Observational Study

Identification of Preoperative Serum Metabolites Associated With Postoperative Opioid Consumption in Gastric Cancer Patients by Extreme Phenotype Sampling

Jianing Li, PhD, Shuo Li, MM, Ling Yu, MD, Jin Wei, MD, Hongwei Sun, MM, Caixia Yang, BS, and Hongyu Tan, MD

From: Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education, Beijing), Department of Anesthesiology, Peking University Cancer Hospital & Institute, Beijing, China

Address Correspondence:
Hongyu Tan, MD
Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education, Beijing)
Department of Anesthesiology
Peking University Cancer Hospital & Institute
Beijing, 100142, China
E-mail: maggitan@yeah.net

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Background: Postoperative pain increases patients' risk and opioids remain the main analgesics to relieve it. However, improper use of opioids causes many side effects and identification of suitable preoperative biomarkers that predict postoperative opioid consumption may aid clinicians in improving analgesic strategies for patients. The activity of metabolites modulates multiple phenotypes and can function as biomarkers for disease prediction and diagnosis.

Objectives: In this study, we explore whether preoperative serum metabolites are associated with postoperative opioid consumption in gastric cancer patients by extreme phenotype sampling.

Study Design: This was a case-control, observational study.

Setting: This study was conducted at Beijing Cancer Hospital.

Methods: One hundred and sixty-nine gastric cancer patients participated in this study. After exclusion of 51 patients, postoperative pain intensity and opioid consumption data of 118 patients were collected. Patients were sorted by gender and classified into 2 groups based on opioid consumption during the 24h postoperative period. Patients in the sufentanil high consumption (SHC) group and patients in the sufentanil low consumption (SLC) group were ranked in the top or bottom 30% of sufentanil consumption, respectively. Untargeted metabolomic analysis of preoperative serum samples from both groups was performed by ultra-performance liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) and orthogonal partial least square discriminant analysis. Allele frequencies of DAO rs10156191 and MAOB rs1799836 SNPs in both groups were detected by Sanger sequencing.

Results: Thirty-five metabolites in preoperative serum were significantly different between the SLC and SHC groups. Hydrogen phosphate had the highest area under the curve in a ROC analysis (0.98), suggesting that it may serve as a predictive biomarker for postoperative opioid consumption. Differential metabolites unique to the male and female subgroups were also identified. Histidine metabolism was the most altered pathway between the SLC and SHC groups. There were no significant differences in the allele frequencies of 2 SNPs associated with histamine degradation; however, 2 metabolites of histamine degradation, imidazole-4-acetaldehyde, and methylimidazole acetaldehyde, showed different trends in the 2 groups.

Limitations: Our study was restricted to gastric cancer patients with strict exclusion criteria, which may limit the generalizability to other groups.

Conclusion: Preoperative serum metabolites were associated with postoperative opioid consumption. Different efficiencies of histamine degradation may be one cause of the variable sensitivity of patients to acute pain and warrants further study.

Key words: Opioids, sufentanil, postoperative pain, metabolome, gene polymorphisms, gastric cancer, hydrogen phosphate, histidine metabolism

Trial registration number: ChiCTR 210-004-6447.

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Postoperative acute pain is an important issue in public health care (1). Although non-opioid analgesics can be applied for acute pain, opioids remain the mainstay of pain management during the postoperative phase (2). There are 2 main clinical issues when considering opioid use: first, excessive use of opioids may produce side effects of respiratory depression, intestinal motility, and vomiting, and second, insufficient opioids may cause severe pain, affect mood, and increase the incidence of complications (2). Patients have different needs for postoperative opioid usage. Recent advances in precision medicine have been used to diagnose and treat diseases at an individual level based on genetic, physiological, and pathological factors, which also can be applied to the use of opioids for postoperative pain management (3).

To avoid out of use opioids and improve postoperative pain management in patients, many studies have been conducted to identify predictors of postoperative opioid consumption and acute pain. Anxiety, preoperative pain, age, and type of surgery were all significant predictors of acute postoperative pain (4). Younger age, smoking, higher body mass index (BMI), female gender, and a history of depression and anxiety symptoms were preoperative predictors of poor postoperative pain control (5). Age and the type of surgery are also strong predictive indicators of postoperative analgesic consumption (4).

Gene polymorphisms are reported to correlate with postoperative pain and opioid consumption. Catechol-O-methyltransferase (COMT) and μ-opioid receptor (OPRM1) have been studied most extensively. Patients who carry a homozygous GG genotype at OPRM1 rs1799971 needed 30% more oxycodone to reduce acute pain, and heterozygous G needed a higher mean opioid dose than AA homozygotes (6-7). COMT polymorphisms are associated with pain perception, sensitivity, persistence, and opioid efficacy (8). The combined effects of OPRM1 and COMT polymorphisms have also been shown to correlate with postoperative morphine consumption (9).

The newly emerging and rapidly developing field of metabolomics can detect a large number of small molecule metabolites and has been used extensively as a reliable tool to examine the physiological state of patients, discover new biomarkers, and analyze metabolic pathways (10). The state of metabolites can be regarded as the response to genotype, phenotype, and environment (11). Metabolomics has recently been used to identify biomarkers of several pain-related symptoms. Metabolites including cholesterol, linoleic acid, and phospholipids were significantly higher in patients who developed chronic postoperative pain compared with others (12). Systemic metabolic differences were found among women who had chronic localized neck-shoulder pain or chronic widespread pain compared to healthy controls (13). Metabolomic studies have also identified lysophosphatidylcholines 26:0 and 28:1 as metabolic biomarkers for multisite musculoskeletal pain (14).

Although studies have shown that gene polymorphisms and patient demography can be used to predict the extent of postoperative opioid consumption, more progress in postoperative pain management is needed to improve patient outcomes. Metabolomics has been used in the field of pain management, but the association between preoperative metabolites and postoperative opioid consumption has not been studied. In this study, we applied metabolomic analysis to patients using an extreme phenotype sampling strategy based on postoperative sufentanil consumption to assess the association between preoperative metabolites and postoperative opioid usage.

Methods

Patients

One hundred sixty-nine total patients who signed the informed consent form were enrolled in this observational study. Inclusion criteria were age 18-80 years, American Society of Anesthesiologists (ASA) physical status of I to III, diagnosed with gastric cancer, and planned to undergo gastrectomy at the Beijing Cancer Hospital from May 2021 to August 2021. Patients with the following diagnoses were excluded: (1) people with schizophrenia, epilepsy, and those who could not communicate due to severe dementia, speech disorder, or end-stage disease; (2) people who have received current and chronic use of opioids, psychiatric medication, hormone therapy, and non-steroidal anti-inflammatories; (3) people who receive multiple surgeries during a single surgical procedure; (4) people with primary malignant tumors in other parts and recurrence of tumors; (5) people with a history of chronic pain and severe liver and kidney dysfunction. The study was approved by the Ethics Committee at the Peking University Cancer Hospital & Institute. The protocol number is LGH2019068, and the trial was registered in the Chinese Clinical Trial Registry (ChiC-

TR-2100046447). Of the 169 total patients enrolled in this study, 118 patients completed the following trials, and 51 patients dropped out.

Preoperative and Perioperative Data Collection

Patient demographic characteristic data, including age, gender, BMI, ethnicity, education level, and history of tobacco and alcohol use, were collected preoperatively. Hospital Anxiety and Depression Scale questionnaires were filled in by patients to evaluate their anxiety and depression state (15). Clinical characteristic data, including the operative duration, method of surgery and anesthesia, range of gastrectomy, and use of anesthetics, were also collected.

Anesthesia and Analgesia

We implemented the same anesthesia plan for all enrolled patients. General anesthesia was induced intravenously as follows: 2 mg/kg of propofol, 0.4 μg/kg of sufentanil, 0.2 mg/kg of cisatracurium. Inhalation of 1% sevoflurane was used to maintain anesthesia. Intraoperative micro-pump infusion of propofol and remifentanil was used to ensure that the Bispectral Index (BIS) was between 40-60. After the suture, infusion of remifentanil and propofol was stopped, and patients were connected to the postoperative analgesia pump. The analgesia pump containers were as follows: 5 μg/kg of sufentanil, 100 μg dextromethorphan, and 20 mg tropisetron in 120 mL 0.9% sodium chloride solution. The initial loading dose was 2 mL; the continuous dose was 0.5 mL/h; the single PCA dose was 1.5 mL; the lock-in time was 10 min; and the limit dose was 13 mL/h.

Pain intensity was evaluated using the Numeric Rating Scale (NRS), with values ranging from 0 to 10 (0 = no pain, 10 = extremely severe). Patients' NRS pain scores were ≤ 3 before leaving the post anesthesia care unit (PACU). Patients were taught to use analgesia pumps before surgery so that the postoperative NRS score was ≤ 3. If the use of sufentanil in the analgesia pump did not effectively relieve postoperative pain, additional analgesics (mainly morphine) were used as rescue analgesics. The total sufentanil consumption, which included sufentanil from the patient-controlled intravenous analgesia (PCIA) pump and postoperative morphine use (converted into sufentanil), was recorded within the 12 h, 24 h, and 48 h post-surgical period and was normalized to patients' body weight. We also collected NRS scores and adverse reactions (nausea and vomiting) after the gastrectomy.

LC-MS/MS

Serum was obtained from patients 1h before the operation. LC-MS/MS analyses were performed using the UHPLC (Ultra High Performance Liquid Chromatography) system (Vanquish, Thermo Fisher Scientific) with a UHPLC BEH Amide column coupled to Q Exactive HFX mass spectrometer (Orbitrap MS, Thermo). Liquid chromatography phase A is the aqueous phase (25 mmol/L ammonium acetate and 25 mmol/L ammonia), and phase B is acetonitrile. The samples were at 4℃ throughout the analysis and were used in UHPLC-MS electrospray (ES)– and ES+ analyses. The spray Voltage of ES+ and ES- conditions were 3.6 kV (positive) or -3.2 kV (negative). QE HFX mass spectrometer was used to obtain MS/MS spectra (Xcalibur, Thermo). The MS resolution was set to 120000, and MS/MS resolution was set to 7500. The raw data were analyzed using ProteoWizard and annotated using the MS2 database.

SHC and SLC Grouping

The diagram shows the flow of patients through the study (Fig. 1). In total, 169 gastric cancer patients met the inclusion criteria, and 51 of these were subsequently excluded from the study. The remaining 118 patients included 80 men and 38 women, and total sufentanil consumption within 24 h was ranked separately by gender. Male and female patients with a total sufentanil consumption per kg in the lowest 30% of their respective gender were included in the sufentanil low consumption (SLC) group. Patients with a total sufentanil consumption per kg in the highest 30% were included in the sufentanil high consumption (SHC) group. The SHC and SLC groups contained 35 patients each, with 24 men and 11 women.

Genotyping

Genomic DNA was isolated from arterial blood samples by conventional phenol and chloroform extraction and amplified by polymerase chain reaction with the following primers: DAO rs10156191 (Forward primer: ATTCCATGGCCCTAACCT; Reverse primer: GGTGGTACTGGAGGGCTG), MAOB rs1799836 (Forward primer: TATACAAGTGTGCTCTTCTT; Reverse primer: ATGATTGGAACCTCTTATAC). The PCR products were separated on an agarose gel, purified, and the single nucleotide polymorphisms (SNPs) at each locus were identified by Sanger sequencing.

Univariate Statistics

Statistical analysis was performed by using Graph-

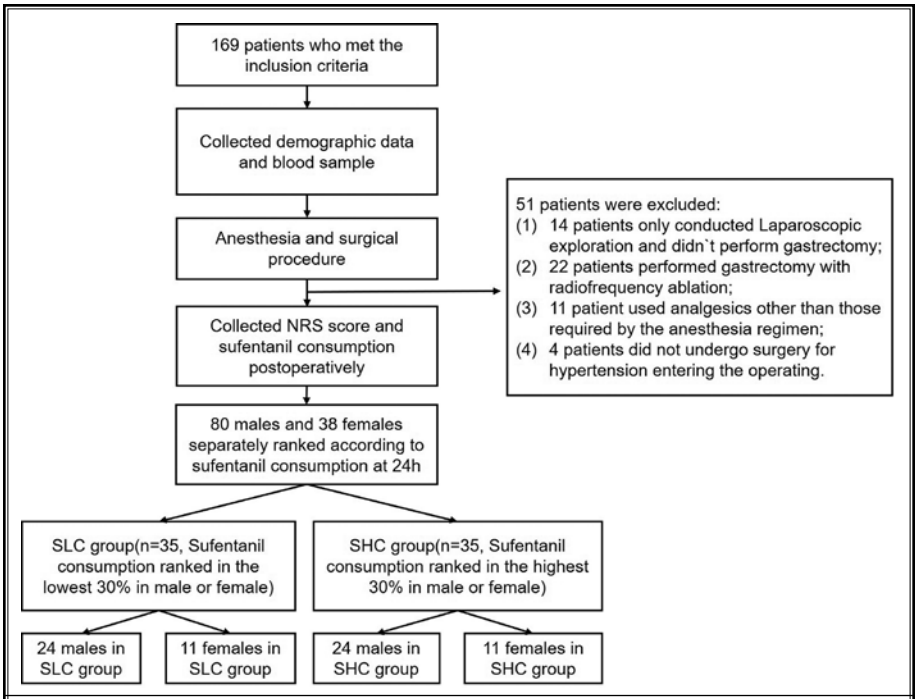

Fig. 1. *Consort diagram showing the study and patient flow.*

Pad Prism 7. A *P*-value < 0.05 was considered statistically significant in all analyses. Specifically, age, BMI, operative duration, the score of preoperative anxiety, and the score of preoperative depression were analyzed by unpaired t-test for the SLC and SHC groups. Education level and NRS score at 3 time points within the 2 groups were compared by the Mann-Whitney U test. Ethnicity (Han), the history of tobacco and alcohol use, the type and range of gastrectomy, adverse reactions (nausea and vomiting), and allele distribution were analyzed by Fisher`s exact test. Hardy–Weinberg equilibrium was evaluated by chi-square test. Welch`s t-test was performed to analyze the total postoperative sufentanil consumption.

Multivariate Statistics

For metabolomics data, metabolites were mapped through the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and the Human Metabolome Database (HMDB) in our study. After obtaining the sorted data, principal component analysis (PCA) and supervised orthogonal projections to latent structures-discriminate analysis (OPLS-DA) were conducted to visualize separation between the SLC and SHC groups. The value of variable importance in the projection (VIP) of the first principal component in OPLS-DA analysis was obtained, which showed the contribution of each variable to the model. The metabolites with VIP > 1 and *P* < 0.05 (student's t-test) were considered significantly changed metabolites between the SHC and SLC groups. A receiver-operating characteristic curve (ROC) was performed for all differential metabolites to examine their potential as prediction indexes. MetaboAnalyst 5.0 was applied to perform the pathway analysis (Fisher`s exact test) and ROC analysis. PCA and OPLS-DA were also carried out in the male and female groups. Differential metabolites in males or females were identified according to the screening criteria described above. The sample size was calculated by using the number of risk factors for postoperative opioids consumption. The sample size was equal to 10 to 20 times the number of variables according to an empirical formula of sample size estimation. Therefore, 169 patients completed our observation who met this sample criterion.

The multivariate linear regression analysis was performed for sufentanil consumption in the first postoperative 24 h to 70 patients of the SLC and SHC groups, with sufentanil usage doses as the dependent variable. Age, gender, BMI, type of procedure, range of gastrectomy, the relative content of imidazole-4-acetaldehyde, hydrogen phosphate, and methylimidazole acetaldehyde were included as independent variables in the model.

Results

Subject and Clinical Characteristics of the SHC and SLC Groups

One hundred sixty-nine patients were enrolled in this study, and 51 patients were removed due to the following reasons: 14 patients only conducted laparoscopic exploration; 22 patients performed gastrectomy with radiofrequency ablation; 11 patients used other analgesics, and 4 patients didn't undergo surgery. The remaining 118 patients were included in the further study (Fig. 1).

Subject and clinical characteristics of the SHC and SLC groups were statistically analyzed. There was no significant difference in demographic characteristics between the SHC and SLC groups, including age, BMI, ethnicity, education level, and history of tobacco and alcohol (Table 1). Clinical characteristics, which included type and range of gastrectomy and operative duration, were not significantly different (Table 1). The score of self-reported preoperative anxiety and depression in the 2 groups weren`t obviously different (Table 1, Supplementary Table 1).

Sufentanil Consumption and Pain Intensity in the SHC and SLC Groups

Within the 12 h, 24 h, and 48 h postoperative periods, the average sufentanil usage dose (normalized to body weight) was significantly different, with the SHC group consuming about twice as much as the SLC group (Table 2). Moreover, NRS scores of both groups averaged 2-3 within 12 h, 24 h, and 48 h after surgery and were not significantly different (Table 2). The adverse reactions (nausea and vomiting) were also not significantly different (Table 2).

Metabolic Profiles and Differential Metabolites of the SHC and SLC Groups

Since there are few studies focused on metabolites in the field of pain and opioids, we used UHPLC-MS/MS to maximally detect metabolites in the 2 groups. Through untargeted metabolomic analysis, we detected 3896 total features with positive-mode features or negative-mode features. Among them, 264 metabolites were accurately identified (Supplementary Table 2). An initial PCA analysis showed that the 2 groups were not separated (Fig. 2A). Next, by using supervised OPLS-DA, there was a clear separation between the SHC group and the SLC group (Fig. 2B). Differential metabolites using the OPLS-DA analysis model were filtered according to VIP > 1 and $P < 0.05$ (student`s t-test). A volcano plot showed up-regulated and down-regulated metabolites with significant differences (Fig. 2C). Among the identified metabolites, 35 were significantly different between the SHC and SLC groups, which are shown by heatmap (Fig. 2D). Differential metabolites mainly consisted of organic compounds (amino acids, amines, carbonyl compounds, pyrrolidones, and imidazoles) and lipids (glycerophosphocholines, phosphosphingolipids, and prenol lipids) (Tables 3, 4).

To explore which metabolites had potential as preoperative biomarkers of sufentanil consumption, we

Table 1. *Patient and clinical characteristics of study groups.*

Variables	SLC group (n = 35)	SHC group (n = 35)	*P*-value
Age (years)	58.34 ± 9.18	54.31 ± 12.73	0.134
BMI (kg/m²)	22.92 ± 3.54	23.45 ± 2.99	0.500
Ethnicity Han	31 (88.6%)	32 (91.4%)	> 0.999
Education level (years)	11 (9-11)	11 (9-16)	0.054
Tobacco use (n)	16 (45.7%)	18 (51.4%)	0.811
Alcohol use (n)	20 (57.1%)	20 (57.1%)	> 0.999
Preoperative anxiety score	4.91 ± 3.65	6.17 ± 3.61	0.152
Preoperative depression score	5.11 ± 3.83	5.40 ± 3.48	0.745
Type of procedure			0.230
Laparoscopic gastrectomy (n)	22 (62.9%)	16 (45.7%)	
Open gastrectomy (n)	13 (37.1%)	19 (54.3%)	
Range of gastrectomy			0.145
Total gastrectomy (n)	11 (31.4%)	18 (51.4%)	
Distal or proximal gastrectomy (n)	24 (68.6%)	17 (48.6%)	
Operative duration (min)	221.3 ± 42.21	215.9 ± 58.53	0.664

Continuous variables are presented as mean ± SD; median (interquartile range), categorical variables are presented as No. (%).
P values were calculated by t-test, Mann-Whitney U test, and Fisher`s exact test based on the data type.
BMI: body mass index.

Table 2. *Postoperative sufentanil consumption, pain intensity, and case of nausea or vomiting.*

Variables	SLC group (n = 35)	SHC group (n = 35)	*P*-value
Sufentanil usage doses-12h(μg/kg)	0.49 ± 0.16	1.07 ± 0.37	< 0.0001
Sufentanil usage doses-24h(μg/kg)	0.76 ± 0.19	1.86 ± 0.47	< 0.0001
Sufentanil usage doses-48h(μg/kg)	1.34 ± 0.41	2.77 ± 0.73	< 0.0001
NRS score-12h	3 (2-3)	3 (3-4)	0.210
NRS score-24h	2 (2-3)	3 (2-3)	0.163
NRS score-48h	2 (1-2)	2 (1-3)	0.548
Nausea or vomiting-12h	5 (14.3%)	5 (14.3%)	> 0.999
Nausea or vomiting-24h	6 (17.1%)	3 (8.6%)	0.477
Nausea or vomiting-48h	3 (8.6%)	4 (11.4%)	> 0.999

Variables are presented as mean ± SD, median (interquartile range), and No. (%).
P-values were calculated by t-test, Mann-Whitney U test, and Fisher`s exact test based on the data type.
NRS: Numeric rating scale.

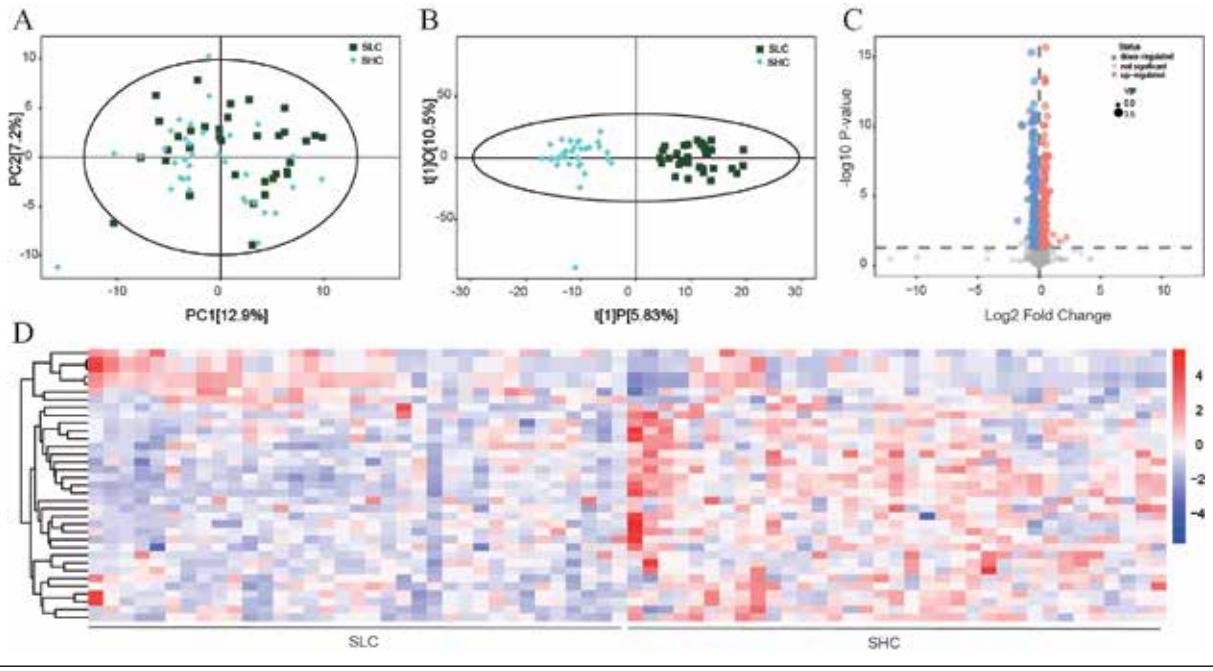

Fig. 2. *Serum metabolic profiling in SLC and SHC groups.* A. *Principal component analysis (PCA).* B. *The partial least squares discriminant analysis (OPLS-DA).* C. *Volcano plot of differential metabolites in both groups.* D. *Hierarchical clustering analysis of differential metabolites in both groups.*

performed ROC analysis of the 35 differential metabolites. The results suggested that hydrogen phosphate, which had the highest area under the curve (AUC) value of 0.98, had potential as a predictive biomarker (Fig. 3). ROC analyses of other metabolites are presented in Supplementary Fig. 1.

Metabolic Profiles of the Male and Female Subgroups Within the SHC and SLC Groups

Postoperative sufentanil consumption was ranked separately by gender, and we explored differential metabolites within the male and female subgroups. The metabolic profiles of 24 male patients in the SHC and SLC groups were analyzed by PCA and OPLS-DA (Supplementary Fig. 2A, 2B), which revealed a clear separation. Meanwhile, the metabolic profiles of 11 female patients were also analyzed by PCA and OPLS-DA (Supplementary Fig. 2C, 2D). The result revealed that 34 metabolites in the male subgroup and 16 metabolites in the female subgroup were significantly different between the SHC and SLC groups by using the OPLS-DA model (Fig. 4A, 4B). The relation of metabolites is shown by chord diagram. There were 8 differential metabolites common to both genders, while the remaining differential metabolites were gender-specific (Fig. 4C). Compared to the female subgroup, differential metabolites in the male subgroup were mainly glycerophosphocholines (PC) which are involved in the lipid metabolism pathway.

Metabolic Pathway Analysis of the SHC and SLC Groups

Using KEGG and MetaboAnalyst 5.0, 5 metabolic pathways were associated with differential metabolites, including histidine metabolism, pentose and glucuronate interconversions, glycerophospholipid metabolism, cysteine and methionine metabolism, and propanoate metabolism (impact factor > 0.04) (Fig. 5A). However, only histidine metabolism was significantly different between the SLC and SHC groups ($P < 0.05$). Furthermore, 2 differential metabolites in the histidine metabolism pathway participate in histamine degradation. We found that imidazole-4-acetaldehyde was down-regulated, and methylimidazole acetaldehyde was up-regulated in the SlC group, relative to the SLC group (Fig. 5B).

Allele Frequencies of DAO rs1799836 and MAOB rs1799836 in 2 Groups

Diamine oxidase (DAO) and monoamine oxidase (MAO) are key enzymes in the production of imidazole-

Table 3. *Differential metabolites between SLC and SHC groups from OPLS-DA modeling (organic compounds).*

Identified metabolite	Subclass	VIP	*P* value	Fold change (SLC/SHC)
N-Methyl-a-aminoisobutyric acid	Amino acids and analogs	2.00	3.13E-05	1.25
4-Guanidinobutanoic acid	Amino acids and analogs	1.68	1.38E-03	0.83
Thiomorpholine 3-carboxylate	Amino acids and analogs	1.34	4.02E-03	0.83
D-Alanine	Amino acids and analogs	1.29	3.14E-02	0.87
Tryptophan	Amino acids and analogs	1.22	3.44E-02	1.46
1-Butylamine	Amines	1.90	6.82E-05	0.86
Isobutylpropylamine	Amines	1.38	4.63E-02	0.87
2-Methylbutylamine	Amines	2.80	1.54E-08	0.79
Rhamnose	Carbohydrates and carbohydrate conjugates	1.27	7.47E-03	1.33
D-Xylulose	Carbohydrates and carbohydrate conjugates	1.23	2.04E-02	0.86
5-Aminopentanal	Carbonyl compounds	1.15	1.06E-02	0.77
6-Methyl-3,5-heptadien-2-one	carbonyl compounds	1.53	2.57E-03	0.60
Pyrrolidine	Pyrrolidones	2.07	1.24E-05	1.29
N-Nitroso-pyrrolidine	Pyrrolidines	1.42	7.52E-03	0.67
Methylimidazole acetaldehyde	Imidazole	1.29	2.77E-02	1.26
Imidazole-4-acetaldehyde	Imidazole	1.16	1.09E-03	0.83
Nervonyl carnitine	Quaternary ammonium salts	1.96	3.40E-04	0.79
Dimethyl dialkyl ammonium chloride	Quaternary ammonium salts	1.73	1.02E-03	0.88
2-Ketobutyric acid	Keto acids and derivatives	1.38	1.88E-02	0.90
N-Nitrosodimethylamine	Organic nitroso compounds	2.34	2.69E-05	0.75
2,5-Dichloro-carboxymethylenebut-2-en-4-olide	Furanones	2.66	3.00E-09	0.80
6-Hydroxy-1H-indole-3-acetamide	Hydroxyindoles	2.37	2.33E-07	0.79
L-Gulonolactone	Gamma butyrolactones	1.25	1.64E-02	0.86
6-Chloro-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine	Aminotriazines	1.24	4.38E-02	1.44

Table 4. *Differential metabolites between the SLC group and SHC group from OPLS-DA modeling (other metabolism).*

Identified metabolite	Subclass	VIP	*P* value	Fold change (SLC/SHC)
Phthalic acid	Benzenoids	3.37	5.73E-14	0.76
Ethylbenzene	Benzenoids	1.58	7.20E-03	0.77
Hydrogen phosphate	phosphates	3.38	2.47E-12	0.74
PC(P-18:1(11Z)/22:4 (7Z,10Z,13Z,16Z))	Glycerophosphocholines	1.67	7.92E-04	0.78
PC(22:5(7Z,10Z,13Z,16Z,19Z)/18:2(9Z,12Z))	Glycerophosphocholines	1.44	3.17E-03	0.75
3-Hydroxymethylglutaric acid	Glycerophosphocholines	1.33	1.76E-02	0.88
PC(P-18:0/22:4 (7Z,10Z,13Z,16Z))	Glycerophosphocholines	1.19	2.74E-03	0.83
PC(P-18:1(11Z)/20:2 (11Z,14Z))	Glycerophosphocholines	1.17	3.58E-03	0.80
SM(d18:1/24:1(15Z))	Phosphosphingolipids	1.26	2.14E-02	0.89
Perillic acid	Prenol lipids	1.12	2.78E-02	0.72
Tetrahydroaldosterone-3-glucuronide	Steroidal glycosides	1.01	4.98E-02	1.16

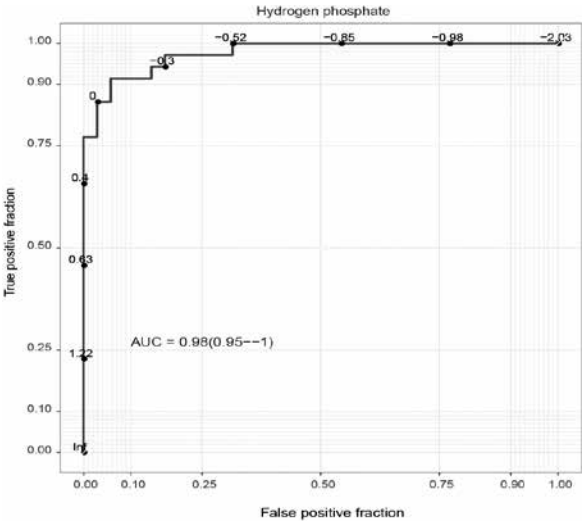

Fig. 3. *ROC curve of hydrogen phosphate for SLC group and SHC group. ROC: receiver-operating characteristic.*

4-acetaldehyde and methylimidazole acetaldehyde. Gene polymorphisms at DAO rs10156191 and MAOB rs1799836 are associated with enzyme activity (16-17). Based on the literature, we analyzed the specific allele distribution of SNPs in the SLC and SHC groups. Since MAOB is located on the X chromosome, we analyzed this data independent of gender. The allele frequencies of DAO rs10156191 and MAOB rs1799836 in the 2 groups were in Hardy–Weinberg equilibrium. There was no significant difference in the allele frequency of either SNPs in men or women for the SLC and SHC groups (Tables 5, 6).

Multivariate Analyses of Postoperative Sufentanil Consumption

The results of multivariate linear regression analyses indicated that BMI (Beta = -0.171, $P = 0.020$), gender (Beta = 0.258, $P = 0.001$), range of gastrectomy (Beta = 0.127, $P = 0.075$), the relative content of imidazole-4-acetaldehyde (Beta = -0.238, $P = 0.017$) and hydrogen phosphate (Beta =

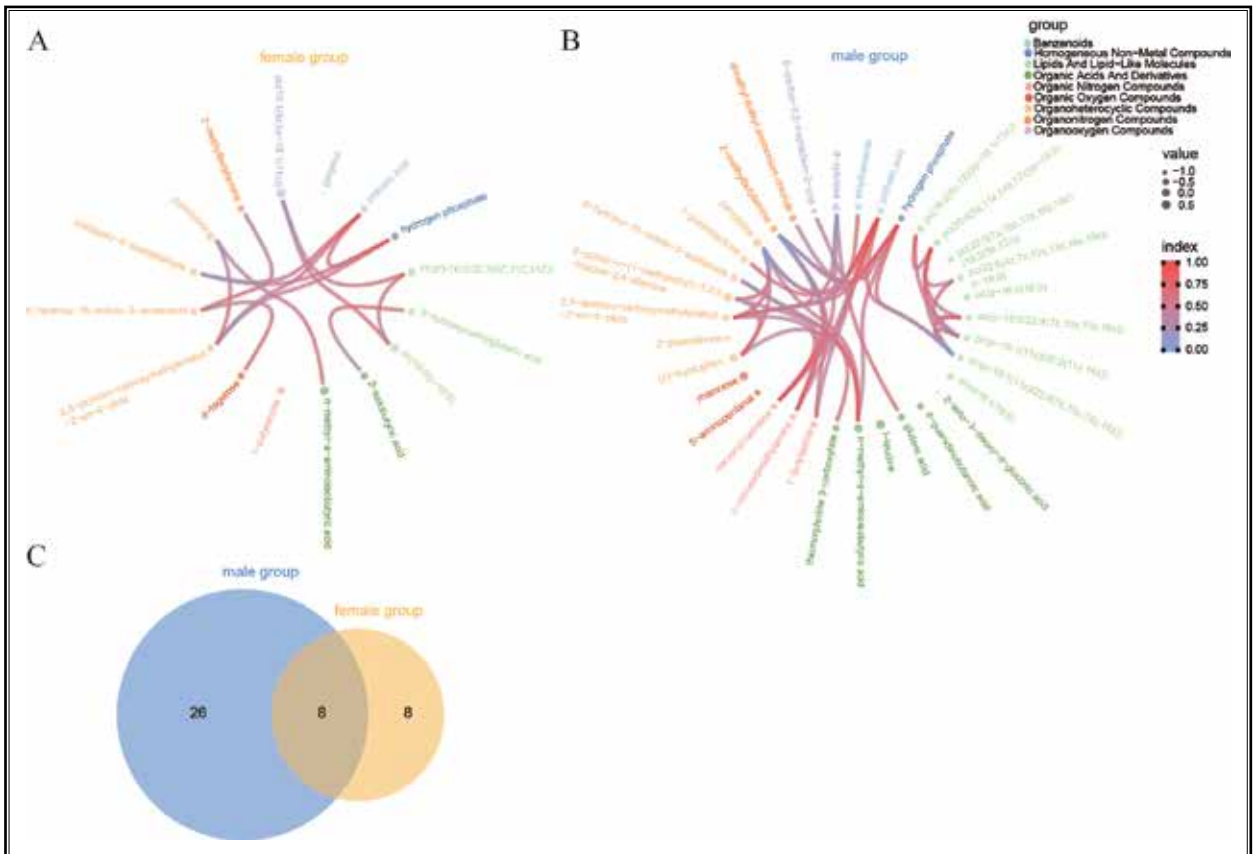

Fig. 4. *Differential metabolites between the male and female subgroups of the SLC and SHC groups. A. Chord plot analysis of different metabolites in the male group. B. Chord plot analysis of different metabolites in the female group. C. Venn diagram showing the different metabolites in males and females.*

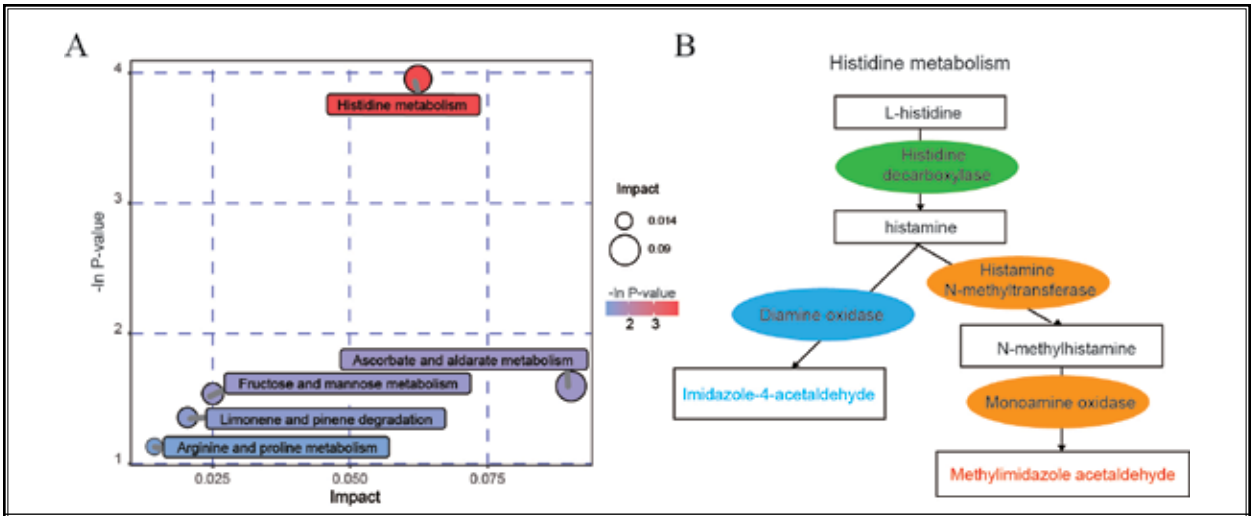

Fig. 5. *Pathway analysis of serum metabolites for SLC and SHC groups.* A. *Matched metabolic pathways by MetaboAnalyst 5.0 (impact factor > 0.04):(1) Histidine metabolism; (2) Pentose and glucuronate interconversions; (3) Glycerophospholipid metabolism; (4) Cysteine and methionine metabolism; (5) Propanoate metabolism.* B. *Diagram of histidine metabolic pathway showing imidazole-4-acetaldehyde and methylimidazole acetaldehyde. Red text indicates metabolite was up-regulated in the SLC group. Blue text indicates metabolite was down-regulated in the SLC group.*

Table 5. *Allele frequencies of DAO rs1799836 in 2 groups.*

	DAO rs10156191	SLC		SHC		OR	95% CI	*P*
		No.	%	No.	%			
male	C	41	85.42	41	85.42	1	0.34 -2.89	> 0.99
	T	7	14.58	7	14.58	1	0.34 -2.89	
female	C	20	90.91	18	81.82	2.22	0.46-12.54	0.66
	T	2	9.09	4	18.18	0.45	0.08-2.18	

P-values were calculated by Fisher`s exact test.

Table 6. *Allele frequencies of MAOB rs1799836 in 2 groups.*

	MAOB rs1799836	SLC		SHC		OR	95% CI	*P*
		No.	%	No.	%			
male	C	8	16.67	6	12.50	1.4	0.46-4.61	0.77
	T	40	83.33	42	87.50	0.71	0.22- 2.2	
female	C	6	27.27	4	18.18	1.69	0.46-5.99	0.72
	T	16	72.73	18	81.82	0.59	0.17-2.19	

P-values were calculated by Fisher`s exact test.

0.902, $P < 0.001$) were associated sufentanil consumption in the first postoperative 24 h (Table 7).

Discussion

To explore whether metabolites can be used as biomarkers to predict postoperative opioid consumption, the total sufentanil consumption of 80 men and 38 women involved in our analysis were separately ranked. They were selected and classified into the SLC and SHC groups, which both contained 24 men and 11 women. Though our results indicated that BMI, gender, and range of gastrectomy were associated with sufentanil usage doses in the first postoperative 24 h, there were no significant differences in subject characteristics, clinical characteristics, or emotion between the SLC and SHC groups (Tables 1, 7). NRS scores and

Table 7. *Multivariate Linear Regression of risk factors for sufentanil usage doses in the first postoperative 24h.*

Variables	Beta	*P*
BMI	-0.171	0.020
Gender	0.258	0.001
Range of gastrectomy	0.127	0.075
Imidazole-4-acetaldehyde	-0.238	0.017
Hydrogen phosphate	0.902	< 0.001

adverse reactions (nausea and vomiting) were also not obviously different (Table 2). The SHC group used about 2-fold more sufentanil than the SLC group after surgery, which revealed 2 extreme phenotype groups for further research.

In this study, the SHC and SLC groups were separated by OPLA-DA analysis, although they were not distinguishable using a PCA model (Fig. 2A, 2B). We found 35 metabolites that were significantly different between the 2 groups with the criterion of VIP > 1 and $P < 0.05$. Most of these were organic compounds and lipid compounds (Tables 3, 4). Among differential metabolites, tryptophan was decreased in the SHC group (Table 3). As the precursor of serotonin, which is associated with pain intensity, tryptophan has been reported to possess analgesic properties (18). Besides, the content of phosphatidylcholine was significantly different in the 2 extreme groups, especially in the male subgroup. Four phosphatidylcholines were increased in the SHC group, which contain PC(P-18:1(11Z)/22:4 (7Z,10Z,13Z,16Z)), PC (22:5(7Z,10Z,13Z,16Z,19Z)/ 18:2 (9Z,12Z)), PC (P-18:0/22:4 (7Z, 10Z, 13Z, 16Z)) and PC(P-18:1(11Z)/20:2 (11Z, 14Z)) (Table 4). Previous research has shown that opioids affect lipid chain length and saturation of phosphatidylcholine, and in turn, phosphatidylcholine can also modulate the μ opioid receptor(19-20). This suggests that these differential phosphatidylcholine metabolites may affect postoperative opioid consumption through the regulation of the μ opioid receptor.

We further analyzed the ROC curves of all differential metabolites and found that hydrogen phosphate had the highest AUC value (0.98), indicating that it might have the potential to predict postoperative sufentanil usage, which was also confirmed by multivariate analyses of sufentanil consumption (Fig. 3, Table 7). The balance of hydrogen phosphate involves sodium/phosphate cotransporters (solute carrier family 17, 20, 34), and as a signaling molecule, it may induce the Akt and Raf/MEK/ERK pathways to regulate gene expression(21). In the gender-specific OPLS-DA model, we analyzed differential metabolites in the male or female subgroups (Supplementary Fig. 2). A considerable number of differential metabolites were only present in males or females (Fig. 4). For example, 8 phosphatidylcholines were significantly different in the male subgroup, while only 3 were significant in the female subgroup. It has been reported that gender differences affect postoperative pain control and opioid consumption (5). For this study, gender-dependent metabolites may reflect their postoperative phenotype considering the pain.

Histidine metabolism was the most significantly different pathway between the SHC and SLC groups (Fig. 5). The relative content of imidazole-4-acetaldehyde and methylimidazole acetaldehyde, which are metabolites of histamine degradation, showed a different trend. Imidazole-4-acetaldehyde was downregulated, and methylimidazole acetaldehyde was upregulated in the SLC group, relative to the SLC group (Fig. 5B). Histamine is synthesized from L-histidine, and its degradation is dependent on 2 pathways: the histamine-N-methyltransferase (HNMT)-mediated pathway (50-80%) and the diamine oxidase (DAO)-mediated pathway (15-30%) (22). DAO is responsible for histamine degradation in the small intestine mucosa, the skin, and the liver (23).Histamine and histamine receptors regulate multiple physiological activities, including pain (22). Histamine acts in an antinociceptive manner in the central nervous system but acts in a nociceptive manner in the peripheral nervous system (24). Previous studies assess postoperative pain by using the NRS score. In our study, we carried out suitable preoperative analgesic preaching and enough analgesic dosage after surgery to make the NRS score less than or equal to 3. Thus, postoperative analgesics consumption could indirectly reflect postoperative pain intensity.

The differential content of imidazole-4-acetaldehyde and methylimidazole acetaldehyde in the extreme phenotype groups suggested that differential efficiencies in these 2 histamine degradation pathways may exist and that DAO and MAOB, which are directly responsible for the production of these metabolites, might have a different distribution of SNP allele frequencies. DAO rs10156191 is associated with DAO activity and has a high risk for migraines (16, 25). MAOB rs1799836 is associated with MAOB enzyme activity, and high-intensity postoperative pain was reported in males with the G allele (26). However, our results indicate that the distribution of allele frequencies of DAO rs10156191 and MAOB rs1799836 in the SHC and SLC groups were not different in either men or women.

This may be due to an insufficient number of people in the 2 groups.

Limitations

There are some limitations to our study. First, though 169 patients met the inclusion criterion, almost 30% of these patients were excluded due to inconsistent perioperative factors related to surgery or anesthesia. Then, we did not collect samples throughout the entire process of pre-operation, peri-operation, and post-operation, which may reveal more dynamic changes. Lastly, our study was restricted to gastric cancer patients, which may limit our findings. Larger and multiple cohorts of patients should be included in future studies to confirm the biomarkers reported here, as well as the relationship between histamine degradation and acute pain.

Conclusions

In this exploratory study, we identified gender-dependent systemic differential serum profiles between the sufentanil high consumption and sufentanil low consumption groups prior to surgery. Hydrogen phosphate acted as the metabolite with the highest probability to predict postoperative sufentanil consumption. Differential efficiencies in histamine degradation may partially explain pain intensity after surgery, although the distribution of DAO rs10156191 and MAOB rs1799836 allele frequencies were not different. Together these findings suggest that histamine degradation is associated with pain intensity, and these metabolites may be useful to predict postoperative phenotypes of patients before surgery.

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Authors' Contributions

JNL designed the study, collected samples, analyzed clinical and metabolic data, and wrote the manuscript. SL implemented anesthesia with the help of LY, JW, and HWS. CXY assisted with the collection of data. LY revised the manuscript. HYT guided the conduct of this study. All authors read and approved the final manuscript.

References

1. Wu CL, Raja SN. Treatment of acute postoperative pain. *Lancet* 2011; 377:2215-2225.
2. Colvin LA, Bull F, Hales TG. Perioperative opioid analgesia-when is enough too much? A review of opioid-induced tolerance and hyperalgesia. *Lancet* 2019; 393:1558-1568.
3. Ramaswami R, Bayer R, Galea S. Precision medicine from a public health perspective. *Annu Rev Public Health* 2018; 39:153-168.
4. Ip HY, Abrishami A, Peng PW, Chung F. Predictors of postoperative pain and analgesic consumption: A qualitative systematic review. *Anesthesiology* 2009; 111:657-677.
5. Yang MMH, Hartley RL, Leung AA, et al. Preoperative predictors of poor acute postoperative pain control: A systematic review and meta-analysis. *BMJ Open* 2019; 9:e025091.
6. Cajanus K, Holmström EJ, Wessman M, Anttila V, Kaunisto MA, Kalso E. Effect of endocannabinoid degradation on pain: Role of FAAH polymorphisms in experimental and postoperative pain in women treated for breast cancer. *Pain* 2016; 157:361-369.
7. Hwang IC, Park JY, Myung SK, Ahn HY, Fukuda K, Liao Q. OPRM1 A118G gene variant and postoperative opioid requirement: A systematic review and meta-analysis. *Anesthesiology* 2014; 121:825-834.
8. Belfer I, Segall S. COMT genetic variants and pain. *Drugs Today (Barc)* 2011; 47:457-467.
9. Kolesnikov Y, Gabovits B, Levin A, Voiko E, Veske A. Combined catechol-O-methyltransferase and mu-opioid receptor gene polymorphisms affect morphine postoperative analgesia and central side effects. *Anesth Analg* 2011; 112:448-453.
10. Carneiro G, Radcenco AL, Evaristo J, Monnerat G. Novel strategies for clinical investigation and biomarker discovery: A guide to applied metabolomics. *Horm Mol Biol Clin Investig* 2019; 38.
11. Zhai G, Randell EW, Rahman P. Metabolomics of osteoarthritis: Emerging novel markers and their potential clinical utility. *Rheumatology (Oxford)* 2018; 57:2087-2095.
12. Lunde S, Nguyen HT, Petersen KK, Arendt-Nielsen L, Krarup HB, Søgaard-Andersen E. Chronic postoperative pain after hysterectomy for endometrial cancer: A metabolic profiling study. *Mol Pain* 2020; 16:1744806920923885.
13. Hadrévi J, Björklund M, Kosek E, et al. Systemic differences in serum metabolome: A cross sectional comparison of women with localised and widespread pain and controls. *Sci Rep* 2015 ;5:15925.
14. Liu M, Xie Z, Costello CA, et al. Metabolomic analysis coupled with extreme phenotype sampling identified that lysophosphatidylcholines are associated with multisite musculoskeletal pain. *Pain* 2021; 162:600-608.
15. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. *J*

Psychosom Res 2002; 52:69-77.

16. Maintz L, Yu CF, Rodriguez E, et al. Association of single nucleotide polymorphisms in the diamine oxidase gene with diamine oxidase serum activities. *Allergy* 2011; 66:893-902.
17. Tunbridge EM, Narajos M, Harrison CH, Beresford C, Cipriani A, Harrison PJ. Which dopamine polymorphisms are functional? Systematic review and meta-analysis of COMT, DAT, DBH, DDC, DRD1-5, MAOA, MAOB, TH, VMAT1, and VMAT2. *Biol Psychiatry* 2019; 86:608-620.
18. Heyliger SO, Goodman CB, Ngong JM, Soliman K. The analgesic effects of tryptophan and its metabolites in the rat. *Pharmacol Res* 1998; 38:243-250.
19. Faulkner C, Santos-Carballal D, Plant DF, de Leeuw NH. Atomistic molecular dynamics simulations of propofol and fentanyl in phosphatidylcholine lipid bilayers. *ACS Omega* 2020; 5:14340-14353.
20. Angladon MA, Fossépré M, Leherte L, Vercauteren DP. Interaction of POPC, DPPC, and POPE with the mu opioid receptor: A coarse-grained molecular dynamics study. *PLoS One* 2019; 14:e0213646.
21. Michigami T, Kawai M, Yamazaki M, Ozono K. Phosphate as a signaling molecule and its sensing mechanism. *Physiol Rev* 2018; 98:2317-2348.
22. Lieberman P. The basics of histamine biology. *Ann Allergy Asthma Immunol* 2011; 106:S2-S5.
23. Patel RH, Mohiuddin SS. Biochemistry, Histamine. In: StatPearls. StatPearls Publishing,Treasure Island, FL, 2021.
24. Khalilzadeh E, Azarpey F, Hazrati R, Vafaei Saiah G. Evaluation of different classes of histamine H1 and H2 receptor antagonist effects on neuropathic nociceptive behavior following tibial nerve transection in rats. *Eur J Pharmacol* 2018; 834:221-229.
25. García-Martín E, Martinez C, Serrador M, et al. Diamine oxidase rs10156191 and rs2052129 variants are associated with the risk for migraine. *Headache* 2015; 55:276-286.
26. Serý O, Hrazdilová O, Didden W, et al. The association of monoamine oxidase B functional polymorphism with postoperative pain intensity. *Neuro Endocrinol Lett* 2006; 27:333-337.

Supplementary Table 1. *Preoperative anxiety and depression score.*

	Preoperative anxiety score		total	Preoperative depression score		total
	SLC group	SHC group		SLC group	SHC group	
0-7	27(77.2%)	23(65.7%)	50	27(77.1%)	27(77.1%)	54
8-10	6(17.1%)	7(20.0%)	13	5(14.3%)	5(14.3%)	10
11-14	2(5.7%)	5(14.3%)	7	2(5.7%)	3(8.6%)	5
15-21	0(0.0%)	0(0.0%)	0	1(2.9%)	0(0.0%)	1

Categorical variables are presented as No. (%).
Scores less than 7, between 8 –10, between 11–14, and greater than 15 indicate normal, mild, moderate, and severe states, respectively.

Supplementary Table 2. *The list of identified metabolites*.

No	Metabolites	Class
1	Vanillic acid	Benzene and substituted derivatives
2	Valyl-Leucine	Carboxylic acids and derivatives
3	Uridine	Pyrimidine nucleosides
4	Tromethamine	Organic nitrogen compounds
5	Trimethylaminoacetone	Organooxygen compounds
6	Trimethylamine N-oxide	Organonitrogen compounds
7	Traumatic acid	Fatty Acyls
8	trans-Hexadec-2-enoyl carnitine	Fatty Acyls
9	Thiomorpholine 3-carboxylate	Carboxylic acids and derivatives
10	Tetrahydroaldosterone-3-glucuronide	Steroids and steroid derivatives
11	Tauroursodeoxycholic acid	Steroids and steroid derivatives
12	Taurocholic acid	Steroids and steroid derivatives
13	Succinic anhydride	Oxolanes
14	Succinic acid semialdehyde	Fatty Acyls
15	SM(d18:1/24:1(15Z))	Sphingolipids
16	SM(d18:1/16:0)	Sphingolipids
17	SM(d16:1/24:1(15Z))	Sphingolipids
18	Sepiapterin	Pteridines and derivatives
19	Saccharin	Benzothiazoles
20	Ribothymidine	Pyrimidine nucleosides
21	Rhamnose	Organooxygen compounds
22	Pyruvic acid	Keto acids and derivatives
23	Pyrrolidonecarboxylic acid	Carboxylic acids and derivatives
24	Pyrrolidine	Pyrrolidines
25	Pyroglutamic acid	Carboxylic acids and derivatives
26	Pyrimidine	Diazines
27	Prostaglandin D2	Fatty Acyls
28	Proline betaine	Carboxylic acids and derivatives
29	Piperidine	Piperidines
30	Pipecolic acid	Carboxylic acids and derivatives
31	Phytosphingosine	Organonitrogen compounds
32	Phthalic acid	Benzene and substituted derivatives
33	Phenylpyruvic acid	Benzene and substituted derivatives
34	Phenyllactic acid	Phenylpropanoic acids

Supplementary Table 2 (cont.). ***The list of identified metabolites***.

35	Phenylalanyl-Tryptophan	Carboxylic acids and derivatives
36	Phenylalanyl-Serine	Carboxylic acids and derivatives
37	Perillic acid	Prenol lipids
38	Pelargonic acid	Fatty Acyls
39	PC(P-18:1(11Z)/22:4(7Z,10Z,13Z,16Z))	Glycerophospholipids
40	PC(P-18:1(11Z)/20:2(11Z,14Z))	Glycerophospholipids
41	PC(P-18:1(11Z)/16:0)	Glycerophospholipids
42	PC(P-18:0/22:4(7Z,10Z,13Z,16Z))	Glycerophospholipids
43	PC(P-16:0/16:0)	Glycerophospholipids
44	PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/P-18:0)	Glycerophospholipids
45	PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/20:1(11Z))	Glycerophospholipids
46	PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/18:0)	Glycerophospholipids
47	PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/16:0)	Glycerophospholipids
48	PC(22:5(7Z,10Z,13Z,16Z,19Z)/18:2(9Z,12Z))	Glycerophospholipids
49	PC(22:5(4Z,7Z,10Z,13Z,16Z)/P-18:0)	Glycerophospholipids
50	PC(22:5(4Z,7Z,10Z,13Z,16Z)/14:0)	Glycerophospholipids
51	PC(22:4(7Z,10Z,13Z,16Z)/P-16:0)	Glycerophospholipids
52	PC(22:2(13Z,16Z)/14:1(9Z))	Glycerophospholipids
53	PC(20:4(8Z,11Z,14Z,17Z)/P-18:0)	Glycerophospholipids
54	PC(20:2(11Z,14Z)/15:0)	Glycerophospholipids
55	PC(18:4(6Z,9Z,12Z,15Z)/15:0)	Glycerophospholipids
56	PC(18:3(6Z,9Z,12Z)/18:1(11Z))	Glycerophospholipids
57	PC(18:2(9Z,12Z)/P-18:1(11Z))	Glycerophospholipids
58	PC(18:2(9Z,12Z)/14:0)	Glycerophospholipids
59	PC(18:0/P-16:0)	Glycerophospholipids
60	PC(16:1(9Z)/P-18:1(11Z))	Glycerophospholipids
61	PC(16:1(9Z)/16:1(9Z))	Glycerophospholipids
62	Pantothenol	Fatty Acyls
63	Palmitoleic acid	Fatty Acyls
64	Paliperidone	Pyridopyrimidines
65	Oleic acid	Fatty Acyls
66	Octadecylamine	Amines
67	Nicotine	Pyridines and derivatives
68	Nervonyl carnitine	Organonitrogen compounds
69	N4-Acetylcytidine	Pyrimidine nucleosides
70	N2,N2-Dimethylguanosine	Purine nucleosides
71	N1-Methyl-2-pyridone-5-carboxamide	Pyridines and derivatives
72	N-Nitrosodimethylamine	Organonitrogen compounds
73	N-Nitroso-pyrrolidine	Pyrrolidines
74	N-methylvaline	Carboxylic acids and derivatives
75	N-Methylnicotinium	Pyridines and derivatives
76	N-Methylnicotinamide	Pyridines and derivatives
77	N-Methylhydantoin	Azolines
78	N-Methyl-a-aminoisobutyric acid	Carboxylic acids and derivatives
79	N-Cyclopropyl-trans-2-cis-6-nonadienamide	Fatty Acyls

Supplementary Table 2 (cont.). ***The list of identified metabolites***.

80	N-Acetylmuramoyl-Ala	Organooxygen compounds
81	N-Acetyl-L-alanine	Carboxylic acids and derivatives
82	Morpholine	Oxazinanes
83	Monoethylglycinexylidide	Carboxylic acids and derivatives
84	Methylsuccinic acid	Fatty Acyls
85	Methylimidazole acetaldehyde	Azoles
86	Methylgingerol	Benzene and substituted derivatives
87	Methyl jasmonate	Fatty Acyls
88	Methyl (2R*,3S*)-2,3-dihydro-3-hydroxy-2-isopropenyl-5-benzofurancarboxylate	Coumarans
89	Maslinic acid	Prenol lipids
90	Mannitol	Organooxygen compounds
91	Mangiferdesmethylursanone	Organooxygen compounds
92	m-Coumaric acid	Cinnamic acids and derivatives
93	LysoPE(18:1(9Z)/0:0)	Glycerophospholipids
94	LysoPC(P-18:1(9Z))	Glycerophospholipids
95	LysoPC(16:0)	Glycerophospholipids
96	Linoelaidic acid	Fatty Acyls
97	Lepidiumterpenoid	Prenol lipids
98	L-Valine	Carboxylic acids and derivatives
99	L-Proline	Carboxylic acids and derivatives
100	L-Phenylalanine	Carboxylic acids and derivatives
101	L-Norleucine	Carboxylic acids and derivatives
102	L-Methionine	Carboxylic acids and derivatives
103	L-Leucine	Carboxylic acids and derivatives
104	L-Homoserine	Carboxylic acids and derivatives
105	L-Hexanoylcarnitine	Fatty Acyls
106	L-Gulonolactone	Lactones
107	L-Erythrulose	Organooxygen compounds
108	L-cis-3-Amino-2-pyrrolidinecarboxylic acid	Carboxylic acids and derivatives
109	L-Carnitine	Organonitrogen compounds
110	L-Arginine	Carboxylic acids and derivatives
111	L-Acetylcarnitine	Fatty Acyls
112	Kynurenic acid	Quinolines and derivatives
113	Kanzonol I	Isoflavonoids
114	Isopentyl mercaptan	Thiols
115	Isopalmitic acid	Fatty Acyls
116	Isoleucyl-Alanine	Carboxylic acids and derivatives
117	Isokobusone	Organooxygen compounds
118	Isobutyric acid	Carboxylic acids and derivatives
119	Isobutylpropylamine	Organonitrogen compounds
120	Inosine	Purine nucleosides
121	Indoxyl sulfate	Organic sulfuric acids and derivatives
122	Indolelactic acid	Indoles and derivatives
123	Indoleacrylic acid	Indoles and derivatives
124	Indoleacetic acid	Indoles and derivatives

Supplementary Table 2 (cont.). ***The list of identified metabolites***.

125	Indole-5,6-quinone	Indoles and derivatives
126	Indole-3-propionic acid	Indoles and derivatives
127	Indole-3-carbinol	Indoles and derivatives
128	Imidazole-4-acetaldehyde	Azoles
129	Hypoxanthine	Imidazopyrimidines
130	Hypogeic acid	Fatty Acyls
131	Hydrogen phosphate	Non-metal oxoanionic compounds
132	Homocysteine thiolactone	Carboxylic acids and derivatives
133	Histidinyl-Leucine	Carboxylic acids and derivatives
134	Hexylresorcinol	Benzene and substituted derivatives
135	Hexadecanedioic acid	Fatty Acyls
136	Guanosine	Purine nucleosides
137	Guanine	Imidazopyrimidines
138	Guanidoacetic acid	Carboxylic acids and derivatives
139	Glycinexylidide	Benzene and substituted derivatives
140	Glutaric acid	Carboxylic acids and derivatives
141	Gingerol	Phenols
142	Geranic acid	Prenol lipids
143	Furcelleran	Cinnamic acids and derivatives
144	Furanone A	Dihydrofurans
145	Eupatilin	Flavonoids
146	Ethylbenzene	Benzene and substituted derivatives
147	Ethyl dodecanoate	Fatty Acyls
148	Erythrono-1,4-lactone	Lactones
149	Ecgonine	Tropane alkaloids
150	Dodecanoic acid	Fatty Acyls
151	Dimethyl dialkyl ammonium chloride	Quaternary ammonium salts
152	Dihydrothymine	Diazines
153	Dihydrolipoate	Fatty Acyls
154	Dihydrojasmonic acid	Lineolic acids and derivatives
155	Diethanolamine	Organonitrogen compounds
156	Desloratadine	Benzocycloheptapyridines
157	Deoxyuridine	Pyrimidine nucleosides
158	Deoxyribose 5-phosphate	Organooxygen compounds
159	Deoxyguanosine	Purine nucleosides
160	Deoxycholic acid	Steroids and steroid derivatives
161	Demethylated antipyrine	Azoles
162	Dehydroascorbic acid	Lactones
163	D-Xylulose	Carbohydrates and carbohydrate conjugates
164	D-Tagatose	Organooxygen compounds
165	D-Ribose	Organooxygen compounds
166	D-Fructosazine	Diazines
167	D-Alanine	Carboxylic acids and derivatives
168	Creatine	Carboxylic acids and derivatives
169	Cotinine	Pyridines and derivatives

Supplementary Table 2 (cont.). ***The list of identified metabolites***.

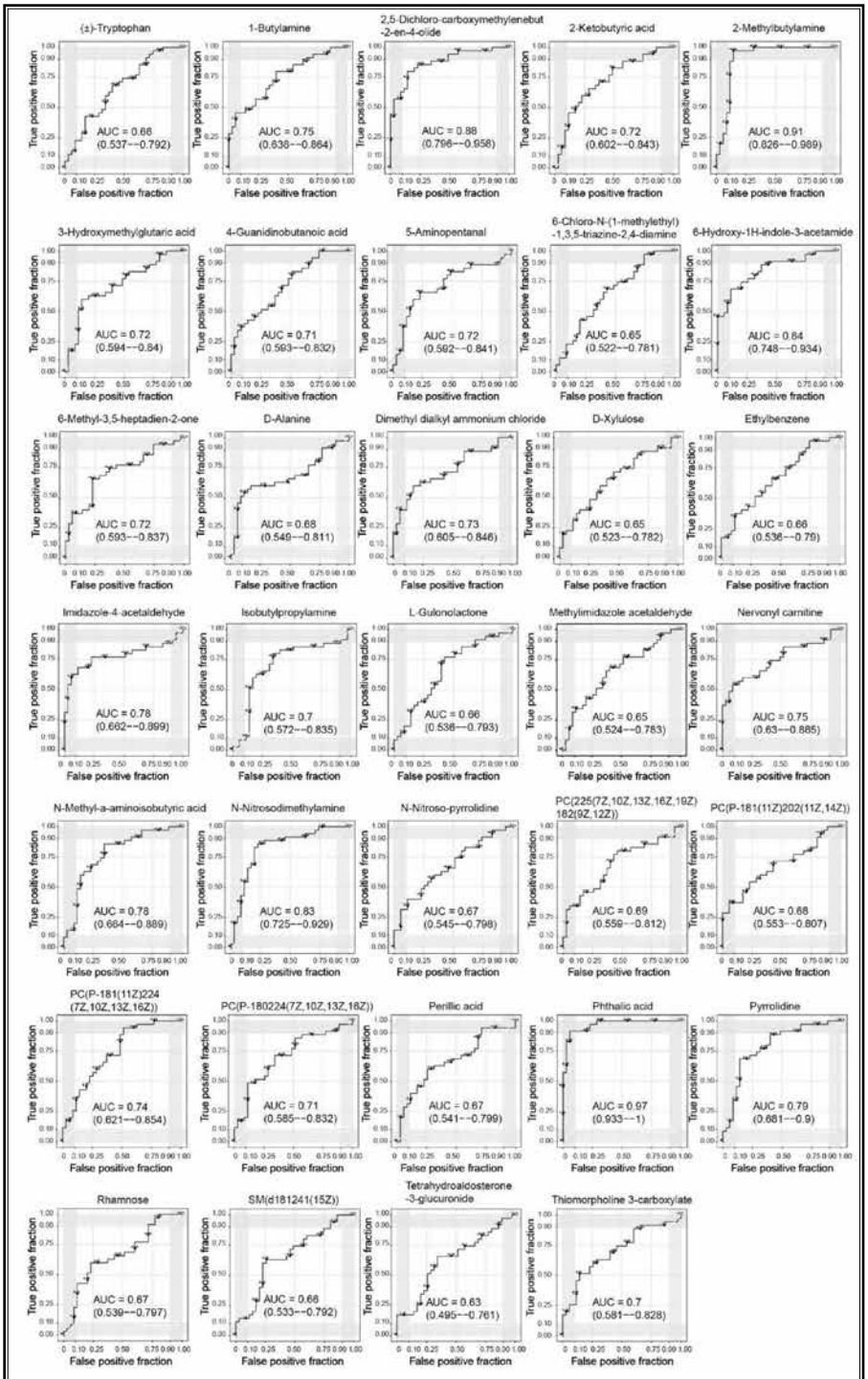
170	Citronellyl beta-sophoroside	Prenol lipids
171	Choline	Organonitrogen compounds
172	Cholic acid	Steroids and steroid derivatives
173	Chavicol	Phenols
174	Caprylic acid	Fatty Acyls
175	Capric acid	Fatty Acyls
176	Caffeine	Imidazopyrimidines
177	Butyrylcarnitine	Fatty Acyls
178	But-2-enoic acid	Fatty Acyls
179	Bovinic acid	Fatty Acyls
180	Betaine	Carboxylic acids and derivatives
181	Beta-Guanidinopropionic acid	Organonitrogen compounds
182	Beta-D-Galactose	Organooxygen compounds
183	benzene-1,2,4-triol	Benzene and substituted derivatives
184	Arecaidine	
185	Arbutin	Organooxygen compounds
186	Arachidonic acid	Fatty Acyls
187	apo-[3-methylcrotonoyl-CoA:carbon-dioxide ligase (ADP-forming)]	Carboxylic acids and derivatives
188	Anofinic acid	Benzopyrans
189	alpha-Methylstyrene	Benzene and substituted derivatives
190	Alpha-Linolenic acid	Fatty Acyls
191	Acetic anhydride	Carboxylic acids and derivatives
192	Abscisic acid	Prenol lipids
193	9-Decenoic acid	Fatty Acyls
194	8-Deoxy-11-hydroxy-13-chlorogrosheimin	Prenol lipids
195	8-Butanoylneosolaniol	Prenol lipids
196	7-Methylguanine	Imidazopyrimidines
197	6-Methyl-3,5-heptadien-2-one	Carbonyl compounds
198	6-Hydroxy-1H-indole-3-acetamide	Indoles and derivatives
199	6-Chloro-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine	Triazines
200	6-Acetyl-2,3-dihydro-2-(hydroxymethyl)-4(1H)-pyridinone	Pyridines and derivatives
201	5a-Tetrahydrocorticosterone	Steroids and steroid derivatives
202	5-Hydroxy-L-tryptophan	Indoles and derivatives
203	5-Ethyl-2,4-dimethyloxazole	Azoles
204	5-Deoxydiplosporin	Pyrans
205	5-Aminopentanal	Organooxygen compounds
206	4-Pyridoxic acid	Pyridines and derivatives
207	4-Hydroxybenzaldehyde	Organooxygen compounds
208	4-Guanidinobutanoic acid	Carboxylic acids and derivatives
209	3,7-Dimethyluric acid	Imidazopyrimidines
210	3,4,5-Trimethoxycinnamic acid	Cinnamic acids and derivatives
211	3-Sulfinoalanine	Carboxylic acids and derivatives
212	3-Methyl-2-oxovaleric acid	Keto acids and derivatives
213	3-Methoxy-4-hydroxyphenylethyleneglycol sulfate	Organic sulfuric acids and derivatives
214	3-Indoleacetonitrile	Indoles and derivatives

Supplementary Table 2 (cont.). ***The list of identified metabolites***.

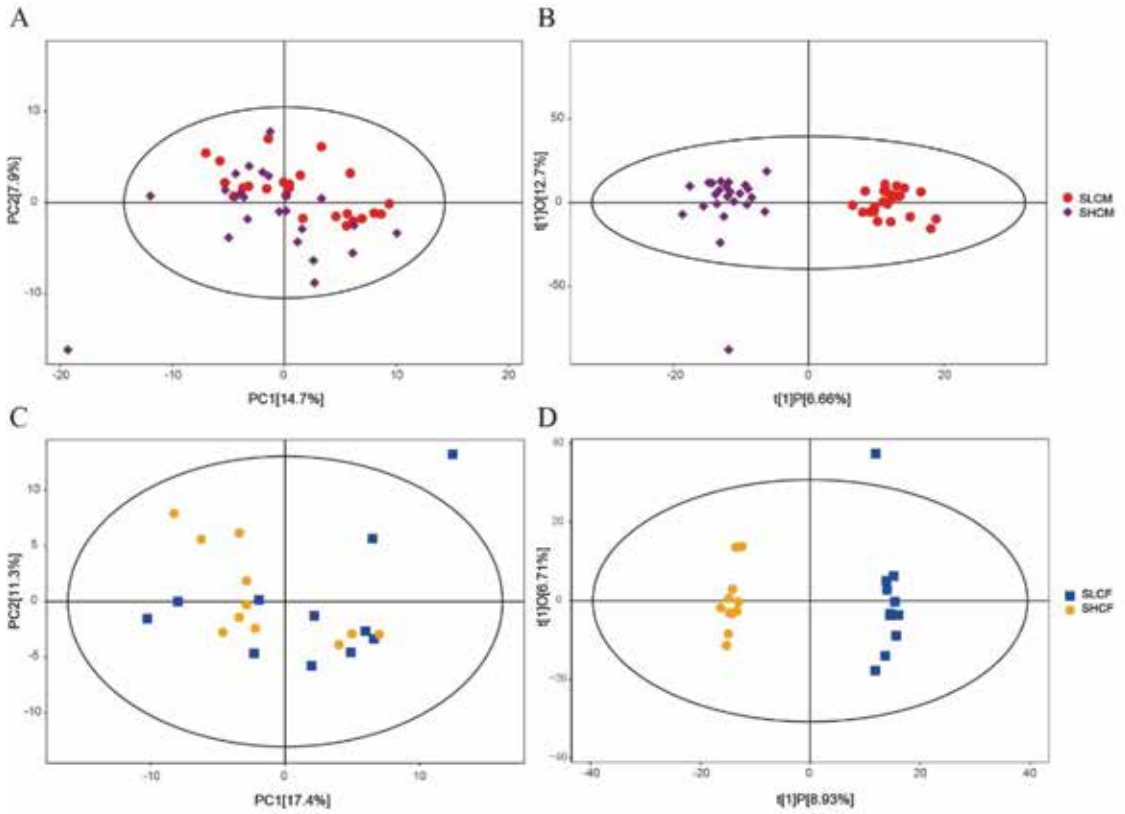
215	3-Hydroxymethylglutaric acid	Fatty Acyls
216	3-Hydroxylidocaine	Carboxylic acids and derivatives
217	3-Hydroxycapric acid	Hydroxy acids and derivatives
218	3-Hydroxybutyric acid	Hydroxy acids and derivatives
219	3-Hydroxybenzyl alcohol	Benzene and substituted derivatives
220	3-Dehydrocarnitine	Keto acids and derivatives
221	3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid	Fatty Acyls
222	3-Aminobutanoic acid	Carboxylic acids and derivatives
223	3-Amino-2-piperidone	Carboxylic acids and derivatives
224	3-Acetyl-2,7-naphthyridine	Naphthyridines
225	3-(3,4-Dihydroxy-5-methoxy)-2-propenoic acid	Cinnamic acids and derivatives
226	2,6 Dimethylheptanoyl carnitine	Fatty Acyls
227	2,5-Dichloro-carboxymethylenebut-2-en-4-olide	Dihydrofurans
228	2-Pyrrolidinone	Pyrrolidines
229	2-Piperidinone	Piperidines
230	2-Oxovaleric acid	Keto acids and derivatives
231	2-Methylbutylamine	Amines
232	2-Methyl-6-(1-propenyl)pyrazine	Diazines
233	2-Methyl-1,4-naphthalenediol bis(dihydrogen phosphate)	Naphthalenes
234	2-Methoxy-3-methylpyrazine	Diazines
235	2-Ketobutyric acid	Keto acids and derivatives
236	2-Keto-3-deoxy-D-gluconic acid	Keto acids and derivatives
237	2-Hydroxystearic acid	Fatty Acyls
238	2-Hydroxy-4-(4-methoxyphenyl)-1H-phenalen-1-one	Naphthalenes
239	2-Hydroxy-3-methylbutyric acid	Fatty Acyls
240	2-Ethyl-2-Hydroxybutyric acid	Fatty Acyls
241	2-Aminoquinoline	Quinolines and derivatives
242	2-Acetyl-3-ethylpyrazine	Carbonyl compounds
243	2-(5,8-Tetradecadienyl)cyclobutanone	Carbonyl compounds
244	2'-Hydroxyacetophenone	Benzene and substituted derivatives
245	1H-Indole-3-acetamide	Indoles and derivatives
246	16-Methylheptadecanoic acid	Fatty Acyls
247	16-Hydroxy hexadecanoic acid	Fatty Acyls
248	15-Keto-13,14-dihydroprostaglandin A2	Fatty Acyls
249	10E,12Z-Octadecadienoic acid	Fatty Acyls
250	1,11-Undecanedicarboxylic acid	Fatty Acyls
251	1-Pyrroline	Pyrrolines
252	1-O-Hexadecyl-2-O-dihomogammalinolenoylglycero-3-phosphocholine	Glycerophospholipids
253	1-Methylnicotinamide	Pyridines and derivatives
254	1-Methylinosine	Purine nucleosides
255	1-Methylhistidine	Carboxylic acids and derivatives
256	1-Butylamine	Organonitrogen compounds
257	[12]-Gingerol	Phenols
258	(R)-Pelletierine	Piperidines
259	(R)-3-Hydroxy-tetradecanoic acid	Fatty Acyls

Supplementary Table 2 (cont.). ***The list of identified metabolites***.

260	(9xi,10xi,12xi)-9,10-Dihydroxy-12-octadecenoic acid	Fatty Acyls
261	(2R,6x)-7-Methyl-3-methylene-1,2,6,7-octanetetrol 2-glucoside	Fatty Acyls
262	(2R)-2-Hydroxy-2-methylbutanenitrile	Organooxygen compounds
263	(2E)-Decenoyl-ACP	Carboxylic acids and derivatives
264	(10E,12Z)-(9S)-9-Hydroperoxyoctadeca-10,12-dienoic acid	Fatty Acyls



Supplementary Fig. 1. ***ROC curves of differential metabolites for the SLC and SHC groups.***



Supplementary Fig. 2. *Serum metabolic profiling in males and females in the SLC and SHC groups. A-B. Principal component analysis (PCA) (A) and partial least squares discriminant analysis (OPLS-DA) (B) in males. C-D. Principal component analysis (PCA) (C) and partial least squares discriminant analysis (OPLS-DA) (D) in females.*