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Original Article

^{1}H-NMR metabolomics identifies significant changes in hypermetabolism after glutamine administration in burned rats

Gang Yang[1,2], Yong Zhang[2], Dan Wu[1,2], Chao Wang[2], Yong-Jun Yang[1], Shi-Jun Fan[1], Lin Xia[1], Yan Wei[1], Xi Peng[1,2,3]

[1]Clinical Medical Research Center, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, PR China; [2]Institute of Burn Research, State Key Laboratory of Trauma, Burns and Combined Injury, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, PR China; [3]Shriners Burns Hospital, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, US

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Abstract: Glutamine is an important amino acid that plays a crucial role in nutritional therapy for patients with burns, but its effects on post-burn metabolism and the underlying mechanisms are unclear. In this study, ^{1}H nuclear magnetic resonance spectroscopy (^{1}H-NMR) was used to examine the effects of glutamine on plasma metabolites in burned rats and to explore the underlying mechanisms. After burn injury, the rats exhibited significant increases in resting energy expenditure (REE) and hypercatabolism, and anabolism was inhibited. The levels of metabolites that reflect the proteolysis of skeletal muscle, such as alanine, histidine, leucine, valine, 3-methylhistidine and creatine, were significantly increased. In addition, the burned rats exhibited energy synthesis dysfunction, as evidenced by a decrease in the ATP concentration and increased levels of lactic acid. Notably, the concentration of α-ketoisovalerate, which reflects the function of the mitochondrial membrane, was significantly increased, suggesting an impairment in mitochondrial function and inhibition of oxidative phosphorylation. Glutamine administration significantly alleviated post-burn hypermetabolism and inhibited proteolysis in skeletal muscle. Consequently, the levels of glutamine metabolites, such as glutamic acid and α-ketoglutarate, along with ATP synthesis were significantly increased, whereas alanine, leucine, 3-methylhistidine and lactic acid were significantly depleted. Furthermore, after glutamine administration, the synthesis of reductive compounds was increased, leading to significantly increased levels of reduced glutathione and NADPH. This process may be an important mechanism by which glutamine alleviates oxidative stress, promotes ATP synthesis, and reduces hypermetabolism after burn.

Keywords: Glutamine, burn, metabolomics, nuclear magnetic resonance (NMR)

Introduction

Persistent hypermetabolic reactions are the most prominent metabolic feature of severe burns, such that the metabolism of material and energy metabolism undergo dramatic changes after the injury. In terms of energy metabolism, the main manifestation is a significant increase in energy consumption. The metabolic rate of the patients increases by 50-80% within one month after injury and then gradually decreases but is still significantly higher than the pre-injury level one year later [1]. In terms of material metabolism, abnormal metabolism of glucose, amino acids and fat, particularly glucose and amino acids, has been observed. This hypermetabolism results in the exuberant proteolysis of skeletal muscle and release of large amounts of amino acids, which conversion to glucose by gluconeogenesis, and synthesis the acute phase proteins to meet the demands of the body to resist injury and promote repair [2, 3]. Therefore, amino acids play important roles in the post-burn alterations in energy and substances metabolism. In particular, the glutamine, a specific amino acid, are worth further study.

As early as in 1935, Nobel laureate Hans Krebs clearly stated that although most amino acids

are multifunctional, glutamine is the most complex one [4]. In 1955, Harry Eagle discovered the dependence of metabolically active cells on glutamine and reported decreased proliferation of glutamine deficient [5]. In the 1970s, Windmueller and Spaeth revealed that glutamine, not glucose, is the main energy source in intestinal epithelial cells, thus establishing the clinical importance of this amino acid [6]. The physiological function of glutamine is mainly determined by its specific molecular structure. Unlike other amino acids, glutamine contains two amino groups: an α amino group and an amide group. The latter is the functional group of glutamine, which provides the nitrogen source for the synthesis of proteins and nucleic acids and is referred to as available nitrogen during the mutual conversion of amino acids [7]. Glutamine is converted into a variety of amino acids via transamination and participates in the tricarboxylic acid cycle. The carbon frame of glutamine is gradually oxidized and releases energy in the mitochondria to generate ATP, thereby partially substituting for glucose [8]. In recent years, great importance has been attached to studies of this amino acid in the tumor, immunity, critical illness, and surgery fields, among others, precisely because glutamine has such an important physiological function. Many basic and clinical studies have revealed that glutamine helps maintain the intestinal barrier [9], sustains immune homeostasis [10], promotes protein synthesis, and inhibits skeletal muscle atrophy [11]. Although controversy exists regarding whether glutamine regulates post-burn hypermetabolism, previous studies by our group have established that glutamine administration to patients with burns significantly alleviates protein proteolysis for 14 days [12] and significantly reduces skeletal muscle consumption in burned mice [13]. Other basic and clinical studies have also confirmed that glutamine regulates post-burn hypermetabolism [14, 15], but the specific pathways and mechanisms by which glutamine regulates post-burn metabolism remain unclear. In this study, metabolomics was employed to analyze metabolites as markers of post-burn metabolic changes and to determine the relevant roles of glutamine. Furthermore, we examined the regulatory mechanisms of glutamine metabolism, thereby providing a theoretical basis for the rational use of glutamine in the clinic.

Materials and methods

Animals

Male Sprague-Dawley rats (250 ± 10 g) were obtained from the Animal Center of Daping Hospital of the Third Military Medical University and raised in a controlled environment. Specifically, the animals were housed in a specific pathogen-free (SPF) facility with a temperature of 23-25°C, 40-60% humidity, and a 12:12 h light-dark cycle. All animals were housed in standard animal laboratories that allowed free movement and were provided food and water ad libitum. Procedures using animals were performed in strict accordance with the national requirements for animal ethics and the guidelines for the use of medical experimental animals at the Third Military Medical University. (NIH publication number 8023, revised 1978).

Measurement of resting energy expenditure (REE)

REE was measured using indirect calorimetry, as described [16]. REE was measured by gauging respiratory gas exchange using an open-circuit respirometer (Columbus Instruments, OH). Before measuring REE, food was removed from the morning to noon (6 h). Rats were placed in a Plexiglas metabolic chamber (4 L volume). The air inlet and outlet contained a calcium sulfate column to desiccate the intake and exhaled air. The airflow rate was monitored continuously for 10 min, which covered 6 cycles (lasting for 60 min), and oxygen consumption and CO_2 production were calculated by multiplying the airflow rate by the differences in entry and exit O_2 and CO_2 concentrations, respectively. Based on these values, the difference in O_2 intake (DO_2) was employed to calculate CO_2 (DCO_2) output and REE using the Oxymax software (Columbia Instruments, OH). REE was calculated before the injury and on post-burn days 1, 3, 7, 10, and 14.

Animal burn model

Thirty rats were individually weighed and recorded; their body surface area was calculated. Rats were randomly divided into three groups, namely, the control group (group C, n = 10), burn group (group B, n = 10), and glutamine treatment group (group Gln, n = 10). Rats were anesthetized intraabdominally with 1% pento-

barbital sodium (40 mg/kg). The hair on the back was completely removed with an electric shaver and hair removal cream. Afterwards, 30% of the total surface area of the back was fixed and then treated as described below. In the control group, the exposed area was placed in 37°C water for 15 s, which was the sham burn treatment, whereas the exposed area of the burn group and glutamine group was placed in 95°C water for 15 s to induce a full-thickness burn. An intraabdominal injection of Ringer's lactate solution (40 mL/kg) was immediately performed for fluid resuscitation. After resuscitation, the rats in the glutamine treatment group received intragastric glutamine (1 g/kg/d), whereas animals in the other two groups were administered the same amount of glutamine-free amino acid powder (He'an, Shenzhen Wanhe Pharmaceutical).

Collection of plasma samples

On post-burn day 14, animals in the three groups were sacrificed. Because, only 6 rats in both groups B and Gln had survived on this day, the same number of rats from group C were euthanized. The 18 rats were used for the subsequent metabolomics study. Rats were anesthetized intraabdominally with 1% pentobarbital sodium (40 mg/kg). Six milliliters of blood were drawn from abdominal aortic and injected into a tube containing sodium citrate anticoagulant and a tube containing a procoagulant. The blood samples were centrifuged at 3000 rpm for 10 min. The resulting supernatant was pipetted into 1.5 mL Eppendorf tubes, labeled, and then stored in a -80°C freezer.

Sample preparation for NMR spectroscopy

Plasma samples from burn-treated rats that had been frozen at -80°C were thawed at 4°C and centrifuged at 16000 rpm for 10 min. Four hundred fifty microliters of the resulting supernatant were transferred to an NMR tube, which was supplemented with 50 ul of heavy water (D_2O), thoroughly vortexed for approximately 120 s, allowed to rest for 10 min, and stored at 4°C until further testing.

^{1}H NMR spectral analysis

One-dimensional spectra were obtained at 600.13 MHz on a Bruker Avance DRx600 NMR instrument (Bruker Biospin Rheinstetten, Germany) and recorded at a room temperature of 300 K, during which standard one-dimensional pulse sequences and Carr-Purcell-Meiboom-Gill (CPMG) sequences were used. A spin-spin relaxation of 64 ms was used for all samples. During the relaxation time of 2 s, water was used to suppress radiation. Two hundred fifty-six transients were collected into 32000 data points across a 20 ppm spectral width. Prior to Fourier transformation, the free induction decay (FID) was multiplied by the exponential function, which had a linewidth broadening factor of 0.3 Hz.

Data processing

The acquired raw data were imported into MestReNova 12.0.1 software (Mestrelab Research, Santiago de Compostela, Galicia, Spain, 12.0.1). All spectra were subjected to baseline and phase correction before the chemical shifts were assigned. The chemical shifts of plasma metabolite spectrum were determined by referencing the data to the CH_3 resonance peak of lactic acid at δ 1.32. The chemical shift region between 0 and 8 ppm was subdivided into 4000 intervals with a width of 0.002 ppm. The absorption spectra of water between the chemical shifts of 4.7 to 5.1 ppm were removed. Finally, the data were normalized to eliminate dilution-, volume- or mass-related differences between samples. The same total integral value was assigned to each spectrum before analysis. Total detailed NMR raw data is shown in Supplementary Data.

Analysis of pattern recognition (PR)

The acquired data were imported into SIMCA-P 14.1 software (Umetrics, Umea, Sweden) for further processing. All metabolites analyzed here were reported in previous studies and human metabolite databases (HMDB, http://www.hmdb.ca/). A principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) were used to analyze the datasets. The OPLS-DA model was subjected to 200 random permutations, and R^2 and Q^2 were calculated to evaluate the variables. The metabolites with significant differences were identified according to the variable importance in projection (VIP > 1) in the OPLS-DA model.

Statistical analysis

In order to compare the body weight and REE among groups, we used Welch two-sample

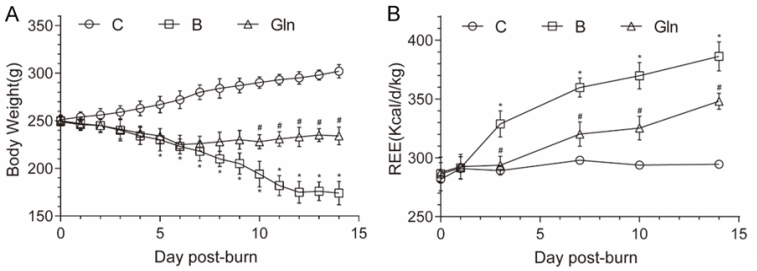

Figure 1. A. Body weights (g) of group C, B and Gln. B. Resting energy expenditures (REE, kcal/d/kg) of burn group and glutamine group compared with control group. Date are mean ± SEM. *: P < 0.05 group B vs. group C. #: P < 0.05 group Gln vs. group B.

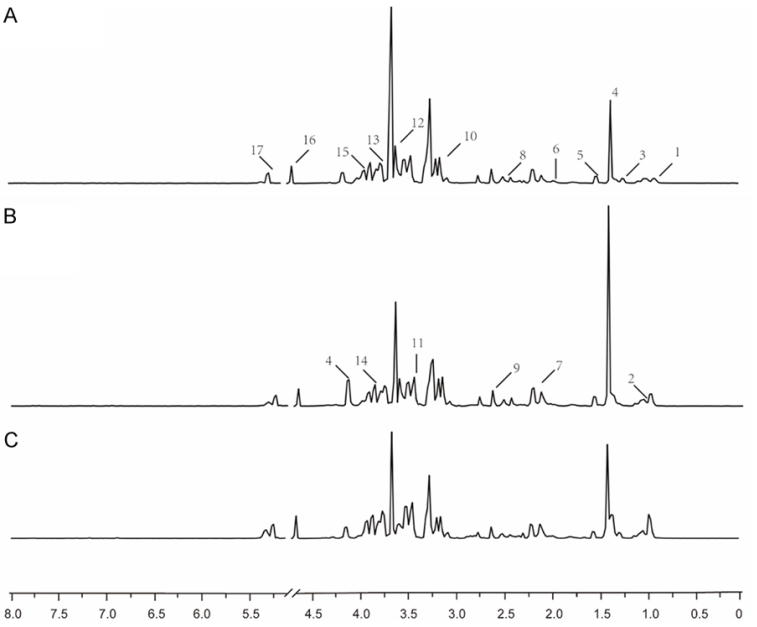

Figure 2. Typical ^{1}H NMR spectra of plasma. A. group C; B. group B; C. group Gln. Plasma main metabolites: 1. low density lipoprotein; 2. leucine; 3. 3-hydroxybutyric acid; 4. lactic acid; 5. alanine; 6. acetate; 7. glutamic acid; 8. glutamine; 9. citric acid; 10. creatine; 11. taurine; 12. glycine; 13. citrulline; 14. methionine; 15. tyrosine; 16. β-glucose; 17. α-glucose.

t-tests for the data. In OPLS-DA models, CV-ANOVA (analysis of variance testing of cross-validated predictive residuals) was used to identify the significantly different characteristics between three groups. P < 0.05 was considered a significant difference to distinguish metabolites. The Mann-Whitney test was employed to analyze abnormally distributed data, while Student's T test was used to analyze normally distributed data among three groups. Data analysis was performed using GraphPad Prism 7.0. The statistical data are presented as the means ± standard errors of the means (SEM).

Analysis of metabolic pathways

MetaboAnalyst 4.0 (http://www.metaboanalyst.ca/) was used to identify the metabolic pathways involved in the mechanism underlying the effects of glutamine and to generate a heat map.

Results

Effects of glutamine on REE in burned rats

The body weight and REE of rats after burn and glutamine treatments are shown in **Figure 1**. Compared with the control group, the burned rats exhibited a significant reduction in body weight; the extent of weight loss was particularly prominent during the first 10 days and was less apparent between days 11-14 (**Figure 1A**). Notably, the weight loss was significantly alleviated by the glutamine treatment, and the body weight began to increase on day 7 and was significantly higher than the weight of group B from days 10-14 (P < 0.05). After burn, the REE was not significantly altered on day 1, but increased beginning on day 3 (**Figure 1B**). On day 14, the REE of group B was increased by 30% compared with the control group. After 3 days of glutamine treatment, the REE was clearly decreased and was consistently lower than group B until day 14, with an average decrease of 10%. In group B and group Gln, 4 animals died and 6 survived, indicating that the glutamine treatment did not affect the survival rate of burned rats.

Metabolic analysis of samples

The ^{1}H-NMR spectra of plasma samples provide an overview of all metabolites from the groups C, B, and Gln (**Figure 2**). The metabolites in the spectrum were derived from previ-

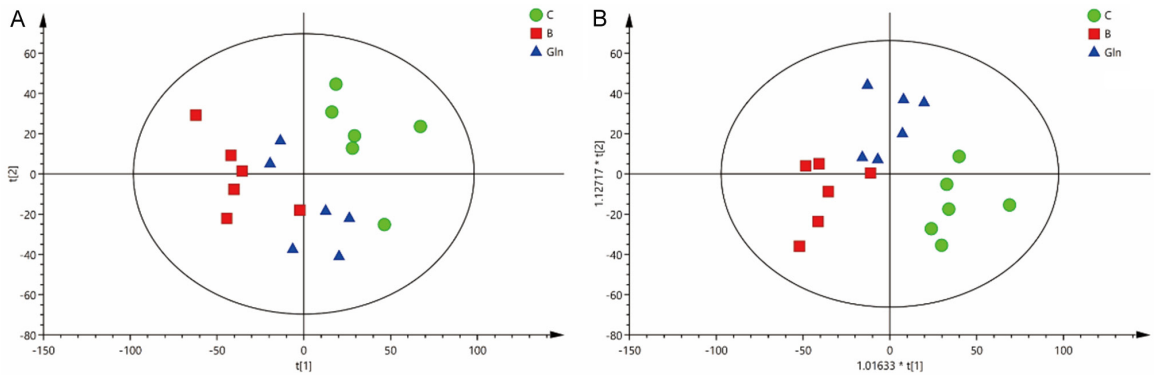

Figure 3. A. PCA score scatter plot based on the ^{1}H NMR spectra of the plasma metabolites from the control (green circles), burn (red squares), and glutamine (blue triangles) groups ($R^2X = 0.574$, $Q^2 = 0.344$). B. OPLS-DA scatter plot based on the ^{1}H NMR spectra of the plasma metabolites from the C, B and Gln groups ($R^2X = 0.457$, $R^2Y = 0.721$, $Q^2 = 0.428$).

ous studies and the human metabolite database (http://www.hmdb.ca/). Compared with the control group, a series of changes in metabolites were observed in the burn group on day 14. These metabolites are mainly involved in amino acid metabolism, bile acid metabolism, and energy metabolism.

PCA and multivariate statistical analysis

The differentially altered metabolites between the control group and the burn group were identified to characterize the post-burn metabolic changes and therapeutic effects of glutamine. First, PCA and OPLS-DA analyses showed that all data points were within the confidence interval. PC1 and PC2 scores revealed clear separation of the groups C, B and Gln, and the group Gln was relatively close to the group B, but not the group C (**Figure 3A**).

By maximizing intergroup differences, an OPLS-DA was used to further separate the group B from the group C or the group B from the group Gln. The quality of the model was described by the cross-validation parameter Q^2, which revealed the predictive ability of the model. The OPLS-DA parameters of the group B and group C were $R^2X = 0.506$, $R^2Y = 0.966$, and $Q^2 = 0.824$ (**Figure 4B**). The parameters of the burn group and the glutamine treatment group were $R^2X = 0.72$, $R^2Y = 1$, and $Q^2 = 0.675$ (**Figure 4E**). A standard seven rounds cross-validation and permutations (200 cycles) were performed to gauge the stability of the model (**Figure 4C**, **4F**). After burn, serum metabolite levels exhibited substantial changes, and the glutamine treatment altered post-burn metabolomic profiles.

Effects of glutamine on the metabolism of various substances in burned rats

The discriminant analysis revealed significant changes in the concentrations of 25 metabolites involved in amino acid metabolism, bile acid metabolism and energy metabolism after burning (**Table 1**). Box plot was used to show the relative levels of metabolites in each group (**Figure 5**). Specifically, the levels of bile acid metabolites (cholic acid, taurocholic acid, and glycine deoxycholic acid), alanine, leucine, valine, histidine, phenylalanine, 3-methylhistidic acid, creatine, lactic acid and succinic acid were significantly increased in the burn group. On the other hand, the levels of urea, glutamine, glutamic acid, glutathione and α-ketoglutarate were decreased in the burn group. Notably, the glutamine treatment reversed the post-burn reductions in the concentrations of urea, glutamine, glutamic acid, glutathione and α-ketoglutarate and, at the same time, decreased the post-burn levels of creatine, lactic acid, succinic acid, histidine, and 3-methylhistidine. We built a heat map to increase the discrimination of the biomarkers among three groups (**Figure 6**).

Effects of glutamine on the main post-burn changes in metabolic pathways and functions

MetaboAnalyst 4.0 was used to investigate the metabolic pathways involved in the functional mechanism of glutamine. The results of the pathway analysis are summarized in **Figure 7** and **Table 2**. A pathway with a P value < 0.05 and impact value > 0.1 was selected as a potential functional pathway. Nine pathways,

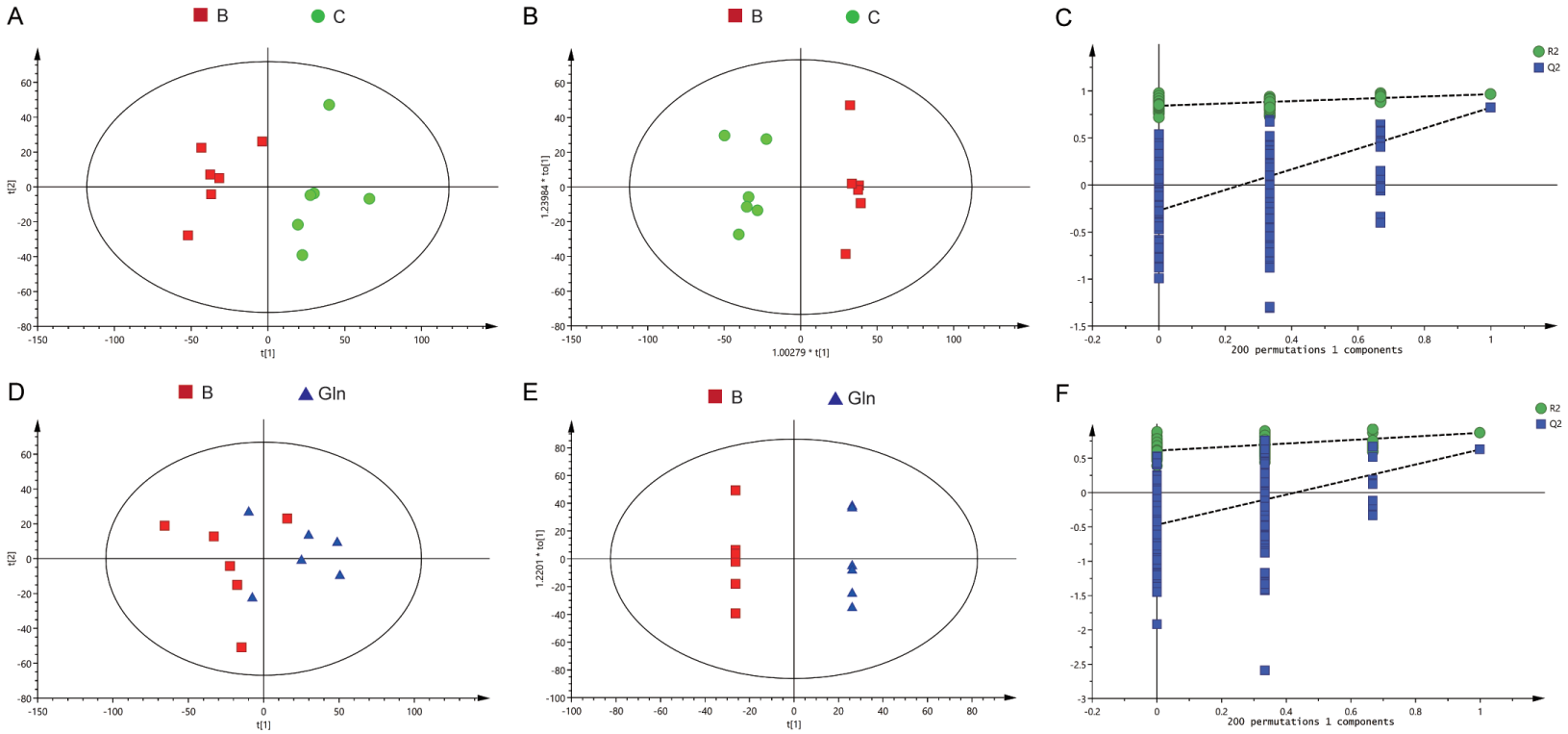

Figure 4. PCA score plot (A), OPLS-DA score plot (B) and OPLS-DA validation plots (C) (permutation number: 200) of plasma samples collected from group C and group Gln. PCA score plot (D), OPLS-DA score plot (E) and OPLS-DA validation plots (F) (permutation number: 200) of plasma samples collected from group B and group Gln.

Table 1. Differential metabolites derived from the OPLS-DA model of ^{1}H-NMR analysis between group C and group B and between group B and group Gln

Metabolites	Chemical shifts	Type[a]	B vs. C			Gln vs. B		
			VIP[b]	P[c]	FC[d]	VIP[b]	P[c]	FC[d]
Urea	5.78	s	1.08	0.0006	-1.84	1.05	0.0034	1.39
3-methylhistidine	3.7	s	1.15	0.0171	0.35	1.84	0.0308	-0.36
Adenosine triphosphate	6.127	d	1.02	0.0002	-1.75	0.98	0.0059	1.25
Alanine	1.46	d	1.92	0.0051	0.81	1.79	0.022	-0.50
α-Ketoglutaric acid	3.00	t	3.17	0.0045	-0.60	2.26	0.0029	0.59
α-Ketoisovaleric acid	1.11	d	2.15	< 0.0001	0.88	1.51	0.0018	-0.48
Citric acid	2.52	d	1.56	0.0031	0.36	1.09	0.0228	-0.25
Creatine	3.92	s	1.74	< 0.0001	0.76	4.31	0.0226	-0.49
Glutamate	2.34	m	1.44	0.002	-0.76	1.14	0.003	0.62
Glutamine	2.446	m	2.52	0.0022	-0.55	1.64	0.026	0.39
Glutathione	3.78	m	1.04	0.0056	-0.18	2.13	0.0143	0.25
Glycine	3.54	s	1.18	0.0019	-0.51	2.24	0.0212	0.32
Glycodeoxycholic acid	0.68	s	1.51	0.007	1.94	1.25	0.014	-1.35
Histidine	7.08	d	1.03	0.0002	1.54	1.21	0.0217	-0.59
Lactic acid	1.32	d	2.71	0.0019	0.92	1.06	0.0216	-0.60
Leucine	1.72	m	1.32	0.0023	1.05	1.14	0.0423	-0.53
Lithocholic acid	0.93	d	3.51	0.0056	0.88	0.87	0.0439	-0.42
NADPH	6.936	s	1.35	0.003	-0.96	1.07	0.0055	0.58
Phenylalanine	7.32	d	1.14	0.0093	1.21	0.94	0.0389	-0.68
Proline	1.99	m	2.87	0.0002	0.47	1.03	0.0164	-0.19
Pyruvic acid	2.46	s	3.13	0.0018	0.62	1.77	0.0295	-0.33
Succinate	2.39	s	1.89	0.002	1.19	1.29	0.041	-0.61
Taurocholic acid	0.58	s	2.09	0.008	1.59	1.12	0.0038	-1.45
α-Glucose	5.23	d	1.38	0.0059	0.50	1.75	> 0.05	/
β-Glucose	4.63	d	1.87	0.0414	0.41	1.47	> 0.05	/

[a]Type: s, singlet; d, doublet; t, triplet; m, multiplet. [b]Variable importance in the projection was obtained from OPLS-DA model with a threshold of 1.0. [c]*P*-value obtained from Students t-test. [d]Fold-change (FC).

including alanine synthesis, leucine synthesis, isoleucine synthesis, Krebs cycle, and glutathione metabolism, exhibited significant changes. Therefore, these pathways potentially represent pathways targeted by glutamine therapy in patients with burns.

Discussion

The metabolism of the body changes dramatically after a burn. Regarding energy metabolism, energy consumption is significantly increased. The maximum energy consumption of patients with severe burns was reported to be 180% of the pre-injury level and subsequently decreased but the hypermetabolic state persisted for approximately one year [17]. In the present study, the REE of burned rats began to increase significantly at 3 days after injury and was continuously elevated until day 14, with an average increase of approximately 15-20% and a maximum increase of 35%. Although the changes in the REE in burned rats observed in this study were not as dramatic as the changes observed in humans, the increasing trends in the metabolic rate were consistent. This difference may be related to differences in metabolic responses among different species. Furthermore, glutamine administration significantly reduced the energy consumption of burned rats by approximately 10%. In addition, glutamine administration significantly inhibited the weight loss of burned rats. After 7 days of administration, the body weight of treated rats was significantly greater than the burn group, which persisted for 14 days after the burn, and the body showed signs of enhanced anabolism. Based on these results, glutamine administra-

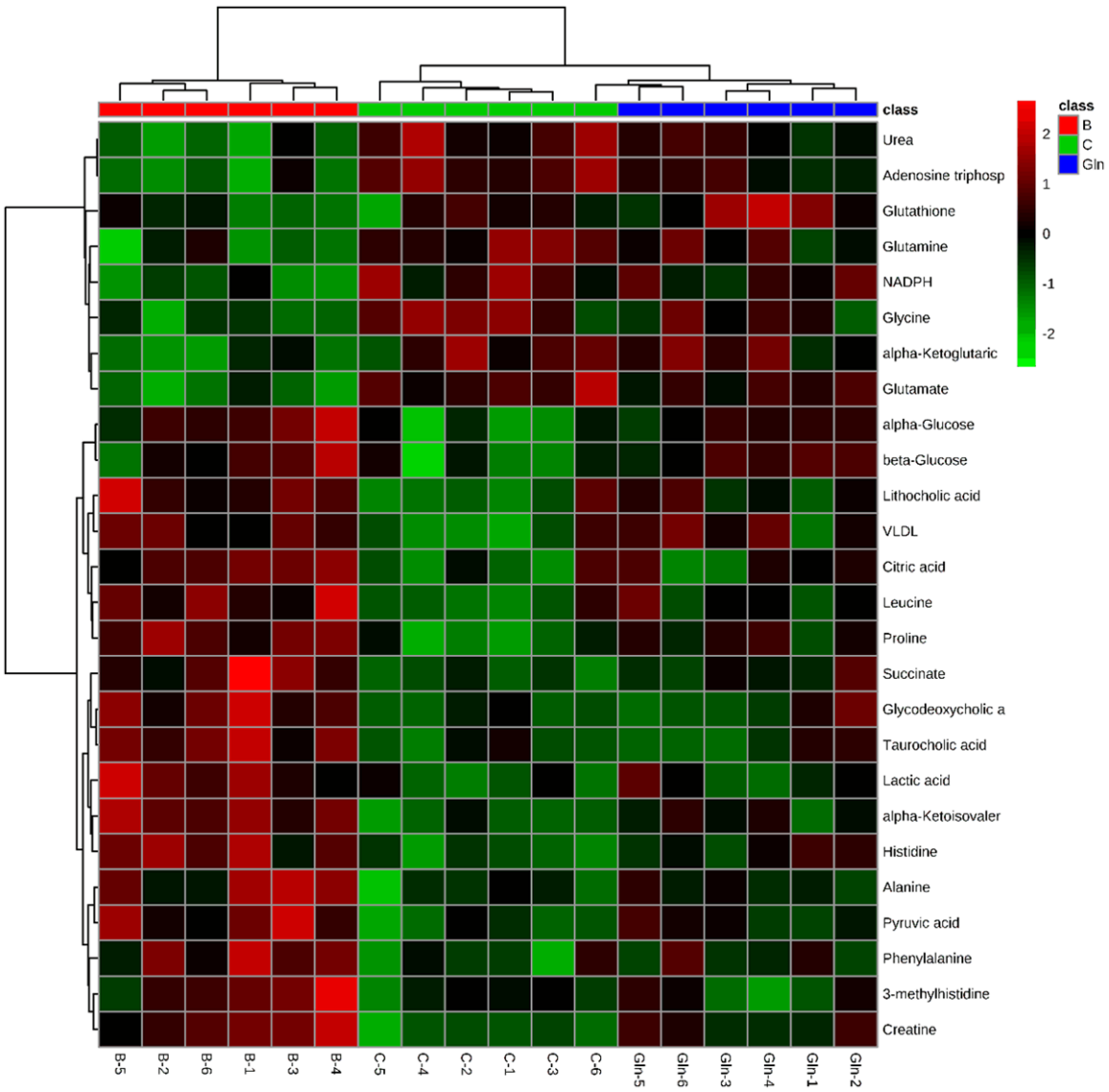

Figure 5. Hierarchical cluster analysis of serum metabolic profile for distinguishing group B and group Gln from group C. Each column represents one plasma sample, and each row represents a single metabolite. The expression values are represented by the color scale. The intensity increases from green (relatively down-regulated) to red (relatively up-regulated). Keys: NADPH: nicotinamide adenine dinucleotide phosphate; VLDL: very low density lipoprotein.

tion reduces energy consumption and high catabolism after a burn. This conclusion is consistent with previous studies and results reported [12, 18, 19]. However, due to the limitations of the research techniques, the mechanism by which glutamine regulates metabolism remains unclear. Therefore, metabolomics was employed to analyze the effect of glutamine on metabolism changes postburn, particularly anabolism, from the perspective of the metabolism of various substances to accurately determine the mechanisms by which glutamine promotes anabolism. After burn, the catabolism of the body was noticeably increased, as manifested by progressive weight loss (particularly the continuous consumption of lean body represented by skeletal muscle, which is the main cause of post-burn malnutrition), organ damage, low immune function and a poor prognosis [20]. In the present study, the level of 3-methylhistidine, which reflects the atrophy of skeletal muscle, increased significantly after burn, indicating that the proteolysis of skeletal muscle persisted after the burn. Meanwhile, the levels of amino acids crucial for skeletal muscle, such as alanine, histidine, leucine, and valine, were also

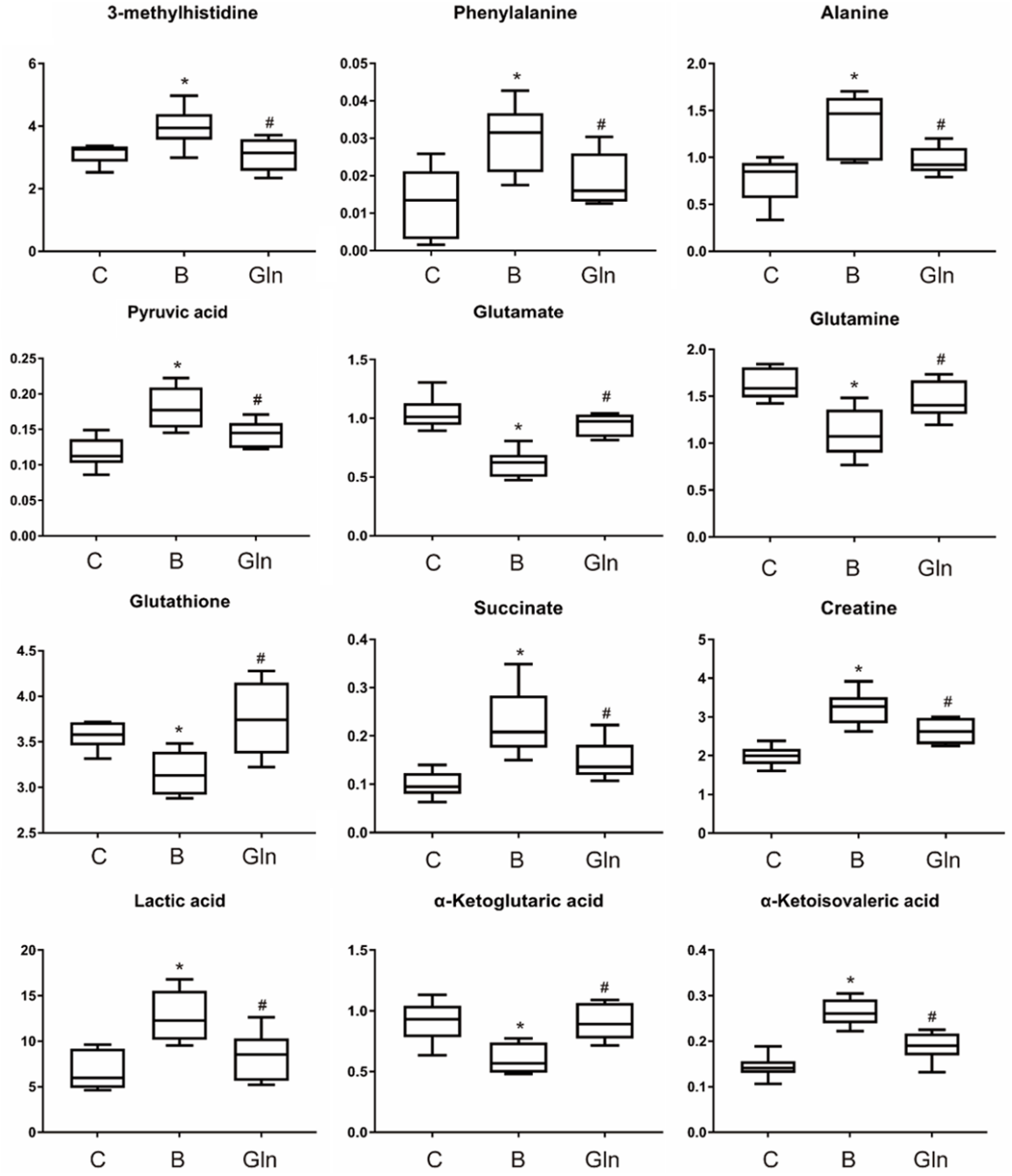

Figure 6. The box plots of the relative quantities of the significant biomarkers in the plasma samples of C, B and Gln groups. *: P < 0.05 B group vs. C group. #: P < 0.05 Gln group vs. B group.

increased. After a burn, a substantial proportion of bone proteins are degraded to release a large quantity of glutamine into the blood, along with alanine and branched-chain amino acids. The latter are involved in gluconeogenesis and the synthesis of acute phase reactive proteins. More than 70% of glutamine is stored in skeletal muscle. Under stress conditions, approximately 3-5 times more glutamine is released by the proteolysis of skeletal than under normal conditions [21]. Intriguingly, the plasma level of glutamine was not increased in the present study, instead its level decreased significantly. This finding is mainly attributed to the uptake of glutamine released by proteolysis into metabolically active cells, including cells in the intes-

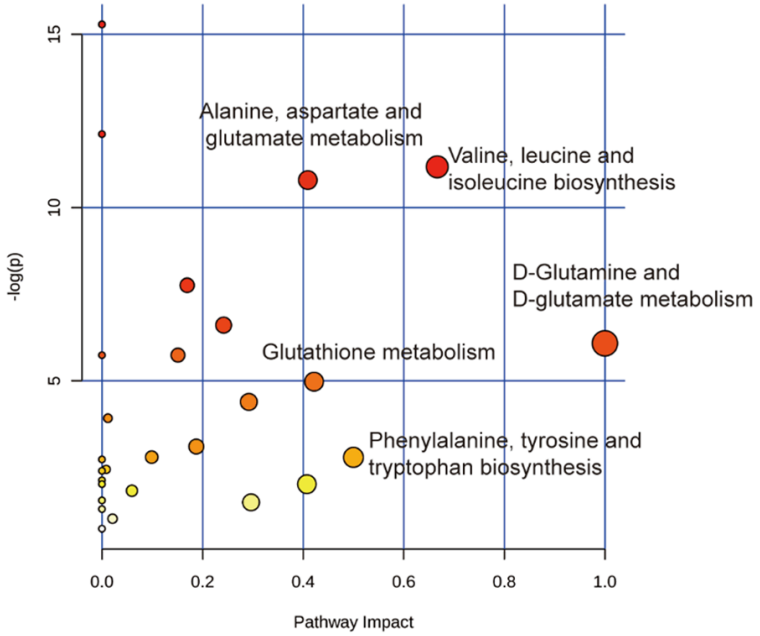

Figure 7. Summary of the altered metabolic pathways with MetPA based on the identified biomarkers in **Table 1**, as analyzed using MetaboAnalyst 4.0. The size and color of each pathway were set according to the *p*-values and pathway impact values.

tine, kidney and immune organs, after it enters the circulation [22]. After burn, the demand for glutamine in these tissues and cells increases substantially, resulting in a decreased plasma glutamine concentration [23]. According to previous studies, after a burn, extensive proteolysis occurs in skeletal muscle to generate glutamine, meeting the requirements of the body and causing extensive skeletal muscle atrophy that results in adverse clinical outcomes. Studies in critically ill patients have also revealed a poor prognosis for patients with hypoglutaminemia [24, 25]. Exogenous glutamine administration alleviates the demand for endogenous glutamine, reduces the catabolism of skeletal muscle, maintains the lean tissue, reduces metabolic expenditure, and improves the prognosis [26-29]. After glutamine administration, burned rats exhibited a significant increase in the plasma glutamine concentration and a significant reduction in the levels of 3-methylhistidine and branched chain amino acids in the present study, all of which are important signs of a decrease in the proteolysis of skeletal muscle. Glutamine not only inhibits catabolism and reduces skeletal muscle proteolysis but also promotes anabolism [30]. In tumor cells, glutamine was recently shown to be an important energy source and partial substituted for glucose [31]. This finding provides new insights into the regulation of post-burn anabolism.

In most organisms, the main energy synthesis pathway is the Krebs cycle, where glucose is converted from pyruvate to acetylcoenzyme A, which enters the tricarboxylic acid cycle to generate the energy source ATP via oxidative phosphorylation [32]. Recently, glutamine was reported to partially compensate for a glucose deficiency or metabolic disorder, which is referred to as the glucose substitution pathway in ATP synthesis. Glutamine is converted to α-ketoglutarate via the transamination pathway and subsequently enters the Krebs cycle, in which it is oxidized to produce energy [33]. Based on our data, after glutamine administration, the plasma concentrations of ATP, glutamic acid and α-ketoglutarate, metabolites of glutamine, were increased. α-Ketoglutarate is the key link between glutamine and mitochondrial metabolism. The increase in the glutamine content suggested that glutamine administration to burned animals resulted in substantial changes in the metabolic pathway of glutamine and that the pathways of glutamine utilization were completely functional. As shown in our previous study of burned rats, the transintestinal administration of glutamine promotes the absorption and transport of glutamine by intestinal epithelial cells [34]. The results of the two studies cross-validated each other. The present study confirmed the effectiveness of glutamine administration in treating burn injury, providing a basis for the clinical use of glutamine in patients with burns.

The importance of glutamine is not only reflected by the synthesis of ATP but also by the generation of reduced coenzyme II (NADPH) [35]. NADPH is the most important reductive compound in the mitochondria and a key substance required to maintain the mitochondrial redox balance [36]. Hence, the regulation of glutamine in energy synthesis is not simply reflected

Table 2. The associated metabolic pathways of the differential metabolites between group B and group Gln in **Table 1**

Pathway Name	Total	Hits	Raw p	-Log (P)	Holm P	FDR	Impact
Valine, leucine and isoleucine biosynthesis	11	4	< 0.001	11.18	0.001	< 0.001	0.67
Alanine, aspartate and glutamate metabolism	24	5	< 0.001	10.79	0.002	< 0.001	0.41
Arginine and proline metabolism	44	5	< 0.001	7.76	0.03	0.007	0.17
Histidine metabolism	15	3	0.001	6.61	0.10	0.02	0.24
Citrate cycle (TCA cycle)	20	3	0.003	5.74	0.24	0.03	0.15
Glutathione metabolism	26	3	0.007	4.98	0.50	0.06	0.42
Glycine, serine and threonine metabolism	32	3	0.012	4.39	0.88	0.09	0.30
Pyruvate metabolism	22	2	0.045	3.10	1.00	0.28	0.19

Note: P is the original *P*-value calculated from the enrichment analysis; the total is the total number of compounds in the pathway; Hits is the actually matched number from the user-uploaded data; Holm P is the *P* value adjusted by the Holm-Bonferroni method; FDR P is the *P* value adjusted using false discovery rate; Impact is the pathway impact value calculated from pathway topology analysis.

in its ability to serve as an energy source (i.e., synthesis of ATP). More importantly, the amino acid maintains the mitochondrial redox balance through its metabolites, thereby ensuring the smooth progression of oxidative phosphorylation. Therefore, glutamine is a critical regulatory factor in energy synthesis. The results of the analysis of metabolic pathways in this study showed that the administered glutamine was mainly converted into a variety of amino acids via transamination. Notably, α-ketoglutarate, which enters the mitochondria to participate in oxidative phosphorylation, is particularly important. In addition, glutamine metabolism promotes the production of reductive substances. An increase in the levels of reduced glutathione and its hydrogen donor NADPH effectively inhibit post-burn cell damage caused by oxidative stress, particularly mitochondrial damage, thereby maintaining its function. The α-ketoisovalerate levels were significantly increased after a burn in the present study. α-Ketoisovalerate is a metabolite of valine that enters the mitochondria to participate in anabolism under physiological conditions. However, mitochondrial membrane damage potentially hinders the transport of α-ketoisovalerate, resulting in its intracellular and plasma accumulation [37]. Thus, α-ketoisovalerate has become a sensitive index of mitochondrial structure and function. Notably, the content of α-ketoisovalerate was significantly reduced after glutamine administration, suggesting an improvement in mitochondrial structure and function, which was an important factor leading to the increased ATP content. Based on these results, glutamine administration partially alleviated the disruption in energy synthesis.

During the metabolite analysis, the levels of a variety of bile acids were significantly increased in burned rats, indicating an abnormal post-burn bile acid metabolism. This finding has not been reported previously. The liver of dead patients exhibited several pathological signs, including apparent enlargement (i.e., the volume was doubled), fat deposition and bile duct dilatation, in a previous study [38]. In a subsequent study, the liver was clearly damaged after a burn, and clear increases in transaminase activity and the bilirubin content were observed [39]. Based on the results from the present study, after the burn, the levels of taurocholic acid, lithocholic acid, and glycodeoxycholic acid were significantly increased. Under physiological conditions, bile acids are concentrated in the gallbladder and intestine and are rarely detected in the blood. The bile acids generated in the liver enter the intestine with bile and participate in fat absorption. Following the actions of intestinal bacteria, secondary bile acids are generated and return to the liver through the portal vein, thereby forming the hepatointestinal circulation of bile acids. This process improves the efficient utilization of bile acids and effectively prevents their cytotoxicity [40]. We argue that the post-burn increase in lithocholic acid levels should receive adequate attention. Bile acids are divided into hydrophilic and hydrophobic bile acids. Hydrophobic bile acids integrate with the cell membrane and thus exert a detergent-like effect that damages the cell membrane. Among all bile acids, litho-

cholic acid has the highest hydrophobicity and is the most toxic, but its concentration is minimal under physiological conditions [41]. The increased plasma level of lithocholic acid in burned rats suggests that the detoxifying conversion of lithocholic acid to taurocholic acid in the liver is inhibited. This finding not only reflects the abnormal intrahepatic metabolism of bile acids but also indicates serious damage to the liver. After glutamine administration, the concentrations of the three bile acids listed above decreased significantly, suggesting that the metabolism of bile acids in the liver approached the normal state, particularly the reduced level of lithocholic acid. The results corroborated our previous findings that administration of glutamine alleviated liver injury. Its mechanism may be related to the glutamine-dependent maintenance of the redox balance, suppression of cellular lipid peroxidation, and maintenance of the normal metabolism of bile acids [42]. In addition, glutamine promotes the expression of Farnesoid-X Receptor (FXR) and increases the synthesis, transformation and metabolism of hepatic bile acids in the liver, which are also components of its biological mechanisms [43].

In summary, after burn, the body undergoes substantial metabolic alterations, which are characterized by increased energy consumption and insufficient energy production. Glutamine administration substantially suppresses hypercatabolism and promotes anabolism. On the one hand, the underlying mechanism is associated with the involvement of glutamine in mitochondrial energy production. On the other hand, glutamine promotes the synthesis of reductive substances, reduces postburn oxidative stress, improves mitochondrial function, and promotes anabolism. An improvement in bile acid metabolism induced by glutamine is not only conducive to the absorption of fat and the utilization of nutrients but also reduces liver injury and maintains the normal physiological function of the liver. Therefore, glutamine is very important in sustaining the normal metabolism of various substances in the body.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xi Peng, Clinical Medical Research Center, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, PR China; Institute of Burn Research, State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Burns of PLA, Southwest Hospital, Third Military Medical University, Chongqing 400038, PR China. Tel: +86-23-68754435; Fax: +86-23-65461696; E-mail: pxlrmm@tmmu.edu.cn; pxlrmm@163.com

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