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Review

Metabolomic-based clinical studies and murine models for acute pancreatitis disease: A review

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ABSTRACT

Acute pancreatitis (AP) is one of the most common gastroenterological disorders requiring hospitalization and is associated with substantial morbidity and mortality. Metabolomics nowadays not only help us to understand cellular metabolism to a degree that was not previously obtainable, but also to reveal the importance of the metabolites in physiological control, disease onset and development. An in-depth understanding of metabolic phenotyping would be therefore crucial for accurate diagnosis, prognosis and precise treatment of AP. In this review, we summarized and addressed the metabolomics design and workflow in AP studies, as well as the results and analysis of the in-depth of research. Based on the metabolic profiling work in both clinical populations and experimental AP models, we described the metabolites with potential utility as biomarkers and the correlation between the altered metabolites and AP status. Moreover, the disturbed metabolic pathways correlated with biological function were discussed in the end. A practical understanding of current and emerging metabolomic approaches applicable to AP and use of the metabolite information presented will aid in designing robust metabolomics and biological experiments that result in identification of unique biomarkers and mechanisms, and ultimately enhanced clinical decision-making.

Abbreviations: AAs, arachidonic acids; AAP, alcoholic acute pancreatitis; ALT, alanine aminotransferase; AP, acute pancreatitis; AST, aspartate aminotransferase; AUC, area under the curve; AUROCs, area under the receiver operating characteristic curves; BAs, bile acids; BAP, biliary acute pancreatitis; BCAAs, branched-chain amino acids; CA, cholic acid; CBS, cystathionine-β-synthase; CCK, cholecystokinin; CDCA, chenodeoxycholic acid; CER, cerulein; DCA, deoxycholic acid; ER, endoplasmic reticulum; ERCP, endoscopic retrograde cholangiopancreatography; GC, gas chromatography; 3-HBA, 3-hydroxybutyrate; HC, healthy control; 3-HK, 3-hydroxykynurenine; HLP, hyperlipidemic acute pancreatitis; ^{1}H MAS, proton magic angle spinning; HMDB, human metabolome database; HR, high resolution; HTG, hypertriglyceridemia; IDH, isocitrate dehydrogenase; IP_3, inositol 1,4,5-triphosphate; KMO, kynurenine 3-monooxygenase; LC, liquid chromatography; LCA, lithocholic acid; LPC, lysophosphotidylcholine; MAP, mild acute pancreatitis; MODS, multiple organ dysfunction syndrome; MPTP, mitochondrial permeability transition pore; MRM, multiple reaction monitoring; MS, mass spectrometry; MSAP, moderate to severe acute pancreatitis; NAD+, oxidized nicotinamide adenine dinucleotide; NaT, sodium taurocholate; NIST, national institute of standards and technology; NMR, nuclear magnetic resonance; OPLS-DA, orthogonal projection latent structure discriminant analysis; PC, phosphatidylcholine; PCA, principal component analysis; PI, phosphatidylinositol; PLA_2, phospholipase A_2; PLSDA, partial least squares discriminant analysis; QC, quality control; QqQ, triple quadrupole; RF, random forest; ROS, reactive oxygen species; SAP, severe acute pancreatitis; SFA, sphinganine; SFO, sphingosine; SIRS, systemic inflammatory response syndrome; SOP, standard operation procedure; S1P, sphingosine-1-phosphate; TAG, triacylglycerol; TCA, tricarboxylic acid; TLCS, taurolithocholic acid 3-sulfate; TMAO, trimethylamine N-oxide; TOF, time of flight; UFA, unsaturated fatty acid.

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1. Background

Acute pancreatitis (AP) is an inflammatory disorder of the pancreas, primarily precipitated by biliary pathologies, alcohol, and hypertriglyceridemia (HTG) [1–4]. Most patients will develop a mild disease course (self-limited), in which moderate fluid resuscitation, management of pain and nausea, and early oral feeding result in rapid clinical improvement [5]. Around 20% of patients will have a severe form of AP, with a mortality rate from 19 to 52% [6–9]. The most commonly used severity classification system for AP is the 2012 revision of the Atlanta classification that offers three categories of mild, moderate, or severe [10]. Severe AP (SAP) is initiated by acinar cell injury-mediated local inflammation and progresses to the systemic inflammatory response syndrome (SIRS), accompanied by the inevitable multiple organ injury, and eventually this leads to multiple organ dysfunction syndrome (MODS) [11,12]. Over the years, advances have been made in various aspects of AP research – for examples, biomarkers identification for diagnosis [13–16], etiology definition [4,17–21], severity grading [22–32] (Table 1); development of management [15] guideline to mitigate the risk and improve performance; as well as discovery potential therapeutic agents such as ORAI1 inhibitors GSK-7975A [33] and CM4620 [34], and the mitochondrial permeability transition pore (MPTP) inhibitor TRO40303 [35]. However, there remain major challenges in AP diagnosis and treatment, including (1) the lack of laboratory biomarkers for accurate prediction of SAP on admission; (2) the determination of risk factors [gallstones, obesity, alcohol, smoking,

Table 1
Summary of markers for diagnosis, etiology and severity of acute pancreatitis.

Application	Biomarkers		Time points	Cut-off or threshold	Type of study	Number of studies	Number of participants	AUC	Sensitivity	Specificity	PPV	NPV	Ref.
Diagnosis	Serum amylase		On admission	>3 times normal	Meta-analysis	3	605	NA	0.72	0.93	0.74	0.92	[16]
	Serum lipase		On admission	>3 times normal	Meta-analysis	4	678	NA	0.79	0.89	0.68	0.93	
	Urinary trypsinogen-2		On admission	>50 ng/mL	Meta-analysis	5	841	NA	0.72	0.90	0.67	0.92	
Etiology	HTG-AP	Serum triglyceride	NA	>1000 mg/dL or >500 mg/dL and lipaemic serum or history of HTG	Systematic review	38	1979	NA	NA	NA	NA	NA	[4]
	BAP	Serum ALT	NA	≥150 IU/L	Meta-analysis	NA	NA	NA	NA	NA	0.95	NA	[17]
			Within 24 h of admission	>80 IU/L	Prospective study	1	68	NA	0.91	1	1	0.86	[18]
			On admission	>2 times normal	Prospective study	1	213	NA	0.74	0.84	0.88	0.66	[19]
				>3 times normal				NA	0.61	0.91	0.92	0.59	
			On admission	>150 IU/L	Prospective database	1	464	NA	0.51	0.97	0.80	0.96	[20]
	AAP	Serum CDT	Within 72 h of admission	≥22.5 U/L	Retrospective study	1	70	NA	0.875	0.852	NA	NA	[21]
		Serum trypsin	Within 72 h of admission	152 U/L	Retrospective study	1	70	NA	0.60	1	NA	NA	
Severity	C-reactive protein		Within 72 h of admission	NA	Meta-analysis	6	869	0.73	0.71	0.87	NA	NA	[24]
	Blood urea nitrogen		On admission	≥20 mg/dL	Prospective database	1	1612	0.65	0.55	0.75	0.35	0.87	[25]
	Hematocrit		On admission	≥44%	Prospective database	1	1612	0.67	0.59	0.74	0.37	0.88	[25]
	Procalcitonin		With 48 h or highest value	NA	Systematic review	12	826	0.87	0.72	0.86	NA	NA	[26]
	Interleukin-6		Day 1	>50 ng/mL	Systematic review	4	NA	0.93	0.87	0.88	NA	NA	[27]
	Triglyceride		Within 2 days of admission	>2648 mg/dL	Retrospective study	1	144	0.71	0.75	0.65	NA	NA	[28]
	TyG index		On admission	4.92	Prospective study	1	395	0.79	0.92	0.374	NA	NA	[29]
	HOMA-IR		On admission	NA	Prospective study	1	269	0.72	NA	NA	NA	NA	[30]
	NLR		1 day after admission	NA	Meta-analysis	10	1713	0.82	0.79	0.71	NA	NA	[31]
	Calcium		On admission	1.97 mmol/L	Retrospective study	1	128	0.89	0.89	0.75	0.51	0.96	[32]

Abbreviations: AAP, alcoholic acute pancreatitis; ALT, alanine aminotransferase; AUC, area under curve; BAP, biliary acute pancreatitis; CDT, carbohydrate-deficient transferrin; HOMA-IR, homeostatic model assessment of insulin resistance; HTG-AP, hypertriglyceridemia induced acute pancreatitis; NA, not available; NLR, neutrophil-lymphocyte ratio; NPV, negative predictive value; PPV, positive predictive value; TyG, triglyceride and glucose.

genetics, endoscopic retrograde cholangiopancreatography (ERCP)] that contribute to the episode of AP; (3) poorly understood pathogenesis of AP, mostly derived from animal models and relative lack of knowledge in human beings; and (4) no effective therapeutic agent exists to ameliorate or prevent AP. Therefore, new approaches dedicated to the discovery of specific biomarkers that offer more accurate diagnosis, etiological definition and severity stratification in AP, as well as the identification of reliable targets that could lead to the development of novel drugs are in urgent need [36–38].

The recently developed "omics" techniques facilitate high-throughput and high-content screening of disease related biomarkers in biological samples, of which metabolomics is one of the most promising approaches for precision medicine [39]. Although genetic variation in populations exists at birth, metabolic profiling is responsive to environmental factors such as dietary intake, gut microbiota variation, physical activity, and environmental exposures, and thus provides a highly integrated profile that is the end result of genomic, epigenetic, transcriptomic, and proteomic variation [40]. Metabolomics research, the comprehensive profiling of small molecule metabolites in biofluids or tissues, is aimed at investigating the global changes of numerous metabolites (substrates and products of metabolism) in a given sample type (e.g., cell, tissue, serum) with various analytical platform technologies, followed by deep data mining and bioinformatic analysis [41,42]. Metabolites have various functions in disease development, such as producing energies [43], synthesizing or modifying macromolecules [44,45], and serving as signaling molecules and hormones [46,47]. Metabolite biomarker discovery efforts have shown that the elevated levels of plasma trimethylamine N-oxide (TMAO) increased atherosclerosis risk [48], oncometabolites including 2-hydroxyglutarated [49], sarcosine [50], and glutamine [49] linked to tumors development, as well as branched-chain amino acids (BCAAs) that precede insulin resistance and diabetes [51]. Increasingly, dysregulated metabolism is also being recognized as a major contributor to a disease not traditionally considered as "metabolic" in origin, and even in critical illness such as sepsis [52]. Metabolomics has shown the potential to predict severity and differentiate sepsis patients from SIRS, and indicated energy production pathways that play vital roles in the pathophysiology of sepsis [53]. Thus, metabolomics can serve as a precise and noninvasive tool not only to define biomarkers that predict disease incidence, severity, and progression, but also to cast new light on underlying mechanistic abnormalities [52].

With the continuous development of instruments and sample preparation methods, advancements have been made in AP metabolomics studies over the past two decades. A mini-review recently discussed the application of metabolomics in the clinical diagnosis, prognosis, treatment, and evaluation of pancreatic diseases [54]; however, it did not fully summarize the altered metabolites and dysregulated metabolic pathways related to the underlying mechanisms of AP. Therefore, we have undertaken a comprehensive review of 22 articles carried out so far on AP metabolomics (Fig. S-1). In this review, we addressed and discussed the metabolomics study design and workflows used on AP, and outlined the alterations of the metabolome of blood, urine, and pancreatic tissue in clinical populations and experimental models. Finally, we focused on several abnormal metabolic pathways in AP, which may hopefully improve our knowledge on the molecular mechanisms mediating AP development and aid in the identification of new therapeutic strategies for this sudden inflammatory disease.

2. Metabolomics design and workflows

The field of metabolomics has undergone a rapid technological evolution in the past decades [42,52,55–57]. These advancements in metabolomics can lead to an increase in our understanding of pathophysiological mechanisms and identifying potential biomarkers for AP diagnosis and prognosis. A good metabolomics study begins with a cogitative hypothesis and careful study design scheme to ensure reliable results [58] (Fig. 1). Metabolomics can be divided into non-targeted and targeted metabolomics according to its research purpose [59,60]. Non-targeted metabolomics is best suited as a discovery tool for identifying metabolites that change in response to manipulation of a biological system rather than providing the exact concentration of a known metabolite [61]. It is generally used for hypothesis generation. Targeted metabolomics is often used for detecting predefined metabolites driven by a specific biochemical question [59]. Nowadays, these two metabolomics approaches are often combined to achieve the goals of better metabolite coverage and reproducibility [62,63]. Generally, the metabolomics workflow consists of sample collection, sample preparation, data acquisition, data analysis and biological interpretation/validation [64–67], as depicted in Fig. 1.

Proper sample collection is one of the most important steps, which directly determines the reliability of the entire study [68,69]. A standard operation procedure (SOP) for sampling and clinical information recording is essential to collecting valid samples of patients and control subjects. The blood collection tubes should be carefully selected, since the citrate or EDTA additives could influence the measurement of some metabolites [70]. A critical individual demographic and clinical information involves admission time, ethnicity, age, gender, body mass index, alcohol and smoking use, diet, medical history, clinical diagnosis and severity classification for patients and similar matched information for controls. Normally, patient samples collected within the first 24 h of admission to hospital (or as soon as possible) and follow-up samples collected afterwards at a fix interval for each patient is typically the best approach. Early sample acquisition and analysis of metabolic alteration in the early phase of AP with different severity can potentially be used for assessing risk stratification as well as for elucidating mechanism in the acute phase. In comparison to human cohorts, animal models enable easier implementation of study protocols for biofluids and tissues harvesting under anesthesia or euthanasia [40]. It should be emphasized that all the samples require rapid collection, aliquoting, quenching, and storage at −80 °C or lower, as well as avoidance of multiple freeze-thaw cycles [68]. A pooled sample generated by mixing a small aliquot of the study specimens can serve as a quality control (QC) to assess the technique precision and correct baseline drift and signal intensity across the analysis [71]. Unfortunately, there is no single extraction technique that can capture the entire metabolome [72]. As a result, the choice of extraction conditions (e.g., liquid-liquid extraction and deproteination) is influenced by sample matrix interference and the specific analytical platforms used [72–74]. Detailed extraction methods used for AP metabolomics have been summarized in Table 2.

Nuclear magnetic resonance (NMR) and mass spectrometry (MS) are two of the most popular analytical tools used in metabolomics [67,75–77]. They have different extraction, data acquisition and processing methods because of various physical and chemical properties [77–79]. NMR is non-destructive and requires minimal sample preparation, detects compounds difficult to ionize or derivatize, and is amenable to high-throughput, but is less sensitive and typically require a larger amount (typically 10–200 mg) of sample [80]. Proton magic angle spinning (^{1}H MAS) NMR is specifically designed for intact tissue (pancreas) without sample preparation [81].

In contrast to NMR, MS offers a highly sensitive and selective approach to measure hundreds to thousands of metabolites based on their mass/charge ratio and signal intensity, but it requires sample extraction procedures and is affected by ionization suppression and sample matrix effects [78,82]. Gas chromatography (GC) or liquid chromatography (LC) coupled to MS provide separation techniques to reduce the complexity of bio-samples [83]. GC–MS is easy to operate and has good stability and repeatability; it is most suitable for naturally volatile and thermally stable metabolites as well as metabolites made volatile by derivatization [78,84]. The two-step derivatization method involving oximation and silylation provides for a broad coverage of free fatty acids, steroids, sugars and citric acid cycle metabolites (Table 2). Unlike GC-MS, LC-MS does not require volatile analytes and often

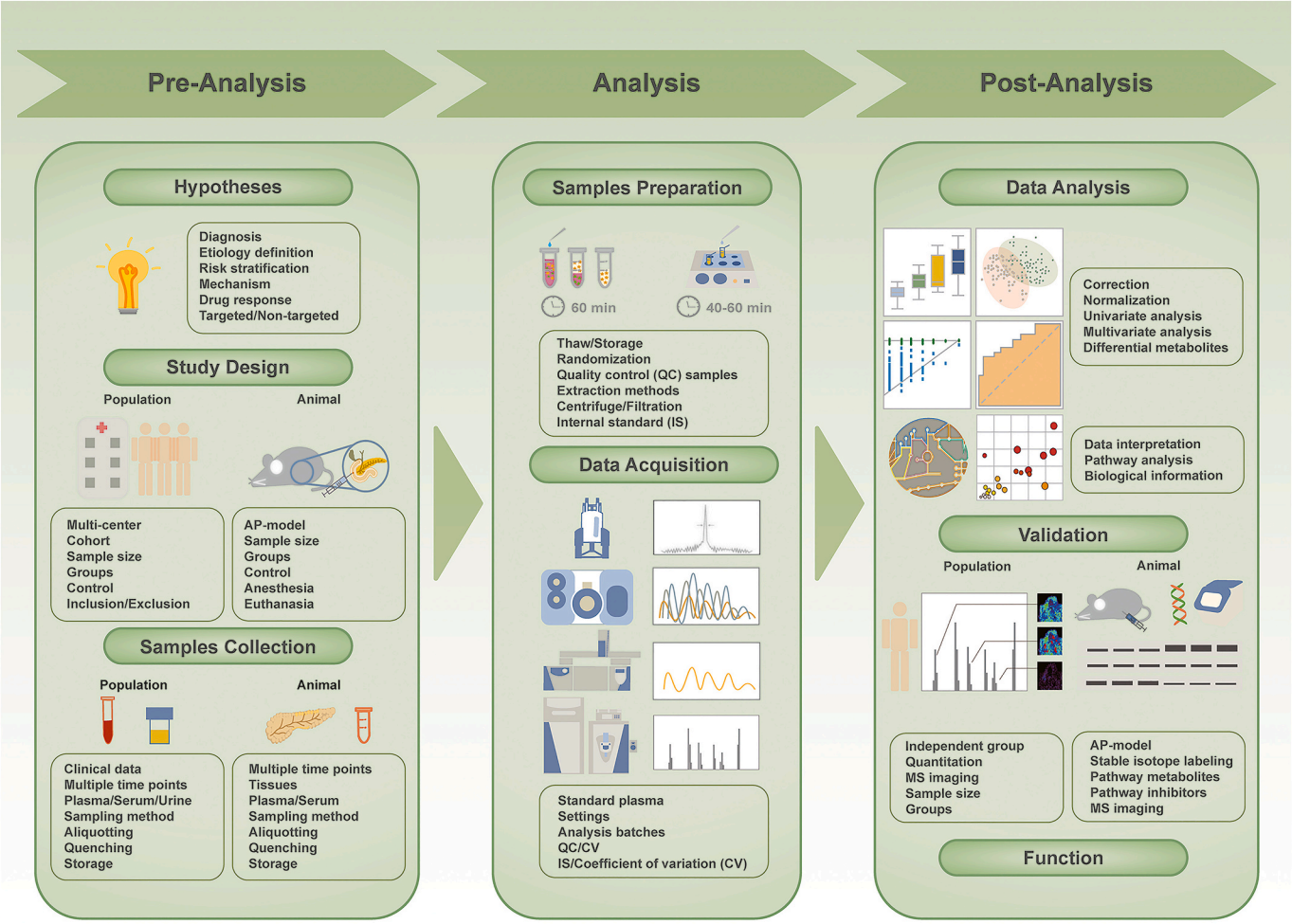

Fig. 1. Overall study design for metabolomics workflow in AP studies.

requires minimal sample preparation [84,85]. Reverse phase and hydrophilic interaction chromatographies are two commonly used stationary phases applied for nonpolar and polar metabolites, respectively [86,87]. Various types of MS such as triple quadrupole (QqQ) and high resolution (HR) MS have been hyphenated with LC systems [75]. Multiple reaction monitoring (MRM)-based targeted metabolite quantitation exhibits high sensitivity, broad dynamic range, and reliable accuracy for limited sets of known metabolites [88]. HRMS instruments, including time of flight (TOF) and Orbitrap platforms are prevalent due to their high resolution and accurate mass determination capabilities [67,75,89]. However, the non-targeted TOF and Orbitrap approaches rely heavily on availability of spectral databases [85]. Identification of unknown features is the major challenge in non-targeted metabolomics [60,90]. Methods for definitive identification and currently available resources are described in a number of published reviews [91–95]. Most of the metabolite feature identifications are putative according to spectral matching or retention times based on in-house databases, the Human Metabolome Database (HMDB) (https://hmdb.ca/), the National Institute of Standards and Technology (NIST) (https://www.nist.gov/), or the Fiehn lab GC-MS database (https://fiehnlab.ucdavis.edu/).

After metabolomic data acquisition, the raw data are processed by run or batch normalization, missing value imputation, data standardization and data transformation or scaling [96,97]. To identify differential metabolites, univariate statistics [98,99] including the parametric test (Student's *t*-test and Analysis of Variance) and nonparametric tests (Kruskal–Wallis and Mann–Whitney *U* test) have been applied for normally distributed and skewed datasets, respectively. Usually, the Benjamini–Hochberg procedure is performed to adjust *p*-values and reduce false discovery errors due to multiple testing. Multivariate statistical analysis [100] including principal component analysis (PCA), partial least squares discriminant analysis (PLSDA), orthogonal projection latent structure discriminant analysis (OPLS-DA), and random forests (RF) also serves as a discriminating tool to find differential metabolite profiles and to build a predictive model. Moreover, use of an external validation cohort is essential for biomarker discovery through assessing accuracy, sensitivity, and specificity of the established model using the parsimonious panel of differential metabolites [101,102]. Lastly, the metabolites are often analyzed from the perspective of their respective metabolic pathways in order to identify perturbations at the pathway level, using software such as MetaboAnalyst (http://www.metaboanalyst.ca) [103]. From the disrupted metabolic pathways and altered metabolite levels, additional functional exploration based on cell biology then need to be performed to verify initial findings. Typical methods for functional metabolomics study have been reviewed recently [67].

3. Metabolomics studies on patients with AP

With the goal of biomarker discovery to improve diagnosis and prognosis, GC-MS and NMR based untargeted metabolomics have been mainly implemented using biofluids from AP patients. Over the past decade, improvements in HRMS analytical technologies have allowed a

Table 2
Summary of extraction methods used in AP metabolomic studies.

Analytic platform	Sample type	Sample volume	Internal standard	Methods	Extraction solvent	Major metabolites	Ref.
^{1}H NMR	Human serum	250 μL	TMSP	(1:1) Dilution	0.2 M Na_2HPO_4	Organic acids, amino acids, lipids	[113]
	Human serum	300 μL	TMSP	(1:1)[b] Dilution	7.5 mM Na_2HPO_4, 20% D_2O, 0.025% NaN_3	Fatty acids, lipids	[119]
	Rat serum	200 μL	NA[a]	(1:2) Dilution	Saline, 20%D_2O	Organic acids, sugars	[135]
	Rat serum	400 μL	NA	(4:1) Dilution	0.2 M Na_2HPO_4, 0.2 M NaH_2PO_4, D_2O	Organic acids, sugars, amino acids, nucleotides, lipids, fatty acids	[136]
	Human plasma	200 μL	NA	(1:2) Dilution	0.9% Saline, 10% D_2O	Organic acids, amino acids, sugars, lipids	[108]
	Rat plasma	150 μL	TMSP	(3:7) Dilution	D_2O	Organic acids, amino acids, sugars, lipids	[133,134]
	Human urine	400 μL	TMSP	(2:1) Dilution	Na_2HPO_4, 20% D_2O	Organic acids, amino acids, lipids	[108]
	Human urine	1 mL	TMSP	(2:1) Dilution	0.2 M Na_2HPO_4	Amino acids, amines, organic acids	[113]
	Human urine	1 mL	TMSP	(2:1) Dilution	0.2 M Na_2HPO_4, D_2O	Organic acids, nucleotides	[125]
	Rat urine	400 μL	TMSP	(2:1) Dilution	0.2 M Na_2HPO_4, 40 mM NaH_2PO_4, 20% D_2O, 3 mM NaN_3	Organic acids, amino acids, amines	[135]
^{1}H MAS NMR	Rat pancreas	15 mg	NA	Excised sections	D_2O	Organic acids, sugars, amino acids, lipids, nucleotides	[132]
GC–MS	Human serum	100 μL	2-IPMA	(1:3) Protein precipitation Oximation, and silylation	MeOH MeOX, BSTFA, 1% TMCS	Organic acids, amino acids, sugars, fatty acids	[106]
	Human serum	100 μL	HDA	(1:3) Protein precipitation Oximation, and silylation	MeOH MeOX, BSTFA, 1% TMCS	Organic acids, amino acids, sugars, fatty acids	[122]
	Mouse serum	50 μL	2-IPMA	(1:5) Liquid-liquid extraction Oximation and silylation	MeOH:H_2O:$CHCl_3$ = 2.5:1:1 MeOX and MSTFA	Organic acids, amino acids, sugars, fatty acids	[131]
	Mouse serum	200 μL	NA	(1:2) Protein precipitation Oximation and silylation	Acetone MeOX, BSTFA, 1% TMCS	Organic acids, fatty acids, amino acids	[139]
	Human plasma	40 μL	Ribitol	(4:155) Liquid-liquid extraction Oximation and silylation	MeOH:MTBE:H_2O = 30:100:25 MeOX, MSTFA	Organic acids, amino acids, sugars	[105]
	Human urine	600 μL	L-2-CPA	(60:55) Derivatization	EtOH:pyridine:ECF = 8:2:1	Organic acids, lipids, benzenoids	[107]
	Mouse pancreas	20 mg	2-IPMA	(1:50) Homogenization Liquid-liquid extraction Oximation and silylation	MeOH:H_2O:$CHCl_3$ = 2.5:1:1 $CHCl_3$:H_2O = 9:4 MeOX, MSTFA	Organic acids, amino acids, sugars, lipids	[131]
LC-MS	Human serum	100 μL	NA	(1:3) Protein precipitation	MeOH	Organic acids, fatty acids, lipids	[117]
	Rat serum	150 μL	NA	(1:4) Protein precipitation	MeOH	Organic acids, amino acids, sugars, lipids	[137]
	Human plasma	40 μL	NA	(4:155) Liquid-liquid extraction	MeOH:MTBE:H_2O = 30:100:25	Lipids	[105]
	Human plasma	5 μL 100 μL	d5-Try NA	(5:20) Protein precipitation (10:13) Protein precipitation	CCl_3COOH ACN:H_2O = 4:6	Amino acids, organic acids 3-Hydroxykynurenine	[124]
	Mouse pancreas	NA	NA	Fixing (1:4) Homogenization (100:4) Protein precipitation	0.1% HCOOH PBS + 10 mM NEM $HClO_4$	Amino acids	[104]

Abbreviations: ACN: acetonitrile, BSTFA: *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide, CPA: chlorophenylalanine, ECF: ethyl chloroformate, GC-MS: gas chromatography-mass spectrometry, HDA: heptadecanoic acid, IPMA: isopropylmalic acid, L-2-CPA: L-2-chlorophenylalanine, LC-MS: liquid chromatography-mass spectrometry, MAS NMR: magic angle spinning nuclear magnetic resonance, MeOX: methoxyamine, MSTFA: *N*-methyl-*N*-trimethylsilyltrifluoroacetamide, MTBE: methyl tert-butyl ether, NEM: N-ethylmaleimide, NMR: nuclear magnetic resonance, PBS: phosphate buffered saline, TMCS: trimethylchlorosilane, TMSP: trimethylsilylpropionic acid, Try: tryptophan.

[a] NA refers to not available or not mentioned in the literature.

[b] Ratio in the brackets refers to the volume-volume ratio or weight-volume ratio of the sample to the solvent.

broader coverage of the metabolome. Most of these studies examined blood, e.g., serum accounts for 53%, plasma 20%, followed by urine 27% of the published literature (Fig. S-1). In the following section, we review differential metabolites in blood and urine matrices obtained from AP patients that may be useful for diagnosis, disease etiology classification, and severity prediction (Table 3).

3.1. Blood metabolome

Due to the minimally invasive nature and relatively easy availability, most of the untargeted metabolomics studies and one targeted metabolomic study [104] have been performed using plasma/serum samples. In general, the differential metabolites identified as useful for diagnosis and prognosis of AP could be classified into five categories: (1) energy-related sugars, ketones, and organic acids; (2) amino acids; (3) lipids; (4) purines; and (5) gut microbiome metabolites.

Citrate, a precursor of pyruvate, provides feedback inhibition of the tricarboxylic acid (TCA) cycle. An elevation of citrate in blood was detected in some AP clinical metabolomics studies [105,106], which suggested an impaired TCA cycle and mitochondrial dysfunction. Recently, Xiao et al. [106] used GC-MS combined with RF analysis to accurately distinguish mild acute pancreatitis (MAP) from SAP [discovery group accuracy = 87.50%, sensitivity = 94.44%, and specificity = 92.30%; and validation group accuracy = 87.50%, sensitivity = 100%, and specificity = 92.85%] and identified citrate as the metabolite with the largest feature importance. Altered levels of citrate have also

Table 3
Metabolomic studies on AP patients.

Sample types	Purpose	Statistical tools	Groups & number	Main differentiateial metabolites from AP vs control[a]	Ref.
Serum	Diagnosis	M-W *U* test, PCA, RF, AUROC = 0.99, accuracy (98.31%), sensitivity (96.15%), specificity (100%)	AP (n = 40, validation set = 14), HC (n = 37)	(↑): 3-HBA, citrate, Man, Gal, Glc, PA (↓): Phosphate, glycerol, serotonin	[106]
	Diagnosis	PCA, OPLS-DA, SVM, two independent samples nonparametric test, CLC: AUROC = 0.865, accuracy (100%)	AP (n = 38), HC (n = 36), CHO (n = 26)	(↑): SFA, MA, CLC, DLC, glycocholate, dodecanol, L-thyronine, 2-TDC AP (↑) vs CHO: SFA, CLC, MA, DLC, dodecanol, 2-TDC AP (↓) vs CHO: Glycocholate, L-thyronine	[117]
	Diagnosis	PCA	AP (n = 17), HC (n = 23)	(↑): Ile, AGly, TAG, inosine (↓): 3-HBA, TMAO, acetate, acetone	[118]
	Diagnosis	K-W test, PCA, OPLS-DA, accuracy (90%)	AP (n = 8), CHC (n = 9) AP (n = 8), CHO (n = 12)	AP (↑) vs CHC: PC, PE, choline AP (↓) vs CHO: PC, PE, choline	[119]
	Etiology	PCA, OPLS-DA	HLP (n = 24), HC (n = 39)	(↑): PA, SA, EA (↓): Gly, Ala, citrate, fumarate	[107]
	Etiology	K-W test, PLS-DA	Post-ERCP: AP (n = 9), non-AP (n = 18)	(↑): Hypoxanthine, Asp	[113]
	Etiology	*t*-Test, RF, accuracy (BAP: 88.6%, AAP: 85.7%, HLP: 90.6%)	BAP (n = 27), HC (n = 15) AAP (n = 20), HC (n = 15) HLP (n = 29), HC (n = 15)	(↑): Lys (↓): Lactate, Val, phosphate, Gly, AGN, Gal, Glc, mannitol, Tyr, Tur, SA, OA, myo-inositol, Cho, G1H (↓): Lactate, Gly, AGN, 3-HBA, Glc, mannitol, Tyr, Tur, SA, myo-inositol, Trp, Cho, G1H (↓): Lactate, butyrate, oxalate, 3-HBA, Gly, Ser, Thr, Pro, ERA, Gln, Phe, Orn, Tyr, SA, PA, Trp, Cho, G1H, AA, LA, OA	[122]
	Etiology	χ^2-test, *F*-test, M-W U test, Wilcoxon signed-rank test	AAP (n = 19), AC (n = 20)	(↑): PA, OA (↓): MA, LA, γ-LOA, HA, α-LOA, MEA	[185]
Plasma	Diagnosis	Student's *t*-test, PLS-DA, OPLS-DA	AP (n = 13), HC (n = 10)	(↑): Urea, oxalate, citrate, Glc, Lyx, Man, myo-inositol, 1,5-AS, 9-HP, inosine, β-Ala, PA, PC, TAG (↓): Pyruvate, lactate, 3-HBA, hydroxylamine, Pro, Gly, Ala, Val, Phe, Orn, phosphate, LPC, CE	[105]
	Diagnosis	PCA, PLS-DA, OPLS-DA, AUROC = 0.86	AP (n = 15), non-AP (n = 21)	(↑): Acetone, 3-HBA, ACA, Glc, choline (↓): Val, Ala	[108]
	Severity	PCA, PLS-DA, OPLS-DA	MAP (n = 8), MSAP (n = 7)	MSAP (↑) vs MAP: Glc MSAP (↓) vs MAP: Ala, Val, choline, Ile, scyllo-inositol	[108]
	Severity	K-W H test, one-way ANOVA, 3-HK: AUC = 21.6 (11.7–28.0) median (IQR), Trp: AUC = 13,782.4 (3,789.8) mean (SD)	SAP (n = 9), MAP (n = 25)	SAP (↑) vs MAP: 3-HK SAP (↓) vs MAP: Trp	[124]
Urine	Diagnosis	PCA, PLS-DA, OPLS-DA, AUROC = 0.91	AP (n = 13), non-AP (n = 18)	(↓): Hippurate, creatine	[108]
	Diagnosis	K-W test, pairwise Wilcoxon rank-sum test, PCA	AP (n = 5), HC (n = 5)	(↑): Acetone, Rib	[125]
	Etiology	PCA, OPLS-DA	HLP (n = 24), HC (n = 39)	(↑): Pro, Leu, tyramine, Phe, Tyr, His, PA, SA (↓): Gly, citrate, p-OPL, hippurate	[107]
	Etiology	PCA, PLS-DA, OPLS-DA	AAP (n = 8), CAP (n = 5), non-AP (n = 21)	AAP (↑) vs non-AP: Glc, acetate, ethanol, acetone, EG AAP (↑) vs CAP: Acetone, ACA, 3-HIL	[108]
	Etiology	K-W test, PLS-DA	Post-ERCP: AP (n = 9), non-AP (n = 18)	(↑): 1-Methylnicotinamide	[113]
	Severity	PCA, PLS-DA, OPLS-DA	MAP (n = 8), MSAP (n = 5)	MSAP (↓) vs MAP: Creatinine, malonate, guanine	[108]

Abbreviations are listed as follows sorted to the sub-catalogs: Statistic tools: ANOVA: analysis of variance, AUROC: area under the receiver operating characteristic curves, *F*-test: Fishers exact test, K-W test: Kruskal-Wallis tests, M-W *U* test: Mann-Whitney U test, OPLS-DA: orthogonal-PLS-DA, PCA: principal component analysis, PLS-DA: partial least-squares discriminant analysis, χ^2-test: chi-square test, RF: random forest, SVM: support vector machine; Groups: AAP: alcoholic acute pancreatitis, AC: alcoholic control, BAP: biliary acute pancreatitis, CAP: cholelithiasis acute pancreatitis, CHC: cholecystitis, CHO: cholelithiasis, ERCP: endoscopic retrograde cholangiopancreatography, HC: healthy control, HLP: hyperlipidemic pancreatitis, MAP: mild acute pancreatitis, MSAP: moderate severe acute pancreatitis, SAP: sever acute pancreatitis; Metabolites (see the references for more details): AA: arachidonic acid, ACA: acetoacetate, AGly: acetylglycine, AGN: *N*-acetyl-D-glucosamine, Ala: alanine, 1,5-AS: 1,5-anhydro-D-sorbitol, Asp: aspartate, CE: ceramide, Cho: cholesterol, CLC: capryloyl choline, DLC: decanoyl choline, EA: eicosanoic acid, EG: ethyl glucuronide, ERA: erythronic acid, Gal: galactose, G1H: glycerol 1-hexadecanoate, Glc: glucose, Gln: glutamine, Gly: glycine, HA: homo-gammalinolenic acid, 3-HBA: 3-hydroxybutyrate, 3-HIL: 3-hydroxyisovaleric acid, His: histidine, 3-HK: 3-Hydroxykynurenine, 9-HP: 9H-purine, Ile: isoleucine, LA: linoleic acid, Leu: leucine, LOA: linolenic acid, LPC: lyso-phosphatidylcholine, Lys: lysine, Lyx: lyxose, MA: myristic acid, Man: mannose, MEA: mead acid, OA: oleic acid, Orn: ornithine, PA: palmitic acid/hexadecanoic acid, PC: phosphatidylcholine, PE: phosphatidylethanolamine, Phe: phenylalanine, p-OPL: *p*-hydroxyphenylacectate, Pro: proline, Rib: ribose, SA: steric acid/octadecanoic acid, Ser: serine, SFA: sphinganine, TAG: triacylglyceride, 2-TDC: 2-tetradecanone, Thr: threonine, TMAO: trimethylamine N-oxide, Trp: tryptophan, Tyr: tyrosine, Tur: turanose; Val: valine.

[a] Unless specified in the subsection, the upward and downward arrows in the table indicate that the number of metabolites is increased and decreased, respectively, compared with the control group.

been found in hyperlipidemic AP (HLP) patients and alcoholic subjects [107,108]. However, due to its non-specificity, the evaluation of plasma citric acid is scarcely used in the diagnosis of human diseases.

Synthesis and utilization of ketone bodies [acetoacetate, 3-hydroxybutyrate (3-HBA), and acetone] is thought to play a key role in AP. Changes in 3-HBA levels have been detected in several AP metabolomics studies (Table 3), and it was elevated in the blood of AP patients in some cases [106,108]. However, excessive 3-HBA would affect the pH in blood and dysregulate the internal environment balance of body [106]. Kabadi showed that ketosis or ketoacidosis secondary to AP is a distinct, though previously unrecognized syndrome, which is induced and maintained by extremely high levels of pancreatic lipase in the circulation [109]. In accordance with these results, Xiao et al. reported 3-HBA as a potential prediction marker for SAP [106]. Ketones are also

predictive for alcohol-induced AP for which high concentrations of ketones have been reported [108]. Ketones are products of the oxidative pathway of alcohol metabolites [110], and ketoacidosis is frequently attributed to ethanol ingestion [111]. In the case of other etiologies, ERCP is the most common cause of iatrogenic AP [112]. Notably, levels of two ketones, acetoacetate and 3-HBA, were significantly elevated after ERCP in serum, suggesting alterations in pancreatic function and insulin resistance resulted from AP [113].

The choline metabolism pathway is known to be involved in the inflammation response [114]. Choline is the initial point of the synthesis of lipids such as lysophosphotidylcholine (LPC) and phosphatidylcholine (PC) [115], is important as a precursor of acetylcholine, and acts as a methyl donor in various biological processes [115]. Both capryloyl choline and decanoyl choline are the esterification products of choline and fatty acids created by choline acetyltransferase [116]. Their serum levels were significantly increased in MAP patients compared with the control group , and area under the receiver operating characteristic curves (AUROCs) of these two metabolites are higher than other routine laboratory indicators including amylase and lipase [117]. The area under the curve (AUC) for capryloyl choline was as high as 0.865 [117]. A series of LPC, PC and triacylglycerol (TAG) were differentially present between the control (healthy or cholecystitis) and AP [105,118,119]. This was predictable as the AP progressed with increased energy requirements from lipids [105]. Phospholipids are one of the main components of cell membranes, the change of phospholipid levels in the AP group might reflect the decomposition of tissues [105]. Moreover, the choline concentration was inversely related to disease severity, which could be used as one of the markers for differentiating moderate to severe AP (MSAP) and MAP [108]. Multivariate models were established based on increased glucose and decreased creatinine, alanine, valine, choline and *N*-acetyl signals from α1-acid glycoprotein and generated a strong prediction model for severity of AP ($R^2Y = 0.96$, $Q^2 = 0.64$) [108].

Abnormal concentrations of sugar alcohols have also been demonstrated in AP patients. Inositol is a precursor molecule for a number of secondary messengers including inositol phosphates [120]. Inositol has some important stereoisomers; for example, myo-inositol is synthesized from glucose and works as an important component of phosphatidylinositol [121]. The alteration of inositol not only contributes to distinguishing biliary AP (BAP) and alcoholic AP (AAP) patients from healthy control (HC) [122], but also aids in the prediction of AP severity [108]. Mannitol is a polyol similar to xylitol or sorbitol, which is used to increase diuresis and improve mucus clearance [123]. It was shown that reduced mannitol have been detected in BAP patients compared to the healthy group [122].

Skouras et al. [124] used LC-QqQ based targeted metabolomics to quantify tryptophan metabolites in AP patients up to 168 h after admission to determine the exact timing and magnitude of the activation of the kynurenine pathway to the onset of AP, AP severity and development of MODS. The standardized AUROC for 3-hydroxykynurenine (3-HK) was significantly different between SAP and MAP [124]. Plasma concentrations of 3-HK correlated with the inflammation burden reflected by contemporaneous CRP and cytokine concentrations like IL-1β and IL-6, incidence of organ dysfunction and AP severity [124].

3.2. Urine metabolome

Given its easy, noninvasive sampling, urine is an ideal source of biomarkers for clinical analysis. A pilot study was performed by Lusczek et al. using ^{1}H NMR to determine how the urine metabolome differed between AP, chronic pancreatitis and HC [125]. Level of five metabolites, including acetone, adenosine, citrate, ribose and 3-indoxylsulfate were found to be significantly different, with adenosine and citrate remaining significant after validation by random permutation [125]. Adenosine could be a better marker of pancreatic disease since it possesses known anti-inflammatory properties [126]. The decreased urinary citrate found in pancreatitis cohorts supported the blood citrate changes [125].

Meanwhile, Villaseñor et al. have investigated the urinary and plasma metabolic phenotype of AP using ^{1}H NMR and multivariate modeling [108]. AP could be reliably identified with a high degree of sensitivity and specificity (OPLS-DA model $R^2 = 0.76$ and $Q^2Y = 0.59$) using a panel of discriminatory biomarkers consisting of guanine, hippurate and creatine in urine, and valine, alanine and lipoproteins in plasma [108]. Urine provided a stronger predictor of AP (AUROC = 0.91) than plasma (AUROC = 0.87) [108]. Hippurate is a well-established gut microbial co-metabolite derived from glycine conjugation of benzoic acid [127]. A significant negative correlation was found for urinal hippurate and AP. This also fits with the theory of gut bacterial translocation in AP, which is thought to explain the high rates of enteric bacteria identified in cases of pancreatic necrosis and sepsis [128]. Moreover, ketones (acetoacetate and acetone) and 3-hydroxyoxyisovalerate were found to be predictors for AAP [108]. 3-Hydroxyoxyisovalerate is a byproduct of leucine degradation excreted in the urine. Reduced urinary creatinine, malonic acid, and guanine were also found in MSAP compared to MAP [108].

The urine, which contains abundant polar metabolites, is suitable for derivatization and GC-MS metabolomic profiling. Zhao et al. employed GC-MS combined with multivariate statistical analysis to discriminate HLP from HC, and also found notable changes in hippurate and *p*-hydroxyphenylacectate as phenol metabolites of gut flora [107]. Fatty acids and aromatic amino acids were also increased in urine, suggesting hydrolysis of TAG in or around pancreas for HLP patients [107].

4. Metabolomics studies using AP murine models

In order to understand the biological and patho-physiological mechanisms of AP, various attempts have been made to induce AP in multiple animal models over the past 100 years [129,130]. Three animal models have been used for AP metabolomic studies: endogenous and xenobiotic toxins like cerulein (CER), sodium taurocholate (NaT) and L-arginine are administered to rodents to accumulate in the pancreas and exert an organ toxicity reaction, and the resultant metabolites then cause secondary damage. We have summarized these results in Table 4. In these animal studies, abnormal metabolic profile changes were detectable before the changes in histological structure or function occurred. Notably, altered metabolites in AP experimental models were similar to those found in AP patients.

Sakai et al. first investigated the metabolite levels in both serum and pancreas from CER- and arginine-induced AP models using GC–MS based global profiling [131]. In accordance with the observation from the clinical metabolome, higher level of sugar alcohols were found in serum from both AP models [131]. Significantly increased arabitol and xylitol, and particularly higher ribitol levels were detected in the serum of arginine-induced AP model [131]. Significantly altered metabolites in the pancreas of two models included elevated serine, lysine, histidine, tryptophan, coumaric acid, citric acid/isocitric acid, and adenine; and reduced glutamate, *O*-phosphoethanolamine, and 2-deoxy-D-glucose [131]. They are regarded as vital metabolites associated with development and state of AP [131]. Moreover, supplementation with glutamate and *O*-phosphoethanolamine attenuated the severity of CER-AP, which might be due to an increase in the amount of substrate useable for energy requirements and phospholipid synthesis [131].

Consistently, studies on arginine- and NaT- induced AP models exhibited abnormal choline levels. Ma et al. [132] detected total choline including choline, phosphocholine and PC increased in arginine-induced AP group using ^{1}H MAS NMR on intact pancreas, which might be a result of the blockage of choline kinase and phosphocholine transferase, or from the consumption of PC. Li et al. [133,134] found lower levels of choline, TMAO, and PC in plasma of 3.5% NaT-induced AP rats by NMR.

The alteration of ketones was also obvious in those two AP models. Increased 3-HBA in blood was detected from three different studies

Table 4
Metabolomic studies on AP animal models.

Toxins	Sample types	Time[a]	Statistical tools	Main differential metabolites from AP vs control	Ref.
L-arginine	Urine	−16, 0, 8, 24, 48, 72, 96, 120, 144, and 168 h	PCA, OPLS-DA	8 and 24 h: (↑): Arg, Orn, Cit, Lys, Glu, Gln, Ile, Leu, Val, Ala, Glc, lactate (↓): Citrate, 2-OG, succinate, fumarate, HPPA, hippurate, 2-AMA; 48 h: (↑): 4-CG, 4-CS, PAG, creatine, creatinine	[135]
	Serum	48 and 168 h		48 h: (↑): Creatine, lactate, 3-HBA, acetate (↓): Glc	
	Serum	48 h	Two-tailed Student's *t*-test.	(↑): Arabitol, xylitol, ribitol, 5-HIAA, G2P, glycerol, KVA (↓): Ara, HTau, Phe, Gly, Met, Asn, Ile, β-Ala, AGln, Val, Pro, Tyr, Orn, Sar, 4-OP, 3-HBA, ACA, citrate/isocitrate, succinate, malate, fumarate, aconitate, GCA, hippurate, ATA,Tur, xyl, m-Ery, threitol, Fru, Sor, GAD, creatinine	[131]
		72 h		(↑): Arabitol, xylitol, KVA, catechol, phosphate, Glc, lactate (↓): HDA, malonate, glutarate, Hcy, Glu, Tyr, HTau, Met, Asn, β-Ala, Galn, Rib, sorbitol, Sor, Fru, Tur, citrate/isocitrate, malate, succinate, aconitate, fumarate, ACA	
	Pancreas	48 h		(↑): Trp, His, Leu, CSA, BCys, lys, Ser, Gly, Cys/Cystine, p-CA, laurate, Glc, F6P, glycerol, 3-PLA, G2P, citrate/isocitrate, adenine, Ara, 1,5-AG (↓): Glu, Asp, Orn, Tyr, 4-OP, ALys, lactate, O-phosphoethanolamine, urea, GCA, ACA, m-Ery, 2-HP, 2-dGlc, Man, prolinamide, orotate, creatinine	
		72 h		(↑): Put, Trp, His, Thr, Ile, Gly-Gly, Phe, Glc, Leu, Val, Lys, BCys, Asn, Ala, Gln, p-Glu, F6P, p-CA, 3-PLA, dopa, laurate, POA, citrate/isocitrate, pantothenate, glycerol, urate, 1,5-AG, xylitol, arabitol, Fru, Rha, galactitol, glucarate (↓): β-Ala, ALys, Tyr, *O*-phosphoethanolamine, 4-OP, 2-picolinate, 2-HP, prolinamide, pipecolate, succinate, creatinine, Man, 2-aminoethanol	
	Pancreas	12 h	PCA	(↑): PC, Choline, phosphocholine, Leu, Ile, Val (↓): Lactate, betaine, Gly	[132]
CER	Serum	8 h	Two-tailed Student's *t*-test.	(↑): Arabitol, O-phosphoethanolamine, xylitol (↓): 1,5-AG, 2-DHG, citrate/isocitrate, aconitate, β-Ala, Cysn, Tyr, Glc, Man, Glcn, Fru, Rib, Galn, PA, DHA, CAD	[131]
	Pancreas			(↑): Put, Cit, Ile, Lys, CTH, Phe, Pro, Tyr, Hcy, Eth, His, β-Ala, Gly-Gly, Trp, Gln, Asn, Orn, Ser, Asp, Thr, Lac, aconitate, citrate/isocitrate, ACA, p-CA, urate, allantoin, adenine, glycerol, 2-HP, creatinine, 2-AMA (↓): Ala, Glu, Gly, phosphate, G2P, *O*-phosphoethanolamine, suberate, pyruvate, oxalacetate, 2-dGlc, DOMA	
	Pancreas	1, 3, 5, and 7 h	One way ANOVA, Bonferroni test	(↓): Met, SAM, MTA, CTH, Cys, GSH	[104]
	Pancreas	24 h	Student's *t*-test, M-W U test	(↑): SPA, SFO, S1P, PA, POA, OA, α-LOA, TAG (↓): CE, AA, DHA	[193]
CER + HTG	Serum	12 h	Student's *t*-test, PCA, OPLS-DA	(↑): Val, Leu, Pro, MA, OA (↓): Citrate, malate, Gln	[139]
NaT	Plasma	12 h	PCA, OPLS-DA	(↑): Lactate, Val, Gln, Ala, succinate, 3-HBA, creatine (↓): PC, glycerol, choline, TMAO, Glc, Gly, Glu	[133,134]
	Serum	6, 12, and 24 h	PCA, PLS-DA	(↑): NAD^+, 3-HBA, ACA, lactate, PAG, PEP, Val, His, creatinine, UN, adenine, deoxyadenosine, pyrimidine, TMAO, hippurate, IAA (↓): Glc, NADH, succinate, formate, pteridine, serotonin	[136]
	Serum	12 h	Student's *t*-test, PCA, PLS-DA, OPLS-DA	(↑): Arabitol, Man, Tyr, Arg, Val, Phe, urate, PAM, AA, 3-OPA, DHA, 3-HBA, citrate, succinate (↓): Isocitrate, AGly, Hcy, Gln, NAS	[137]

Abbreviations were listed as follows sorted to the sub-catalogs: Toxins: CER: cerulein, HTG: hypertriglyceridemia, NAT: sodium taurocholate; Methods: ^{1}H MAS NMR: high-resolution proton magic angle spinning nuclear magnetic resonance, HPLC: high-performance liquid chromatography; Metabolites: 1,5-AG: 1,5-anhydro-D-glucitol, AGln: *N*-acetyl-L-glutamate, ALys: N-β-acetyl-L-lysine, 2-AMA: 2-amino-adipate, ATA: anthranilic acid, Ara: arabinose, Arg: arginine, Asn: asparagine, BCys: S-benzyl-L-cysteine, CAD: coniferyl aldehyde, 4-CG: 4-cresol-glucuronide, Cit: citrulline, 4-CS, 4-cresol-sulfate, CSA: cysteine sulfonic acid, CTH: cystathionine, Cys: cysteine, Cysn: cysteamine, 2-dGlc: 2-deoxy-glucose, DHA: docozahekaenoic acid, 2-DHG: 2-dehydro-D-gluconate, DOA: dihydroxyacetone, DOMA: 3,4-dihydroxymandelic acid, Eth: ethionine, F6P: fructose-6-phosphate, Fru: fructose, GAD: glyceraldehyde, Galn: galactosamine, GCA: glycolic acid, Glcn: glucoronate, Glu: glutamic acid, G2P: glycerol-2-phosphate, GSH: reduced glutathione, Hcy: homocysteine, HDA: heptadecanoate, 5-HIAA: 5-hydroxyindoleacetate, 2-HP: 2-hydroxypyridine, HPPA: 3/4-hydroxyphenylpropionic acid, HTau: hypotaurine, IAA: indole-acetic acid, KVA: ketovaline, Lac: lactose, Met: methione, m-Ery: meso-erythritol, MTA: methylthioadenosine, NAD^+: oxidized nicotinamide adenine dinucleotide, NADH: reduced nicotinamide adenine dinucleotide, NAS: *N*-acetyl-serotonin, 2-OG: 2-oxoglutaric acid, 4-OP: trans-4-hydroxy-L-proline, 3-OPA: (*R*)-3-hydroxy-hexadecanoic acid, PAG: phenylacetylglycine, PAM: palmitic amide, p-CA: p-coumaric acid, PEP: phosphoenolpyruvate, p-Glu: pyroglutamic acid, 3-PLA: D-3-phenyllactic acid, POA: palmitoleic acid, Put: putrescine, Rha: rhamnose, SAM: S-adenosylmethionine, Sar: sarcosine, Sor: α-sorbopyranose, S1P: sphingosine-1-phosphate, SFO: sphingosine, TAG: triglyceride, UN: urea nitrogen, Xyl: xylose; other abbreviations as defined in Table 1.

[a] For CER-AP, samples were collected at the time which was calculated from first injection of CER, while for the other models, samples were collected at the time calculated from the last dosing of toxins or operation.

[135–137], indicating that fatty acids are decomposed to ketones and transferred to acetyl CoA in the liver [138]. In addition, serum TCA cycle metabolites and fatty acids were significantly changed in lipoprotein lipase-deficient heterozygous mice with CER injections in support of similar metabolic pathway impacted in HLP patients [139].

Creatinine and urea nitrogen levels are standard indicators for the evaluation of renal function [140]. SAP is often associated with the dysfunction of organs distant form pancreas, like the kidney [141]. Acute kidney injury is one of the main complications of SAP, with an incidence of 14–16% [142]. Increased levels of creatinine and creatine were found in blood or in the pancreas from metabolic profiling in all three AP models [131,133–136], and corroborated the alteration of these metabolites found in human urine [108]. Arginine is a central amino acid in the urea cycle, which serves to continuously eliminate potentially toxic ammonia from the body, and is also required for the synthesis of creatine [143,144]. Serum urea nitrogen levels were significantly elevated 24 h after administration of 4 g/kg arginine, indicating its direct impact on the urea cycle; the ammonia generated by deamination was directed into increased urea synthesis [135]. In urine, the levels of arginine, ornithine, and citrulline from urea cycle, lysine, glutamine, and glutamate from transamination were expected to be elevated during the first 24 h of arginine-induced AP [135], while high levels of creatine and creatinine were presented in urine from arginine-administrated rats at 48 h [135].

Another potentially important feature of the metabolic derangement found in AP models was the gut microbial co-metabolites. Phenylpropanoic acid contains a benzene ring conjugated to a propanoic acid, and is a phenolic acid metabolite formed by the gut microflora [145]. 4-Cresol metabolites and phenylacetylglycine are derived from metabolism of the aromatic amino acids phenylalanine and tyrosine as a part of the putrefactive processes mediated by the colonic microflora [146]. Increased levels of hippurate and phenylacetylglycine have been detected in biofluids from two necrosis AP models [135,136]. The alteration of urinary 3- and 4-hydroxyphenylpropionic acids as well as 4-cresol-glucuronide and 4-cresol-sulfate appeared in different phases in arginine-induced AP [135]. These observations suggest that compromised pancreatic function impacts the activity of the gut microbiota and gives rise to potential biomarkers for diagnosis [135].

5. Altered pathways related to phenotype of AP

AP causes serious digestive system disturbances, including production of digestive juices that digest protein, starch, and fat as well as balancing the acid in the duodenum. This situation is associated with a disruption in the homeostatic metabolism of sugars, amino acids, organic acids and lipids in mammalian cells and gut bacteria. The relevant systemic impact includes oxidative stress substances, pro-inflammatory intermediates, and gut microbiota metabolites which may also contribute to secondary damages to pancreas and distant tissues. Meanwhile, the inflammatory response of AP triggers a hypercatabolic state, resulting in increased energy requirements for protein, lipid and nucleotide synthesis, and catabolism of major nutrient substrates [147]. Although the alteration trends of some metabolites in reports (Tables 3 and 4) were contradictory, the pathway topology analysis based on differential metabolites provides clues for dysregulation of metabolic pathways and biological function.

Based on the reported ~203 metabolites summarized in AP metabolomics, we respectively analyzed the metabolic pathways using *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus* databases from MetaboAnalyst 4.0 (www.metaboanalyst.ca) to indicate the most impacted pathways in AP patients and murine models. The results are presented in Fig. 2, Figs. S-2, and S-3, as well as detailed in Tables S-1, S-2, and S-3. More than 30 metabolic pathways with impact values larger than 0 have been detected in AP clinical and murine studies. The representative metabolic pathways are presented in Fig. 3, and are sorted into three major nutrient pathway types 'carbohydrate metabolism', 'amino acid metabolism', and 'lipid metabolism', which will be discussed further below. Overall, these results gave us a glimpse of the potential metabolic alterations in AP and may shed some light on the pathogenesis of AP at molecular level.

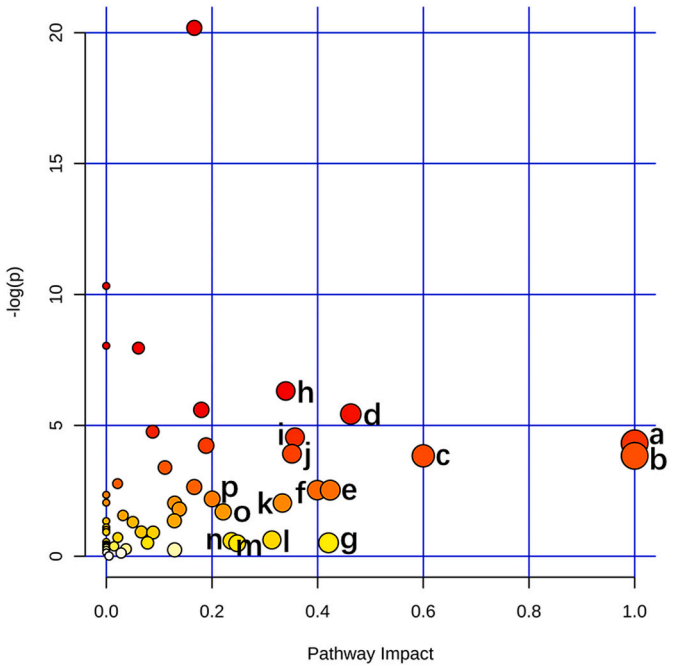

Fig. 2. Summary of the metabolomics pathway analysis based on the data from AP metabolomic studies of human cohorts performed by MetaboAnalyst 4.0. Sixteen important metabolic pathways selected based on impact values ≥0.2 were identified: (a) Phenylalanine, tyrosine and tryptophan biosynthesis, (b) Linoleic acid metabolism, (c) Synthesis and degradation of ketone bodies, (d) Glycine, serine and threonine metabolism, (e) Sphingolipid metabolism, (f) beta-Alanine metabolism, (g) Starch and sucrose metabolism, (h) Alanine, aspartate and glutamate metabolism, (i) Phenylalanine metabolism, (j) Pyruvate metabolism, (k) alpha-Linolenic acid metabolism, (l) Arachidonic acid metabolism, (m) Tryptophan metabolism, (n) Glycerolipid metabolism, (o) Histidine metabolism, and (p) Arginine and proline metabolism.

5.1. Carbohydrate metabolism

Hyperglycemia is common in AP, resulting in insulin resistance and altered pancreatic function [148]. However, reduced levels of plasma/serum glucose were unexpectedly found in most AP rodents [135], and this finding deserves further study. An enhanced anaerobic glycolysis state accompanied by increased lactate might occur in the early stage of AP due to the decreased energy supply under reduced aerobic respiration. The glycolysis pathway could be partially retardant or retrocomplementate glucose via gluconeogenesis with the development of disease. Mizushima et al. reported the increases in gluconeogenesis, combined with lower glucose clearance and oxidation, leading to glucose intolerance in 40% to 90% of AP patients [149]. Strikingly altered lactate, pyruvate, phosphoenolpyruvate, and oxalacetate, as well as glucogenic amino acids were closely associated with glycolysis or gluconeogenesis. Lactate is the end product of glycolysis under anaerobic condition and considered an important indicator of ischemia and hypoxia in tissue [150]. Recently, Xia et al. reported that elevated arterial lactate levels were independently associated with pancreatic infection in 503 patients with MSAP [151] and poor outcomes in 329 patients with SAP [152]. Further, external validation of this finding would likely establish elevated lactate as a risk factor.

AP in the acute phase seems to depend on energy metabolism. Pyruvate is one of the end products of glycolysis, which is then transported to the mitochondria for participation in the TCA cycle [153]. Pyruvate

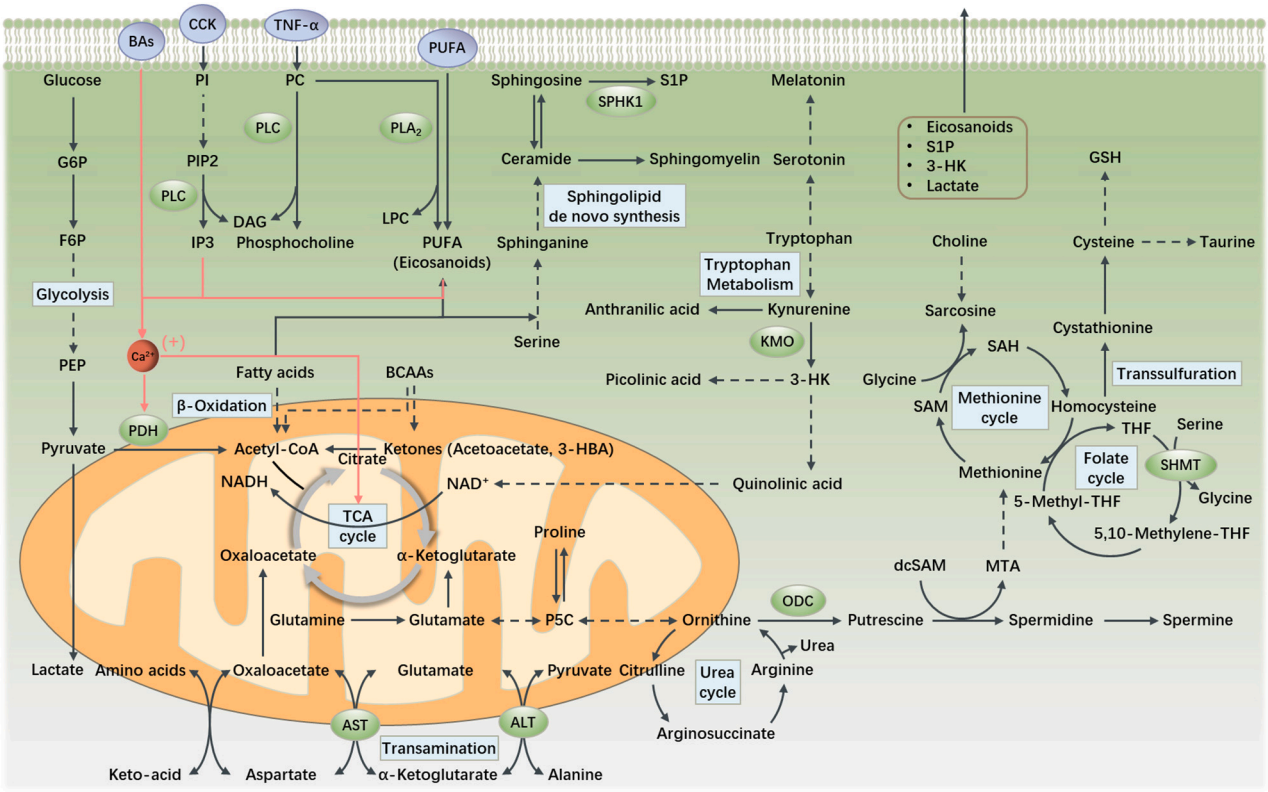

Fig. 3. Overview of major metabolic pathways involved in AP. Cells utilize absorbed glucose, fatty acids, branched-chain amino acids (BCAAs), glutamine, and tryptophan, and divert them to the tricarboxylic acid (TCA) cycle. Blue rectangles elucidate vital altered pathways. Green ovals signify enzymes. Purple ovals represent toxins taken up by acinar cells, which lead to mitochondrial injury through activation of calcium and generation of excess reactive oxygen species. The increased generation of some active metabolites are not limited to eicosanoids, S1P, 3-hydroxy-kynurenine (3-HK) and lactate, and such metabolites are released outside the cell or into circulation. The solid arrows represent one-step reactions between the two metabolites, while the dashed arrows represent a multistep reaction. The red solid line indicates an enhanced process. 3-HBA, 3-hydroxybutyric acid; 3-HK, 3-hydroxykynurenine; acetyl-CoA, acetyl coenzyme A; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAs, bile acids; BCAAs, branched-chain amino acids; CCK, cholecystokinin; DAG, diacylglycerol; dcSAM, decarboxylated SAM; F6P, fructose 6-phosphate; G6P, glucose 6-phosphate; IP3, inositol 1,4,5-trisphosphate; KMO, kynurenine 3-monooxygenase; LPC, lysophosphatidylcholine; MTA, methylthioadenosine; NAD, nicotinamide adenine dinucleotide; ODC, ornithine decarboxylase; P5C, pyrroline-5-carboxylate; PC, phosphatidylcholine; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PI, phosphatidylinositol; PIP2, phosphatidylinositol (4,5)-bisphosphate; PLA2, phospholipase A2; PLC, phospholipase C; PUFA, polyunsaturated fatty acid; S1P, sphingosine-1-phosphate; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; SPHK1, sphingosine kinase 1; TCA cycle, tricarboxylic acid cycle; THF, tetrahydrofolate; TNF-α, tumor necrosis factor-alpha.

has been shown to improve organ function in animal models of oxidant-mediated cellular injury, and its analogue ethyl pyruvate is regarded as a metabolic substrate to fuel the TCA cycle, scavenge reactive oxygen species (ROS) and provide additional ATP [154]. Calcium is used as a regulator in the TCA cycle. Physiological calcium mediated signaling activates the pyruvate dehydrogenase complex, isocitrate dehydrogenase (IDH) and α-ketoglutarate dehydrogenase, and increases ATP production [155]. Conversely, toxic cellular calcium overload or increased ROS production can cause mitochondrial damage and a concomitant drop in ATP levels [156]. An accumulation of citrate and reduced levels of other TCA cycle intermediates in biofluids and the pancreas have been indicated in some AP metabolomic studies [105,131], which confirm the increased energy demand and reduced ATP levels.

5.2. Amino acid metabolism

Disturbed amino acid balances may have negative effects on pancreatitis and other severe disease conditions [157–159]. BCAAs can be glucogenic (valine), ketogenic (leucine and isoleucine) or both (isoleucine), since their end products, succinyl-CoA and/or acetyl-CoA can enter the TCA cycle for energy generation and gluconeogenesis or act as precursors for lipogenesis and ketone body production through acetyl-CoA and acetoacetate [160]. Therefore, apart from an increase in ketones in AP patients, elevated BCAAs have been observed in AP patients or murine models (see Tables 3 and 4). BCAA deficiency in the diet makes it difficult for animals to maintain pancreatic enzyme activities and BCAA-deficient animals were less able to recover pancreatic enzyme activity after a 23-h fast [161].

Glutamate, aspartate, and alanine are important amino acids for transamination. They are catalyzed by two common transaminases alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [162]. Elevated ALT in BAP has been described in previous clinical studies [163,164], which led to a high degree of deamination for most amino acids. Glutamine is a precursor for a number of biosynthetic pathways required for growth and cell division [165]. It is hydrolyzed to glutamate and ammonia, which ultimately serve as substrates for gluconeogenesis and urea synthesis. Reduced glutamine levels were detected in HLP patients [122], while higher levels of glutamine and lower glutamate were found in pancreatic tissue of two murine models [131]. It was speculated that there was a large uptake of glutamate in pancreas during AP, resulting in large amounts of available glutamine.

Glycine, mainly synthesized from threonine, choline, and serine, is required for multiple metabolic pathways, such as glutathione synthesis and regulation of one-carbon metabolism. Several enzymes, including sarcosine dehydrogenase and dimethylglycine dehydrogenase, are highly present in the pancreas and crucial for glycine synthesis [166]. A

reduced level of choline and glycine in the pancreas or biofluids is evident in the AP studies included in this review (Tables 3 and 4). Notably, prophylactic glycine administration has been shown to attenuate pancreatic damage and inflammation in experimental AP [167].

The oxidation of sulfur in the sulfur containing amino acids, methionine and cysteine, occurs by both direct oxidation and desulfhydration-oxidation pathways in mammals [168]. AP caused dysregulated trans-sulfuration mediated by nitration of cystathionine-β-synthase (CBS), leading to depletion of methionine, cystathionine and cysteine [104] (Table 4). Taurine, derived from cysteine, has shown beneficial effects in CER-AP and related lung injuries by preventing the production of oxygen free radicals [169]. In addition, glutathione is synthesized from glutamate, glycine, and cysteine, and shown to neutralize ROS in AP [170].

L-tryptophan administration prevented pancreatic damage through activation of central receptors for locally produced melatonin, which improved the antioxidant status and reduced inflammation of the tissue [171]. Accordingly, the retardation of serotonin formation from tryptophan was revealed in both AP clinical and murine model studies [106,136]. However, in fact, only a minor fraction (<3%) of the tryptophan is utilized for serotonin and melatonin biosynthesis [172]. The majority (over 95%) of tryptophan enters the kynurenic pathway and is ultimately used for oxidized nicotinamide adenine dinucleotide (NAD^+) synthesis through the key intermediates 3-HK and aminocarboxymuconic semialdehyde [173,174]. Early depletion of tryptophan and contemporaneous 3-HK were found in SAP patients by the same group [124]. Treatment with specific inhibitors of kynurenine 3-monooxygenase (KMO) resulted in therapeutic protection against AP-MODS in a NaT-induced rat model via deprivation of 3-HK [175].

Large doses of basic amino acids including L-arginine, L-ornithine, L-lysine and L-histidine are widely used in inducing severe pancreatic inflammation, however the mechanism is not well understood [176]. Possible explanations might be related to early endoplasmic reticulum (ER) stress [177], mitochondrial damage [178] and/or polyamine homeostasis [179]. However, only arginine-induced AP models have been used in AP metabolomics research [131,132,135]. There are two key enzymes involved in the metabolism of arginine: nitric oxide synthase and arginase. Arginase is a key enzyme in the urea cycle, which hydrolyzes L-arginine to L-ornithine and urea [180]. Of note, L-ornithine is a precursor of polyamines that are aliphatic polycations involved in numerous cellular processes [181]. Extensive excretion of arginine, ornithine and urea (indicative of enhanced urea cycle activity) were observed during the first 24 h in urine [135] of rats administrated with arginine. The altered concentration of these basic amino acids was not obvious in AP patients; however, these results may have been somewhat limited by the sample preparation and the analytical platform used (Table 2).

5.3. Lipid metabolism

Several metabolomic studies identified disturbed lipid class changes, including arachidonic acids (AAs), glycerophospholipids, glycerolipids, sphingolipids as well as bile acids (BAs) during AP (see Tables 3 and 4). HTG might play a key role in aggravating AP [182]; however, a direct relationship between lipid metabolism and AP has not yet been established. Unsaturated fatty acid (UFA) elevations have been found in AP patients [183]. The hydrolysis by lipases causes UFA release from the damaged adipocytes adjacent to acinar cells, leading to an inflammatory response and necrosis of the pancreas [184]. Toxicity depends on fatty acid type, as unsaturated linoleate, oleate and linolenate were found to be toxic to acinar cells [184]. Levels of saturated C-16 and C-18 fatty acids were elevated in two etiology related studies [107,185], probably due to the hypercatabolic state or excessive alcohol metabolism to acetyl-CoA. In contrast, reduced UFAs and saturated fatty acids were found to be important in the development of AP in a study by Huang et al. [122], but the authors did not give a percentage contribution of all free fatty acids.

Phosphatidylinositol (PI) and PC may act as sources of intracellular signals in response to extracellular signals which interact with receptors on the outer layer of plasma membrane [186]. Both cholecystokinin (CCK) hyperstimulation and TNF-α binding to the plasma membrane receptor led to activation of phospholipase C [187]. The resultant inositol 1,4,5-triphosphate (IP_3) and diacylglycerol from PI and/or PC are involved in calcium release from the ER and activation of enzyme protein kinase C that induce NF-κB activation [188].

Eicosanoids are one of the most important precursors of lipid metabolism [186]. Intracellular (cytosolic) phospholipase A_2 (PLA_2) participates in cellular eicosanoid metabolism, liberating n-3 series eicosapentaenoic acid and n-6 series AAs from the sn-2 position of phospholipids [189]. AAs released from pancreas during AP were reported in early studies [190]. Major findings by Sztefko et al. concluded that the level of AA in the free fatty acid fraction was related to the severity of AP, and that elevated AA was correlated with increased PLA_2 at the early stage of the disease [183].

Ceramide downstream metabolites including sphingosine (SFO) and sphingosine-1-phosphate (S1P) from sphingomyelin pathway may be useful for diagnosis in the early stages of AP. Li et al. [191] reported a positive correlation between sphingosine-1-kinase (catalyzing SFO to S1P) expression and the disease severity in 22 patients via a radiometric assay and PCR determination. In Konończuk et al. study, elevated SFO and S1P were found in patients with MAP and MSAP [192], and consistent results (+3.8 fold sphinganine (SFA), +4 fold SFO, and +3 fold S1P) were observed in CER-AP model too [193] (Table 4).

Various primary and secondary BAs have been used to induce AP [194]. The most abundant BAs in mammals include the primary BAs cholic acid (CA) and chenodeoxycholic acid (CDCA), and secondary BAs deoxycholic acid (DCA) and lithocholic acid (LCA). In humans, BAs are primarily synthesized in the liver, conjugated with glycine or taurine before secretion into bile, deconjugated in the intestine, reabsorbed into enterocytes at the terminal ileum and lastly secreted into the portal circulation system [195]. Principally, hydrophobic bile salts exert higher cytotoxicity than hydrophilic bile salts [196]. Taurolithocholic acid 3-sulfate (TLCS) was demonstrated to cause large abnormal calcium signals in acinar cells via a mechanism that involves depletion of intracellular calcium stores and activation of calcium entry as well as depolarization of mitochondria [197]. Taurocholic acid elicit dramatic calcium responses and necrosis in pancreatic stellate cells [198]. Marked elevation of fecal DCA was reported in a recurrent AP case, which was resolved by rebalancing the abnormal microbiome ecology [199].

6. Summary and future perspectives

6.1. Merits and limitations in AP metabolomics studies

Significant advances in analytical methods now enable the investigation of AP-related pathological processes and metabolic pathways. As a result of genomic, transcriptomic, and proteomic variations caused by AP, the metabolome provides a set of altered metabolites in multiple biofluids and tissues, which could be used for further biomarker validation and development, biological function studies, and therapeutic drug development. Currently, no successful drugs have been reported for preventing AP. Thus, an alternative and more effective strategy might be the development of specific inhibitors or activators designed to directly target the metabolic processes. Nevertheless, metabolomics in AP research is still in its infancy, and the identification of unknown compounds and accurate detection of an expanded set of metabolites remains a huge challenge. Another important issue inherent to the metabolomics approach is the heterogeneous nature of the individuals, which can affect metabolite levels. The differing elevation and reduction

of metabolites might also be attributed in part to the inconsistency of experimental designs, analytical platforms and statistic methods used in the research to date. The proposed workflow of Fig. 1 is an optimized strategy for resolving such inconsistencies. Additionally, limitations for AP clinical metabolomics include the relatively small sample size and non-inclusion of controls that can masquerade as AP, such as cholelithiasis.

6.2. Future perspectives

We propose that future clinical metabolomics in AP should aim at recruiting larger cohorts from multiple centers with sufficient statistic power to identify and validate potential biomarkers. A direct relevance analysis of the metabolome between human AP and animal models could be performed through MS imaging. Also, a joint analysis of tissue and biofluid samples, which were collected over multiple time points from AP rodent models, using complementary analytical platforms should be employed in parallel to reveal a holistic view of fingerprints for more in-depth biological investigations. It is noteworthy that metabolite changes caused by microbiome dysbiosis and bacterial translocation are strongly associated with progression of AP. New and unanticipated metabolites from host-microbe interactions may lead to new insights in AP. Additionally, the ultimate challenge is to move beyond simply identifying biomarkers, and to start establishing the direct functional role of metabolites and their involvement in phenotypic outcomes, such that better treatments can be proposed and evaluated. The first steps along this path, require follow-up experiments including the use of pathway inhibitors, multi-omics, and stable isotope labeling as well as flux analysis. These are valid strategies to define novel disease mechanisms and targets. Unambiguous understanding of the active metabolites in AP is certain to aid in the translation of metabolomic findings to clinical precision medicine.

CRediT authorship contribution statement

Dan Du and Qing Xia had the idea for the article, Dan Du and Yang Peng drafted the article, performed the literature search and data analysis, as well as finished the tables, figures and supplementary data, Jiwon Hong and Daniel Raftery critically revised the work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2021.166123.

References

[1] A.Y. Xiao, M.L.Y. Tan, L.M. Wu, V.M. Asrani, J.A. Windsor, D. Yadav, M.S. Petrov, Global incidence and mortality of pancreatic diseases: a systematic review, meta-analysis, and meta-regression of population-based cohort studies, Lancet Gastroenterol Hepatol 1 (2016) 45–55, https://doi.org/10.1016/S2468-1253(16)30004-8.

[2] A.F. Peery, S.D. Crockett, C.C. Murphy, J.L. Lund, E.S. Dellon, J.L. Williams, E. T. Jensen, N.J. Shaheen, A.S. Barritt, S.R. Lieber, B. Kochar, E.L. Barnes, Y.C. Fan, V. Pate, J. Galanko, T.H. Baron, R.S. Sandler, Burden and cost of gastrointestinal, liver, and pancreatic diseases in the United States: update 2018, Gastroenterol 156 (2019) 254–272 (e211), https://doi.org/10.1053/j.gastro.2018.08.063.

[3] P. Valdivielso, A. Ramírez-Bueno, N. Ewald, Current knowledge of hypertriglyceridemic pancreatitis, Eur J Intern Med 25 (2014) 689–694, https://doi.org/10.1016/j.ejim.2014.08.008.

[4] A. Adiamah, E. Psaltis, M. Crook, D.N. Lobo, A systematic review of the epidemiology, pathophysiology and current management of hyperlipidaemic pancreatitis, Clin. Nutr. 37 (2018) 1810–1822, https://doi.org/10.1016/j.clnu.2017.09.028.

[5] S.D. Crockett, S. Wani, B.T. Gardner, Y. Falck-Ytter, A.N. Barkun, American Gastroenterological Association Institute Guideline on Initial Management of Acute Pancreatitis, Gastroenterol 154 (4) (2018) 1096–1101, https://doi.org/10.1053/j.gastro.2018.01.032.

[6] R.K. Padhan, S. Jain, S. Agarwal, S. Harikrishnan, P. Vadiraja, S. Behera, S. K. Jain, R. Dhingra, N.R. Dash, P. Sahni, P.K. Garg, Primary and secondary organ failures cause mortality differentially in acute pancreatitis and should be distinguished, Pancreas 47 (2018) 302–307, https://doi.org/10.1097/MPA.0000000000000998.

[7] H. Sternby, F. Bolado, H.J. Canaval-Zuleta, C. Marra-López, A.I. Hernando-Alonso, A. Del-Val-Antoñana, G. García-Rayado, R. Rivera-Irigoin, F.J. Grau-García, L. Oms, J. Millastre-Bocos, I. Pascual-Moreno, D. Martínez-Ares, J. A. Rodríguez-Oballe, A. López-Serrano, M.L. Ruiz-Rebollo, A. Viejo-Almanzor, B. González-de-la-Higuera, A. Orive-Calzada, I. Gómez-Anta, J. Pamies-Guilabert, F. Fernández-Gutiérrez-Del-Álamo, I. Iranzo-González-Cruz, M.E. Pérez-Muñante, M.D. Esteba, A. Pardillos-Tomé, P. Zapater, E. de Madaria, Determinants of severity in acute pancreatitis: a nation-wide multicenter prospective cohort study, Ann. Surg. 270 (2019) 348–355, https://doi.org/10.1097/SLA.0000000000002766.

[8] N.J. Schepers, O.J. Bakker, M.G. Besselink, U.A. Ali, T.L. Bollen, H.G. Gooszen, H. C.v. Santvoort, M.J. Bruno, Impact of characteristics of organ failure and infected necrosis on mortality in necrotising pancreatitis, Gut 68 (2019) 1044–1051, https://doi.org/10.1136/gutjnl-2017-314657.

[9] N. Shi, T. Liu, D. Iglesia-Garcia, L. Deng, T. Jin, L. Lan, P. Zhu, W. Hu, Z. Zhou, V. Singh, J.E. Dominguez-Munoz, J. Windsor, W. Huang, Q. Xia, R. Sutton, Duration of organ failure impacts mortality in acute pancreatitis, Gut 69 (2020) 604–605, https://doi.org/10.1136/gutjnl-2019-318241.

[10] P.A. Banks, T.L. Bollen, C. Dervenis, H.G. Gooszen, C.D. Johnson, M.G. Sarr, G. G. Tsiotos, S.S. Vege, Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus, Gut 62 (2013) 102–111, https://doi.org/10.1136/gutjnl-2012-302779.

[11] P.K. Garg, V.P. Singh, Organ failure due to systemic injury in acute pancreatitis, Gastroenterol 156 (2019) 2008–2023, https://doi.org/10.1053/j.gastro.2018.12.041.

[12] M. Bhatia, F.L. Wong, Y. Cao, H.Y. Lau, J.L. Huang, P. Puneet, L. Chevali, Pathophysiology of acute pancreatitis, Pancreatol 5 (2–3) (2005), https://doi.org/10.1159/000085265.

[13] S. Tenner, J. Baillie, J. DeWitt, S.S. Vege, American college of gastroenterology guideline: management of acute pancreatitis, Am. J. Gastroenterol. 108 (2013) 1400–1415 (1416), https://doi.org/10.1038/ajg.2013.218.

[14] M. Yokoe, T. Takada, T. Mayumi, M. Yoshida, S. Isaji, K. Wada, T. Itoi, N. Sata, T. Gabata, H. Igarashi, K. Kataoka, M. Hirota, M. Kadoya, N. Kitamura, Y. Kimura, S. Kiriyama, K. Shirai, T. Hattori, K. Takeda, Y. Takeyama, M. Hirota, M. Sekimoto, S. Shikata, S. Arata, K. Hirata, Japanese guidelines for the management of acute pancreatitis: Japanese guidelines 2015, J Hepatobiliary Pancreat Sci 22 (2015) 405–432, https://doi.org/10.1002/jhbp.259.

[15] A. Leppaniemi, M. Tolonen, A. Tarasconi, H. Segovia-Lohse, E. Gamberini, A. W. Kirkpatrick, C.G. Ball, N. Parry, M. Sartelli, D. Wolbrink, H. van Goor, G. Baiocchi, L. Ansaloni, W. Biffl, F. Coccolini, S. Di Saverio, Y. Kluger, E. Moore, F. Catena, 2019 WSES guidelines for the management of severe acute pancreatitis, World J Emerg Surg 14 (2019) 27, https://doi.org/10.1186/s13017-019-0247-0.

[16] G. Rompianesi, A. Hann, O. Komolafe, S.P. Pereira, B.R. Davidson, K. S. Gurusamy, Serum amylase and lipase and urinary trypsinogen and amylase for diagnosis of acute pancreatitis, Cochrane Database Syst. Rev. 4 (2017), CD012010, https://doi.org/10.1002/14651858.CD012010.pub2.

[17] S. Tenner, H. Dubner, W. Steinberg, Predicting gallstone pancreatitis with laboratory parameters: a meta-analysis, Am. J. Gastroenterol. 89 (1994) 1863–1866.

[18] B.J. Ammori, B. Boreham, P. Lewis, S.A. Roberts, The biochemical detection of biliary etiology of acute pancreatitis on admission: a revisit in the modern era of biliary imaging, Pancreas 26 (2003), https://doi.org/10.1097/00006676-200303000-00023 (e32-35).

[19] P. Lévy, A. Boruchowicz, P. Hastier, A. Pariente, T. Thévenot, J.L. Frossard, L. Buscail, F. Mauvais, J.C. Duchmann, A. Courrier, P. Bulois, J.L. Gineston, M. Barthet, H. Licht, D. O'Toole, P. Ruszniewski, Diagnostic criteria in predicting a biliary origin of acute pancreatitis in the era of endoscopic ultrasound:

multicentre prospective evaluation of 213 patients, Pancreatol 5 (2005) 450–456, https://doi.org/10.1159/000086547.
[20] Z. Moolla, F. Anderson, S.R. Thomson, Use of amylase and alanine transaminase to predict acute gallstone pancreatitis in a population with high HIV prevalence, World J. Surg. 37 (2013) 156–161, https://doi.org/10.1007/s00268-012-1801-z.
[21] J.R. Aparicio, J.A. Viedma, L. Aparisi, S. Navarro, J. Martínez, M. Pérez-Mateo, Usefulness of carbohydrate-deficient transferrin and trypsin activity in the diagnosis of acute alcoholic pancreatitis, Am. J. Gastroenterol. 96 (2001) 1777–1781, https://doi.org/10.1111/j.1572-0241.2001.03870.x.
[22] C.J. Yang, J. Chen, A.R.J. Phillips, J.A. Windsor, M.S. Petrov, Predictors of severe and critical acute pancreatitis: a systematic review, Dig. Liver Dis. 46 (2014) 446–451, https://doi.org/10.1016/j.dld.2014.01.158.
[23] M. Otsuki, K. Takeda, S. Matsuno, Y. Kihara, M. Koizumi, M. Hirota, T. Ito, K. Kataoka, M. Kitagawa, K. Inui, Y. Takeyama, Criteria for the diagnosis and severity stratification of acute pancreatitis, World J. Gastroenterol. 19 (2013) 5798–5805, https://doi.org/10.3748/wjg.v19.i35.5798.
[24] A. Mikó, É. Vigh, P. Mátrai, A. Soós, A. Garami, M. Balaskó, L. Czakó, B. Mosdósi, P. Sarlós, B. Erőss, J. Tenk, I. Rostás, P. Hegyi, Computed tomography severity index vs. other indices in the prediction of severity and mortality in acute pancreatitis: a predictive accuracy meta-analysis, Front. Physiol. 10 (2019) 1002, https://doi.org/10.3389/fphys.2019.01002.)eCollection 2019).
[25] E. Koutroumpakis, B.U. Wu, O.J. Bakker, A. Dudekula, V.K. Singh, M.G. Besselink, D. Yadav, R. Mounzer, H.C.v. Santvoort, D.C. Whitcomb, H.G. Gooszen, P. A. Banks, G.I. Papachristou, Admission hematocrit and rise in blood urea nitrogen at 24 h outperform other laboratory markers in predicting persistent organ failure and pancreatic necrosis in acute pancreatitis: a post hoc analysis of three large prospective databases, Am. J. Gastroenterol. 110 (2015) 1707–1716, https://doi.org/10.1038/ajg.2015.370.
[26] R. Mofidi, S.A. Suttie, P.V. Patil, S. Ogston, R.W. Parks, The value of procalcitonin at predicting the severity of acute pancreatitis and development of infected pancreatic necrosis: systematic review, Surgery 146 (2009) 72–81, https://doi.org/10.1016/j.surg.2009.02.013.
[27] F.F.v.d. Berg, A.C.d. Bruijn, H.C.v. Santvoort, Y. Issa, M.A. Boermeester, Early laboratory biomarkers for severity in acute pancreatitis; a systematic review and meta-analysis, Pancreatol 20 (2020) 1302–1311, https://doi.org/10.1016/j.pan.2020.09.007.
[28] S.H. Wang, Y.C. Chou, W.C. Shangkuan, K.Y. Wei, Y.H. Pan, H.C. Lin, Relationship between plasma triglyceride level and severity of hypertriglyceridemic pancreatitis, PLoS One 11 (2016), e0163984, https://doi.org/10.1371/journal.pone.0163984.
[29] J.M. Park, S.P. Shin, S.K. Cho, J.H. Lee, J.W. Kim, C.D. Kang, J.H. Huh, K.J. Lee, Triglyceride and glucose (TyG) index is an effective biomarker to identify severe acute pancreatitis, Pancreatol 20 (2020) 1587–1591, https://doi.org/10.1016/j.pan.2020.09.018.
[30] S.K. Cho, J.H. Huh, J.S. Yoo, J.W. Kim, K.J. Lee, HOMA-estimated insulin resistance as an independent prognostic factor in patients with acute pancreatitis, Sci. Rep. 9 (2019), 14894, https://doi.org/10.1038/s41598-019-51466-5.
[31] W. Kong, Y. He, H. Bao, W. Zhang, X. Wang, Diagnostic value of neutrophil-lymphocyte ratio for predicting the severity of acute pancreatitis: a meta-analysis, Dis. Markers 2020 (2020), 9731854, https://doi.org/10.1155/2020/9731854 (eCollection 2020).
[32] T. Peng, X. Peng, M. Huang, J. Cui, Y. Zhang, H. Wu, C. Wang, Serum calcium as an indicator of persistent organ failure in acute pancreatitis, Am. J. Emerg. Med. 35 (2017) 978–982, https://doi.org/10.1016/j.ajem.2017.02.006.
[33] L. Wen, S. Voronina, M.A. Javed, M. Awais, P. Szatmary, D. Latawiec, M. Chvanov, D. Collier, W. Huang, J. Barrett, M. Begg, K. Stauderman, J. Roos, S. Grigoryev, S. Ramos, E. Rogers, J. Whitten, G. Velicelebi, M. Dunn, A. V. Tepikin, D.N. Criddle, R. Sutton, Inhibitors of ORAI1 prevent cytosolic calcium-associated injury of human pancreatic acinar cells and acute pancreatitis in 3 mouse models, Gastroenterol 149 (2015) 481–492 (e487), https://doi.org/10.1053/j.gastro.2015.04.015.
[34] I. CalciMedica, CM4620 Injectable Emulsion Versus Supportive Care in Patients With Acute Pancreatitis and SIRS, US National Library of Medicine, 2018–2019 (ClinicalTrials.gov Identifier: NCT03401190).
[35] M.A. Javed, L. Wen, M. Awais, D. Latawiec, W. Huang, M. Chvanov, S. Schaller, T. Bordet, M. Michaud, R. Pruss, A. Tepikin, D. Criddle, R. Sutton, TRO40303 ameliorates alcohol-induced pancreatitis through reduction of fatty acid ethyl ester-induced mitochondrial injury and necrotic cell death, Pancreas 47 (2018) 18–24, https://doi.org/10.1097/MPA.0000000000000953.
[36] P. Silva-Vaz, A.M. Abrantes, M. Castelo-Branco, A. Gouveia, M.F. Botelho, J. G. Tralhao, Multifactorial scores and biomarkers of prognosis of acute pancreatitis: applications to research and practice, Int. J. Mol. Sci. 21 (2020) 338, https://doi.org/10.3390/ijms21010338.
[37] S. Meher, T.S. Mishra, P.K. Sasmal, S. Rath, R. Sharma, B. Rout, M.K. Sahu, Role of biomarkers in diagnosis and prognostic evaluation of acute pancreatitis, J Biomark 2015 (2015), 519534, https://doi.org/10.1155/2015/519534.
[38] S.G. Barreto, A. Habtezion, A. Gukovskaya, A. Lugea, C. Jeon, D. Yadav, P. Hegyi, V. Venglovecz, R. Sutton, S.J. Pandol, Critical thresholds: key to unlocking the door to the prevention and specific treatments for acute pancreatitis, Gut 70 (2021) 194–203, https://doi.org/10.1136/gutjnl-2020-322163.
[39] N.P. Long, T.D. Nghi, Y.P. Kang, N.H. Anh, H.M. Kim, S.K. Park, S.W. Kwon, Toward a standardized strategy of clinical metabolomics for the advancement of precision medicine, Metabolites 10 (2020) 51, https://doi.org/10.3390/metabo10020051.
[40] R.W. McGarrah, S.B. Crown, G.F. Zhang, S.H. Shah, C.B. Newgard, Cardiovascular metabolomics, Circ. Res. 122 (2018) 1238–1258, https://doi.org/10.1161/CIRCRESAHA.117.311002.
[41] C. Guijas, J.R. Montenegro Burke, B. Warth, M.E. Spilker, G. Siuzdak, Metabolomics activity screening for identifying metabolites that modulate phenotype, Nat. Biotechnol. 36 (2018) 316–320, https://doi.org/10.1038/nbt.4101.
[42] G.J. Patti, O. Yanes, G. Siuzdak, Innovation: metabolomics: the apogee of the omics trilogy, Nat Rev Mol Cell Biol 13 (2012) 263–269, https://doi.org/10.1038/nrm3314.
[43] S.M. Fendt, P. Verstreken, Neurons eat glutamate to stay alive, J. Cell Biol. 216 (2017) 863–865, https://doi.org/10.1083/jcb.201702003.
[44] C. Choudhary, B.T. Weinert, Y. Nishida, E. Verdin, M. Mann, The growing landscape of lysine acetylation links metabolism and cell signalling, Nat Rev Mol Cell Biol 15 (2014) 536–550, https://doi.org/10.1038/nrm3841.
[45] D.A. Petkovich, D.I. Podolskiy, A.V. Lobanov, S.G. Lee, R.A. Miller, V. N. Gladyshev, Using DNA methylation profiling to evaluate biological age and longevity interventions, Cell Metab. 25 (2017) 954–960 (e956), https://doi.org/10.1016/j.cmet.2017.03.016.
[46] I. Toma, J.J. Kang, A. Sipos, S. Vargas, E. Bansal, F. Hanner, E. Meer, J. Peti-Peterdi, Succinate receptor GPR91 provides a direct link between high glucose levels and renin release in murine and rabbit kidney, J. Clin. Invest. 118 (2008) 2526–2534, https://doi.org/10.1172/JCI33293.
[47] L. Schiffer, L. Barnard, E.S. Baranowski, L.C. Gilligan, A.E. Taylor, W. Arlt, C.H. L. Shackleton, K.H. Storbeck, Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: a comprehensive review, J. Steroid Biochem. Mol. Biol. 194 (2019), 105439, https://doi.org/10.1016/j.jsbmb.2019.105439.
[48] R.A. Koeth, Z. Wang, B.S. Levison, J.A. Buffa, E. Org, B.T. Sheehy, E.B. Britt, X. Fu, Y. Wu, L. Li, J.D. Smith, J.A. DiDonato, J. Chen, H. Li, G.D. Wu, J.D. Lewis, M. Warrier, J.M. Brown, R.M. Krauss, W.H. Tang, F.D. Bushman, A.J. Lusis, S. L. Hazen, Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis, Nat. Med. 19 (2013) 576–585, https://doi.org/10.1038/nm.3145.
[49] A. Terunuma, N. Putluri, P. Mishra, E.A. Mathe, T.H. Dorsey, M. Yi, T.A. Wallace, H.J. Issaq, M. Zhou, J.K. Killian, H.S. Stevenson, E.D. Karoly, K. Chan, S. Samanta, D. Prieto, T.Y. Hsu, S.J. Kurley, V. Putluri, R. Sonavane, D.C. Edelman, J. Wulff, A.M. Starks, Y. Yang, R.A. Kittles, H.G. Yfantis, D.H. Lee, O.B. Ioffe, R. Schiff, R. M. Stephens, P.S. Meltzer, T.D. Veenstra, T.F. Westbrook, A. Sreekumar, S. Ambs, MYC-driven accumulation of 2-hydroxyglutarate is associated with breast cancer prognosis, J. Clin. Invest. 124 (2014) 398–412, https://doi.org/10.1172/JCI71180.
[50] A.P. Khan, T.M. Rajendiran, B. Ateeq, I.A. Asangani, J.N. Athanikar, A.K. Yocum, R. Mehra, J. Siddiqui, G. Palapattu, J.T. Wei, G. Michailidis, A. Sreekumar, A. M. Chinnaiyan, The role of sarcosine metabolism in prostate cancer progression, Neoplasia 15 (2013) 491–501, https://doi.org/10.1593/neo.13314.
[51] Y. Karusheva, T. Koessler, K. Strassburger, D. Markgraf, L. Mastrototaro, T. Jelenik, M.C. Simon, D. Pesta, O.P. Zaharia, K. Bodis, F. Barenz, D. Schmoll, M. Wolkersdorfer, A. Tura, G. Pacini, V. Burkart, K. Mussig, J. Szendroedi, M. Roden, Short-term dietary reduction of branched-chain amino acids reduces meal-induced insulin secretion and modifies microbiome composition in type 2 diabetes: a randomized controlled crossover trial, Am. J. Clin. Nutr. 110 (2019) 1098–1107, https://doi.org/10.1093/ajcn/nqz191.
[52] C.B. Newgard, Metabolomics and metabolic diseases: where do we stand? Cell Metab. 25 (2017) 43–56, https://doi.org/10.1016/j.cmet.2016.09.018.
[53] M. Eckerle, L. Ambroggio, M.A. Puskarich, B. Winston, A.E. Jones, T. J. Standiford, K.A. Stringer, Metabolomics as a driver in advancing precision medicine in sepsis, Pharmacotherapy 37 (2017) 1023–1032, https://doi.org/10.1002/phar.1974.
[54] W. Gu, Z. Tong, Clinical application of metabolomics in pancreatic diseases: a mini-review, Lab. Med. 51 (2020) 116–121, https://doi.org/10.1093/labmed/lmz046.
[55] W. Lv, X. Shi, S. Wang, G. Xu, Multidimensional liquid chromatography-mass spectrometry for metabolomic and lipidomic analyses, TrAC 120 (2019), 115302, https://doi.org/10.1016/j.trac.2018.11.001.
[56] P. Yin, R. Lehmann, G. Xu, Effects of pre-analytical processes on blood samples used in metabolomics studies, Anal. Bioanal. Chem. 407 (2015) 4879–4892, https://doi.org/10.1007/s00216-015-8565-x.
[57] X. Liu, L. Zhou, X. Shi, G. Xu, New advances in analytical methods for mass spectrometry-based large-scale metabolomics study, TrAC 121 (2019), 115665, https://doi.org/10.1016/j.trac.2019.115665.
[58] C.H. Johnson, J. Ivanisevic, G. Siuzdak, Metabolomics: beyond biomarkers and towards mechanisms, Nat Rev Mol Cell Biol 17 (2016) 451–459, https://doi.org/10.1038/nrm.2016.25.
[59] L.D. Roberts, A.L. Souza, R.E. Gerszten, C.B. Clish, Targeted Metabolomics, Curr Protoc Mol Biol Chapter 30, 2012, https://doi.org/10.1002/0471142727.mb3002s98 (Unit 30.32.31-24).
[60] A.C.S. Rutledge, S.G. Codreanu, S.D. Sherrod, J.A. McLean, Untargeted metabolomics strategies-challenges and emerging directions, J. Am. Soc. Mass Spectrom. 27 (2016) 1897–1905, https://doi.org/10.1007/s13361-016-1469-y.
[61] N. Vinayavekhin, A. Saghatelian, Untargeted Metabolomics, Curr Protoc Mol Biol Chapter 30, 2010, https://doi.org/10.1002/0471142727.mb3001s90 (Unit 30.31.31-24).
[62] T. Cajka, O. Fiehn, Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics, Anal. Chem. 88 (2016) 524–545, https://doi.org/10.1021/acs.analchem.5b04491.

[63] S. Chen, H. Kong, X. Lu, Y. Li, P. Yin, Z. Zeng, G. Xu, Pseudotargeted metabolomics method and its application in serum biomarker discovery for hepatocellular carcinoma based on ultra high-performance liquid chromatography/triple quadrupole mass spectrometry, Anal. Chem. 85 (2013) 8326–8333, https://doi.org/10.1021/ac4016787.
[64] A. Verhoeven, M. Giera, O.A. Mayboroda, Scientific workflow managers in metabolomics: an overview, Analyst 145 (2020) 3801–3808, https://doi.org/10.1039/D0AN00272K.
[65] R. Tautenhahn, K. Cho, W. Uritboonthai, Z. Zhu, G.J. Patti, G. Siuzdak, An accelerated workflow for untargeted metabolomics using the METLIN database, Nat. Biotechnol. 30 (2012) 826–828, https://doi.org/10.1038/nbt.2348.
[66] O. Fiehn, Metabolomics by gas chromatography-mass spectrometry: combined targeted and untargeted profiling, Curr Protoc Mol Biol 114 (2016), https://doi.org/10.1002/0471142727.mb3004s114 (30.34.31-30.34.32).
[67] M. Yan, G. Xu, Current and future perspectives of functional metabolomics in disease studies - a review, Anal Chim Acta 1037 (2018) 41–54, https://doi.org/10.1016/j.aca.2018.04.006.
[68] A.J. Chetwynd, W.B. Dunn, G.R. Blanco, Collection and preparation of clinical samples for metabolomics, Adv. Exp. Med. Biol. 965 (2017) 19–44, https://doi.org/10.1007/978-3-319-47656-8_2.
[69] H. Bi, Z. Guo, X. Jia, H. Liu, L. Ma, L. Xue, The key points in the pre-analytical procedures of blood and urine samples in metabolomics studies, Metabolomics 16 (2020) 68, https://doi.org/10.1007/s11306-020-01666-2.
[70] M. Khadka, A. Todor, K.M. Maner-Smith, J.K. Colucci, V. Tran, D.A. Gaul, E. J. Anderson, M.S. Natrajan, N. Rouphael, M.J. Mulligan, C.E. McDonald, M. Suthar, S. Li, E.A. Ortlund, The effect of anticoagulants, temperature, and time on the human plasma metabolome and lipidome from healthy donors as determined by liquid chromatography-mass spectrometry, Biomolecules 9 (2019) 200, https://doi.org/10.3390/biom9050200.
[71] D. Broadhurst, R. Goodacre, S.N. Reinke, J. Kuligowski, I.D. Wilson, M.R. Lewis, W.B. Dunn, Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies, Metabolomics 14 (2018), https://doi.org/10.1007/s11306-018-1367-3.
[72] M.Y. Mushtaq, Y.H. Choi, R. Verpoorte, E.G. Wilson, Extraction for metabolomics: access to the metabolome, Phytochem. Anal. 25 (2014) 291–306, https://doi.org/10.1002/pca.2505.
[73] D.G. Sitnikov, C.S. Monnin, D. Vuckovic, Systematic assessment of seven solvent and solid-phase extraction methods for metabolomics analysis of human plasma by LC-MS, Sci. Rep. 6 (2016), 38885, https://doi.org/10.1038/srep38885.
[74] Y. Yang, Y. Yin, X. Chen, C. Chen, Y. Xia, H. Qi, P.N. Baker, H. Zhang, T.L. Han, Evaluating different extraction solvents for GC-MS based metabolomic analysis of the fecal metabolome of adult and baby giant pandas, Sci. Rep. 9 (2019), 12017, https://doi.org/10.1038/s41598-019-48453-1.
[75] G.A.N. Gowda, D. Djukovic, Overview of mass spectrometry-based metabolomics: opportunities and challenges, Methods Mol. Biol. 1198 (2014) 3–12, https://doi.org/10.1007/978-1-4939-1258-2_1.
[76] K. Segers, S. Declerck, D. Mangelings, Y.V. Heyden, A.V. Eeckhaut, Analytical techniques for metabolomic studies: a review, Bioanalysis 11 (2019) 2297–2318, https://doi.org/10.4155/bio-2019-0014.
[77] A.H. Emwas, R. Roy, R.T. McKay, L. Tenori, E. Saccenti, G.A.N. Gowda, D. Raftery, F. Alahmari, L. Jaremko, M. Jaremko, D.S. Wishart, NMR spectroscopy for metabolomics research, Metabolites 9 (2019) 123, https://doi.org/10.3390/metabo9070123.
[78] K. Dettmer, P.A. Aronov, B.D. Hammock, Mass spectrometry-based metabolomics, Mass Spectrom. Rev. 26 (2007) 51–78, https://doi.org/10.1002/mas.20108.
[79] A. Alonso, S. Marsal, A. Julià, Analytical methods in untargeted metabolomics: state of the art in 2015, Front. Bioeng. Biotechnol 3 (2015), https://doi.org/10.3389/fbioe.2015.00023.
[80] J.L. Markley, R. Bruschweiler, A.S. Edison, H.R. Eghbalnia, R. Powers, D. Raftery, D.S. Wishart, The future of NMR-based metabolomics, Curr. Opin. Biotechnol. 43 (2017) 34–40, https://doi.org/10.1016/j.copbio.2016.08.001.
[81] O. Beckonert, M. Coen, H.C. Keun, Y. Wang, T.M. Ebbels, E. Holmes, J.C. Lindon, J.K. Nicholson, High-resolution magic-angle-spinning NMR spectroscopy for metabolic profiling of intact tissues, Nat. Protoc. 5 (2010) 1019–1032, https://doi.org/10.1038/nprot.2010.45.
[82] A. Zhang, H. Sun, G. Yan, P. Wang, X. Wang, Mass spectrometry-based metabolomics: applications to biomarker and metabolic pathway research, Biomed. Chromatogr. 30 (2016) 7–12, https://doi.org/10.1002/bmc.3453.
[83] Y. Iwasaki, T. Sawada, K. Hatayama, A. Ohyagi, Y. Tsukuda, K. Namekawa, R. Ito, K. Saito, H. Nakazawa, Separation technique for the determination of highly polar metabolites in biological samples, Metabolites 2 (2012) 496–515, https://doi.org/10.3390/metabo2030496.
[84] J.M. Halket, D. Waterman, A.M. Przyborowska, R.K.P. Patel, P.D. Fraser, P. M. Bramley, Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS, J. Exp. Bot. 56 (2004) 219–243, https://doi.org/10.1093/jxb/eri069.
[85] B. Zhou, J.F. Xiao, L. Tuli, H.W. Ressom, LC-MS-based metabolomics, Mol. BioSyst. 8 (2012) 470–481, https://doi.org/10.1039/c1mb05350g.
[86] T. Zhang, D.J. Creek, M.P. Barrett, G. Blackburn, D.G. Watson, Evaluation of coupling reversed phase, aqueous normal phase, and hydrophilic interaction liquid chromatography with orbitrap mass spectrometry for metabolomic studies of human urine, Anal. Chem. 84 (2012) 1994–2001, https://doi.org/10.1021/ac2030738.
[87] W. Lv, L. Guo, F. Zheng, Q. Wang, W. Wang, L. Cui, Y. Ouyang, X. Liu, E. Li, X. Shi, G. Xu, Alternate reversed-phase and hydrophilic interaction liquid chromatography coupled with mass spectrometry for broad coverage in metabolomics analysis, J. Chromatogr. B 1152 (2020), 122266, https://doi.org/10.1016/j.jchromb.2020.122266.
[88] J. Zhou, Y. Yin, Strategies for large-scale targeted metabolomics quantification by liquid chromatography-mass spectrometry, Analyst 141 (2016) 6362–6373, https://doi.org/10.1039/c6an01753c.
[89] W. Jia, X. Chu, J. Chang, P.G. Wang, Y. Chen, F. Zhang, High-throughput untargeted screening of veterinary drug residues and metabolites in tilapia using high resolution orbitrap mass spectrometry, Anal. Chim. Acta 957 (2017) 29–39, https://doi.org/10.1016/j.aca.2016.12.038.
[90] L. Cui, H. Lu, Y.H. Lee, Challenges and emergent solutions for LC-MS/MS based untargeted metabolomics in diseases, Mass Spectrom. Rev. 37 (2018) 772–792, https://doi.org/10.1002/mas.21562.
[91] J.F. Xiao, B. Zhou, H.W. Ressom, Metabolite identification and quantitation in LC-MS/MS-based metabolomics, Trends Analyt Chem 32 (2012) 1–14, https://doi.org/10.1016/j.trac.2011.08.009.
[92] R. Spicer, R.M. Salek, P. Moreno, D. Cañueto, C. Steinbeck, Navigating freely-available software tools for metabolomics analysis, Metabolomics 13 (2017), https://doi.org/10.1007/s11306-017-1242-7.
[93] B.B. Misra, J.F. Fahrmann, D. Grapov, Review of emerging metabolomic tools and resources: 2015–2016, Electrophoresis 38 (2017) 2257–2274, https://doi.org/10.1002/elps.201700110.
[94] B.B. Misra, New tools and resources in metabolomics: 2016–2017, Electrophoresis 39 (2018) 909–923, https://doi.org/10.1002/elps.201700441.
[95] B.B. Misra, J.J. van der Hooft, Updates in metabolomics tools and resources: 2014–2015, Electrophoresis 37 (2016) 86–110, https://doi.org/10.1002/elps.201500417.
[96] J. Bartel, J. Krumsiek, F.J. Theis, Statistical methods for the analysis of high-throughput metabolomics data, Comput Struct Biotechnol J 22 (2013), e201301009, https://doi.org/10.5936/csbj.201301009 (eCollection 2013).
[97] D. Balding, I. Moltke, J. Marioni, Handbook of Statistical Genomics: Two Volume Set, fourth edition, John Wiley & Sons, Inc, 2019.
[98] E. Saccenti, H.C.J. Hoefsloot, A.K. Smilde, J.A. Westerhuis, M.M.W.B. Hendriks, Reflections on univariate and multivariate analysis of metabolomics data, Metabolomics 10 (2013) 361–374, https://doi.org/10.1007/s11306-013-0598-6.
[99] M. Vinaixa, S. Samino, I. Saez, J. Duran, J.J. Guinovart, O. Yanes, A guideline to univariate statistical analysis for LC/MS-based untargeted metabolomics-derived data, Metabolites 2 (2012) 775–795, https://doi.org/10.3390/metabo2040775.
[100] B. Worley, R. Powers, Multivariate analysis in metabolomics, Curr Metabolomics 1 (2013) 92–107, https://doi.org/10.2174/2213235X11301010092.
[101] F. Farshidfar, A.M. Weljie, K.A. Kopciuk, R. Hilsden, S.E. McGregor, W.D. Buie, A. MacLean, H.J. Vogel, O.F. Bathe, A validated metabolomic signature for colorectal cancer: exploration of the clinical value of metabolomics, Br. J. Cancer 115 (2016) 848–857, https://doi.org/10.1038/bjc.2016.243.
[102] I. Tzoulaki, T.M.D. Ebbels, A. Valdes, P. Elliott, J.P.A. Ioannidis, Design and analysis of metabolomics studies in epidemiologic research: a primer on omic technologies, Am. J. Epidemiol. 180 (2014) 129–139, https://doi.org/10.1093/aje/kwu143.
[103] J. Xia, D.S. Wishart, MetPA: a web-based metabolomics tool for pathway analysis and visualization, Bioinformatics 26 (2010) 2342–2344, https://doi.org/10.1093/bioinformatics/btq418.
[104] S. Rius Perez, S. Perez, I. Torres Cuevas, P. Marti Andres, R. Talens Visconti, A. Paradela, L. Guerrero, L. Franco, G. Lopez Rodas, L. Torres, F. Corrales, J. Sastre, Blockade of the trans-sulfuration pathway in acute pancreatitis due to nitration of cystathionine beta-synthase, Redox Biol. 28 (2020), 101324, https://doi.org/10.1016/j.redox.2019.101324.
[105] Q. Yang, J. Sun, Y.Q. Chen, Multi-dimensional, comprehensive sample extraction combined with LC-GC/MS analysis for complex biological samples: application in the metabolomics study of acute pancreatitis, RSC Adv. 6 (2016) 25837–25849, https://doi.org/10.1039/c5ra26708k.
[106] H. Xiao, J.H. Huang, X.W. Zhang, R. Ahmed, Q.L. Xie, B. Li, Y.M. Zhu, X. Cai, Q. H. Peng, Y.H. Qin, H.Y. Huang, W. Wang, Identification of potential diagnostic biomarkers of acute pancreatitis by serum metabolomic profiles, Pancreatol 17 (2017) 543–549, https://doi.org/10.1016/j.pan.2017.04.015.
[107] Y. Zhao, W. Jia, M. Su, Y. Qiu, X. Wang, Novel biomarkers of hyperlipidemic acute pancreatitis: metabolomic identification, Asian Biomed 6 (2012) 765–769, https://doi.org/10.5372/1905-7415.0605.119.
[108] A. Villaseñor, J.M. Kinross, J.V. Li, N. Penney, R.H. Barton, J.K. Nicholson, A. Darzi, C. Barbas, E. Holmes, 1H NMR global metabolic phenotyping of acute pancreatitis in the emergency unit, J. Proteome Res. 13 (2014) 5362–5375, https://doi.org/10.1021/pr500161w.
[109] U.M. Kabadi, Pancreatic ketoacidosis: ketonemia associated with acute pancreatitis, Postgrad. Med. J. 71 (1995) 32–35, https://doi.org/10.1136/pgmj.71.831.32.
[110] P.C. Nicholas, D. Kim, F.T. Crews, J.M. Macdonald, 1H NMR-based metabolomic analysis of liver, serum, and brain following ethanol, Chem. Res. Toxicol. 21 (2008) 408–420, https://doi.org/10.1021/tx700324t.
[111] L.C. McGuire, A.M. Cruickshank, P.T. Munro, Alcoholic ketoacidosis, Emerg. Med. J. 23 (2006) 417–420, https://doi.org/10.1136/emj.2004.017590.
[112] M.L. Freeman, Understanding risk factors and avoiding complications with endoscopic retrograde, Curr Gastroenterol Rep 5 (2003) 145–153, https://doi.org/10.1007/s11894-003-0084-9.
[113] E.R. Lusczek, K. Colling, S. Muratore, D. Conwell, M. Freeman, G. Beilman, Stereotypical metabolic response to endoscopic retrograde

cholangiopancreatography show alterations in pancreatic function regardless of post-procedure pancreatitis, Clin Transl Gastroen 7 (2016), e169, https://doi.org/10.1038/ctg.2016.26.
[114] E. Sanchez-Lopez, Z. Zhong, A. Stubelius, S.R. Sweeney, L.M. Booshehri, L. Antonucci, R. Liu-Bryan, A. Lodi, R. Terkeltaub, J.C. Lacal, A.N. Murphy, H. M. Hoffman, S. Tiziani, M. Guma, M. Karin, Choline uptake and metabolism modulate macrophage IL-1β and IL-18 production, Cell Metab. 29 (2019) 1350–1362 (e1357), https://doi.org/10.1016/j.cmet.2019.03.011.
[115] S.H. Zeisel, K.A.d. Costa, Choline: an essential nutrient for public health, Nutr. Rev. 67 (2009) 615–623, https://doi.org/10.1111/j.1753-4887.2009.00246.x.
[116] C. Schorsch, E. Boles, S. Schaffer, Biotechnological production of sphingoid bases and their applications, Appl. Microbiol. Biotechnol. 97 (2013) 4301–4308, https://doi.org/10.1007/s00253-013-4878-x.
[117] H. Xu, L. Zhang, H. Kang, J. Zhang, J. Liu, S. Liu, Serum metabonomics of mild acute pancreatitis, J. Clin. Lab. Anal. 30 (2016) 990–998, https://doi.org/10.1002/jcla.21969.
[118] D. Ouyang, Metabolomic characterization of human pancreatitis by 1H-NMR spectroscopy, Hepato-Gastroenterol 59 (2012) 2314–2317, https://doi.org/10.5754/hge11839.
[119] P. Takis, A. Taddei, R. Pini, S. Grifoni, F. Tarantini, P. Bechi, C. Luchinat, Fingerprinting acute digestive diseases by untargeted NMR based metabolomics, Int. J. Mol. Sci. 19 (2018) 3288, https://doi.org/10.3390/ijms19113288.
[120] G.E. Gillaspy, The cellular language of myo-inositol signaling, New Phytol. 192 (2011) 823–839, https://doi.org/10.1111/j.1469-8137.2011.03939.x.
[121] F. Gonzalez-Uarquin, M. Rodehutscord, K. Huber, Myo-inositol: its metabolism and potential implications for poultry nutrition-a review, Poult. Sci. 99 (2020) 893–905, https://doi.org/10.1016/j.psj.2019.10.014.
[122] J.H. Huang, D. He, L. Chen, C.Y. Dong, S.H. Zhang, Y.H. Qin, R. Yu, R. Ahmed, J. J. Kuang, X.W. Zhang, GC-MS based metabolomics strategy to distinguish three types of acute pancreatitis, Pancreatol 19 (2019) 630–637, https://doi.org/10.1016/j.pan.2019.05.456.
[123] M. Grembecka, Sugar alcohols - their role in the modern world of sweeteners: a review, Eur. Food Res. Technol. 241 (2015) 1–14, https://doi.org/10.1007/s00217-015-2437-7.
[124] C. Skouras, X. Zheng, M. Binnie, N.Z. Homer, T.B. Murray, D. Robertson, L. Briody, F. Paterson, H. Spence, L. Derr, A.J. Hayes, A. Tsoumanis, D. Lyster, R. W. Parks, O.J. Garden, J.P. Iredale, I.J. Uings, J. Liddle, W.L. Wright, G. Dukes, S. P. Webster, D.J. Mole, Increased levels of 3-hydroxykynurenine parallel disease severity in human acute pancreatitis, Sci. Rep. 6 (2016), 33951, https://doi.org/10.1038/srep33951.
[125] E.R. Lusczek, J.A. Paulo, J.R. Saltzman, V. Kadiyala, P.A. Banks, G. Beilman, D. L. Conwell, Urinary 1H-NMR metabolomics can distinguish pancreatitis patients from healthy controls, JOP 14 (2013) 161–170, https://doi.org/10.6092/1590-8577/1294.
[126] T. Noji, K.i.N. Ya, M. Mizutani, C. Katagiri, J.i. Sano, C. Takada, S. Nishikawa, A. Karasawa, H. Kusaka, KF24345, an adenosine uptake inhibitor, ameliorates the severity and mortality of lethal acute pancreatitis via endogenous adenosine in mice, Eur. J. Pharmacol. 454 (2002) 85–93, https://doi.org/10.1016/s0014-2999(02)02476-7.
[127] X. Zheng, G. Xie, A. Zhao, L. Zhao, C. Yao, N.H. Chiu, Z. Zhou, Y. Bao, W. Jia, J. K. Nicholson, W. Jia, The footprints of gut microbial-mammalian co-metabolism, J. Proteome Res. 10 (2011) 5512–5522, https://doi.org/10.1021/pr2007945.
[128] Q. Li, C. Wang, C. Tang, Q. He, N. Li, J. Li, Bacteremia in patients with acute pancreatitis as revealed by 16S ribosomal RNA gene-based techniques, Crit. Care Med. 41 (2013) 1938–1950, https://doi.org/10.1097/CCM.0b013e31828a3dba.
[129] J.J. Hyun, H.S. Lee, Experimental models of pancreatitis, Clin Endosc 47 (2014) 212–216, https://doi.org/10.5946/ce.2014.47.3.212.
[130] X. Yang, L. Yao, X. Fu, R. Mukherjee, Q. Xia, M.A. Jakubowska, P.E. Ferdek, W. Huang, Experimental acute pancreatitis models: history, current status, and role in translational research, Front. Physiol. 11 (2020), https://doi.org/10.3389/fphys.2020.614591.
[131] A. Sakai, S. Nishiumi, Y. Shiomi, T. Kobayashi, Y. Izumi, H. Kutsumi, T. Hayakumo, T. Azuma, M. Yoshida, Metabolomic analysis to discover candidate therapeutic agents against acute pancreatitis, Arch. Biochem. Biophys. 522 (2012) 107–120, https://doi.org/10.1016/j.abb.2012.03.025.
[132] C. Ma, B. Tian, J. Wang, G.J. Yang, C.S. Pan, J.P. Lu, Metabolic characteristics of acute necrotizing pancreatitis and chronic pancreatitis, Mol. Med. Rep. 6 (2012) 57–62, https://doi.org/10.3892/mmr.2012.881.
[133] J. Li, S.F. Zhu, X.L. Zhao, Y.X. Liu, M.H. Wan, H. Guo, Y.L. Liu, H.L. Gong, G. Y. Chen, W.F. Tang, Metabolomic profiles illuminate the efficacy of Chinese herbal Da-Cheng-Qi decoction on acute pancreatitis in rats, Pancreatol 15 (2015) 337–343, https://doi.org/10.1016/j.pan.2015.04.011.
[134] J. Li, X.l. Zhao, Y.X. Liu, X.h. Peng, S.f. Zhu, H. Guo, Y.l. Liu, M.h. Wan, W.f. Tang, 1HNMR-based metabolomic profile of rats with experimental acute pancreatitis, BMC Gastroenterol. 14 (2014) 115, https://doi.org/10.1186/1471-230x-14-115.
[135] E. Bohus, M. Coen, H.C. Keun, T.M.D. Ebbels, O. Beckonert, J.C. Lindon, E. Holmes, B. Noszal, J.K. Nicholson, Temporal metabonomic modeling of L-arginine-induced exocrine pancreatitis, J. Proteome Res. 7 (2008) 4435–4445, https://doi.org/10.1021/pr800407j.
[136] Z. Chen, L. Shen, C. Yang, J. Jiang, X. Liu, Metabolic analysis of the effect of rheum on a taurocholate-induced acute pancreatitis rat model, Braz J Pharm Sci 53 (2017), e15022, https://doi.org/10.1590/s2175-97902017000215022.
[137] J. Guo, X. Li, D. Wang, Y. Guo, T. Cao, Exploring metabolic biomarkers and regulation pathways of acute pancreatitis using ultra-performance liquid chromatography combined with a mass spectrometry-based metabolomics strategy, RSC Adv. 9 (2019) 12162–12173, https://doi.org/10.1039/c9ra02186h.
[138] J.M. Berg, J.L. Tymoczko, L. Stryer, Biochemistry, Available from. https://www.ncbi.nlm.nih.gov/books/NBK21154/, 5th edition, W H Freeman, New York, .
[139] M. Tang, G. Hu, Y.A.N. Zhao, M. Su, Y. Wang, W.E.I. Jia, Y. Qiu, G. Liu, X. Wang, A serum metabolomic investigation on lipoprotein lipase-deficient mice with hyperlipidemic pancreatitis using gas chromatography/mass spectrometry, Biomed Rep 1 (2013) 469–473, https://doi.org/10.3892/br.2013.78.
[140] A.O. Hosten, Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Chapter 193: BUN and Creatinine, Butterworths, Boston, 1990. Available from: https://www.ncbi.nlm.nih.gov/books/NBK305/.
[141] T.I. Nassar, W.Y. Qunibi, AKI associated with acute pancreatitis, Clin. J. Am. Soc. Nephrol. 14 (2019) 1106–1115, https://doi.org/10.2215/CJN.13191118.
[142] Q. Ruan, H. Lu, H. Zhu, Y. Guo, Y. Bai, A network-regulative pattern in the pathogenesis of kidney injury following severe acute pancreatitis, Biomed. Pharmacother. 125 (2020), 109978, https://doi.org/10.1016/j.biopha.2020.109978.
[143] S.M.M. Jr, Regulation of enzymes of the urea cycle and arginine metabolism, Annu. Rev. Nutr. 22 (2002) 87–105, https://doi.org/10.1146/annurev.nutr.22.110801.140547.
[144] R.P.d. Silva, I. Nissim, M.E. Brosnan, J.T. Brosnan, Creatine synthesis: hepatic metabolism of guanidinoacetate and creatine in the rat in vitro and in vivo, Am. J. Physiol. Endocrinol. Metab. 296 (2009), https://doi.org/10.1152/ajpendo.90547.2008 (E256–261).
[145] J. Han, J. Meng, S. Chen, C. Li, Integrative analysis of the gut microbiota and metabolome in rats treated with rice straw biochar by 16S rRNA gene sequencing and LC/MS-based metabolomics, Sci. Rep. 9 (2019), 17860, https://doi.org/10.1038/s41598-019-54467-6.
[146] E.A. Smith, G.T. Macfarlane, Enumeration of human colonic bacteria producing phenolic and indolic compounds, J Appl Bacteriol 81 (1996) 288–302, https://doi.org/10.1111/j.1365-2672.1996.tb04331.x.
[147] M. Bhatia, Acute pancreatitis as a model of SIRS, Front Biosci (Landmark Ed) vol. 14 (2009) 2042–2050, https://doi.org/10.2741/3362.
[148] Y.Y. Zuo, Y. Kang, W.H. Yin, B. Wang, Y. Chen, The association of mean glucose level and glucose variability with intensive care unit mortality in patients with severe acute pancreatitis, J. Crit. Care 27 (2012) 146–152, https://doi.org/10.1016/j.jcrc.2011.12.004.
[149] T. Mizushima, K. Ochi, N. Koide, Metabolic disorders of patients with acute pancreatitis: carbohydrate, lipid and protein metabolic disorders, Nihon Rinsho 62 (2004) 1989–1992.
[150] J. Bakker, S.J. Schieveld, W. Brinkert, Serum lactate level as a indicator of tissue hypoxia in severely ill patients, Ned. Tijdschr. Geneeskd. 144 (2000) 737–741.
[151] W. Shu, J. Wan, J. Chen, W. He, Y. Zhu, N. Lu, L. Xia, Elevated arterial lactate level as an independent risk factor for pancreatic infection in moderately severe acute pancreatitis, Pancreatol 19 (2019) 653–657, https://doi.org/10.1016/j.pan.2019.06.001.
[152] W. Shu, J. Wan, J. Chen, W. He, Y. Zhu, H. Zeng, P. Liu, Y. Zhu, L. Xia, N. Lu, Initially elevated arterial lactate as an independent predictor of poor outcomes in severe acute pancreatitis, BMC Gastroenterol. 20 (2020) 116, https://doi.org/10.1186/s12876-020-01268-1.
[153] I.M. Reyes, N.S. Chandel, Mitochondrial TCA cycle metabolites control physiology and disease, Nat. Commun. 11 (2020) 102, https://doi.org/10.1038/s41467-019-13668-3.
[154] L. Yao, C. Cheng, X. Yang, C. Han, D. Du, T. Liu, M. Chvanov, J. Windsor, R. Sutton, W. Huang, Q. Xia, Ethyl pyruvate and analogs as potential treatments for acute pancreatitis: a review of in vitro and in vivo studies, Pancreatol 19 (2019) 209–216, https://doi.org/10.1016/j.pan.2018.12.007.
[155] R.M. Denton, P.J. Randle, B.J. Bridges, R.H. Cooper, A.L. Kerbey, H.T. Pask, D. L. Severson, D. Stansbie, S. Whitehouse, Regulation of mammalian pyruvate dehydrogenase, Mol. Cell. Biochem. 9 (1975) 27–53, https://doi.org/10.1007/bf01731731.
[156] J. Maleth, P. Hegyi, Ca2+ toxicity and mitochondrial damage in acute pancreatitis: translational overview, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 371 (2016), 20150425, https://doi.org/10.1098/rstb.2015.0425.
[157] P. Sandstrom, L. Trulsson, T. Gasslander, T. Sundqvist, U. von Dobeln, J. Svanvik, Serum amino acid profile in patients with acute pancreatitis, Amino Acids 35 (2008) 225–231, https://doi.org/10.1007/s00726-007-0557-5.
[158] W. Druml, G. Heinzel, G. Kleinberger, Amino acid kinetics in patients with sepsis, Am. J. Clin. Nutr. 73 (2001) 908–913, https://doi.org/10.1093/ajcn/73.5.908.
[159] I.F. Btaiche, R.A. Mohammad, C. Alaniz, B.A. Mueller, Amino acid requirements in critically ill patients with acute kidney injury treated with continuous renal replacement therapy, Pharmacotherapy 28 (2008) 600–613, https://doi.org/10.1592/phco.28.5.600.
[160] I. Manoli, C.P. Venditti, Disorders of branched chain amino acid metabolism, Transl Sci Rare Dis 1 (2016) 91–110, https://doi.org/10.3233/TRD-160009.
[161] R.L. Lyman, S.S. Wilcox, Effect of acute amino acid deficiencies on carcass composition and pancreatic function in the force-fed rat. II. Deficiencies of valine, lysine, tryptophan, leucine and isoleucine, J. Nutr. 79 (1963) 37–44, https://doi.org/10.1093/jn/79.1.37.
[162] C. Burtis, E. Ashwood, D. Bruns, Tietz textbook of clinical chemistry and molecular diagnostics, in: Enzyme and Rate Analyses, Saunders, 5th ed., 2012.
[163] M.J. McMahon, I.R. Pickford, Biochemical prediction of gallstones early in an attack of acute pancreatitis, Lancet 2 (1979) 541–543, https://doi.org/10.1016/s0140-6736(79)91610-6.
[164] D.C. Díaz, W. Otero Regino, M. Gómez Zuleta, Acute pancreatitis and elevated aminotransferases: what to think? A case report and literature review, Rev Col Gastroenterol 30 (2015) 479–484.

[165] M. Watford, Glutamine and glutamate: nonessential or essential amino acids? Anim Nutr 1 (2015) 119–122, https://doi.org/10.1016/j.aninu.2015.08.008.
[166] F. Bergeron, A. Otto, P. Blache, R. Day, L. Denoroy, R. Brandsch, D. Bataille, Molecular cloning and tissue distribution of rat sarcosine dehydrogenase, Eur. J. Biochem. 257 (1998) 556–561, https://doi.org/10.1046/j.1432-1327.1998.2570556.x.
[167] G.O. Ceyhan, A.K. Timm, F. Bergmann, A. Guenther, A.A. Aghdassi, I.E. Demir, J. Mayerle, M. Kern, M.M. Lerch, M.W. Buechler, H. Friess, P. Schemmer, Prophylactic glycine administration attenuates pancreatic damage and inflammation in experimental acute pancreatitis, Pancreatol 11 (2011) 57–67, https://doi.org/10.1159/000325972.
[168] M.H. Stipanuk, I. Ueki, Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur, J. Inherit. Metab. Dis. 34 (2011) 17–32, https://doi.org/10.1007/s10545-009-9006-9.
[169] B.O. Ahn, K.H. Kim, G. Lee, H.S. Lee, C.D. Kim, Y.S. Kim, M.W. Son, W.B. Kim, T. Y. Oh, J.H. Hyun, Effects of taurine on cerulein-induced acute pancreatitis in the rat, Pharmacol 63 (2001) 1–7, https://doi.org/10.1159/000056106.
[170] E. Koster, R. Graf, F. Verrey, S.M. Camargo, Acute pancreatitis involves alterations in the transport of amino acids necessary for glutathione synthesis, FASEB J. 30 (2016) (968.967).
[171] A. Leja Szpak, J. Jaworek, R. Tomaszewska, K. Nawrot, J. Bonior, M. Kot, M. Palonek, J. Stachura, A. Czupryna, S.J. Konturek, W.W. Pawlik, Melatonin precursor, L-tryptophan protects the pancreas from development of acute pancreatitis through the central site of action, J. Physiol. Pharmacol. 55 (2004) 239–254.
[172] D.M. Richard, M.A. Dawes, C.W. Mathias, A. Acheson, N. Hill-Kapturczak, D. M. Dougherty, L-tryptophan: basic metabolic functions, behavioral research and therapeutic indications, Int J Tryptophan Res 23 (2009) 45–60, https://doi.org/10.4137/ijtr.s2129.
[173] M. Platten, E.A.A. Nollen, U.F. Röhrig, F. Fallarino, C.A. Opitz, Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond, Nat. Rev. Drug Discov. 18 (2019) 379–401, https://doi.org/10.1038/s41573-019-0016-5.
[174] N.L. Floc'h, W. Otten, E. Merlot, Tryptophan metabolism, from nutrition to potential therapeutic applications, Amino Acids 41 (2011) 1195–1205, https://doi.org/10.1007/s00726-010-0752-7.
[175] D.J. Mole, S.P. Webster, I. Uings, X. Zheng, M. Binnie, K. Wilson, J.P. Hutchinson, O. Mirguet, A. Walker, B. Beaufils, N. Ancellin, L. Trottet, V. Bénéton, C. G. Mowat, M. Wilkinson, P. Rowland, C. Haslam, A. McBride, N.Z.M. Homer, J. E. Baily, M.G.F. Sharp, O.J. Garden, J. Hughes, S.E.M. Howie, D.S. Holmes, J. Liddle, J.P. Iredale, Kynurenine-3-monooxygenase inhibition prevents multiple organ failure in rodent models of acute pancreatitis, Nat. Med. 22 (2016) 202–209, https://doi.org/10.1038/nm.4020.
[176] B. Kui, Z. Balla, E.T. Vegh, P. Pallagi, V. Venglovecz, B. Ivanyi, T. Takacs, P. Hegyi, Z. Rakonczay Jr., Recent advances in the investigation of pancreatic inflammation induced by large doses of basic amino acids in rodents, Lab. Investig. 94 (2014) 138–149, https://doi.org/10.1038/labinvest.2013.143.
[177] C.H. Kubisch, M.D. Sans, T. Arumugam, S.A. Ernst, J.A. Williams, C.D. Logsdon, Early activation of endoplasmic reticulum stress is associated with arginine-induced acute pancreatitis, Am. J. Physiol. Gastrointest. Liver Physiol. 291 (2006), https://doi.org/10.1152/ajpgi.00471.2005 (G238–245).
[178] Z. Rakonczay Jr., P. Hegyi, S. Dosa, B. Ivanyi, K. Jarmay, G. Biczo, Z. Hracsko, I. S. Varga, E. Karg, J. Kaszaki, A. Varro, J. Lonovics, I. Boros, I. Gukovsky, A. S. Gukovskaya, S.J. Pandol, T. Takacs, A new severe acute necrotizing pancreatitis model induced by L-ornithine in rats, Crit. Care Med. 36 (2008) 2117–2127, https://doi.org/10.1097/CCM.0b013e31817d7f5c.
[179] G. Biczo, P. Hegyi, R. Sinervirta, S. Berczi, S. Dosa, A. Siska, B. Ivanyi, V. Venglovecz, T. Takacs, L. Alhonen, Z. Rakonczay Jr., Characterization of polyamine homeostasis in L-ornithine-induced acute pancreatitis in rats, Pancreas 39 (2010) 1047–1056, https://doi.org/10.1097/MPA.0b013e3181d3cdf0.
[180] R. Keshet, A. Erez, Arginine and the metabolic regulation of nitric oxide synthesis in cancer, Dis. Model. Mech. 11 (2018), dmm033332, https://doi.org/10.1242/dmm.033332.
[181] R. Majumdar, L. Shao, R. Minocha, S. Long, S.C. Minocha, Ornithine: the overlooked molecule in the regulation of polyamine metabolism, Plant Cell Physiol 54 (2013) 990–1004, https://doi.org/10.1093/pcp/pct053.
[182] J.E. Dominguezmunoz, F. Junemann, P. Malfertheiner, Hyperlipidemia in acute-pancreatitis - cause or epiphenomenon, Int. J. Pancreatol. 18 (1995) 101–106, https://doi.org/10.1007/BF02785883.
[183] K. Sztefko, J. Panek, Serum free fatty acid concentration in patients with acute pancreatitis, Pancreatol 1 (2001) 230–236, https://doi.org/10.1159/000055816.
[184] S. Navina, C. Acharya, J.P. DeLany, L.S. Orlichenko, C.J. Baty, S.S. Shiva, C. Durgampudi, J.M. Karlsson, K. Lee, K.T. Bae, A. Furlan, J. Behari, S. Liu, T. McHale, L. Nichols, G.I. Papachristou, D. Yadav, V.P. Singh, Lipotoxicity causes multisystem organ failure and exacerbates acute pancreatitis in obesity, Sci. Transl. Med. 3 (2011), 107ra110, https://doi.org/10.1126/scitranslmed.3002573.
[185] J. Khan, T. Solakivi, H. Seppanen, R. Lappalainen-Lehto, S. Jarvinen, J. Ronkainen, J. Sand, I. Nordback, Serum lipid and fatty acid profiles are highly changed in patients with alcohol induced acute pancreatitis, Pancreatol 12 (2012) 44–48, https://doi.org/10.1016/j.pan.2011.12.006.
[186] L.S. Kremmyda, E. Tvrzicka, B. Stankova, A. Zak, Fatty acids as biocompounds: their role in human metabolism, health and disease - a review. Part 2: fatty acid physiological roles and applications in human health and disease, Biomed Pap 155 (2011) 195–218, https://doi.org/10.5507/bp.2011.052.
[187] K. Fukami, S. Inanobe, K. Kanemaru, Y. Nakamura, Phospholipase C is a key enzyme regulating intracellular calcium and modulating the phosphoinositide balance, Prog. Lipid Res. 49 (2010) 429–437, https://doi.org/10.1016/j.plipres.2010.06.001.
[188] R.P. Sah, P. Garg, A.K. Saluja, Pathogenic mechanisms of acute pancreatitis, Curr. Opin. Gastroenterol. 28 (2012) 507–515, https://doi.org/10.1097/MOG.0b013e3283567f52.
[189] J.E. Burke, E.A. Dennis, Phospholipase A2 biochemistry, Cardiovasc. Drugs Ther. 23 (2009) 49–59, https://doi.org/10.1007/s10557-008-6132-9.
[190] B. Vollmar, H. Waldner, J. Schmand, P.F. Conzen, A.E. Goetz, H. Habazettl, L. Schweiberer, W. Brendel, Release of arachidonic acid metabolites during acute pancreatitis in pigs, Scand. J. Gastroenterol. 24 (1989) 1253–1264, https://doi.org/10.3109/00365528909090796.
[191] Q. Li, C. Wang, Q. Zhang, C. Tang, N. Li, J. Li, The role of sphingosine kinase 1 in patients with severe acute pancreatitis, Ann. Surg. 255 (2012) 954–962, https://doi.org/10.1097/SLA.0b013e31824d2ca4.
[192] T. Konończuk, B. Łukaszuk, M.Ż. Piotrowska, A. Dąbrowski, M. Krzyżak, L. Ostrowska, K. Kurek, Plasma sphingolipids in acute pancreatitis, Int. J. Mol. Sci. 18 (2017) 2606, https://doi.org/10.3390/ijms18122606.
[193] T. Konończuk, B. Lukaszuk, A. Miklosz, A. Chabowski, M. Zendzian-Piotrowska, K. Kurek, Cerulein-induced acute pancreatitis affects sphingomyelin signaling pathway in rats, Pancreas 47 (2018) 898–903, https://doi.org/10.1097/MPA.0000000000001086.
[194] M.H. Wan, W. Huang, D. Latawiec, K. Jiang, D.M. Booth, V. Elliott, R. Mukherjee, Q. Xia, Review of experimental animal models of biliary acute pancreatitis and recent advances in basic research, HPB (Oxford) 14 (2012) 73–81, https://doi.org/10.1111/j.1477-2574.2011.00408.x.
[195] C. Thomas, R. Pellicciari, M. Pruzanski, J. Auwerx, K. Schoonjans, Targeting bile-acid signalling for metabolic diseases, Nat. Rev. Drug Discov. 7 (2008) 678–693, https://doi.org/10.1038/nrd2619.
[196] L.P. van Minnen, N.G. Venneman, J.E. van Dijk, A. Verheem, H.G. Gooszen, L.M. A. Akermans, K.J. van Erpecum, Cholesterol crystals enhance and phospholipids protect against pancreatitis induced by hydrophobic bile salts: a rat model study, Pancreas 32 (2006) 369–375, https://doi.org/10.1097/01.mpa.0000220861.78248.1f.
[197] B.W. Lau, M. Colella, W.C. Ruder, M. Ranieri, S. Curci, A.M. Hofer, Deoxycholic acid activates protein kinase C and phospholipase C via increased Ca2+ entry at plasma membrane, Gastroenterol 128 (2005) 695–707, https://doi.org/10.1053/j.gastro.2004.12.046.
[198] P.E. Ferdek, M.A. Jakubowska, J.V. Gerasimenko, O.V. Gerasimenko, O. H. Petersen, Bile acids induce necrosis in pancreatic stellate cells dependent on calcium entry and sodium-driven bile uptake, J. Physiol. 594 (2016) 6147–6164, https://doi.org/10.1113/JP272774.
[199] G.A. Plotnikoff, Elevated deoxycholic acid and idiopathic recurrent acute pancreatitis: a case report with 48 months of follow-up, Glob Adv Health Med 3 (2014) 70–72, https://doi.org/10.7453/gahmj.2014.016.