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Applied nutritional investigation

Perioperative serum and urine metabolome analyses in patients with hepatocellular carcinoma undergoing partial hepatectomy

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ABSTRACT

Objectives: Perioperative nutritional management is essential for early recovery after liver surgery. The aim of this study was to assess changes in amino acid levels in serum and urine after hepatectomy.
Methods: Serum samples were collected from 16 patients with hepatocellular carcinoma before and 1, 3, and 14 d after hepatectomy (S0, S1, S3, and S14, respectively). Spot urine samples were collected before and 3 d after the hepatectomy (U0 and U3). Metabolites in the serum and urine were analyzed.
Results: Compared with S0, insulin levels significantly increased in the S1 and S3 samples. Valine levels significantly decreased in S1 and S14, and leucine levels significantly decreased in S14. Phenylalanine levels significantly increased in S1 and S3, and tyrosine levels significantly increased in S1. The Fischer ratio (branched-chain/aromatic amino acids) significantly decreased in S1 and S3. In multiple regression analysis, changes in serum taurine levels were related to the white blood cell count in S1 and S3, and inversely related to alanine aminotransferase levels in S14. Changes in serum glutamine levels were negatively related to C-reactive protein levels in S3. Serum glutamine levels decreased in S3 and S14, and tended to increase in U3, suggesting a deficiency of glutamate resulting from the invasive surgical procedure.
Conclusions: These findings highlight the usefulness of metabolome analysis for characterizing perioperative patterns after liver resection. The observed amino acid pattern, including the reduction in Fischer ratio, underscores the need for specialized nutritional support.

Introduction

Advances in surgical and anesthetic techniques have resulted in extensive hepatic resection becoming a useful treatment modality for patients with hepatocellular carcinoma (HCC) [1]. Most patients with HCC have underlying liver cirrhosis, and the hepatic resection is performed under rather severe conditions. Despite the advances in surgical techniques and perioperative management, hepatic resection remains associated with a high incidence of complications [2]. To reduce morbidity, it is important to gain a full understanding of each patient's condition and to plan the perioperative management in detail.

The liver plays a central role in nutritional metabolism, and protein-energy malnutrition is common in patients with liver disease due to their abnormal energy metabolism [3]. The metabolic conditions in liver cirrhosis after a normal overnight fast are similar to those resulting from prolonged starvation in healthy individuals. When a patient with liver cirrhosis fasts, the rate of glucose

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oxidation decreases and the rate of fat oxidation increases. As a result of the abnormal energy metabolism of these patients, their non-protein respiratory quotient (npRQ) is reduced after an overnight fast; when severe, this can result in protein-energy malnutrition. Protein-energy malnutrition and reduced npRQ are closely correlated to the prognosis in patients with liver cirrhosis [4,5], who are recommended to consume frequent meals and a late evening snack to compensate for their abnormal energy metabolism [6,7]. In more severe cases, such as patients with HCC undergoing liver resection, the metabolism is severely disrupted by both the background liver disease and the reduction in liver volume due to the operative procedure. These patients require careful management with regard to their energy metabolism.

The postoperative period after hepatic resection is characterized by a catabolic state, often with glucose and electrolyte imbalances, as the body attempts to meet the high demand of the regenerating liver [8]. Nutritional support during this critical period is of paramount importance to enable adequate hepatic regeneration and postoperative recovery. Much attention has been paid to patients' tolerance of early oral feeding and immunonutrition in the preoperative period. Immune-enhancing nutrients include ω-3 fatty acids, glutamine (Gln), arginine (Arg), and nucleic acids, with the administration of Arg and Gln reported to promote liver regeneration [9,10]. Administering branched-chain amino acids (BCAAs) to surgical patients as postoperative nutritional support promotes rapid improvement of albumin levels [11]. A previous article described the practice of administering parenteral nutrition of dextrose and amino acids (AAs; consisting of 35%–50% BCAAs) to patients from the first postoperative day (POD) after liver resection to moderate the catabolic drive and provide support for liver regeneration [12]. Nevertheless, parenteral nutrition for liver resection patients remains a poorly defined field. Additionally, few studies have examined the relationship between the administration of nutrients to this patient group and perioperative outcomes or detailed changes in metabolites during the perioperative period.

Advances in analytical chemistry and computational processes have led to the recent development of metabolomics [13], enabling the measurement in a single drop of blood of metabolites that could not previously be identified. Similar to phenotyping in postgenomic research, metabolomics is an analytical method used both to detect diseases and as a research biomarker [14,15]. It makes possible the detailed evaluation of perioperative variations in metabolites in the living body. In this study, we used metabolomics methods to evaluate changes in metabolites in HCC patients after liver resection, and to examine their blood biochemical data.

Materials and methods

Participants

Sixteen patients with HCC from the Tokushima University Hospital were enrolled; their clinical and laboratory data are shown in Table 1. All were classified as Child–Pugh grade A at entry. They had the following disease etiologies: hepatitis B virus (n = 7), hepatitis C virus (n = 3), alcoholic liver disease (n = 2), hepatitis C virus plus alcoholic liver disease (n = 1), and non-B, non-C hepatitis (n = 3). All patients underwent liver resection: three patients underwent segmental resection, five had resection of one liver segment, and eight had lobectomy. All of the patients had a normal clinical course, with no complications. Informed consent was obtained from all patients. Study design was approved by the Ethical Committee of Tokushima University Hospital.

AAs were not administered perioperatively. Glucose (10%) was administered according to the following routine: POD 0, 2000 mL; POD 1, 1500 mL; and POD 2, 1000 mL starting with a soft meal. On POD 3, the peripheral feeding was stopped and the energy intake of the patients' meals was gradually increased. The patients' body weight and body mass index were measured with a PC-320 body composition meter (Tanita Corp., Tokyo, Japan) under fasting conditions.

Laboratory examination

The following blood biochemical parameters were measured before surgery, after surgery on the same day, and on POD 1, 3, and 14: white blood cells (WBC), C-reactive protein (CRP), platelets (PLT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil), total protein (TP), albumin (Alb), total cholesterol, triacylglycerol, immunoreactive insulin, ammonia, interleukin (IL)-6, tumor necrosis factor-α, indocyanine green retention rate at 15 min, cholinesterase, 8-hydroxy-2′-deoxyguanosine, protein induced by vitamin K absence or antagonist II, alpha-fetoprotein, type IV collagen (IV collagen), and hyaluronic acid. Blood samples also were obtained to determine non-esterified fatty acids and blood sugar concentrations immediately after the indirect calorimetric measurements. Urinary 3-methylhistidine (3-MH) was measured before surgery, after surgery on the same day, and on POD 3.

Indirect calorimetry

Resting energy expenditure (REE) and the npRQ were measured by indirect calorimetry before and on POD 14 using an AE-300 S respiratory gas analyzer (Minato Medical Science Corp., Ltd., Osaka, Japan). Patients were instructed to avoid eating or drinking anything except non-energy water or tea from 1900 the day before the measurements. Urinary urea nitrogen was measured using 24-h urine samples. After overnight fasting, the indirect calorimetric measurements were made at 0730. Oxygen consumption and carbon dioxide production rates were measured for 15 min, with the average values from the final 10 min used in the analysis. REE and npRQ were calculated from the measured oxygen consumption, rates of carbon dioxide production, and urinary urea nitrogen. Patients' basal energy expenditures were estimated from the Harris–Benedict equation, and the ratio of REE to the basal energy expenditure was expressed as the %REE.

Sample collection and metabolite measurement

All blood samples were drawn after a 12-h fast, and urine samples were collected on the same day. The serum was obtained by centrifugation of the blood samples at 3000*g* at 4°C for 10 min. The samples were divided into aliquots and stored at −80°C until extraction of metabolites.

Table 1
Clinical profiles of the hepatectomy patients

Hepatocellular carcinoma patients (N = 16)						
Age (y)	67	± 2	ICGR15 (%)	11.2	± 0.2	
Male/Female	13	/3	ChE (IU/l)	245	± 21	
BW (kg)	55.9	± 2.2	8-OH-dG (ng/mL)	5.1	± 0.8	
BMI (kg/m^2)	22.2	± 0.6	PIVKA-II (mAU/mL)	18937	± 16596	
Etiology			AFP (ng/mL)	10257	± 9550	
HBV		7	IV collagen (ng/mL)	198	± 23	3
HCV		3	Hyaluronic acid (ng/mL)	133	± 52	
Alcoholic		2				
HCV + alcoholic		1				
Non-B/Non-C		3				
Child–Pugh A/B	16	/0				

AFP, α-fetoprotein; Alb, albumin; BMI, body mass index; BW, body weight; ChE, cholinesterase; HBV, hepatitis B virus; HCV, hepatitis C virus; ICGR15, indocyanine green retention rate at 15 min; non-B/non-C, non-B, non-C hepatitis; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; PIVKA-II, protein induced by vitamin K absence or antagonist-2.
Values are mean ± SEM

Table 2
Serum profiles of hepatocellular carcinoma patients before (S0) and 1, 3, and 14 d after hepatectomy (S1, S3, and S14)

	Hepatocellular carcinoma patients											
	S0			S1			S3			S14		
WBC (/μL)	4819	±	342	12831	±	823*	8594	±	778*	6731	±	405
CRP (mg/mL)	0.26	±	0.19	2.23	±	0.29*	5.87	±	1.06*	1.47	±	0.32
PLT ($\times 10^4$/μL)	17.7	±	1.3	14.0	±	3.1*	11.6	±	4.2*	23.4	±	2*
AST (IU/l)	52	±	10	542	±	66*	165	±	12*	44	±	12
ALT (IU/l)	51	±	8	493	±	81*	315	±	48*	58	±	14
T-Bil (mg/dL)	0.8	±	0.1	1.0	±	0.1	1.6	±	0.2*	0.9	±	0.1
TP (g/dL)	7.4	±	0.2	5.4	±	0.1*	5.7	±	0.2*	6.7	±	0.1
Alb (g/dL)	3.8	±	0.1	2.7	±	0.1*	2.8	±	0.1*	3.2	±	0.1*
T-CHO (mg/dL)	171	±	11	120	±	9*	112	±	8*	125	±	8*
TG (mg/dL)	117	±	14	28	±	3*	49	±	4*	77	±	7
BS (mg/dL)	103	±	4	198	±	14*	141	±	6*	115	±	9
IRI (μU/mL)	7.6	±	0.8	42.8	±	8.8*	27.1	±	4.2*	19.7	±	7.2
NH_3 (μg/dL)	43.2	±	3.2	83.2	±	6.3*	53.1	±	4.4	40.1	±	3.1
IL-6 (pg/mL)	3.6	±	1.4	227.4	±	164.7*	38.7	±	7.5*		–	
TNF-α (pg/mL)	1.6	±	0.2	7.4	±	5.9	3.5	±	1.4*		–	
NEFA (μEq/l)	512	±	61		–			–		607	±	69
npRQ	0.90	±	0.01		–			–		0.86	±	0.02*
REE (kcal)	1171	±	53		–			–		1153	±	53
%REE (%)	98.3	±	3.4		–			–		99.2	±	3.5

Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BS, blood sugar; CRP, C-reactive protein; IL-6, interleukin- 6; IRI, immunoreactive insulin; NEFA, non-esterified fatty acids; NH_3, ammonia; npRQ, non-protein respiratory quotient; REE, resting energy expenditure; PLT, platelets; T-Bil, total bilirubin; T-CHO, total cholesterol; TNF-α, tumor necrosis factor-α; TG, triacylglycerol; TP, total protein; WBC, white blood cells. Values are means ± SEM.
*$P < 0.05$ compared with preoperative value

The metabolites in the fasting serum and urine samples were analyzed by mass spectrometry-based metabolome profiling using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) and capillary electrophoresis tandem mass spectrometry (CE-MS/MS). To minimize the effects of metabolic changes in the serum, the metabolites were extracted from the serum within 6 h of collection; the extraction method has been described in detail elsewhere [16]. The CE-TOFMS and CE-MS/MS analyses of the cationic and anionic metabolites were performed as described previously [17,18], and the raw data were processed with our proprietary software (MasterHands) [17,19]. We decided a priori to measure absolute concentrations of 555 metabolites (274 cations, 241 anions, and 40 peptides) that could be expected to be stably observed in most human serum and urine samples, and that had matched standards.

Statistical analysis

The results are expressed as mean ± SEM. Statistical analysis was performed using Prism 6.07 for Windows (GraphPad Software Inc.). $P < 0.05$ was considered to indicate statistically significant differences. Values after surgery were compared with the preprocedural values using the Wilcoxon matched-pairs signed-rank test and the Friedman test with Dunn's test. Heat maps of metabolite levels were generated using hierarchical clustering based on Pearson correlation coefficients, using the MultiExperiment Viewer software (Institute for Genomic Research; [20].

Regression analysis was performed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA). Multiple regression analysis was used to identify clinical variables that were significantly associated with AAs and taurine (Tau) levels preoperatively and on PODs 1, 3, and 14, including WBC, CRP, AST, ALT, PLT, TP, Alb, and T-Bil as the possible predictive variables. Multivariate analysis comprised the stepwise selection of multiple linear regression models. To visualize the postoperative metabolic changes in comparison with the preoperative condition, the data were exported and analyzed by principal components analysis (PCA) using SIMCA-P software 12.0.1 (Umetrics AB, Umea, Sweden) after centering the means and scaling to unit variance.

Results

Blood biochemistry

Laboratory findings before and after hepatectomy are shown in Tables 1 and 2. Levels of inflammation markers (WBC, CRP, IL-6, and tumor necrosis factor-α) significantly increased and serum albumin levels significantly decreased after liver resection. Most laboratory findings were significantly higher or lower (Friedman test) on PODs 1 and 3 in the serum compared with baseline, with a return to near baseline levels on POD 14.

Multivariate analysis: Correlations between changes in serum AAs and Tau levels and laboratory parameters

Correlations between changes from baseline (i.e., preoperative day 0) in AA and Tau levels and laboratory parameters were investigated using stepwise multiple regression analysis (Table 3). Stepwise multivariate regression models found that changes in serum levels of Gln correlated negatively with changes in CRP, and changes in serum levels of Tau correlated positively with changes in WBC and negatively with changes in ALT (Table 3).

Metabolic profiling of the samples

The PCA showed a trend toward intergroup separation on the score plot (Fig. 1). The results showed that the posthepatectomy serum samples from PODs 1 and 3, but not POD 14, were separated from the prehepatectomy sample (day 0). Considering variations in metabolomics, these changes are further visualized on heat maps, as are results of hierarchical cluster analysis (Fig. 2), providing an intuitive visualization of the metabolic remodeling on PODs 1, 3, and 14 for serum and POD 3 for urine compared with the baseline levels (day 0 for both serum and urine). Both PCA and hierarchical cluster analysis showed a clear separation between day 0 and PODs 1 and 3 for serum and between day 0 and POD 3 for urine, indicating that metabolic changes occurred perioperatively in the patients who underwent hepatectomy.

The analyses of serum levels showed that valine (Val) significantly decreased on PODs 1 and 14 and leucine (Leu) significantly decreased on POD 14, whereas phenylalanine significantly increased on PODs 1 and 3 and tyrosine significantly increased on POD 1. Thus, the serum Fischer ratio (i.e., BCAA/aromatic AAs) as an integrated parameter significantly decreased on PODs 1 and 3 (Fig. 3A). Serum levels of Tau significantly increased on PODs 1, 3, and 14, and urine levels of Tau and 3-MH decreased on POD 3 (Figs. 3B,C).

We investigated the 20 metabolites that showed the greatest relative postoperative increases or decreases from baseline in the serum or urine (Fig. 4). Many of the metabolites showed greater relative changes in urine than in serum from baseline. The

Table 3
Multivariate analysis of parameters correlating with the changes from baseline (Δ) in serum levels of amino acids and taurine, measured 1, 3, and 14 d after surgery in 16 hepatocellular carcinoma patients

	Dependent variable	Independent variable	Regression coefficient	Standardized regression coefficient	*P*-value
S1		–	–	–	–
S3	ΔAla	ΔWBC	0.004	0.584	0.018
S14		–	–	–	–
S1		–	–	–	–
S3	ΔArg	ΔALT	−0.056	−0.592	0.016
S14		ΔAST	−0.221	−0.572	0.021
S1		–	–	–	–
S3	ΔAsn	ΔALT	−0.029	−0.503	0.047
S14		–	–	–	–
S1		–	–	–	–
S3	ΔCys	–	–	–	–
S14		ΔPLT	20.219	−0.608	0.012
S1		–	–	–	–
S3	ΔGln	ΔCRP	−7.742	−0.502	0.048
S14		–	–	–	–
S1	ΔGlu	ΔT-Bil, ΔWBC, ΔPLT	−165.831, 0.016, −13.397	−0.576, 0.719, −5.111	0.005, 0.002, 0.015
S3		ΔCRP	−10.097	−0.549	0.028
S14		–	–	–	–
S1		–	–	–	–
S3	ΔGly	–	–	–	–
S14		ΔWBC	0.006	0.514	0.041
S1		–	–	–	–
S3	ΔHis	–	–	–	–
S14		ΔAlb	−18.996	−0.512	0.043
S1		–	–	–	–
S3	ΔIle	ΔCRP	−4.161	−0.534	0.033
S14		–	–	–	–
S1		–	–	–	–
S3	ΔLeu	ΔALT	−0.200	−0.530	0.035
S14		–	–	–	–
S1		–	–	–	–
S3	ΔLys	ΔALT	−0.051	−0.601	0.014
S14		–	–	–	–
S1		ΔWBC	0.008	0.638	0.008
S3	ΔMet	ΔALT	−0.077	−0.539	0.035
S14		–	–	–	–
S1		–	–	–	–
S3	ΔPhe	–	–	–	–
S14		–	–	–	–
S1		–	–	–	–
S3	ΔPro	ΔCRP, ΔALT	−4.045, −0.085	−0.497, −0.444	0.027, 0.045
S14		–	–	–	–
S1		–	–	–	–
S3	ΔSer	ΔALT	−0.153	−0.574	0.02
S14		ΔPLT	5.103	0.554	0.026
S1	ΔThr	ΔT-Bil, ΔWBC	52.335, −0.002	3.706, −2.175	0.003, 0.049
S3		–	–	–	–
S14		–	–	–	–
S1		–	–	–	–
S3	ΔTrp	ΔTP, ΔALT	27.854, −0.065	0.593, −0.408	0.007, 0.045
S14		ΔAST	−0.397	−0.628	0.009
S1		–	–	–	–
S3	ΔTyr	ΔCRP,	−12.335, 48.394	−0.790,	0.002, 0.043
S14		–	–	–	–
S1		–	–	–	–
S3	ΔVal	ΔTP	69.268	0.618	0.011
S14		–	–	–	–

(*continued*)

Table 3 (*Continued*)

	Dependent variable	Independent variable	Regression coefficient	Standardized regression coefficient	*P*-value
S1		ΔWBC	0.008	0.536	0.032
S3	ΔTau	ΔWBC	0.02	0.775	0.001
S14		ΔALT	−0.081	−0.516	0.043

Ala, Alanine; Alb, albumin; ALT, alanine aminotransferase; Arg, arginine; Asn, asparagine; AST, aspartate aminotransferase; CRP, C-reactive protein; Cys, cysteine; Gln, glutamine; Glu, glutamate; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; PLT, platelets; Pro, proline; Ser, serine; T-Bil, total bilirubin; Tau, taurine; Thr, threonine; TP, total protein; Trp, tryptophan; Tyr, tyrosine; Val, valine; WBC, white blood cells

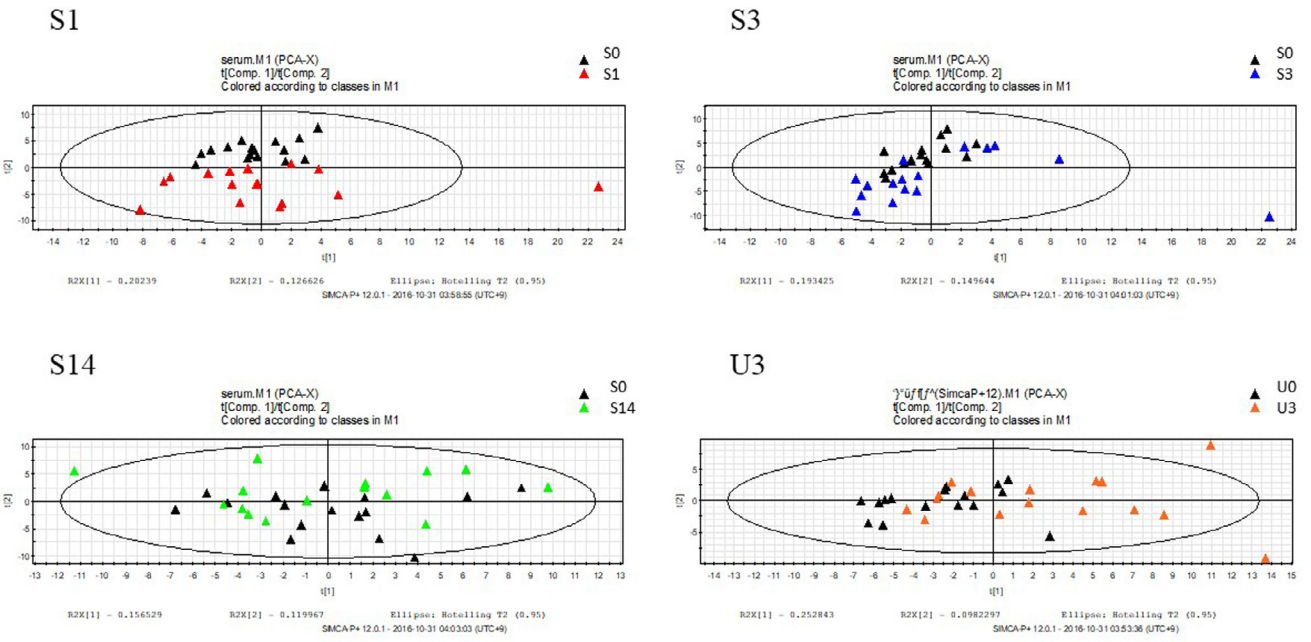

Fig. 1. Principal component analysis (PCA) of metabolomics data reveals clustering of data into separate cohort. PCA score plot generated from metabolites measured with samples. Scores plots from PCA classifying serum and urine sample with control group (black triangle), POD1 group (red triangle), POD3 (blue and orange triangle), POD14 group (green triangle). POD, postoperative day.

metabolites in the serum returned to their baseline values on POD 14. Hypoxanthine showed the greatest relative change in serum in the early postoperative period (PODs 1 and 3), and o-hydroxybutyrate appeared in the urine. Serum 3-hydroxybutyrate was included on PODs 1 and 3 in decreased metabolites and on POD 14 in increased metabolites.

A map of the tricarboxylic acid cycle was constructed from the measured serum metabolites (Fig. 5). Citrate, cis-aconitate, isocitrate, 2-oxoglutarate, succinate, fumarate, and malate were observed. Cis-aconitate and isocitrate significantly increased and 2-oxoglutarate significantly decreased on POD 14.

Discussion

Appropriate management of the patient's nutritional metabolism is essential during the perioperative period [20]. In this study of patients undergoing hepatectomy, many metabolites in the serum and urine increased at the early postoperative stage, whereas serum BCAA concentrations decreased on POD 1. Urinary Val and Leu concentrations increased on POD 3, which was thought to be due to enhanced muscle breakdown. Concentrations of aromatic AAs (phenylalanine and tyrosine) in the serum and urine tended to increase on PODs 1 and 3, with the serum Fischer ratio showing a decrease on POD 1 or 3. T-Bil levels increased postoperatively and returned to baseline levels on POD 14. Alb levels, indicating protein synthesis in the liver (and acute phase response), had not recovered by POD 14, and CRP did not increase.

A decrease in Alb may be one of the causes of postoperative ascites. In previous studies, survival was significantly greater in patients without postoperative ascites than in those with the condition [21]. The administration of BCAAs has been shown to increase Alb synthesis in rat hepatocytes via the mechanistic target of rapamycin signaling pathway [22], and to promote rapid improvement of Alb levels in HCC patients with liver cirrhosis after hepatectomy [11].

BCAAs promote protein synthesis [11] and improve insulin resistance [23]. AAs in the BCAA amino group are changed into glycogenic amino acids, such as alanine and Gln, via the glucosealanine cycle [24]; BCAAs are therefore involved in gluconeogenesis and become sources of energy [25]. Insulin sensitivity is reduced by the postoperative increase in hormones such as cortisol, catecholamine, and glucagon. Insulin by oral glucose tolerance test infusion results in a decrease of AAs in the blood [26]. Our results showed that the concentration of BCAAs decreased on PODs 1 and 3. We believe this decrease was a result of the BCAAs being transported into the tissues by insulin, and assume that the Fischer ratio decreased postoperatively as a result of BCAAs being used as the energy source in skeletal muscles [27] and ammonia detoxification. Therefore, we conclude that administration of

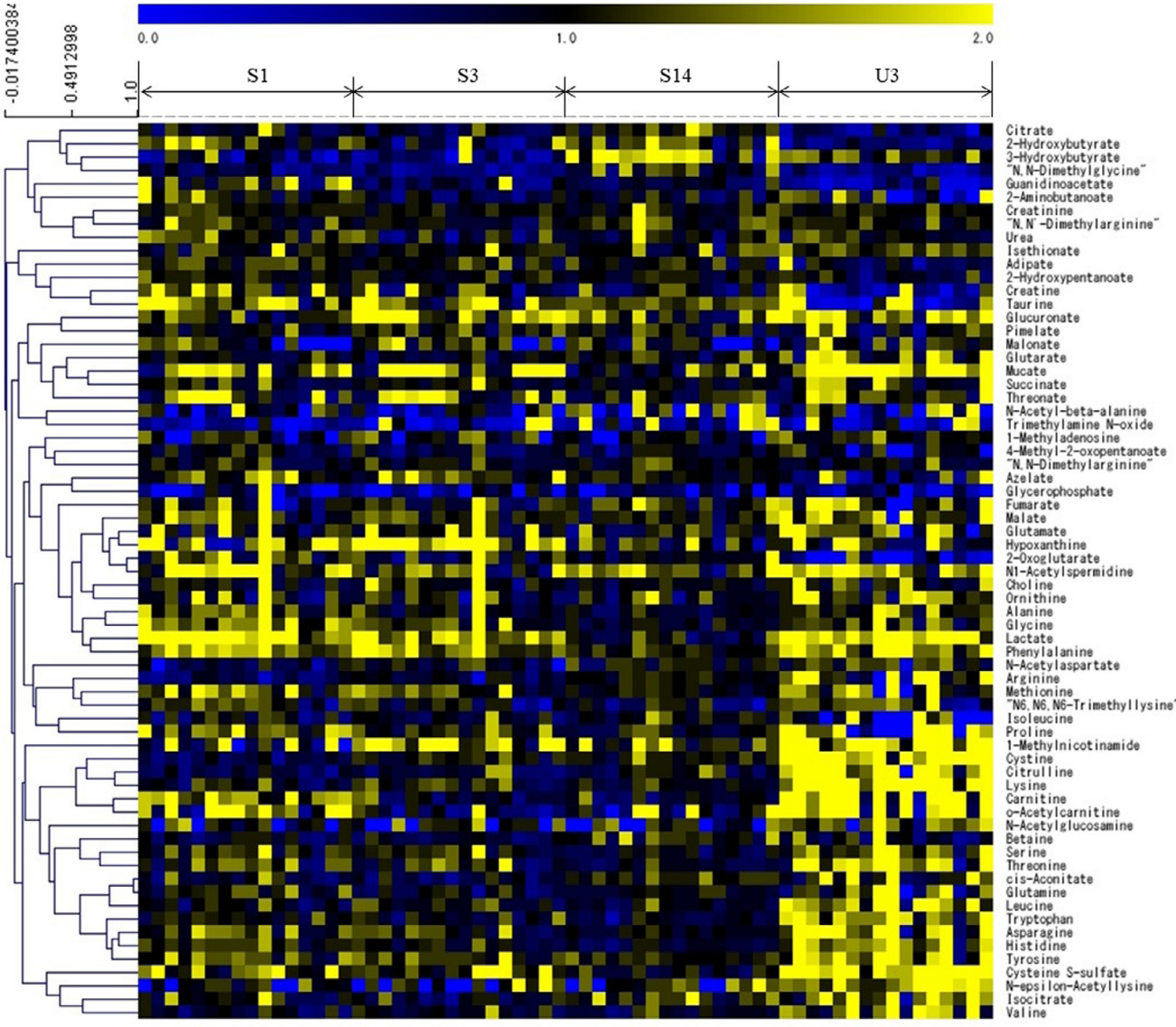

Fig. 2. Heat map representing hierarchical clustering of 67 compounds in serum and urine samples from patients at various numbers of postoperative days. Each row shows data for a specific metabolite, and each column shows data for the patients undergoing liver resection. The concentration of the compound in each individual was divided by the average preoperative concentration, and the obtained values were averaged again for each postoperative day. The compounds were clustered based on elucidation distances. Blue and yellow denote high and low concentrations, respectively, compared with preoperative concentrations.

BCAAs could be beneficial for the outcomes for patients with abnormal metabolism after liver resection.

Multivariate analyses were conducted to examine associations between changes in AA and Tau levels and changes in the eight variables (WBC, CRP, AST, ALT, PLT, TP, Alb, and T-Bil) included in the biochemical analysis of the serum samples. On POD 3, levels of Gln and Tau were independently associated with the degree of inflammation. Postoperative catabolism of skeletal muscle has a pivotal role in regulating the availability of Gln and Tau, due to the high content of these AAs in muscle cells [28]. Gln levels tended to decrease in S3 compared with S0 and were inversely related with CRP. Gln is highly consumed by several tissues in the postoperative state for various purposes; in particular, Gln utilization has been linked to functional activities of cells of the immune system. BCAA are precursors for glutamate (and Gln), and a key function of the BCAA in the skeletal muscle is to provide the nitrogen needed to maintain muscle pools of glutamate, alanine, and Gln [29]. On the other hand, Tau levels were significantly higher in S3 than in S0 and directly related to WBC count, whereas urinary excretion in U3 decreased: Indeed in WBC, large amounts of Tau are needed intracellularly because of its antioxidant activity [30]; however, the mechanism cannot be explained based on the present data.

It has been found that, in the acute phase of a hyperglycemic state under the influence of surgery known as surgical diabetes. In the present study, the levels of 3-MH in urine significantly increased on POD 3. 3-MH, a constitutive AA of myosin and actin (a muscle fiber protein), is known to be a muscle proteolysis marker and is used to determine the efficacy of nutritional management [31,32]. 3-MH isolated by muscle fiber proteolysis is not used for protein synthesis and is excreted in the urine [33]; thus, a change in 3-MH concentration reflects metabolic turnover of the muscle fiber protein, providing a good index of internal protein metabolism [33]. The catabolism of muscle protein is enhanced under severe conditions, such as starvation, burns, and sepsis, resulting in the increased excretion of 3-MH in the urine [34–37].

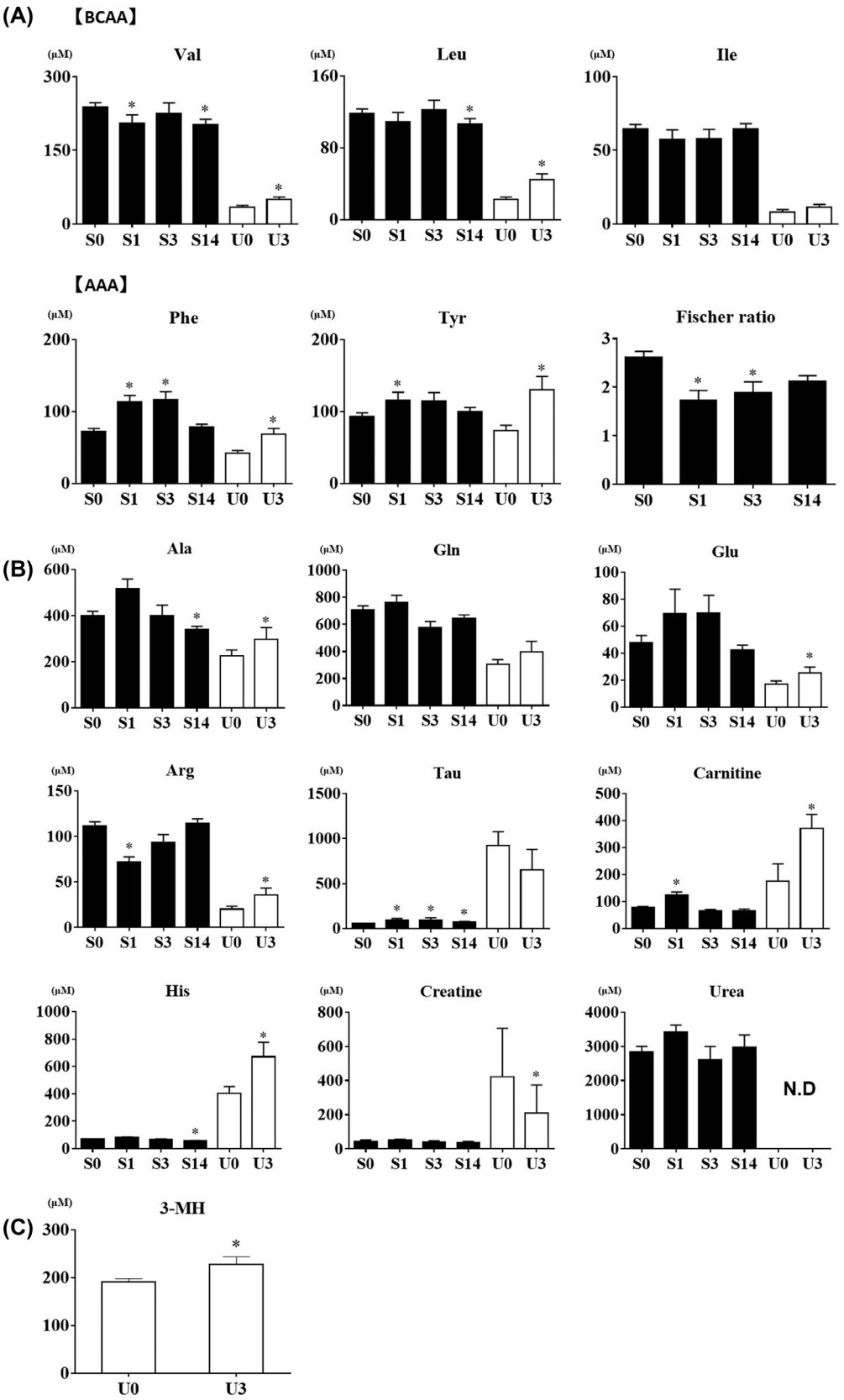

Fig. 3. (**A**) Changes in metabolite levels in serum 0, 1, 3, and 14 d and in urine 0 and 3 d after liver resection. (**B**) Changes in metabolite levels in serum 0, 1, 3, and 14 d and in urine 0 and 3 d after liver resection. (**C**) Changes in metabolite levels in urine 0 and 3 d after liver resection. *Significant difference from preoperative value ($P < 0.05$; Wilcoxon matched-pairs signed-rank test). 3-MH, 3-Methylhistidine; AAA, aromatic amino acids; Ala, alanine; Arg, arginine; BCAA, branched-chain amino acids; Gln, glutamine; Glu, glutamate; His, histidine; Ile, isoleucine; Leu, leucine; Tau, taurine; Tyr, tyrosine; Val, valine.

	Ranking	Metabolite			
		UP	FC	DOWN	FC
S1	1	Hypoxanthine	4.74	Trimethylamine N-oxide	0.31
	2	Pyruvate	2.62	3-Indoxyl sulfate	0.43
	3	5-Oxoproline	2.57	3-Hydroxybutyrate	0.47
	4	Lactate	2.47	Cysteine-glutathione disulphide -Divalent	0.47
	5	Azelate	2.41	3-Methylhistidine	0.56
	6	Asp	2.10	Proline betaine	0.58
	7	2-Oxoglutarate	2.10	g-Glu-Glu	0.59
	8	Cysteine S-sulfate	2.04	Arg	0.64
	9	N1-Acetylspermidine	1.95	g-Glu-Ornithine	0.66
	10	Sebacate	1.92	2-Hydroxyisobutyrate	0.66
S3	1	Hypoxanthine	11.50	Proline betaine	0.27
	2	3-Phenyllactate	3.11	3-Hydroxybutyrate	0.47
	3	Phe-Phe	3.02	N,N-Dimethylglycine	0.49
	4	Azelate	2.51	3-Methylhistidine	0.50
	5	5-Oxoproline	2.46	3-Indoxyl sulfate	0.54
	6	Lactate	2.37	Glycerophosphate	0.58
	7	Asp	2.35	Urate	0.61
	8	Glucuronate	2.22	Cysteine-glutathione disulphide -Divalent	0.66
	9	2-Oxoglutarate	2.21	N-Acetyl-beta-alanine	0.66
	10	Sebacate	1.92	g-Glu-Ornithine	0.67
S14	1	3-Hydroxybutyrate	1.81	Glycerophosphorylcholine	0.58
	2	Cytidine	1.50	3-Indoxyl sulfate	0.60
	3	Pipecolate	1.50	Glycerophosphate	0.64
	4	N-epsilon-Acetyllysine	1.45	N6,N6,N6-Trimethyllysine	0.73
	5	5-Hydroxylysine	1.45	Guanidinoacetate	0.76
	6	Hydroxyproline	1.39	Trp	0.80
	7	3-Phenyllactate	1.38	Thr	0.80
	8	3-Aminoisobutyrate	1.35	His	0.80
	9	Proline betaine	1.34	2-Hydroxyglutarate	0.81
	10	Taurine	1.31	4-Methyl-2-oxopentanoate	0.81
U3	1	Mucate	9.36	Quinate	0.10
	2	2-Hydroxyhippurate	5.22	Trigonelline	0.11
	3	Citrulline	3.64	Hippurate	0.32
	4	Lys	3.59	Piperidine	0.32
	5	Cysteine S-sulfate	3.59	Guanidinoacetate	0.34
	6	Cystine	3.08	2-Oxoglutarate	0.35
	7	Pro	2.92	Glycerophosphate	0.38
	8	p-Hydroxyphenylacetate	2.76	3-Methylhistidine	0.43
	9	trans-Aconitate	2.46	N,N-Dimethylglycine	0.45
	10	Lactate	2.25	Creatine	0.50

Fig. 4. FC >1 indicate higher levels of serum or urinary metabolites relative to preoperative levels (S0, U0), whereas fold-change (FC) <1.0 indicate lower levels. Arg, arginine; Asp, asparagine; FC, fold-change; g-Glu-Glu, gamma-glutamylglutamate; His, histidine; Lys, lysine; Pro, proline; Thr, threonine; Trp, tryptophan.

We previously reported that npRQ was recovered by POD 14 in young liver transplant donors (<40 y of age) but had not improved in patients with HCC or in older donors (≥40 y of age) [38]. After liver resection, npRQ is influenced by decreased glycogen accumulation due to existing metabolic abnormalities. The present results showed that npRQ decreased and non-esterified fatty acids increased on POD 14 compared with baseline. In the present study, HCC patients showed catabolic state until POD 14.

Characteristic movements of upstream metabolites of 2-oxoglutarate were observed. The present results showed that cis-aconitate and isocitrate significantly increased and 2-oxoglutarate significantly decreased on POD 14. This may be explained by activation of the tricarboxylic acid cycle metabolism, requiring ATP for liver regeneration [39], with some of the metabolite in tissue appearing to have leaked into the blood.

By using an artificial pancreas system, it has been shown that perioperative glycemic control in patients undergoing surgery is beneficial to reduce postoperative infections [40]. Actually, in a randomized study in patients undergoing hepatic resection, the control of postoperative glucose levels using insulin therapy contributed to a reduction in the incidence of surgical site infections [41]. Previous studies have indicated the importance of administering a highly concentrated, BCAA-rich AA preparation during the perioperative period [42]. Adequate and timely nutrition care to compensate for reduced levels of AAs such as BCAAs in the perioperative period has been shown also to contribute in maintaining postoperative glycemic control, besides modulating the acute phase response and reducing muscle proteolysis: Okabayashi found that preoperative oral administration of carbohydrate and BCAA reduces postoperative insulin resistance in patients

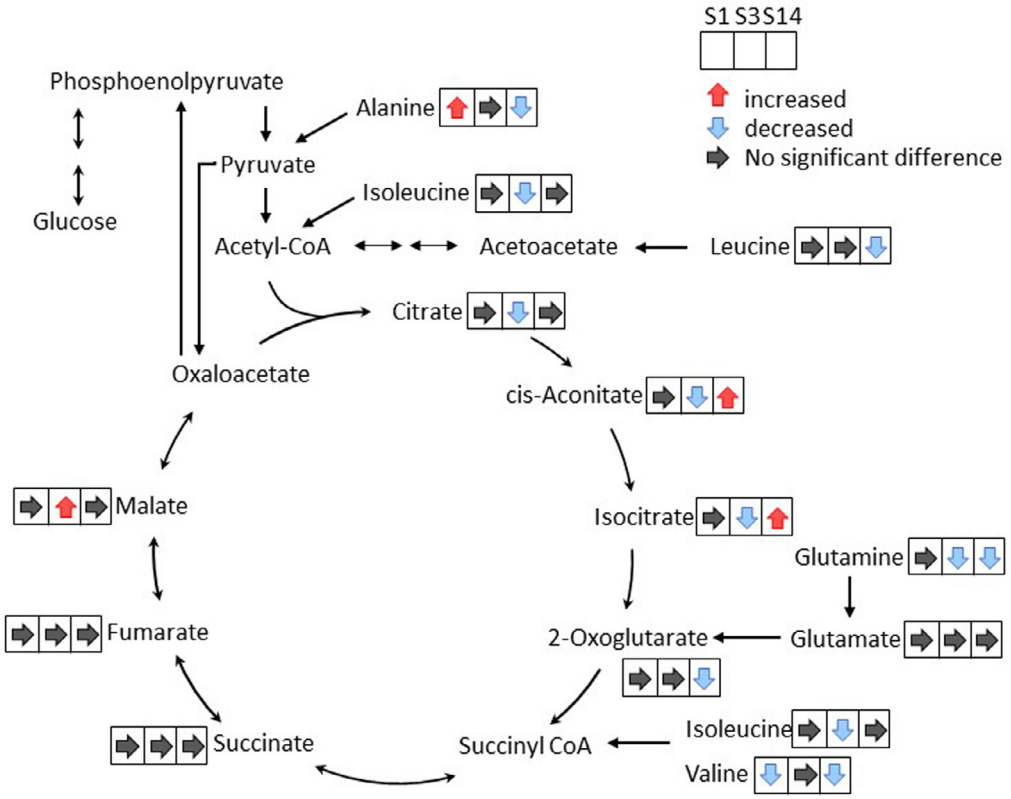

Fig. 5. Schematic overview of altered serum metabolites in glucose utilization and TCA cycle changes in after surgery compared to before. Significant test were used Wilcoxon matched-pairs signed-rank test. It represents POD 1, 3 and 14 after liver resection from the left. The metabolites are shown in color: Red represents significantly increased metabolites, blue represents significantly decreased metabolites and black represents no significant difference metabolites, respectively. CoA, coenzyme-A; POD, postoperative day; TCA, tricarboxylic acid.

undergoing hepatic resection [43]. The results of the present study may be useful for addressing an excess or deficiency of metabolites because the greatest changes in the various serum and urinary metabolites were observed on different PODs.

However, a limitation of the study was that the study period was not long enough to observe metabolite levels returning to baseline levels. Furthermore a wider study should be performed to allow comparisons with other types of patients.

This study provides an example of the potential benefits of metabolomics when applied to the characterization of patients undergoing liver resection for HCC. The suitability of the method was verified in this study by reproducing the changes in plasma AA pattern known to occur in postoperative liver dysfunction in this type of patient and that may have nutritional implications. The analysis of additional specific aspects will be the objective of further studies that include a larger number of patients. In the future, perioperative nutrition care should be determined according to disease type.

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References

[1] Redaelli CA, Wagner M, Krähenbühl L, Gloor B, Schilling MK, Dufour J-F, et al. Liver surgery in the era of tissue-preserving resections: early and late outcome in patients with primary and secondary hepatic tumors. World J Surg 2002;26:1126–32.
[2] Wiezer MJ, Meijer C, Vuylsteke R, Pullens RH, Prins HA, Cuesta MA, et al. Is major liver surgery associated with an increased systemic inflammatory response? A prospective comparison of hemihepatectomy and other major abdominal surgery. Liver Int 1999;19:220–7.
[3] Sidiq T, Khan N. Nutrition and food sciences protein calorie malnutrition in liver cirrhosis. J Nutr Food Sci 2014;5.
[4] Yamanaka H, Genjida K, Yokota K, Taketani Y, Morita K, Miyamoto KI, et al. Daily pattern of energy metabolism in cirrhosis. Nutrition 1999;15:749–54.
[5] Tajika M, Kato M, Mohri H, Miwa Y, Kato T, Ohnishi H, et al. Prognostic value of energy metabolism in patients with viral liver cirrhosis. Nutrition 2002;18:229–34.
[6] ASPEN Board of Directors and the Clinical Guidelines Task Force. Guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients. JPEN J Parenter Enteral Nutr 2002;26(suppl 1). 1SA–138SA.
[7] Plauth M, Cabré E, Campillo B, Kondrup J, Marchesini G, Schü Tz T, et al. ESPEN guidelines on parenteral nutrition: hepatology. Clin Nutr 2009;28:436–44.
[8] Ciuni R, Biondi A, Grosso G, Nunnari G, Panascia E, Randisi L, et al. Nutritional aspects in patient undergoing liver resection. Updates Surg 2011;63:249–52.
[9] Ito A, Higashiguchi T. Effects of glutamine administration on liver regeneration following hepatectomy. Nutrition 1999;15:23–8.
[10] Kurokawa T, An J, Tsunekawa K, Shimomura Y, Kazama S, Ishikawa N, et al. Effect of L-arginine supplement on liver regeneration after partial hepatectomy in rats. World J Surg Oncol 2012;10:99.
[11] Togo S, Tanaka K, Morioka D, Sugita M, Ueda M, Miura Y, et al. Usefulness of granular BCAA after hepatectomy for liver cancer complicated with liver cirrhosis. Nutrition 2005;21:480–6.
[12] Chiarla C, Giovannini I, Giuliante F, Ardito F, Vellone M, De Rose AM, et al. Parenteral nutrition in liver resection. J Nutr Metab 2012;2012:508103.

[13] Theodoridis G, Gika HG, Wilson ID. Mass spectrometry-based holistic analytical approaches for metabolite profiling in systems biology studies. Mass Spectrom Rev 2011;30:884–906.
[14] Zhao X, Fritsche J, Wang J, Chen J, Rittig K, Schmitt-Kopplin P, et al. Metabonomic fingerprints of fasting plasma and spot urine reveal human pre-diabetic metabolic traits. Metabolomics 2010;6:362–74.
[15] Rubio-Aliaga I, de Roos B, Duthie SJ, Crosley LK, Mayer C, Horgan G, et al. Metabolomics of prolonged fasting in humans reveals new catabolic markers. Metabolomics 2011;7:375–87.
[16] Hirayama A, Nakashima E, Sugimoto M, Akiyama S, Sato W, Maruyama S, et al. Metabolic profiling reveals new serum biomarkers for differentiating diabetic nephropathy. Anal Bioanal Chem 2012;404:3101–9.
[17] Sugimoto M, Wong DT, Hirayama A, Soga T, Tomita M. Capillary electrophoresis mass spectrometry-based saliva metabolomics identified oral, breast and pancreatic cancer-specific profiles. Metabolomics 2010;6:78–95.
[18] Soga T, Igarashi K, Ito C, Mizobuchi K, Zimmermann H-P, Tomita M. Metabolomic profiling of anionic metabolites by capillary electrophoresis mass spectrometry. Anal Chem 2009;81:6165–74.
[19] Hirayama A, Kami K, Sugimoto M, Sugawara M, Toki N, Onozuka H, et al. Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. Cancer Res 2009;69:4918–25.
[20] Fan S-T, Lo C-M, Lai E, Chu K-M, Liu C-L, Wong J. Perioperative nutritional support in patients undergoing hepatectomy for hepatocellular carcinoma. N Engl J Med 1994;331:1547–52.
[21] Chan K-M, Lee C-F, Wu T-J, Chou H-S, Yu M-C, Lee W-C, et al. Adverse outcomes in patients with postoperative ascites after liver resection for hepatocellular carcinoma. World J Surg 2012;36:392–400.
[22] Ijichi C, Matsumura T, Tsuji T, Eto Y. Branched-chain amino acids promote albumin synthesis in rat primary hepatocytes through the mTOR signal transduction system. Biochem Biophys Res Commun 2003;303:59–64.
[23] Valerio A, D'Antona G, Nisoli E. Branched-chain amino acids, mitochondrial biogenesis, and healthspan: An evolutionary perspective. Aging 2011;3:464–78.
[24] Felig P. The glucose-alanine cycle. Metabolism 1973;22:179–207.
[25] O'Connell TM. The complex role of branched chain amino acids in diabetes and cancer. Metabolites 2013;3:931–45.
[26] Skurk T, Rubio-Aliaga I, Stamfort A, Hauner H, Daniel H. New metabolic interdependencies revealed by plasma metabolite profiling after two dietary challenges. Metabolomics https://doi.org/10.1007/s11306-010-0258-z.
[27] Tom A, Nair KS. Assessment of branched-chain amino acid status and potential for biomarkers. J Nutr 2006;136:324.. S–30 S.
[28] Rennie MJ. Influence of exercise on protein and amino acid metabolism. Comprehensive physiology. Hoboken, NJ: John Wiley & Sons, Inc.; 2011.
[29] Cole J. Metabolism of BCAAs. In: Rajendram R, Preedy VR, Patel VB, eds. Branched chain amino acids in clinical nutrition: vol. 1, New York, NY: Springer New York; 2015:13–24.
[30] Stapleton PP, O'Flaherty L, Redmond HP, Bouchier-Hayes DJ. Host defense—a role for the amino acid taurine? JPEN 1998;22:42–8.
[31] Neuhäuser M, Bergström J, Chao L, Holmström J, Nordlund L, Vinnars E, et al. Urinary excretion of 3-methylhistidine as an index of muscle protein catabolism in postoperative trauma: the effect of parenteral nutrition. Metabolism 1980;29:1206–13.
[32] Tashiro T. Contribution by skeletal muscle to whole-body protein catabolism in critical illness: usefulness of urinary 3-methylhisitidine excretion. Nutrition 1998;14:708–10.
[33] Young VR, Munro HN. Ntau-methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. Fed Proc 1978;37:2291–300.
[34] De Bandt JP, Coudray-Lucas C, Lioret N, Lim SK, Saizy R, Giboudeau J, et al. A randomized controlled trial of the influence of the mode of enteral ornithine alpha-ketoglutarate administration in burn patients. J Nutr 1998;128:563–9.
[35] Tiao G, Hobler S, Wang JJ, Meyer TA, Luchette FA, Fischer JE, et al. Sepsis is associated with increased mRNAs of the ubiquitin-proteasome proteolytic pathway in human skeletal muscle. J Clin Invest 1997;99:163–8.
[36] Yoshizawa F, Nagasawa T, Nishizawa N, Funabiki R. Nutrient metabolism protein synthesis and degradation change rapidly in response to food intake in muscle of food-deprived mice. J Nutr 1997;127:1156–9.
[37] Kuhls DA, Rathmacher JA, Musngi MD, Frisch DA, Nielson J, Barber A, et al. Beta-hydroxy-beta-methylbutyrate supplementation in critically ill trauma patients. J Trauma Inj Infect Crit Care 2007;62:125–32.
[38] Sugihara K, Yamanaka-Okumura H, Teramoto A, Urano E, Katayama T, Mori H, et al. Recovery pattern of non-protein respiratory quotient and non-esterified fatty acids after liver resection. Nutrition 2014;30:443–8.
[39] Ozawa K. Hepatic function and liver resection. J Gastroenterol Hepatol 1990;5:296–309.
[40] Hanazaki K, Maeda H, Okabayashi T. Relationship between perioperative glycemic control and postoperative infections. World J Gastroenterol 2009;15:4122–5.
[41] Okabayashi T, Nishimori I, Maeda H, Yamashita K, Yatabe T, Hanazaki K. Effect of intensive insulin therapy using a closed-loop glycemic control system in hepatic resection patients: a prospective randomized clinical trial. Diabetes Care 2009;32:1425–7.
[42] Weimann A, Braga M, Harsanyi L, Laviano A, Ljungqvist O, Soeters P, et al. ESPEN guidelines on enteral nutrition: surgery including organ transplantation. Clin Nutr 2006;25:224–44.
[43] Okabayashi T, Nishimori I, Yamashita K, Sugimoto T, Namikawa T, Maeda H, et al. Preoperative oral supplementation with carbohydrate and branched-chain amino acid-enriched nutrient improves insulin resistance in patients undergoing a hepatectomy: a randomized clinical trial using an artificial pancreas. Amino Acids 2010;38:901–7.