Research Letter

# Plasma metabolomic analysis of patients with different severities acute pancreatitis

**Shenglan Liu[1], Hua Xu[1], Zhengyan Wu[2], Yanxia Guo[1], Zheng Fan[1], Yuanyuan Zhang[1], Lichao Fang[3], Weichang Chen[4]**

[1]*Department of Critical Care Medicine, the First Affiliated Hospital of Soochow University, Suzhou 215006, China*
[2]*Department of Health Management Center, the First Affiliated Hospital of Soochow University, Suzhou 215006, China*
[3]*Department of Emergency and Intensive Care Unit, Suzhou Xiangcheng People's Hospital, Suzhou 215131, China*
[4]*Department of Gastroenterology, the First Affiliated Hospital of Soochow University, Suzhou 215006, China*

***Corresponding Authors:*** *Weichang Chen, Email: chenweichangsuda@126.com; Lichao Fang, Email: lichaofang76@163.com*

*World J Emerg Med 2025;16(6):597–602*
DOI: 10.5847/wjem.j.1920-8642.2025.117

Acute pancreatitis (AP) is a prevalent gastrointestinal disease necessitating hospitalization globally, with an annual incidence ranging from 13 to 45 per 100,000 individuals[1] and a mortality rate of 5%–10%.[2] While most cases follow a self-limiting course, approximately 20%–30% of cases progress to severe acute pancreatitis (SAP), characterized by pancreatic necrosis and multiorgan failure, with the mortality rate increasing to 36%–50%.[2–6] Early and precise severity assessment is essential for guiding individualized therapeutic interventions to improve outcomes in critical cases and for optimizing resource allocation for mild patients. Despite the wide acceptance of several scoring systems such as Accuracy of Acute Physiology and Chronic Health Evaluation (APACHE) II, Ranson, and Computed Tomography Severity Index (CTSI), their clinical efficacy is constrained by inadequate prediction accuracy, sensitivity, and specificity.[7] Therefore, the identification of potential biomarkers for AP severity is urgently needed.

Metabolomics, defined as "the quantitative analysis of dynamic metabolic responses to pathophysiological stimuli or genetic modifications in biological systems",[8] has attracted major interest in medical research during the past decade.[9] However, only a limited number of metabolomics studies have focused on pancreatitis. Furthermore, these previous studies emphasized the differences between patients and healthy controls rather than those between severe and mild cases, and were limited to small sample sizes or rely on animal models.[9]

In this study, liquid chromatography–mass spectrometry (LC–MS) was used to analyze the plasma metabolism of patients with AP of varying severities, with the aim of identifying potential metabolites for assessing the severity of AP.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. Written information was provided, and informed consent was obtained from all the subjects. All patients and healthy volunteers were informed before their plasma samples were collected. The diagnosis of moderate and severe acute pancreatitis (MAP/SAP) followed the revised Atlanta classification.[1] The exclusion criteria for patients were as follows: (1) presence of cardiovascular, cerebrovascular, liver, kidney or hematopoietic system diseases or other comorbidities; (2) diagnosis of diabetes, hyperthyroidism, hypothyroidism or other metabolic disorders; (3) had a history of alcohol or drug abuse; (4) long-term use of specific medications (such as antibiotics, hormones, etc.); (5) occurrence of major life events (such as psychological trauma, extensive burns, etc.) within the prior two weeks; and (6) lactation or pregnancy.

From August 2022 to February 2023, 87 patients diagnosed with AP in the Intensive Care Unit, Emergency Department or Gastroenterology Department of the First Affiliated Hospital of Soochow University were selected. Of the participants, 48 patients had MAP (21 males, 27 females; mean age 53 ± 15 years), while 39 patients had SAP (23 males, 16 females; mean age 46 ± 16 years). Furthermore, 30 age-matched healthy volunteers were chosen as controls (16 males, 14 females; mean age 47 ± 13 years).

Peripheral blood samples were collected within 24

 

h of admission following a 12-hour fasting period. The peripheral blood of healthy volunteers was collected after an overnight fasting. Healthy volunteers were instructed to consume a light diet the day before blood collection, avoiding seafood, smoking and alcohol consumption. The samples were placed into polyethylene tubes prerinsed with EDTA and then centrifuged at 3,000 rpm for 10 min. The plasma samples were carefully gathered and promptly frozen and stored at −80 °C for subsequent analysis. Before LC-MS analysis, the plasma samples (100 μL) were protein-precipitated with 200 μL of cold methanol, centrifuged (12,000 rpm, 4 °C), and reconstituted in 80% methanol before filtration (0.22 μm). Quality control (QC) samples were analysed for system monitoring.

All the plasma samples were analysed using untargeted LC–MS (Thermo LTQ Orbitrap XL, USA). Chromatographic separation was performed on an ACQUITY UPLC® HSS T3 column (150 mm×2.1 mm, 1.8 μm) at 40 °C with 0.1% formic acid/water (A) and 0.1% formic acid/acetonitrile (B) mobile phases (flow rate: 0.25 mL/min). The gradient program was as follows: 2% B (0–1 min), 2%–50% B (1–9.5 min), 50%–98% B (9.5–14 min), 98% B (14–15 min), and return to 2% B at 17 min. The MS parameters were as follows: ESI source: ±4.8/4.5 kV spray voltage; Orbitrap resolution: 60,000 (*m/z* 50–1,000); CID-MS/MS: 30 eV collision energy; and dynamic exclusion: 15 s.

The raw mass data were processed via Proteowizard (v3.0.8789) software and R (v3.1.3) XCMS. Normalized datasets were imported into SIMCA-P 13.0 software (Umetrics, Sweden) for multivariate data analysis, including principal component analysis (PCA) and orthogonal projection to latent structures squares-discriminant analysis (OPLS-DA). The corresponding variable importance in the projection (VIP) was calculated via the OPLS-DA model. The Mann-Whitney-Wilcoxon test and *P*-values were used to identify significant differences between groups. The first principal component VIP value >1 was combined with the Mann-Whitney-Wilcoxon test *P*-value < 0.05 to screen potentially differentially abundant metabolites.

The differentially abundant metabolites were initially identified based on their exact molecular weights (mass error < 10 ppm) with putative molecular formulas. Structural confirmation was subsequently accomplished by matching MS/MS fragmentation patterns with public databases, including METLIN (https://metlin.scripps.edu), Human Metabolome Database (HMDB; http://www.hmdb.ca), MassBank (http://www.massbank.jp), LipidMaps (http://www.lipidmaps.org), and mzCloud (https://www.mzcloud.org). Metabolic pathway analysis was performed via the KEGG (https://www.kegg.jp) and MetaboAnalyst (https://www.metaboanalyst.ca) platforms.

The general ion flow base peak chromatograms of typical samples are shown in Supplementary Figure 1. There were 1,314 precursor molecules in positive ion mode and 1,110 precursor molecules in negative ion mode. Unsupervised hierarchical clustering and metabolite correlation analysis were used to reveal the relationships between metabolites and patients in different groups (Supplementary Figure 2).

PCA was used to investigate the general relationships among the MAP, SAP and control groups (Supplementary Figure 3). The quality of the model was evaluated by the cross-validation parameters Q2 and R2X. The PCA model constructed with the three groups of plasma samples revealed 16 primary ingredients (R2X=0.596, Q2=0.323) in negative ion mode and 15 primary ingredients (R2X=0.545, Q2=0.279) in positive ion mode, indicating that the plasma metabolites of the SAP, MAP and control groups tended to be clustered.

OPLS-DA allows a straightforward interpretation of the results by separating the predictive variation from orthogonal sources of systematic variability and was performed to maximize class discrimination. To generate a training set for the biomarker signature that discriminates well among each group, an identification study was performed. The dataset was randomly split into a training set and a test set. The training set comprised 20 controls, 22 SAP patients, and 30 MAP patients, while the test set comprised the remaining 10 controls, 17 SAP patients, and 18 MAP patients. The OPLS-DA model with 5 latent variables (2 predictive and 3 orthogonal) was obtained, and a clear separation distinguishing clustering was observed on the score plot, as were good fitting and predictive abilities (R2X=0.357, R2Y=0.948, and Q2=0.839; Figure 1A). Moreover, the samples in the test set were correctly predicted (Figure 1B) on the basis of the T-prediction score.

Thirty-five metabolites were selected as biomarkers and were identified and confirmed by reference standards (Supplementary Table 1). Variations in these altered metabolites are expressed as the fold change (FC) in each comparison. The clinical diagnostic value of metabolites was evaluated by the receiver operating characteristic (ROC) curves. The area under the ROC curve (AUC) values compared between groups are shown in Table 1.

Fifteen of the 35 metabolites, including (R)-(+)-2-pyrrolidone-5-carboxylic acid, (S)-(-)-2-hydroxyisocaproic acid, acetylcarnitine, beta-alanine, creatine, indolelactic

acid, L-carnitine, kynurenine, L-palmitoyl carnitine, octanoylcarnitine, L-phenylalanine, malic acid, oxoglutaric acid, threonate, and uric acid, showed progressive increases in correlation with the severity of AP, suggesting that they might be potential biomarkers of the severity of AP. Thirteen metabolites (2-aminoisobutyric acid, 3-buten-1-amine, angelic acid, D-beta-hydroxy butyric acid, L-glutamate, L-glutamine, L-histidine, L-valine, L-leucine, nonanedioic acid, succinic aldehyde, N,N-dimethylglycine, and sphingosine-1-phosphate) significantly increased in MAP and subsequently decreased in SAP, suggesting that these metabolites might be potential indicators for the early diagnosis of AP or characteristic indicators of MAP. The level of indoxyl sulfate in SAP patients was significantly greater than those in the other two groups, which might be a predictor of SAP.

The levels of intermediates involved in the tricarboxylic acid (TCA) cycle, such as oxoglutaric acid and L-malic acid, tend to increase progressively with the severity of AP. These metabolic changes indicate that

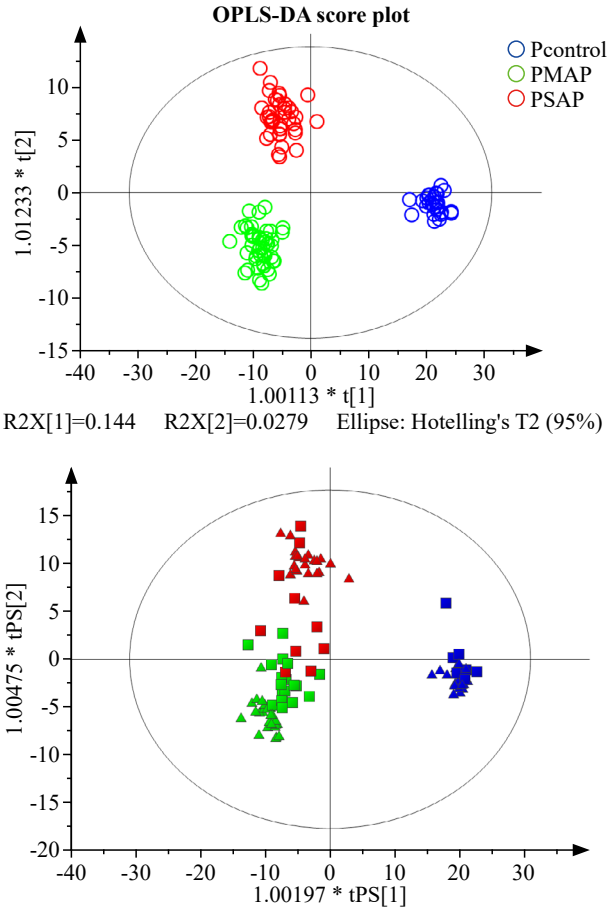


**Figure 1.** Orthogonal projection to latent structures squares-discriminant analysis (OPLS-DA) score plot (A) and the T-prediction score of the test set (B). Blue triangle: training set of Pcontrol; red triangle: training set of PSAP; green triangle: training set of PMAP; blue square: test set of Pcontrol; red square: test set of PSAP; green square: test set of PMAP.

pancreatitis leads to a higher energy demand or short energy supply and tends to display a hyperdynamic and hypermetabolic state.[10] This deficiency may result from a lack of acetyl-CoA in the mitochondria due to a reduction in the rates of oxidative phosphorylation and fatty acid β-oxidation.[11]

Carnitine is responsible for transporting fat into the mitochondria of muscle cells for oxidation.[12] L-acetylcarnitine, which has neuroprotective, neuromodulatory and neurotrophic properties, is an acetic acid ester of carnitine. L-palmitoylcarnitine is a long-chain acyl fatty acid derivative ester of carnitine that facilitates the transfer of long-chain fatty acids from the cytoplasm into mitochondria during the oxidation of fatty acids. It can regulate the activity of several enzymes and transporters located in the membrane, and its level increases during apoptosis.[13] Palmitoylcarnitine significantly increases the amount of palmitate covalently bound to GAP-43, which can regulate signal transduction pathways.[14] Octanoylcarnitine is a type of medium-chain acylcarnitine, the high level of which can be attributed to either high incorporation of medium-chain fatty acids or high production of medium-chain fatty acids from long-chain fatty acids.[15] Pancreatic lipase, which acts on circulating or tissue triglycerides, might generate nonesterified fatty acids that could promote pancreatic and fat tissue necrosis.[16] L-carnitine may be protective against pancreatic tissue degeneration, oxidative stress or lipid peroxidation in different AP model mice.[17,18] Carnitine and its esters may protect pancreatic tissue against the debilitating effects of nonesterified fatty acids in a murine AP model, probably via regulating the oxidant/antioxidant balance and modulating the myeloperoxidase and nitric oxide pathways.[19] Thus, the increase in L-carnitine and its derivatives with increasing severity of AP might be the result of self-regulation in the organism and could be a metabolic indicator of AP progression.

This study identified abnormalities in various essential amino acids and derivatives. For instance, beta-alanine, L-phenylalanine, 2-pyrrolidone-5-carboxylic acid and creatine were progressively elevated, whereas L-glutamate, L-glutamine, L-histidine, L-valine, L-leucine, N,N-dimethylglycine and 2-aminoisobutyric acid were significantly elevated in MAP and subsequently decreased in SAP.

The accumulation of 2-pyrrolidone-5-carboxylic acid has been reported as a cause of severe high anion gap metabolic acidosis.[20] Elevated pyroglutamic acid may be associated with problems with glutamine or glutathione metabolism. N,N-dimethylglycine, a glycine derivative and a byproduct of choline and homocysteine metabolism, is increased in renal failure in rats and

chronic kidney disease (CKD) patients.[21,22] The increase in N,N-dimethylglycine may result from disturbances in membrane phospholipid metabolism. Glutamic acid can be metabolised to α-oxoglutaric acid, from which sugar is produced through the TCA cycle and other pathways. Increased gluconeogenesis in patients with AP probably results in increased levels of amino acids such as glutamic acid. With the aggravation of AP, the function of the liver and kidney is often impaired, accompanied by a decrease in gluconeogenesis. Moreover, the changes in the identified amino acids also indicate that patients experience systemic complications due to impaired exocrine pancreatic function and digestive insufficiency.

Kynurenine is an essential compound in the tryptophan metabolism pathway. It can be transformed to the neuroprotective agent kynurenic acid or the neurotoxic agent quinolinic acid. Disruption of this balance can be observed in many disorders. Mole et al[23] demonstrated that plasma kynurenine concentrations in APs correlated with multiple organ failure scores and preceded a requirement for mechanical ventilation and hemodialysis, and they concluded that mesenteric lymph-borne kynurenines may contribute to pancreatitis-associated multiple organ failure. Kynurenine has also been reported to contribute to acute lung injury in rats with AP and patients with SAP.[23] The inhibition of kynurenine-3-monooxygenase could be a novel treatment approach for multiple organ dysfunction syndrome in acute pancreatitis.[24-26] On the basis of previous studies and our findings, kynurenine might be a potential metabolic indicator of the severity of AP.

Elevated levels of (S)-(-)-2-hydroxyisocaproic acid, angelic acid, and nonanedioic acid were detected in patients with AP, indicating abnormal fatty acid catabolism. The metabolic stress and lipase release induced by AP can lead to accelerated fat mobilization, increased lipolysis or oxidative disturbances, and elevated free fatty acid and triglyceride levels in the plasma.[27]

D(-)-beta-hydroxybutyric acid is involved in the synthesis and degradation of ketone bodies. Like other ketone bodies (acetoacetate and acetone), beta-hydroxybutyrate is elevated in patients with ketosis.

**Table 1.** The clinical diagnostic value of identified metabolites

| Metabolites | SAP vs control | | | | MAP vs control | | | | SAP vs MAP | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | VIP | *P*-value | AUC | Fold change | VIP | *P*-value | AUC | Fold change | VIP | *P*-value | AUC | Fold change |
| D(-)-beta-hydroxybutyric acid | 1.150 | 0.000 | 0.863 | 7.600 | 1.020 | 0.000 | 0.845 | 18.200 | 1.570 | 0.123 | 0.583 | 0.417 |
| L-leucine | 0.413 | 0.568 | 0.546 | 1.180 | 0.987 | 0.000 | 0.793 | 1.610 | 1.300 | 0.004 | 0.687 | 0.731 |
| Indolelactic acid | 0.965 | 0.000 | 0.816 | 3.210 | 0.671 | 0.028 | 0.642 | 1.660 | 1.560 | 0.017 | 0.646 | 1.940 |
| Nonanedioic acid | 1.270 | 0.000 | 0.826 | 3.050 | 1.260 | 0.000 | 0.844 | 3.610 | 0.670 | 0.269 | 0.573 | 0.845 |
| Glyceric acid | 1.080 | 0.000 | 0.868 | 3.960 | 0.967 | 0.000 | 0.867 | 3.920 | 0.028 | 0.983 | 0.498 | 1.010 |
| Threonate | 0.948 | 0.000 | 0.795 | 7.320 | 0.454 | 0.300 | 0.573 | 2.190 | 1.760 | 0.001 | 0.717 | 3.340 |
| 3-buten-1-amine | 0.511 | 0.110 | 0.611 | 1.150 | 0.987 | 0.000 | 0.769 | 1.550 | 1.450 | 0.008 | 0.652 | 0.741 |
| 4-hydroxy benzenesulfonic acid | 0.641 | 0.315 | 0.554 | 3.110 | 0.187 | 0.052 | 0.617 | 0.750 | 1.640 | 0.009 | 0.666 | 4.150 |
| L-glutamine | 1.190 | 0.000 | 0.800 | 1.950 | 1.260 | 0.000 | 0.848 | 2.040 | 0.230 | 0.542 | 0.541 | 0.954 |
| L-histidine | 1.080 | 0.000 | 0.821 | 2.190 | 0.903 | 0.000 | 0.780 | 2.540 | 0.416 | 0.814 | 0.527 | 0.861 |
| L-glutamate | 1.030 | 0.000 | 0.798 | 3.850 | 1.130 | 0.000 | 0.837 | 3.940 | 0.062 | 0.606 | 0.561 | 0.976 |
| Sphinganine | 0.982 | 0.001 | 0.760 | 0.879 | 0.042 | 0.478 | 0.542 | 0.995 | 1.680 | 0.002 | 0.689 | 0.884 |
| Hexadecanedioic acid | 1.040 | 0.000 | 0.900 | 12.700 | 0.634 | 0.000 | 0.794 | 12.200 | 0.051 | 0.059 | 0.619 | 1.030 |
| Creatinine | 0.430 | 0.449 | 0.569 | 1.720 | 0.804 | 0.005 | 0.705 | 0.769 | 1.230 | 0.001 | 0.705 | 2.230 |
| Oxoglutaric acid | 1.070 | 0.000 | 0.958 | 15.500 | 0.954 | 0.000 | 0.925 | 11.800 | 0.569 | 0.324 | 0.575 | 1.320 |
| L-tryptophan | 0.676 | 0.005 | 0.702 | 0.722 | 0.280 | 0.618 | 0.529 | 1.200 | 1.500 | 0.004 | 0.666 | 0.602 |
| Indoxylsulfuric acid/3-Indoxyl sulfate | 0.482 | 0.420 | 0.558 | 8.240 | 0.313 | 0.033 | 0.648 | 0.696 | 1.200 | 0.003 | 0.688 | 11.800 |
| Angelic acid | 1.160 | 0.000 | 0.812 | 1.810 | 1.100 | 0.000 | 0.798 | 2.540 | 1.120 | 0.202 | 0.577 | 0.715 |
| Malic acid | 1.360 | 0.000 | 0.974 | 17.400 | 1.090 | 0.000 | 0.944 | 10.000 | 1.410 | 0.048 | 0.637 | 1.740 |
| Creatine | 0.754 | 0.001 | 0.742 | 3.250 | 1.250 | 0.000 | 0.835 | 2.290 | 0.708 | 0.466 | 0.548 | 1.420 |
| L-valine | 0.625 | 0.052 | 0.629 | 1.720 | 0.939 | 0.000 | 0.798 | 3.200 | 1.270 | 0.010 | 0.649 | 0.538 |
| Acetylcarnitine | 1.390 | 0.000 | 0.924 | 3.780 | 1.540 | 0.000 | 0.934 | 3.220 | 0.630 | 0.405 | 0.548 | 1.170 |
| beta-Alanine | 1.190 | 0.000 | 0.773 | 1.880 | 0.785 | 0.009 | 0.685 | 1.710 | 0.352 | 0.148 | 0.588 | 1.100 |
| (R)-(+)-2-pyrrolidone-5-carboxylic acid | 1.440 | 0.000 | 0.968 | 4.090 | 1.530 | 0.000 | 0.957 | 3.410 | 0.712 | 0.386 | 0.576 | 1.200 |
| L-carnitine | 0.934 | 0.000 | 0.818 | 1.990 | 1.010 | 0.001 | 0.734 | 1.350 | 1.360 | 0.040 | 0.633 | 1.470 |
| Succinic aldehyde | 1.020 | 0.000 | 0.929 | 7.180 | 1.080 | 0.000 | 0.897 | 12.900 | 1.050 | 0.168 | 0.597 | 0.559 |
| L-phenylalanine | 1.290 | 0.000 | 0.910 | 1.840 | 1.000 | 0.000 | 0.811 | 1.670 | 0.434 | 0.094 | 0.592 | 1.100 |
| 2-aminoisobutyric acid | 0.851 | 0.007 | 0.699 | 1.740 | 1.200 | 0.000 | 0.858 | 2.570 | 1.260 | 0.003 | 0.685 | 0.677 |
| Uric acid | 1.680 | 0.000 | 0.946 | 4.340 | 1.470 | 0.000 | 0.920 | 3.100 | 1.640 | 0.007 | 0.678 | 1.400 |
| (S)-(-)-2-hydroxy isocaproic acid | 0.961 | 0.000 | 0.878 | 5.510 | 1.120 | 0.000 | 0.856 | 4.190 | 0.640 | 0.801 | 0.504 | 1.310 |
| Kynurenine | 1.270 | 0.000 | 0.833 | 3.100 | 0.620 | 0.013 | 0.680 | 1.830 | 1.400 | 0.001 | 0.722 | 1.690 |
| N,N-dimethylglycine | 0.851 | 0.007 | 0.698 | 1.740 | 1.200 | 0.000 | 0.866 | 2.570 | 1.260 | 0.003 | 0.690 | 0.677 |
| Sphingosine-1-phosphate | 0.823 | 0.006 | 0.695 | 1.850 | 1.350 | 0.000 | 0.838 | 2.370 | 0.884 | 0.016 | 0.674 | 0.780 |
| L-palmitoylcarnitine | 1.170 | 0.000 | 0.922 | 10.900 | 1.090 | 0.000 | 0.900 | 6.910 | 0.979 | 0.064 | 0.625 | 1.580 |
| Octanoylcarnitine | 0.972 | 0.000 | 0.850 | 7.870 | 0.527 | 0.000 | 0.805 | 2.100 | 1.790 | 0.000 | 0.766 | 3.750 |

Variable importance in the projection (VIP) was obtained from OPLS-DA model with a threshold of 1.0. *P*-values were calculated from Mann-Whitney-Wilcoxon test. Fold change (FC) was obtained by comparing those metabolites with each other group. AUC: area under the ROC curve. MAP: moderate acute pancreatitis; SAP: severe acute pancreatitis.

Beta-hydroxybutyrate is synthesised in the liver from acetyl-CoA and can be used as an energy source when blood glucose is low.[28] Li et al[27] reported increased serum levels of 3-hydroxybutyric acid in rats with AP; whereas Ouyang[29] reported that 3-hydroxybutyrate levels were significantly lower in patients with pancreatitis than in control individuals. In this study, the level of 3-hydroxybutyric acid was higher in the AP group than in the control group. In addition, 3-hydroxybutyric acid significantly increased in the MAP group but subsequently decreased in the SAP group. This phenomenon indicated a hypercatabolic state with excessive oxidative stress and ketogenesis in pancreatitis and subsequent ketone body depletion for energy.

Sphingosine 1-phosphate (S1P) is a potent sphingolipid mediator that regulates many critical biological processes.[30] S1P elevations in plasma correlate with metabolic abnormalities. S1P is a key endogenous regulator of the response to lung injury, maintaining endothelial barrier integrity through interaction with S1P1.[31] S1P significantly decreased pulmonary inflammation and injury in a rat model of acute lung injury caused by acute necrotizing pancreatitis and may represent a novel therapeutic strategy for acute necrotizing pancreatitis-associated lung injury.[32] The levels of the S1P carrier protein apoM are decreased in patients with sepsis and systemic inflammatory response syndrome, S1P plasma levels are possibly reduced under these conditions and thus may contribute to vascular leakage.[33] The variation in the levels of S1P in this study might be correlated with the severity of the inflammatory response and cell rupture caused by tryptic hydrolysis.

Indoxyl sulfate is a metabolite of tryptophan and a circulating uremic toxin that stimulates glomerular sclerosis and interstitial fibrosis. Indoxyl sulfate is associated with the progression of renal failure, all-cause mortality, cardiovascular events and endothelial dysfunction in patients with kidney disease.[34-36] It induces oxidative stress by modifying the balance between pro- and antioxidant mechanisms in endothelial cells and decreases the levels of glutathione.[37] Compared with those in the other two groups, the elevated levels of indoxyl sulfate in the SAP patients observed in this study may be correlated with high oxidative stress and renal insufficiency.

This study has several limitations that should be acknowledged. First, we did not perform etiological classification of patients, which may introduce confounders from diverse underlying causes on metabolic profiles. Second, certain AP patients had received pharmacological treatment before the collection of blood samples, potentially influencing our findings. Future studies should expand the sample sizes to provide stratified analysis by etiology and perform targeted analyses of samples prior to medication administration.

In conclusion, plasma metabolites in patients with AP of varied severity exhibited significant metabolic disturbances. Plasma samples from patients with SAP, MAP and healthy controls were differentiated using OPLS-DA. Thirty-five metabolites were identified as metabolic indicators, while 15 metabolites might be considered indicators of AP progression. These findings highlight the pathophysiology and metabolic changes of AP and may facilitate the diagnosis and intervention.

**Funding:** This work was supported by National Natural Science Foundation of China (81272737).

**Ethical approval:** This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University. Written information was provided, and informed consent was obtained from all the subjects.

**Conflicts of interest:** The authors declare no competing financial interests.

**Author contributions:** SLL, HX and ZYW have equal contribution to this article, and share the co-first authorship. SLL performed the experiments and analysed data; ZYW, YXG, ZF and YYZ provided clinical samples; SLL and HX wrote the paper; WCC designed the study. LCF reviewed and edit the paper.

All the supplementary files in this paper are available at http://wjem.com.cn.

## REFERENCES

1 Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1): 102-11.
2 Tenner S, Baillie J, DeWitt J, Vege SS; American College of Gastroenterology. American College of Gastroenterology guideline: management of acute pancreatitis. Am J Gastroenterol. 2013;108(9): 1400-15;1416.
3 Padhan RK, Jain S, Agarwal S, Harikrishnan S, Vadiraja P, Behera S, et al. Primary and secondary organ failures cause mortality differentially in acute pancreatitis and should be distinguished. Pancreas. 2018;47(3): 302-7.
4 Sternby H, Bolado F, Canaval-Zuleta HJ, Marra-López C, Hernando-Alonso AI, Del-Val-Antoñana A, et al. Determinants of severity in acute pancreatitis: a nation-wide multicenter prospective cohort study. Ann Surg. 2019;270(2): 348-55.
5 Schepers NJ, Bakker OJ, Besselink MG, Ali UA, Bollen TL, Gooszen HG, et al. Impact of characteristics of organ failure and infected necrosis on mortality in necrotising pancreatitis. Gut. 2019;68(6): 1044-51.
6 Shi N, Liu TT, de la Iglesia-Garcia D, Deng LH, Jin T, Lan L, et al. Duration of organ failure impacts mortality in acute pancreatitis. Gut. 2020;69(3): 604-5.
7 van den Berg FF, de Bruijn AC, van Santvoort HC, Issa Y, Boermeester MA. Early laboratory biomarkers for severity

in acute pancreatitis; A systematic review and meta-analysis. Pancreatology. 2020;20(7): 1302-11.
8 Ramirez T, Daneshian M, Kamp H, Bois FY, Clench MR, Coen M, et al. Metabolomics in toxicology and preclinical research. ALTEX. 2013;30(2): 209-25.
9 Peng Y, Hong J, Raftery D, Xia Q, Du D. Metabolomic-based clinical studies and murine models for acute pancreatitis disease: a review. Biochim Biophys Acta Mol Basis Dis. 2021;1867(7): 166123.
10 Xiao H, Huang JH, Zhang XW, Ahmed R, Xie QL, Li B, et al. Identification of potential diagnostic biomarkers of acute pancreatitis by serum metabolomic profiles. Pancreatology. 2017;17(4): 543-9.
11 Li MJ, Wang XF, Aa JY, Qin WS, Zha WB, Ge YC, et al. GC/TOFMS analysis of metabolites in serum and urine reveals metabolic perturbation of TCA cycle in db/db mice involved in diabetic nephropathy. Am J Physiol Renal Physiol. 2013;304(11): F1317-F1324.
12 Houten SM, Wanders RJA, Ranea-Robles P. Metabolic interactions between peroxisomes and mitochondria with a special focus on acylcarnitine metabolism. Biochim Biophys Acta Mol Basis Dis. 2020;1866(5): 165720.
13 McCoin CS, Knotts TA, Adams SH. Acylcarnitines—old actors auditioning for new roles in metabolic physiology. Nat Rev Endocrinol. 2015;11(10): 617-25.
14 Ge J, Guo JJ, Zhang X, Yang WZ, Han LS, Sun HZ, et al. L-palmitoylcarnitine supplementation improves oocyte quality and embryo development derived from obese mice. Fundamental Research. 2024.
15 Bando M, Iwakura H, Koyama H, Hosoda H, Shigematsu Y, Ariyasu H, et al. High incorporation of long-chain fatty acids contributes to the efficient production of acylated ghrelin in ghrelin-producing cells. FEBS Lett. 2016;590(7): 992-1001.
16 Dholakia S, Sharples EJ, Ploeg RJ, Friend PJ. Significance of steatosis in pancreatic transplantation. Transplant Rev (Orlando). 2017;31(4): 225-31.
17 Kaya I, Citil M, Sozmen M, Karapehlivan M, Cigsar G. Investigation of protective effect of L-carnitine on L-asparaginase-induced acute pancreatic injury in male Balb/c mice. Dig Dis Sci. 2015;60(5): 1290-6.
18 Karakahya M, Gül M, Işık S, Aydın C, Yiğitcan B, Otan E, et al. The histopathologic effects of L-carnitine in sodium taurocholate induced severe pancreatitis model. Int Surg. 2016. Online ahead of print.
19 Arafa HMM, Hemeida RAM, Hassan MIA, Abdel-Wahab MH, Badary OA, Hamada FMA. Acetyl-L-carnitine ameliorates caerulein-induced acute pancreatitis in rats. Basic Clin Pharmacol Toxicol. 2009;105(1): 30-6.
20 Kumar A, Bachhawat A. Pyroglutamic acid: throwing light on a lightly studied metabolite. Current Science.2012;102(2):288-97.
21 Peris-Fernández M, Roca-Marugán M, Amengual JL, Balaguer-Timor Á, Viejo-Boyano I, Soldevila-Orient A, et al. Uremic toxins and inflammation: metabolic pathways affected in non-dialysis-dependent stage 5 chronic kidney disease. Biomedicines. 2024;12(3): 607.
22 Mutsaers HAM, Engelke UFH, Wilmer MJG, Wetzels JFM, Wevers RA, van den Heuvel LP, et al. Optimized metabolomic approach to identify uremic solutes in plasma of stage 3-4 chronic kidney disease patients. PLoS One. 2013;8(8): e71199.
23 Mole DJ, McFerran NV, Collett G, O'Neill C, Diamond T, Garden OJ, et al. Tryptophan catabolites in mesenteric lymph may contribute to pancreatitis-associated organ failure. Br J Surg. 2008;95(7): 855-67.
24 Mole DJ, Webster SP, Uings I, Zheng XZ, Binnie M, Wilson K, et al. Kynurenine-3-monooxygenase inhibition prevents multiple organ failure in rodent models of acute pancreatitis. Nat Med. 2016;22(2): 202-9.
25 Liddle J, Beaufils B, Binnie M, Bouillot A, Denis AA, Hann MM, et al. The discovery of potent and selective kynurenine 3-monooxygenase inhibitors for the treatment of acute pancreatitis. Bioorg Med Chem Lett. 2017;27(9): 2023-8.
26 Walker AL, Ancellin N, Beaufils B, Bergeal M, Binnie M, Bouillot A, et al. Development of a series of kynurenine 3-monooxygenase inhibitors leading to a clinical candidate for the treatment of acute pancreatitis. J Med Chem. 2017;60(8): 3383-404.
27 Li J, Zhao XL, Liu YX, Peng XH, Zhu SF, Guo H, et al. 1HNMR-based metabolomic profile of rats with experimental acute pancreatitis. BMC Gastroenterol. 2014;14: 115.
28 Chu YX, Zhang C, Xie M. Beta-hydroxybutyrate, friend or foe for stressed hearts. Front Aging. 2021;2: 681513.
29 Ouyang D. Metabolomic characterization of human pancreatitis by 1H-NMR spectroscopy. Hepatogastroenterology. 2012; 59(118):2314-7.
30 Cartier A, Hla T. Sphingosine 1-phosphate: lipid signaling in pathology and therapy. Science. 2019;366(6463): eaar5551.
31 Fu PF, Ramchandran R, Dudek SM, Parinandi NL, Natarajan V. Regulation of vascular endothelial barrier integrity and function by lipid-derived mediators. Cardiovasc Signal Health Dis. 2022:445-84.
32 Fu F, Li WM, Zheng XY, Wu YL, Du D, Han CX. Role of sphingosine-1-phosphate signaling pathway in pancreatic diseases. Int J Mol Sci. 2024;25(21): 11474.
33 Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. Nature. 2014;510(7503): 58-67.
34 Wang CH, Lai YH, Kuo CH, Lin YL, Tsai JP, Hsu BG. Association between serum indoxyl sulfate levels and endothelial function in non-dialysis chronic kidney disease. Toxins (Basel). 2019;11(10): 589.
35 Lusczek ER, Paulo JA, Saltzman JR, Kadiyala V, Banks PA, Beilman G, et al. Urinary 1H-NMR metabolomics can distinguish pancreatitis patients from healthy controls. JOP. 2013;14(2): 161-70.
36 Melamed ML, Plantinga L, Shafi T, Parekh R, Meyer TW, Hostetter TH, et al. Retained organic solutes, patient characteristics and all-cause and cardiovascular mortality in hemodialysis: results from the retained organic solutes and clinical outcomes (ROSCO) investigators. BMC Nephrol. 2013;14: 134.
37 Lu CL, Zheng CM, Lu KC, Liao MT, Wu KL, Ma MC. Indoxyl-sulfate-induced redox imbalance in chronic kidney disease. Antioxidants (Basel). 2021;10(6): 936.

*Received January 6, 2025*
*Accepted after revision May 26, 2025*