abraham descriptors for each metabolite. Water−gas partition coefficients (log $P_{w/g}$) of each metabolite under physiological conditions (37 °C) were subsequently calculated using linear free-energy relationship equation. This coefficient characterizes the ability of compounds to partition from respiratory tract lining fluid into breath air. A higher log $P_{w/g}$ value indicates a lower tendency to partition into breath air. The equation of log $P_{w/g}$ is as follows:[36]

$$\log P_{w/g} = \log\left(\frac{\text{concentration of solute in the water, mol}}{\text{concentration of solute in the gas phase, mol L}^{-1}}\right)$$

Features in breath and blood with over 50% missing values were excluded. Remaining missing values were imputed with 1/5 of the minimum value of the corresponding feature across all samples. The data was then log transformed and autoscaled. Spearman correlation analysis was used on common features detected in both breath and blood using R (version 4.2.2). Partial least-squares discriminant analysis (PLS-DA) was performed using MetaboAnalyst (https://www.metaboanalyst.ca/) to assess metabolic differences by gender (Figure 1).[37]

3. RESULTS AND DISCUSSION

3.1. Comparison between the Features of Breath and Blood

In sampling and pretreatment processes, SESI-HRMS/MS offers significant advantages over UHPLC-ESI-HRMS/MS. SESI-HRMS allows for real-time analysis of breath samples without any pretreatment and analyses of 12 replicates of a single sample can be completed within 10 min. Moreover, breath is ideal for continuous monitoring studies due to its noninvasiveness and unlimited sampling frequency. Its user-friendly nature provides a distinct advantage for patients, particularly infants and the elderly, who may face challenges with sample collection.[38] In contrast, UHPLC-ESI-HRMS/MS analysis of blood samples involves a complex pretreatment process. The detection of a single sample takes about 16 h and the procedure also requires in-depth training and practice of the operators.

SESI and ESI can ionize similar species. In this study, they were coupled with performance-matched Q Exactive and Q Exactive Plus, respectively. Therefore, the breath features detected by SESI-HRMS/MS and the blood features analyzed by UHPLC-ESI-HRMS/MS were comparable. The features detected in both methods were filtered by the following criteria: (1) Removal of features with missing values exceeding 50% across all samples; (2) Removal of isotope peaks; (3) Selection of features in breath samples with an average intensity greater than 3×10^4. As a result, 735 and 2351 features were extracted from breath and blood, respectively. Then, a preliminary comparison was made based on the exact mass of the features. Exact mass of features were calculated for positive ion mode ($[M + H]^+$) and negative ion mode ($[M-H]^-$). Features detected in breath using SESI-HRMS/MS are predominantly below 300 Da (92.38%), while features found in blood by UHPLC-ESI-HRMS/MS are primarily below 600 Da (92.60%) (Figure 2A). The number of features below 200 Da in breath and blood is similar, which are 457 and 536,

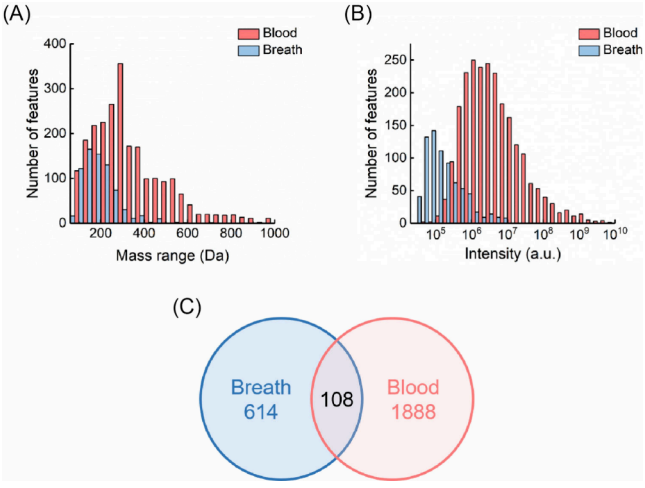

Figure 2. (A) Exact mass distributions of breath and blood features; (B) intensity distributions of breath and blood features; (C) Venn analysis of exact mass of breath and blood features.

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Environ. Health 2025, 3, 596−604

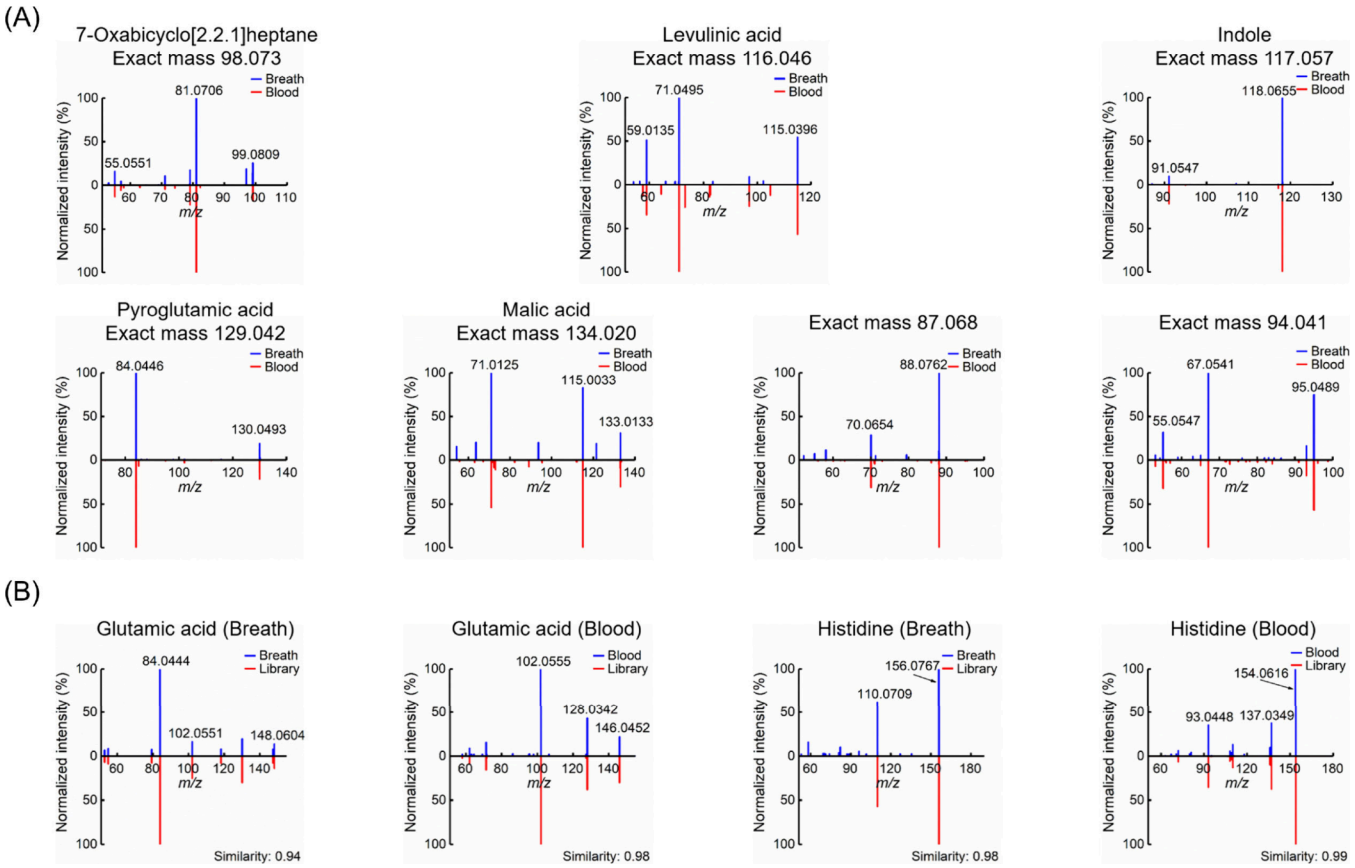

Figure 3. (A) Comparison of seven features with identical MS/MS in breath and blood and (B) MS/MS match results of glutamic acid and histidine in breath and blood with NIST20 and MoNA database.

respectively. The number of features above 200 Da in blood is higher than that in breath, with 1815 features in blood compared to 278 in breath. This result may be attributed to several factors: (1) The concentration by volume of metabolites in breath generally ranges from 10^{-12} to 10^{-6}, while metabolites in blood ranges from 10^{-12} to 10^{-3}.[39,40] This indicates that analytical methods for breath metabolites require higher sensitivity to ensure adequate coverage. (2) Breath samples are directly analyzed using SESI-HRMS/MS, while blood samples undergo sample pretreatment and chromatographic separation, which help to concentrate metabolites and improve the detection limit of UHPLC-ESI-HRMS/MS.

Following the feature comparison between breath and blood, the results from SESI-HRMS/MS indicated that most of the breath features have intensities within the range of 10^4–10^6, while the intensities of blood features are typically in the range of 10^5–10^{10} (Figure 2B). Given that the dynamic linear range of the Q Exactive extends up to 6 orders of magnitude, further improvements in the method performance is required in order to detect breath metabolites across a broader intensity range. Furthermore, Venn analysis was conducted by employing exact mass (with a mass tolerance of 10 ppm) to compare features detected in both breath and blood (Figure 2C). The results demonstrate that 108 features were detected in both breath and blood, representing only 14.7% of the features detected in breath and 4.6% of those in blood. This highlights a disparity between breath features obtained through SESI-HRMS/MS and blood features acquired via UHPLC-ESI-HRMS/MS.

3.2. Comparison between the Metabolites of Breath and Blood

MS/MS was used for the further analysis of 108 common features. Nine features exhibited similar MS/MS in both breath and blood (Figure 3). These features were matched 7-oxabicyclo [2.2.1] heptane, levulinic acid, indole, pyroglutamic acid, malic acid, glutamic acid and histidine in database (Figure 3). It is noteworthy that glutamic acid and histidine were detected in positive ion mode in SESI-HRMS/MS while in negative ion mode in UHPLC-ESI-HRMS/MS (Figure 3B). This implies that in the Super SESI source, glutamic acid and histidine in the gas phase are more prone to form pronated molecular ions ($[M + H]^+$) as their amino groups ($-NH_2$) have a tendency to accept protons from the primary reactant ions.[41] In contrast, in the ESI process the carboxyl groups ($-COOH$) of two compounds in the liquid phase are inclined to lose protons to generate negative ions ($[M-H]^-$).

Additionally, despite having the same exact mass in HRMS, 20 features exhibited distinct MS/MS patterns between breath and blood (Figure S1), indicating that they may correspond to different compounds. This highlights the limitations of relying solely on exact mass for identification.[27] Incorporating MS/MS data into the identification process would greatly improve the accuracy of outcome. Consequently, due to the absence of MS/MS data either in breath or blood, the remaining 79 features cannot be confirmed as the same metabolite in both matrices.

For the seven identified compounds detected in both breath and blood, i.e., 7-oxabicyclo [2.2.1] heptane, levulinic acid, indole, pyroglutamic acid, malic acid, glutamic acid and

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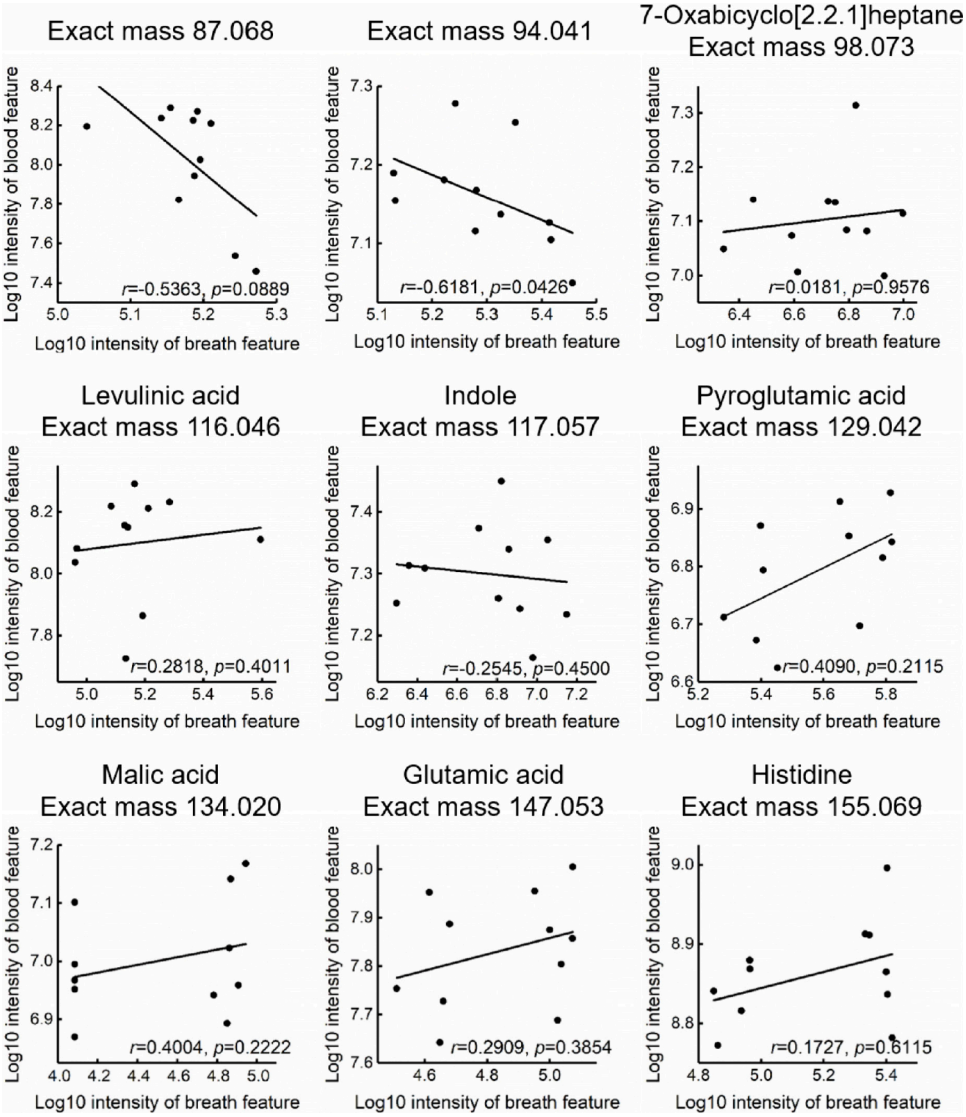

Figure 4. Spearman correlation analysis of nine features (seven identified and two nonidentified) detected in both breath and blood.

histidine (Table S2), which closely intertwined with human metabolism. Indole is derived from tryptophan by gut microbiota and can diffuse through the intestinal epithelial cells into blood. It has been reported to modulate various inflammatory responses and is associated with neurological diseases.[42] Exhaled indole has been found to be useful for monitoring glycemia fluctuations in diabetic patients.[43] Pyroglutamic acid is an intermediate in glutathione metabolism and associated with oxidative stress. An increase of pyroglutamic acid can lead to metabolic acidosis.[44] Malic acid is a key metabolite in tricarboxylic acid cycle and plays an essential role in energy metabolism.[45] Malic acid has been detected in exhaled breath and may serve as a noninvasive marker for investigating energy metabolism and circadian rhythms.[11] Glutamic acid and histidine are two key nonessential amino acids. They are intimately connected to the occurrence and development of cancer, diabetes, cardiovascular diseases, autoimmune diseases and neurological diseases.[46] These two metabolites also demonstrate notable potential in the clinical treatment.

Spearman correlation analysis was conducted on the 9 common features (7 identified and 2 nonidentified) using their intensities in breath and blood (Figure 4). Low correlation coefficients ($r < 0.4$ or $p > 0.05$) was found for all 9 features. This finding is consistent with previous studies, which also reported weak correlations of VOCs across different sample types.[47] Several factors may explain this observation: (1) A delay may occur in the transfer of metabolites from blood into alveolar air. It has been observed that venlafaxine and ketamine exhibit a certain delay in peak concentrations in mouse exhaled breath compared to blood.[8,9] (2) Metabolites present in exhaled breath are not only from blood but can also originate from lung tissue, respiratory tract and oral cavity.[48] (3) Blood sample collection and pretreatment processes may lead to metabolite loss, potentially affecting the correlation results.

A total of 26 compounds were identified using MS/MS in breath-unique features (Table S3 and Figure S2). These include 8 endogenous metabolites and 18 exogenous compounds. To be specific, the 8 endogenous metabolites include glycolic acid, succinic acid semialdehyde, parabanic acid, δ-hexanolactone, 3-hydroxy-2-methylbutyric acid, phenylacetaldehyde, menthone and α-ionone. These metabolites may have potential applications in assessing human health. For instance, succinic acid semialdehyde is generated from γ-

https://doi.org/10.1021/envhealth.4c00248
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aminobutyric acid and can be converted into succinic acid and nicotinamide adenine dinucleotide phosphate (NADPH). Its high concentration in the body suggest succinic semialdehyde dehydrogenase deficiency.[49] 3-Hydroxy-2-methylbutyric acid is a metabolite from isoleucine, serve as a urinary biomarker for β-ketothiolase deficiency.[50] Parabanic acid is a product of uric acid oxidation and has been proposed as a marker for monitoring oxidative stress.[51] The 18 exogenous compounds include N-formylglycine, *N*-methyl aniline, 1-piperidinecarbaldehyde, 1,2-cyclohexanediol, 3-hydroxybenzaldehyde, sulcatone, γ-heptalactone, 4-anisaldehyde, adipic acid, N-oxalylglycine, phthalic anhydride, cinnamic acid, anethole, verbenone, vanillin, thujone, δ-nonolactone and benzophenone. Detection of trace environmental chemicals in exhaled breath demonstrates the potential of this platform as a novel noninvasive approach for exposure risk assessment.

A total of 73 compounds were identified using MS/MS in blood-unique features (Table S4), comprising 60 endogenous metabolites and 13 exogenous compounds. For the endogenous ones, commonly reported metabolites like tryptophan, testosterone, uric acid, aconitic acid, caffeine, various phospholipids and carnitine were encompassed. These metabolites have been widely reported in previous studies. As an example, tryptophan is one of the essential amino acids, plays a critical role in fundamental metabolic pathways and is vital for cognition, behavior and nervous system.[52] Testosterone is a pivotal sex hormone involved in metabolism. Testosterone is associated with metabolic syndrome and type 2 diabetes and it also affects skeletal muscle strength.[53,54] Compared to breath, blood yielded a higher number of identified features and a greater proportion of endogenous metabolites. One major reason for this disparity is the extremely limited proportion of breath metabolites in current mainstream databases. Establishing a comprehensive and authoritative database specifically for breath metabolites would greatly facilitate the development and application of breath metabolomics in life sciences, particularly for biomarker discovery and clinical research.

To better understand the disparity in metabolites between breath and blood, the log $P_{w/g}$ of 26 breath-unique metabolites, 73 blood-unique metabolites and 7 common metabolites were calculated. The results revealed that breath metabolites were mainly within a low log $P_{w/g}$ (<5), whereas blood metabolites were primarily concentrated in a higher log $P_{w/g}$ (>5). For the metabolites detected in both breath and blood, their log $P_{w/g}$ values were distributed in both regions (Figure 5). Metabolites with a lower log $P_{w/g}$ typically have higher volatility and are more likely to appear in exhaled breath.[36] However, a few metabolites with higher log $P_{w/g}$ were also detected in breath, where they likely exist in the form of exhaled breath particles (EBP).[55] For example, glycolic acid (log $P_{w/g}$ 4.7925), 3-hydroxy-2-methybutyric acid (log $P_{w/g}$ 5.4552), phthalic anhydride (log $P_{w/g}$ 4.6826), Cinnamic acid (log $P_{w/g}$ 4.9273), pyroglutamic acid (log $P_{w/g}$ 7.9832), malic acid (log $P_{w/g}$ 8.0038), glutamic acid (log $P_{w/g}$ 9.9857) and histidine (log $P_{w/g}$ 11.2322) have been reported in the exhaled breath condensate.[56] To summarize, from the perspective of metabolite detection, breath provides a valuable complement to blood, especially for volatile metabolites, and thereby provides a broader scope of metabolic information.

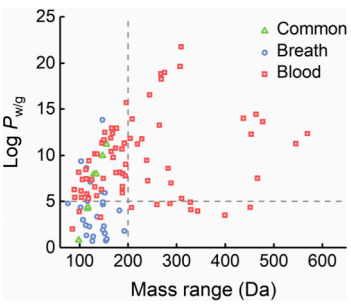

Figure 5. Distribution of exact mass and log $P_{w/g}$ of breath, blood, and common metabolites.

3.3. Differences in Breath Metabolome and Blood Metabolome between Males and Females

A substantial body of literature has shown that gender, age, smoking and body mass index (BMI) can affect blood metabolic profiles.[57−59] Subjects selected for this study have similar age and BMI, and none of them smoke. Thus, the impact of gender on breath and blood metabolites was specifically explored. Breath and blood samples were categorized by gender and analyzed using PLS-DA. As shown in Figure 6, the differentiation between males and females in blood is significantly greater than that observed in breath. A total of 76 breath features and 189 blood features with a variable importance in projection (VIP) value greater than 1.5 were selected. In breath, endogenous metabolite δ-Hexanolactone, identified using MS/MS (Table S5), showed higher intensity in males. δ-Hexanolactone is a substrate for phosphatases and possesses antioxidant properties.[60] In blood, gender-specific metabolites identified in this study are consistent with previous reports. For example, uric acid, testosterone and creatinine exhibit higher intensity in males, whereas creatine shows higher intensity in females (Table S5).[61,62]

Blood metabolome directly reflects sex-specific signals, including sex hormones and their regulatory components. On the other hand, breath metabolome is predominantly composed of volatile metabolites and exhibits information less relevant to the gender. This suggests that using breath samples for biomarker screening may help to reduce the interference caused by the gender. However, a more profound comprehension of gender-related differences in various biosamples is indispensable, because it can aid in the development of more personalized health management plans, tailored drug treatment strategies and more accurate disease risk assessments.

4. CONCLUSIONS

This study explored the similarities and differences between breath metabolomics and blood metabolomics by utilizing SESI-HRMS/MS and UHPLC-ESI-HRMS/MS, respectively. The significant differences observed between breath and blood metabolomics offer a new perspective on metabolic analysis. Integrating of multiple metabolomic approaches will provide deeper insights into human metabolism. Building on the real-time monitoring capabilities of SESI-HRMS for low-concentration volatile metabolites, SESI-HRMS/MS further enhances identification accuracy, making it an ideal tool for noninvasive, convenient and highly sensitive breath analysis. However, the precise identification of breath metabolites and the study of their transfer processes within the body remain key focuses for future research. With continued advancements in identification strategies and the development of breath metabolomics

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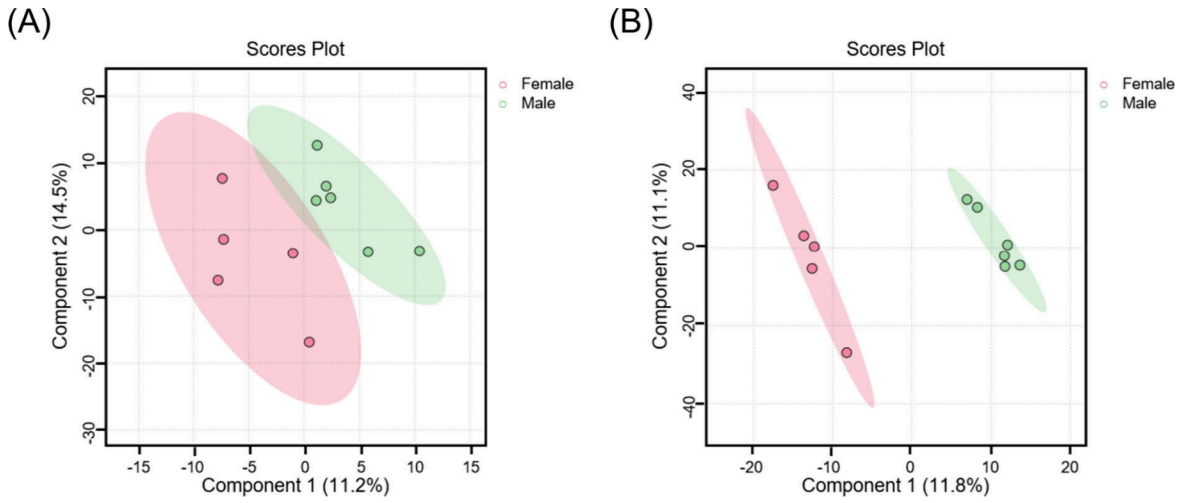

Figure 6. PLS-DA score plot between males and females in (A) breath and (B) blood.

database, a more precise understanding of breath metabolomics can be achieved.

■ ASSOCIATED CONTENT

Data Availability Statement

The raw files for the real-time breath analysis and UHPLC-ESI-HRMS/MS blood analysis, as well as the Spearman correlation analysis code, are available on Zenodo (10.5281/zenodo.14570062). The code related to BreathXplorer can be found on GitHub (https://github.com/HuanLab/breathXplorer).

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00248.

Detailed instrument parameters, blood sample pretreatment procedures, basic subject information, breath and blood metabolite identification results, comparison of features with the same exact mass but different MS/MS in breath and blood, and MS/MS matched results of breath metabolites in the database (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was funded by the Guangdong Major Project of Basic and Applied Basic Research (2023B0303000013), the National Natural Science Foundation of China (22122603), and the Research Fund Program of Guangdong Provincial Key Laboratory of Speed Capability Research (2023B1212010008). We also thank Pablo Sinues for providing the Super SESI.

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https://doi.org/10.1021/envhealth.4c00248
Environ. Health 2025, 3, 596−604