Original Article

Serum metabolite profiles of postoperative fatigue syndrome in rat following partial hepatectomy

Ye Lu,[1,†] Rui Yang,[1,†] Xin Jiang,[1] Yajuan Yang,[2] Fei Peng[2,*] and Hongbin Yuan[1,*]

[1]Department of Anaesthesiology and [2]Department of Nursing, Changzheng Hospital, Second Military Medical University, 415 Fengyang Road, Huangpu district, Shanghai 200003, China

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Postoperative fatigue syndrome is a general complication after surgery. However, there is no "gold standard" for fatigue assessment due to the lack of objective biomarkers. In this study, a rodent model of postoperative fatigue syndrome based on partial hepatectomy was firstly established and serum metabonomic method based on ultra-high performance liquid chromatography coupled with Q-TOF mass spectrometry was applied. Partial least-squares discriminant analysis was used to identify the differential metabolites in 70% partial hepatectomy rats relative to sham rats and 30% partial hepatectomy rats, which showed 70% partial hepatectomy group was significantly distinguishable from 30% partial hepatectomy group and sham group. Eighteen serum metabolites responsible for the discrimination were identified. The levels of hypoxanthine, kynurenine, tryptophan, uric acid, phenylalanine, palmitic acid, arachidonic acid and oleic acid showed progressive elevation from sham group to 30% partial hepatectomy group to 70% partial hepatectomy group, and levels of valine, tyrosine, isoleucine, linoleyl carnitine, palmitoylcarnitine, lysophosphatidylcholine (16:0), lysophosphatidylcholine (20:3), citric acid, succinic acid and hippuric acid showed progressive declining trend from sham group to 30% partial hepatectomy group to 70% partial hepatectomy group. These potential biomarkers help to understand of etiology, pathophysiology and treatment of postoperative fatigue syndrome.

Key Words: **postoperative fatigue syndrome, metabonomics, biomarker, partial hepatectomy, ultra-high performance liquid chromatography-mass spectrometry**

Postoperative fatigue syndrome (POFS) is an unpleasant and distressing symptom and frequently has a major impact on the patient's quality of life. Unsurprisingly, POFS may be a common complication after surgery, especially major abdominal and cardiac procedures.[1] Patients who suffer from POFS had a prolonged recovery to normal daily life. In addition, POFS also increased health-service costs, burdens patients themselves, their families, hospitals and society greatly.[2] Unfortunately, the etiology of the syndrome has not been fully explained.[3] A significant factor that has hindered understanding of POFS has been that there is no ''gold standard'' for fatigue assessment.[3] Although questionnaires such as Christensen's Visual Analogue Scales and the Profile of Mood States can be an effective tool for measuring subjective feelings of POFS,[3–5] clear objective correlates for fatigue have not been identified. Thus, the study of plasma biomarkers for the diagnosis and prognosis of POFS has been the focus of extensive research. However, at present, neither a biomarker nor a biomarker profile is generally accepted in clinical practice.

Additionally, POFS occurs because of a variety of complex factors, including biological, psychological and social factors.[6–10] For this reason, a new approach, such as "omics" technology for POFS, is required. In particular, metabolomics, an emerging field of omics technologies, can investigate the perturbed metabolic pattern in a complete set of metabolites in body fluids or tissue for early diagnosis and therapy monitoring and can clarify the pathogenesis of many diseases.[11–13] To date, however, no metabolomics study for POFS has been reported. In this study, we investigated the perturbed metabolic pattern in serum from rat with POFS induced by partial hepatectomy and identified metabolic markers associated with POFS using UHPLC-Q-TOFMS coupled with multivariate statistical analysis. The finding of metabolic pathways and potential biomarkers related to POFS will be helpful to increase the understanding of etiology, pathophysiology and treatment of this condition.

Materials and Methods

Reagents and materials. HPLC-grade Methanol and acetonitrile (ACN) were purchased from Merk (Darmstadt, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). Lysophosphatidylcholine (16:0) was purchased from Larodan AB (Malmö, Sweden). Valine, hypoxanthine, tyrosine, isoleucine, kynurenine, tryptophan, uric acid, citric acid, phenylalanine, hippuric acid, and palmitic acid were obtained from Shanghai Jingchun Reagent Co. Ultrapure water was prepared with a Milli-Q water purification system (Millipore, Bedford, MA).

Animal protocols. Twenty four male Sprague-Dawley rats (200 ± 15 g) were purchased from the Slac Laboratory Animal Co., LTD (Shanghai, China) and housed in standard conditions. Partial hepatectomy (PHx) of rat liver which resulted in typical characteristics of POFS was used to produce POFS model. The animals were randomly divided into three groups ($n = 8$) including sham group, 30% PHx group and 70% PHx group. 70% PHx was performed during morning hours under anesthesia as described by Mitchell and Willenbring.[14] The animals were maintained postoperatively on the standard chow diet and water, provided ad libitum. Briefly, rats were anesthetized by the subcutaneous injection of 2% pentobarbital sodium (60 mg/kg body weight). The abdomen was opened to allow access to the liver, and the left lateral and medial lobes were ligated individually before removal. The 30% PHx surgical procedure was identical to 70% PHx. For sham surgeries, livers were externalized and gently palpated to mimic the surgical stress of the PHx procedure. The survival

†Both authors have contributed equally to this work.
*To whom correspondence should be addressed.
E-mail: jfjczyy@aliyun.com (HY); zhaozichen03@163.com (PF)

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rates for PHx and sham surgeries were 100%. The body weight and endurance capacity were assessed at different time points. On the 7th day after partial hepatectomy, blood samples were collected into standard vials from the retro-orbital venous sinus. The samples were allowed to clot at room temperature for 1 h, and then the serum was separated by centrifugation at 3,000 × *g* for 10 min at 4°C and stored at –70°C until the analysis. An in-house quality control (QC) was prepared by pooling and mixing the same volume of each sample.[15] All experimental procedures in this study were approved by the institutional animal care and use committee of the Second Military Medical University (Shanghai, China).

Tail suspension test. This test was performed at 24 h and on the 7th day, respectively, after surgery. Rats were suspended for 6 min by tails in the shielded box 30 cm above the floor. The collection of data and analysis of the test were improved, as the mobile energy of rats responding to the inescapable stress was recorded in the form of electronic signal by multiplying a channel biological signal recorder (Medease Technology Company, Nanjing, China). After filtering the threshold, the positive signals represented the objective mobile. The areas of signal wave above the threshold level were added up, which represent the total mobile strength. Prolonged single immobile time and total immobile time represent physical weakness and depressed mood.

Sample preparation. Prior to the analysis, a volume of 400 µl of methanol was added to 100 µl of serum. After vigorous shaking for 1 min and incubation on ice for 10 min, the mixture was centrifuged at 14,000 × *g* for 15 min at 4°C to precipitate the protein. All the supernatant was removed (without removing any particles left at the bottom of the vial). The supernatant was evaporated to dryness with a gentle nitrogen stream. The dry residue was reconstituted in 100 µl of ACN/water (7:3, v/v), then centrifuged again at 14,000 × *g* for 10 min at 4°C.

UHPLC-Q-TOFMS analysis. UHPLC-MS analysis was performed on Agilent 1290 Infinity LC system coupled to Agilent 6530 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Agilent, Palo Alto, CA). Chromatographic separations were performed on an ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.7 µm, Waters, Milford, MA) maintained at 40°C. The mobile phase consisted of 0.1% formic acid (A) and ACN modified with 0.1% formic acid (B). The following gradient program was used: 5% B at 0–2 min, 5–95% B at 2–17 min, 95% B at 17–19 min. the post-time was set as 6 min. The flow rate was 400 ml/min and the injection volume was 2 µl.

An electrospray ionization source (ESI) interface was used, and was set in both positive and negative modes so as to monitor as many ions as possible. The following parameters were employed: capillary voltage, 3.5 kV; drying gas flow, 11 L/min; gas temperature: 350°C; nebulizer pressure, 45 psig. Fragmentor voltage, 120 V; skimmer voltage, 60 V. All analyses were acquired using a mixture of 10 mM purine (*m/z* 121.0508) and 2 mM hexakis phosphazine (*m/z* 922.0097) as internal standards to ensure mass accuracy and reproducibility. Data were collected in centroid mode and the mass range was set at *m/z* 50–1,000 using extended dynamic range. Potential biomarkers were analyzed by MS/MS. MS spectra were collected at 2 spectra/s, and MS/MS spectra were collected at 0.5 spectra/s, with a medium isolation window (~4 *m/z*) and a fixed collision energy of 15 V.

Data handling. The raw data in instrument specific format (.d) were converted to common data format (.mzData) files using a conversion software program (file converter program available in Agilent MassHunter Qualitative software), in which the isotope interferences were eliminated. The program XCMS (http://metlin.scripps.edu/download/) was used for nonlinear alignment of the data in the time domain and automatic integration and extraction of the peak intensities.[16] XCMS parameters were default settings except for the following: full width at half maximum (FWHM) = 8, bandwidth (bw) = 10 and snthresh = 5. The variables presenting in at least 80% of either group were extracted[17] Variables with less than 30% relative standard deviation (RSD) in QC samples[18] were then retained for further multivariate data analysis because they were considered stable enough for prolonged UHPLC–Q-TOFMS analysis. For each chromatogram, the intensity of each ion was normalized to the total ion intensity, in order to partially compensate for the concentration bias of metabolites between samples and to obtain the relative intensity of metabolites. The normalized data and imported into a SIMCA-P (ver. 11.0, Umetrics, Umeå, Sweden), where principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) were performed. All data were mean-centered and pareto-scaled in SIMCA-P. The quality of the models is described by the R^2X or R^2Y and Q^2 values. R^2X or R^2Y is defined as the proportion of variance in the data explained by the models and indicates goodness of fit. Q^2 is defined as the proportion of variance in the data predicted by the model and indicates predictability, calculated by a cross-validation procedure.[19] A default seven-round cross-validation in SIMCA-P was performed throughout to determine the optimal number of principal components and to avoid model overfitting. The PLS-DA models were also validated by apermutation analysis (99 times).

The differential metabolites were selected on the basis of the combination of a statistically significant threshold of variable influence on projection (VIP) values obtained from the PLS-DA model and p values from one-way ANOVA on the normalized peak areas, where metabolites with VIP values larger than 1.0 and p values less than 0.05 were included, respectively. The software MedCalc (ver. 11.4.2.0) was used to perform receiver operating characteristic (ROC) analysis based on binary logistic regression model.

Results

Effects of PHx-induced fatigue on the body weight (BW) time profiles. The body weight time profiles during the animal study period are shown in Supplemental Fig. 1*. There were no differences among these three groups before surgery (Day 0). After partial hepatectomy, the 70% PHx group showed a significantly lower weight as compared with the sham group and the 30% PHx group. As compared with the sham group, the weight of the 30% PHx group gradually increased during the study period. However, the 70% PHx group demonstrated a delay in weight increase during the 7 days of the study period.

Tail suspension test. At the 1st day and 7th day after surgery, the positive struggle signals of 70% PHx group showed discontinuous and lower waves as compared with the sham group and the 30% PHx group. The value of accumulated areas above the threshold level was the lowest in the 70% PHx group (Supplemental Fig. 2A*). The value of the test was still significantly lower in the 70% PHx group than the sham group at the seventh postoperative day. The immobile time of the suspended period significantly increased in the 70% PHx group as compared with the sham and 30% PHx groups, even at the seventh postoperative day (Supplemental Fig. 2B*). The longest mean single immobile time was also observed in the 70% PHx group (Supplemental Fig. 2C*).

Serum metabolic profiling by UHPLC-MS. Using the UHPLC-Q-TOFMS condition described above, the representative profiles of serum metabolomes in ESI positive and negative mode are shown in Fig. 1A and B. The stability of the analytical method is very important to obtain valid metabonomic data. To validate the system performance during the analysis of real samples, an in-house QC sample was applied.[15] The QC sample was prepared by mixing equal volumes (100 ml) from each of the 24 samples as they were being aliquoted for analysis. This "pooled'' serum was used to provide a representative "mean" sample containing all the

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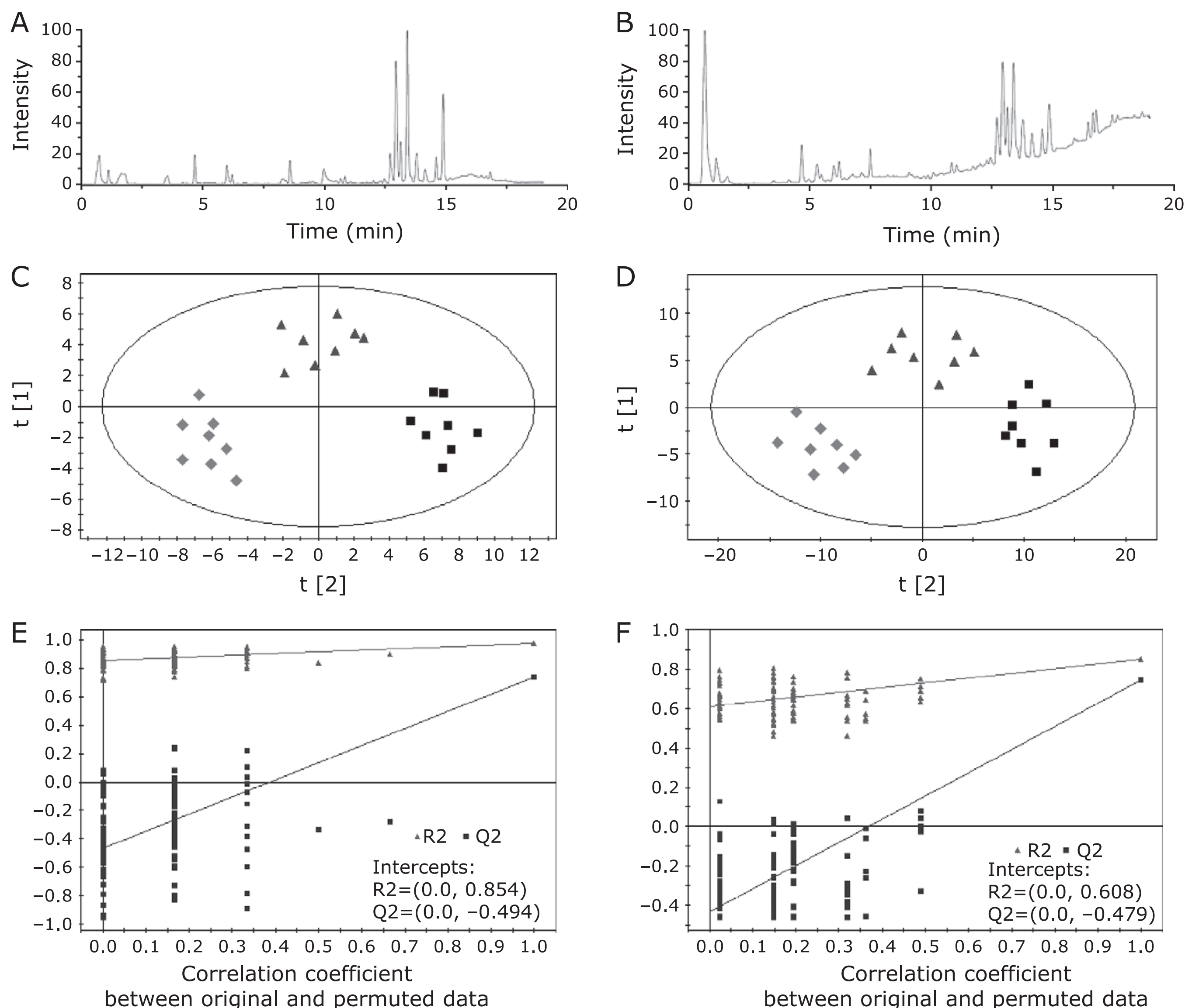

Fig. 1. Representative UHPLC-Q-TOFMS total ion current (TIC) chromatograms of 70% PHx rat serum, scores plots and permutation test from PLS-DA. (A) positive ion mode TIC, (B) negative ion mode TIC, (C) and (D) PLS-DA scores plot derived from UHPLC-(+)ESI-QTOFMS and UHPLC-(–)ESI-Q-TOFMS datasets derived from concerning sham rats (■), 30% PHx rats (▲) and 70% PHx rats (◆), respectively. (F) and (E) Plot of the permutation test of PLS-DA on sham rats (■), 30% PHx rats (▲) and 70% PHx rats (◆) from UHPLC-(+)ESI-QTOFMS and UHPLC-(–)ESI-Q-TOFMS datasets, respectively.

analytes that will be encountered during the analysis. The QC sample was processed as real samples and then was randomly inserted amongst the real sample queue to be analyzed eight times in ESI positive or negative analysis batch. It was found that the relative standard derivations (RSD) of the peak areas are 4.8–13.6% for ions selected from QC samples (see Table 1 for data). The results indicated that the method was robust with good repeatability and stability.

Detection and identification of POFS-related biomarker candidates. For obtaining useful metabolomics results, data analysis strategy is as important as the analytical technique employed. In this study, the UHPLC-MS analysis by ESI in both positive and negative ion modes raw data were converted into mzData format. XCMS was then used to carry out peak discrimination, filtering, and retention time alignment, yielding 1,320 peaks of positive ions and 1,624 peaks of negative ions between retention times of 0.6–21 min. Missing values caused by peaks that were not present in the sample/chromatogram were reduced using the 80% rule. The resulting data were imported into SIMCA-P, and centered and pareto scaled to reduce the impact of noise and artifacts in the models.

To investigate global metabolomic alterations, all observations acquired in both ion modes were integrated and analyzed using PCA. A separation tendency in the PCA score plot was observed among three groups (sham, 30% PHx, and 70% PHx groups) for UHPLC-(±)ESI-QTOFMS analysis ($R^2 = 0.54$ for positive model data and $R^2 = 0.47$ for negative model data) (data not shown), suggesting that metabolic perturbations were evident in the POFS rats, dependent on pathological condition of partial hepatectomy. To further discover potential POFS-related metabolites, PLS-DA model was established in that the supervised approach of PLS-DA was more focused on the actual class discriminating variation in the data compared to the unsupervised approach of PCA. A clear separation among the three groups was observed in the PLS-DA

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Table 1. Identification results of candidate biomarkers in serum for discriminating sham, 30% PHx and 70% PHx rats

No.	RT (min)[a]	m/z	Formula	Metabolite	VIP[d]	Trend[e]	Ratio[f]		Sham group & 70% PHx group			C_{ij}[h]	Related pathway	%RSD[i]
							30% PHx	70% PHx	AUC[g]	Sensitivity	Specificity			
1	0.69	118.0869	$C_5H_{11}NO_2$	Valine[b]	2.7	↓	0.82	0.64	0.87 (0.72–0.98)	0.75	0.87	–0.62	BCAA metabolism	9.6
2	0.72	137.0451	$C_5H_4N_4O$	Hypoxanthine[b]	1.3	↑	1.21	1.66	0.85 (0.59–0.97)	0.87	0.75	0.53	Purine metabolism	13.6
3	1.65	182.0808	$C_9H_{11}NO_3$	Tyrosine[b]	1.6	↓	0.79	0.53	0.83 (0.56–0.96)	0.87	0.87	–0.81	Phenylalanine metabolism	9.5
4	1.79	132.1019	$C_6H_{13}NO_2$	Isoleucine[b]	2.6	↓	0.89	0.69	0.80 (0.54–0.95)	0.75	0.75	–0.56	BCAA metabolism	8.2
5	3.32	209.0926	$C_{10}H_{12}N_2O_3$	Kynurenine[b]	1.2	↑	1.33	1.66	0.97 (0.74–0.99)	0.87	0.87	0.73	Tryptophan metabolism	12.3
6	4.67	205.0973	$C_{11}H_{12}N_2O_2$	Tryptophan[b]	3.1	↑	1.13	1.44	0.83 (0.56–0.96)	0.87	0.75	0.65	Tryptophan metabolism	5.1
7	12.45	424.3435	$C_{25}H_{45}NO_4$	Linoleyl carnitine[c]	3.2	↓	0.88	0.74	0.73 (0.51–0.92)	0.75	0.62	–0.53	Fatty acid transportion	7.8
8	12.79	400.3423	$C_{23}H_{45}NO_4$	Palmitoylcarnitine[c]	1.6	↓	0.84	0.68	0.90 (0.67–0.99)	0.75	0.87	–0.57	Fatty acid transportion	8.5
9	13.4	496.3423	$C_{24}H_{50}NO_7P$	LysoPC(16:0)[b]	5.4	↓	0.89	0.78	0.79 (0.53–0.95)	0.63	0.63	–0.42	Phospholipid metabolism	5.6
10	13.47	546.3565	$C_{28}H_{52}NO_7P$	LysoPC(20:3)[c]	1.4	↓	0.93	0.63	0.81 (0.54–0.96)	0.63	0.75	–0.45	Phospholipid metabolism	6.2
11	1.16	167.0215	$C_5H_4N_4O_3$	Uric acid[b]	3.5	↑	1.15	1.49	0.84 (0.57–0.97)	0.87	0.75	0.58	Purine metabolism	9.7
12	1.23	191.0207	$C_6H_8O_7$	Citric acid[b]	1.3	↓	0.8	0.64	0.79 (0.53–0.95)	0.75	0.63	–0.51	Citrate cycle	10.5
13	1.57	117.0196	$C_4H_6O_4$	Succinic acid[c]	1.3	↓	0.78	0.62	0.87 (0.62–0.98)	0.75	0.75	–0.54	Citrate cycle	10.7
14	3.55	164.0716	$C_9H_{11}NO_2$	Phenylalanine[b]	1.4	↑	1.16	1.48	0.94 (0.70–0.99)	0.87	0.87	0.73	Phenylalanine metabolism	4.8
15	5.49	178.0516	$C_9H_9NO_3$	Hippuric acid[c]	1.6	↓	0.9	0.78	0.79 (0.52–0.95)	0.75	0.63	–0.57	Phenylalanine metabolism	7.6
16	14.46	255.2343	$C_{16}H_{32}O_2$	Palmitic acid[b]	1.3	↑	1.4	1.81	0.94 (0.71–0.99)	0.87	0.75	0.52	Fatty acid metabolism	9.2
17	16.67	303.2346	$C_{20}H_{32}O_2$	Arachidonic acid[c]	1.4	↑	1.41	1.93	0.96 (0.73–0.99)	0.87	0.75	0.61	Fatty acid metabolism	10.3
18	17.67	281.2499	$C_{18}H_{34}O_2$	Oleic acid[c]	1.1	↑	1.26	1.76	0.87 (0.72–0.98)	0.87	0.63	0.55	Fatty acid metabolism	8.2

[a]RT values in italics are potential biomarkers detected in negative ESI mode and those in non-italics detected in positive ESI mode. [b]Metabolites validated with standard sample.[c]Metabolites putatively annotated. [d]Variable importance in the projection (VIP) was obtained from the PLS-DA model. [e]Metabolites showed progressive elevation (↑) or a declining (↓) trend from sham group to 30% PHx group to 70% PHx. [f]The ratio of relative amounts of 30% PHx group or 70% PHx group to control group. [g]Area under the receiver operating characteristic (ROC) curve, with the 95% confidence interval (CI) range in parentheses. [h]The correlation between the identified biomarkers with the fatigue traditional marker data (accumulated immobile time) was performed based on the pearson correlation coefficient (C_{ij}) at the significance level of $p<0.05$. [i]Variation of the biomarker concentrations in QC samples expressed as relative standard deviation (%RSD).

score plot according the degree of PH-induced POFS ($R^2 = 0.91$ and $Q^2 = 0.78$ for positive model data; $R^2 = 0.89$ and $Q^2 = 0.75$ for negative model data) (Fig. 1C and D), which suggested that proper POFS-related patterns could be revealed by the proposed PLS-DA model.

To further guard against model overfitting, a default of seven rounds of cross-validation across four components was applied, with 1/7th of the samples being excluded from the model in each round. Validation with 99 random permutation tests generated intercepts of $R^2 = 0.854$ and $Q^2 = –0.494$ from positive model data (Fig. 1E) and $R^2 = 0.608$ and $Q^2 = –0.479$ from negative model data (Fig. 1F). These results indicate that PLS-DA models derived from the UHPLC-(±)ESI-QTOFMS data had good predictive ability and were reliable.

The VIP value generated form PLS-DA model reflects the influence of every variable on the classification. Variables with a VIP value of >1.0 had an above average influence on the classifcation. Thus, using a combination of the VIP values (>1.0) from PLS-DA with results from one-way ANOVA between the sham, 30% PHx, and 70% PHx groups, ten and eight metabolite ions were selected as potential biomarkers related with PH-induced POFS, based on the UHPLC-(+)ESI-QTOFMS and UHPLC-(–)ESI-Q-TOFMS datasets, respectively. To identify these metabolites, we first searched candidates from the freely accessible databases of HMDB (http://www.hmdb.ca), METLIN (http://metlin.scripps.edu) and KEGG (http://www.kegg.jp) by masses; then, MS/MS analyses were performed, and due to the possible fragment mechanisms, items without the given mass fragment information were removed from the candidate list and only the most probable items survived. By comparing the retention times and mass spectra to the authentic chemicals as well as the standard MS/MS spectrum from the above database, these sixteen POFS-related metabolites were structurally confirmed (Table 1). Among these metabolites, the levels of hypoxanthine, kynurenine, tryptophan, uric acid, phenylalanine, palmitic acid, arachidonic acid and oleic acid showed progressive elevation from sham group to 30% PHx group to 70% PHx group, and the levels of valine, tyrosine, isoleucine, linoleyl carnitine, palmitoylcarnitine, lysoPC(16:0), lysoPC(20:3), citric acid, succinic acid and hippuric acid showed progressive declining trend from sham group to 30% PHx group to 70% PHx group (Supplemental Fig. 3*). To quantitatively assess the capability of the potential marker metabolites to discriminate between sham rats and 70% PHx rats, the area under the ROC curve (AUC) and sensitivity as well as specificity were calculated individually for these 18 metabolites. The results are listed in Table 1, where nine metabolites including hypoxanthine, tyrosine, kynurenine, tryptophan, uric acid, phenylalanine, palmitic acid, arachidonic acid, and oleic acid yielded relatively high sensitivity (>80%), and five metabolites including valine, tyrosine, kynurenine, palmitoylcarnitine, and phenylalanine yielded relatively high specificity (>80%). In order to further assess the relationship between the potential marker metabolites with postoperative fatigue, and evaluate whether the potential marker metabolites in this model was effected by hepatic function itself, The correlation between the potential marker metabolites with the fatigue traditional marker data (accumulated immobile time) was performed based on the pearson correlation coefficient (C_{ij}) (Table 1). It was found that the associations $| C_{ij} |$ of sixteen metabolites were more than 0.50 except for LysoPC(16:0) and LysoPC(20:3), which indirectly confirmed that the potential marker metabolites have good relationship with postoperative fatigue.

Discussion

The POFS is a common complaint after surgery. Generally, major abdominal and cardiac surgeries represent the main types of interventions causing POFS. In this study, we reported a rodent model of POFS where the magnitude of the surgical challenge, based on the degree of partial hepatectomy, predicted POFS. A 70% PHx in rats demonstrated the worst general state of health and lowest muscle strength during the 7 days of study period,

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which indicated that the 70% PHx represents a good model of experimental POFS. Based on the assessment of body weight and muscle strength, the serum metabolite profiling based on UHPLC-Q-TOFMS was used to understand the pathogenesis of POFS and identify the potential biomarkers related POFS. Eighteen differential metabolites were identified that are highly associated with the metabolic changes resulting from PHx-induced POFS.

To explore the underlying molecular functions of these serum metabolite biomarkers, metabolic pathway analysis was performed. These metabolites were found to be primarily involved in citrate cycle, branched-chain amino acids (BCAAs) metabolism, fatty acid transportion and metabolism, phospholipid metabolism, tryptophan metabolism, phenylalanine metabolism and purine metabolism. By relating the metabolic pathways, the metabolic network of POFS-related potential biomarkers was constructed (Supplemental Fig. 4*). The disturbed metabolic pathways are discussed in detail below.

Citrate cycle. Citric acid and succinic acid, two important intermediate in tricarboxylic acid cycle, were observed to be reduced in POFS rats. PHx-induced POFS rat may be lead to depletion of the liver and muscle glycogen,[20,21] which could result in a reduction of tricarboxylic acid cycle intermediates and in impaired aerobic energy formation. These consistent set of findings suggests that PHx-induced POFS is associated with the inhibition of citrate cycle.

BCAAs metabolism. The low levels of valine and isoleucine, two branched-chain amino acids, were observed in fatigue rats. Consistent with these findings, several studies have also demonstrated that the plasma levels of BCAAs were decreased in fatigue humans induced by exhaustive exercise.[22] One possible interpretation of these results might be that fatigue facilitates utilization of amino acids for the synthesis of neurotransmitters in the central nervous system,[23,24] the other might be that the fatigue-induced depletion of glycogen lead to the utilization of branched-chain amino acids as energy compensation. Thus, supplementation of the diet with the branched-chain amino acids may be useful for lowering fatigue and for facilitating recovery from it.

Fatty acid transportion and metabolism. Carnitines, which play an important role in transporting fatty acids across the inner mitochondrial membrane, can improve energy and physical function in old people by reducing fatigue.[25] Here, the lower levels of linoleylcarnitine and palmitoylcarnitine were observed in the 70% PHx-induced POFS rats, suggesting that the fatigue rats following partial hepatectomy alters energy metabolism. In agreement with this presumption, a previous urine and plasma metabonomic study in excessive fatigue rat model has also demonstrated that fatigue is associated with significantly reduced levels of carnitines.[26]

The levels of palmitic acid and arachidonic acid as well as oleic acid were significantly increased in POFS rats following PHx, suggesting the fatty acid metabolism is implicated in POFS. One possible explanation for the high level of free fatty acids was that fatigue increased energy consumption and further increased utilization of fat as an energy source by increasing lipid mobilization.[10,27]

Phospholipid metabolism. The levels of LysoPC(16:0) and LysoPC(20:3) were significantly decreased in POFS rats compared to the control rats. It is conceivable that these alterations may ascribe to the lower activity of plasma lecithin-cholesterol acyltransferase (LCAT), which could mediate the release of LysoPCs.[28] It was reported that partially hepatectomized rats demonstrated the impairment of activity LCAT,[29] which indirectly validated our results. In addition, a diminishment of LysoPCs induced by excess fatigue in rats has also been observed in a recent LC-MS study.[26] These consistent set of findings suggests that the lower LCAT activity were implicated in the pathology of fatigue.

Tryptophan metabolism. The significantly high levels of tryptophan and kynurenine were observed in POFS rats induced by 70% PHx, suggesting the tryptophan metabolism pathway is perturbed in POFS rats. Tryptophan is not only the precursor of serotonin, which is known to have a role in pathways involving sleep and fatigue, but it is also the precursor of the kynurenine, which participate in many physiological and pathological processes.[10,30] Previous studies have consistently reported that this metabolic pathway plays an important role in fatigue pathogenesis. Tryptophan is unique among amino acids in that it can be transported not only freely in solution, but can also be bound to plasma albumin in the blood. Thus, the mechanism responsible for the increase is that an equilibrium which exists within the plasma between bound and unbound tryptophan may be altered significantly by the level change of plasma free fatty acids, since free fatty acids are also bound to albumin.[31] It has been reported that the mobilisation of fatty acids in the blood was increased under the condition of fatigue, which indirectly confirmed our presumption.

Phenylalanine metabolism. Hippuric acid and tyroine are two metabolites of phenylalanine. Here, The increased level of phenylalanine and the decreased levels of hippuric acid and tyroine were significantly observed in PHx-induced fatigue rats. The consistent pattern of decreased levels of metabolites of phenylalanine in the phenylalanine metabolism pathway suggests that this pathway may be inhibited in PHx-induced fatigue. Consistent with these findings, a previous study in an animal model of complex fatigue has also demonstrated that fatigue behavior is associated with significantly reduced levels of metabolites in phenylalanine metabolism pathway.[32]

Purine metabolism. The levels of hypoxanthine and uric acid were significantly increased in POFS rats relative to control group, which suggests that perturbed purine metabolism is implicated in fatigue rats following partial hepatectomy. Hypoxanthine and uric acid are adenosine enzymatic degradation. A number of studies have demonstrated that fatigue could lead to acceleration of adenine nucleotide degradation, followed by increases in the plasma concentrations of hypoxanthine and uric acid,[33,34] which is consistent with our results.

Conclusion

In this study, a rodent model of POFS based on partial hepatectomy (PHx) was established and a novel serum metabonomic method based on ultra-high performance liquid chromatography coupled with Q-TOF mass spectrometry was applied to study the metabolic changes of serum in PHx-induced POFS rats. The result showed that citrate cycle, branched-chain amino acids metabolism, fatty acid transportion and metabolism, phospholipid metabolism, tryptophan metabolism, phenylalanine metabolism and purine metabolism were abnormal in PHx-induced POFS rats. Our findings may be helpful for further understanding of POFS. Furthermore the detected pathway alteration may also be useful for the development of new drug targets for the treatment of patients suffering from POFS.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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