The metabolic effects of multi-trace elements on parenteral nutrition for pediatric patients: A Randomized Control Trial and Metabolomic Research

Qingti Tan
Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital
Yu Wang
Sichuan Provincial People's Hospital: Sichuan Academy of Medical Sciences and Sichuan People's Hospital
Guoying Zhang
Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital
Bin Lu
Chengdu Women's and Children's Central hospital
Tao Wang
Chengdu Women's and Children's Central Hospital
Tao Tao
Chengdu Women's and Children's Central Hospital: Chengdu Women and Children's Central Hospital
He Wang
Shanghai University of Traditional Chinese medicine
Hua Jiang (cdjianghua@qq.com)
Sichuan Provincial People's Hospital: Sichuan Academy of Medical Sciences and Sichuan People's Hospital
Wei Chen
Chinese Academy of Medical Science & Peking Union Medical College Hospital

Research

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Abstract

Objectives: The recommended dose of multi-trace element injection □ (MTEI-(□)), and the effects of MTEI-(□) on nutrient metabolism were investigated by supplementing different doses of multi-trace element parenteral nutrition (PN) to severe pediatric subjects.

Methods: Enrolled subjects were randomly divided into two groups: Group A (low-dose group) received MTEI-(I) at 1 mL/kg/d, and Group B (high-dose group) received MTEI-(I) at 2 mL/kg/d, with a maximum dose of 15 mL/d. Patient blood samples before PN, and five day after treatment were collected for trace element detection and metabolomics analyses.

Results: After 5 days treatment, white blood counts (WBC), nitrogen (N), chromium (Cr), total bilirubin (TB), direct bilirubin (DB) and albumin (ALB) levels in both groups were variably decreased; of which, WBC ($p=0.011$) and Cr ($p=0.007$) levels of subjects in Group B were significantly decreased; Overall, after 5 days of treatment, manganese (Mn) and copper (Cu) levels were decreased, zinc (Zn) and selenium (Se) levels were increased. The increase of Zn (A: 0.170±0.479 VS B□0.193±0.900)and decrease of Cu (A: -0.240±0.382 VS B: -0.373±0.465) of subjects in group B were particularly greater than those in group A. After 5 days treatment, valine, leucine, isoleucine degradation (α-ketoisovaleric acid) products, taurine and hypotaurine metabolism (hypotaurine), arginine and proline metabolism (phosphocreatine), ketone body metabolism (acetoacetic acid and acetone), and other metabolic outputs were decreased (p□0.05). For Group B, β-oxidation of very long chain fatty acids (hexacosanoic acid), arginine and proline metabolism (phosphocreatine), pentose phosphate metabolism (D-ribose), ketone body metabolism (acetone), citric acid cycle (succinic acid), purine metabolism (adenine), caffeine metabolism (dimethylxanthine) and pyruvate metabolism (acetyl phosphate) were variably decreased (p□0.05) when compared with Group A at T5.

Conclusions: Our work suggested that the high-dose administration of MTEI-(I) is safe for severe pediatric patients. It may alleviate inflammation and antioxidation, relieved hyper caused by stress, improved tissues-based hypoxia, and improved renal function. (Trial registration: ChiCTR-IPR-17013037. Registered 19 October 2017, http://www.chictr.org.cn/showproj.aspx?proj=22327)

1. Introduction

The nutritional status of pediatric Subjects is closely related to disease progression and prognosis. To improve nutritional status and cure rates of associated conditions, guidelines[1] have recommended that complete or partial parenteral nutrition (PN) should supply calories, fluids, and nutrients in situations where there is unavailable or insufficient intestinal nutritional uptake. Trace elements are important components of PN as they effectively utilize glucose and fat to supply energy and synthesize proteins. These molecules are essential components for enzyme cofactors, and they play key roles in immune regulation and antioxidation. Therefore, guideline documents have provided recommendations on trace element-use and doses for pediatric PN[1−3], but no global consensus exists for these measures.

Metabolomics is an emerging research area which reflects body health or disease status via the proteomic analysis of metabolites. Using this technology, we analyzed the effects of different multi-trace element injection (I) doses on the nutritional metabolism of pediatric subjects.

2. Materials And Methods

2.1. Subjects

In total, 40 Subjects requiring PN and hospitalized at the Chengdu Women's and Children's Central Hospital from November 2017 to March 2018 were enrolled. The research protocol is approved by the ethical committee of the hospital (No.2017(21)). The research protocol register number is: ChiCTR-IPR-17013037.

Inclusion criteria:

1. 1. Subjects requiring PN due to gastrointestinal failure, congenital malformation surgery of the digestive tract, other congenital malformation surgeries, gastrointestinal bleeding, and expected to receive PN for > five days,
2. 2. Ages ranging from 29 days to 18 years old,
3. 3. No serious cardiac, hepatic (hepatic function index exceeding twice the normal upper limit), renal (below CKD IV stage) or pulmonary dysfunction,
4. 4. Subjects where the central venous pathway (subclavian, internal jugular, femoral vein or peripherally Inserted Central Catheter) was established, and
5. 5. Informed consent was granted.

Exclusion criteria:

1. 1. Subjects with allergies to PN components,
2. 2. Subjects with diabetes,
3. 3. Subjects with allergies or adverse reactions to known trace elements,
4. 4. Subjects with obvious trace element deficiencies (iron (Fe), zinc (Zn), copper (Cu), etc.),
5. 5. Subjects with congenital metabolic abnormalities, and
6. 6. Subjects with Fe deficient anemia.

General information, such as gender, weight, main diagnosis, vital signs, length of hospital stay, and hospitalization expenses were collected. Simultaneously, we collected routine blood and biochemical data, as well as blood samples from Subjects before PN, and after 1, 3 and 5 days of treatment, for trace element and metabolomics studies.

2.2. Study Design

Random numbers were generated by computer, and Subjects were divided into two groups according to a randomization table. Subjects in both groups were provided with basic standard of care treatment

(including surgery, anti-infection, blood transfusion and other treatments) according to clinical needs, and they were given PN support for at least five consecutive days. Subjects were administrated low dose MTEI-(I) at 1 mL/kg/d, which is defined as Group A, while Subjects were administrated high dose MTEI-(I) at 2 mL/kg/d, with the maximum dose of 15 mL/d, which is defined as Group B.

2.3. Sample Collection

After subject enrolled, bloods were drawn before PN treatment, and after 1, 3 and 5 days of PN treatment. Approximately, 2 × 2 mL samples were taken each time, with one sample taken in a non-anticoagulant blood tube for trace element detection, and the other into an anticoagulant tube for proton nuclear magnetic resonance (^{1}H-NMR) analysis. Samples were centrifuged at 4000 rpm for 10 min, after that, the upper plasma layer were removed and stored at -80℃.

2.4. Analytical Method

Samples were sent to the Beijing QingXi Technology Research Institute for trace element detection.

2.4.1 Method

2.4.1.1 Instruments and Reagents

ICP-MS, NexION 300, PerkinElmer, USA; direct injection system, PerkinElmer, USA; multi-element mixed standard solution: 10 mg/L, CAS#: HNO3 [7697-37-2], PerkinElmer, USA;

Nitric acid: Suprapu, Merck KGaA, Germany; Oxygen: 99.999%; Water: ultrapure water obtained after double treatment by UPR pure water system

2.4.1.2 Instrument Conditions

Power of RF generator: 1600W;

Plasma gas flow rate: 18 L/min

Auxiliary gas flow rate (Ar): 1.2 L/min

Carrier gas flow (Ar): 0.96 L/min

Chamber vacuum: 1 × 10 − 6

Analog voltage: -1700V;

Pulse voltage: 850V

Measurement method: quantitative analysis.

Organic sampling system conditions: Oxygen flow rate of 8 mL/min, temperature of 4℃.

2.4.1.3 Sample Pretreatment and Preparation of Standard Solution

Pretreatment: 0.25 mL serum sample, added with 0.8 mL of 65% HNO3 and 0.2 mL of 29–32% H_2O_2, heated and digested on a heating plate at 90 ℃ for 3 h, then diluted to 10 mL with deionized water.

Preparation of standard solution: Dilute 10 mg/L standard mixture solution to 1 mg/L for future use.

2.4.1.4 Calibration Curve

Accurately pipette multi-element mixed standard solution, prepare standard curve solution, and the final concentrations are 1 μg/L, 5 μg/L, 10 μg/L, 20 μg/L, 50 μg/L and 100 μg/L. The prepared standard solution were tested by the organic injection system to obtain the standard curve.

In the experiment, parallel samples and spike recovery were used as the means of sample quality control to ensure the accuracy of the results.

2.5. Metabolomics

2.5.1. Preparation of plasma samples

After thawing at room temperature, plasma samples were centrifuged at 16000 rpm for 10 min. Then, 50 μl deuterated heavy water (D_2O) was added to an NMR tube, to which 450 μL plasma was added. The sample was shaken for 2 min and incubated at room temperature for 10 min until ^{1}H-NMR (600 MHz) analysis.

2.5.2. ^{1}H-NMR data collection

A one-dimensional hydrogen spectrum was obtained after sample processing. In this study, ^{1}H-NMR analysis was performed using a Bruker Avance DR × 600 MHz model, with a working frequency of 600.13 MHz, equipped with a Bruker inverse broad band probe (rIBB). The addition of 10% D_2O inhibited the solvent peak during sample preparation, and the pulse sequence (zgp) was used to inhibit the water peak during pre-saturation. All spectra were collected at room temperature (i.e., 300 Kelvin (K)), with a spectrum width of 20 ppm, sampling points of 32 K, and a cumulative frequency of 256 times.

2.5.3. ^{1}H-NMR spectrum processing

In plasma samples, the molecular nucleus of a compound resonates in a high magnetic field, and its frequency is gradually decreased. The original decay signal of the tested sample referred to our raw data (free induction decay; FID). The FID signal was imported into MestReNova software (MestreLab Research, Spain) for Fourier transformation, to generate one-dimensional ^{1}H-NMR spectra. These were processed for chemical shift and automatic baseline adjustment. The convolution technique was used to minimize changes in peaks, and to ensure that larger peaks did not cover up smaller ones. Then, for all plasma samples, 0–9 parts per million (ppm) segments of one-dimensional hydrogen spectra were divided into

0.04 ppm sections, and 223 chemical shift value segments were integrated to finally obtain corresponding integral values. The two-dimensional matrix was then exported in .CSV format for analysis.

2.5.4. Data preprocessing

All data matrices were preprocessed - line normalization and standardization. Due to differences in plasma sample dilution, concentration, test temperature, instrument working stability, and other factors during processing and measurement, ^{1}H-NMR spectra from the same types of plasma sample in different batches were not completely consistent. Therefore, line normalization of data matrices was required (it was assumed the highest peak in each ^{1}H-NMR spectrum referred to the same substance with very similar content). The line normalization formula was represented as:

$$x* = \frac{x - min}{max - min}$$

2.5.5. Spectrum data analysis

We used the supervised pattern recognition method, Partial Least Squares-Discriminant Analysis (PLS-DA) to perform data dimension reduction. The variable importance in the projection (VIP) of the PLS-DA model, with corresponding chemical shifts, was calculated. Chemical shifts with VIP values > 1 and P < 0.05 were selected, and corresponding metabolites were investigated using the human metabolome database (HMDB at https://hmdb.ca/).

All clinical data were statistically analyzed using SPSS Version 21.0 software, and described by median (interquartile range) or mean values ± standard deviation (mean ± SD) according to distribution type. Measurement data were first tested for data distribution type. Student's t-test was used for normally distributed data, and the rank-sum test was used for non-normally distributed data. The chi-square test was used for enumeration data. The statistical significance level was set at $p < 0.05$. The mean substitution method was used for missing clinical data.

3. Results

3.1. Subjects status

In total, 40 subjects were enrolled into the study ranging from 29 days to 10 years old, including 18 subjects in Group A and 22 subjects in Group B. Principal diagnoses included: 1) 10 subjects had received fistulation surgery, 2) Six subjects had acute upper gastrointestinal bleeding, 3) Four subjects had congenital mega-colon, 4) Three subjects had acute gangrenous appendicitis with perforation, 5) Two subjects had congenital hypertrophic pyloric stenosis, 6) Two subjects had adhesive intestinal obstruction, and 7) Two subjects had acute intussusception. The remaining diagnoses included; one patient each with Merkel diverticulitis with bleeding, necrotizing enterocolitis, acute descending colon

perforation, small intestine torsion, portal hypertension syndrome, acute severe myocarditis, toxic intestinal paralysis, autotransplantation after splenectomy, congenital anal atresia and traumatic splenic rupture, and oesophageal atresia surgery

Subjects information at admission is shown (Table 1). No significant differences were observed between groups in terms of gender, weight, pediatric critical illness score, vital signs, length of hospital stay, and hospitalization expenses. Similarly, we observed no significant differences in routine blood and biochemical tests, except hemoglobin.

Economic aspects were also considered in this study. After comparing the length of hospital stay and hospitalization expenses between groups, we observed that the high-dose administration of MTEI-(I) did not significantly prolong these factors for subjects, suggesting minimal impact and burden on subjects and their families.

Table 1
General condition of subjects before treatment

	A (n = 18)	B (n = 22)	p
Gender			0.761
Male	10	11	
Female	8	11	
Height (cm, mean ± SD)	73.500 ± 25.91	86.18 ± 27.81	0.147
Weight (kg, mean ± SD)	9.11 ± 6.48	11.90 ± 7.22	0.211
Pediatric critical illness score (mean ± SD)	95.67 ± 4.13	94.36 ± 5.19	0.393
Body temperature (℃; mean ± SD)	36.81 ± 0.38	36.69 ± 0.48	0.413
Respiratory rate (breaths/min; mean ± SD)	33.56 ± 10.70	29.86 ± 7.36	0.205
Heart rate (beats/min; mean ± SD)	130.05 ± 24.08	123.27 ± 22.71	0.366
Testing indices			
WBC ($\times 10^9$/L; mean ± SD)	11.24 ± 5.04	12.93 ± 6.69	0.380
N (%; mean ± SD)	51.59 ± 23.65	55.64 ± 26.86	0.620
LY (%; mean ± SD)	41.51 ± 22.16	35.05 ± 24.86	0.397
HGB (g/L; mean ± SD)	112.67 ± 25.30	94.55 ± 17.37	0.011
PLT ($\times 10^9$/L; mean ± SD)	401.33 ± 166.70	361.14 ± 179.54	0.472
ALT (IU/L; mean ± SD)	27.02 ± 14.70	32.51 ± 20.72	0.355
Cr (mmol/L; mean ± SD)	23.87 ± 11.50	24.48 ± 10.41	0.860
TB (μmol/L median (IQR))	6.65 (7.0)	8.45 (6.9)	0.693
DB (μmol/L; mean ± SD)	3.93 ± 5.09	3.12 ± 2.12	0.495
ALB (g/L; mean ± SD)	42.52 ± 5.17	39.10 ± 7.01	0.093
Received surgery;			0.747
Yes	12	13	
No	6	9	
Transferred to ICU;			1.000
Yes	18	21	
No	0	1	

	A (n = 18)	B (n = 22)	p
Length of hospital stay (days; mean ± SD)	19.28 ± 7.84	21.5 ± 21.17	0.676
Hospitalization expenses (RMB), median (IQR))	25739.62 (18220.26)	27938.09 (128896.89)	0.892

3.2. General condition changes in Subjects before and after treatment

According to results of trace element detection, There was no significant differences observed in subjects after 1, 3 and 5 days of treatment in each group, therefore we focused on and analyzed data before treatment (T0) and after 5 days of treatment (T5). The general condition, routine blood and biochemistry data of both groups after 5 days of treatment are shown (Table 2); No significant differences were observed between two groups. Routine blood and biochemistry data before and after treatments were compared between groups (Table 3). After treatment, WBC, N, Cr, TB, DB, and ALB data in both groups decreased, of which, WBC and Cr in Group B were significantly decreased after 5 days of treatment.

Table 2
General patient data after 5 days of treatment

	A (n = 18)	B (n = 22)	p
Body temperature (℃, median (IQR))	36.6 ± 0.2	36.6 ± 0.3	0.447
Respiratory rate (breaths/min, mean ± SD)	32.59 ± 8.43	29.05 ± 7.22	0.172
Heart rate (beats/min, mean ± SD)	122.18 ± 14.01	111.95 ± 21.39	0.099
Testing index			
WBC ($\times 10^9$/L, mean ± SD)	8.34 ± 3.39	8.70 ± 4.63	0.781
N (%, mean ± SD)	46.35 ± 17.59	54.77 ± 16.43	0.127
LY (%, mean ± SD)	42.08 ± 14.62	33.49 ± 17.35	0.103
HGB (g/L, mean ± SD)	95.98 ± 9.79	100.80 ± 15.93	0.269
PLT ($\times 10^9$/L, mean ± SD)	392.61 ± 94.13	380.50 ± 168.32	0.776
ALT (IU/L, mean ± SD)	34.06 ± 24.73	36.83 ± 21.57	0.707
Cr (mmol/L, mean ± SD)	19.76 ± 5.23	18.10 ± 4.94	0.308
TB (μmol/L, mean ± SD)	7.75 ± 8.18	7.38 ± 3.98	0.852
DB (μmol/L, mean ± SD)	2.53 ± 2.25	2.59 ± 1.45	0.923
ALB (g/L, mean ± SD)	35.86 ± 4.42	36.55 ± 4.89	0.644

Table 3
Routine test data in both groups before and after treatment

Testing index	A (n = 18)		p	B (n = 22)		p
	T0	T5		T0	T5	
WBC ($\times 10^9$/L, mean ± SD)	11.24 ± 5.04	8.34 ± 3.39	0.085	12.93 ± 6.69	8.70 ± 4.63	0.011
N (%, mean ± SD)	51.59 ± 23.65	46.35 ± 17.59	0.267	55.64 ± 26.86	54.77 ± 16.43	0.873
LY (%, mean ± SD)	41.51 ± 22.16	42.08 ± 14.62	0.901	35.05 ± 24.86	33.49 ± 17.35	0.754
HGB (g/L, mean ± SD)	112.67 ± 25.30	95.98 ± 9.79	0.015	94.55 ± 17.37	100.80 ± 15.93	0.272
PLT ($\times 10^9$/L, mean ± SD)	401.33 ± 166.70	392.61 ± 94.13	0.838	361.14 ± 179.54	380.50 ± 168.32	0.642
ALT (IU/L, mean ± SD)	27.02 ± 14.70	34.06 ± 24.73	0.335	32.51 ± 20.72	36.83 ± 21.57	0.505
Cr (mmol/L, mean ± SD)	23.87 ± 11.50	19.76 ± 5.23	0.056	24.48 ± 10.41	18.10 ± 4.94	0.007
TB (μmol/L, median (IQR))	6.65 (4.15–11.28)	4.48 (3.53–7.96)	0.005	8.45 (4.75–11.68)	6.55 (4.8–9.9)	0.112
DB (μmol/L, mean ± SD)	3.93 ± 5.09	2.53 ± 2.25	0.076	3.12 ± 2.12	2.59 ± 1.45	0.296
ALB (g/L, mean ± SD)	42.52 ± 5.17	35.86 ± 4.42	0.000	39.10 ± 7.01	36.55 ± 4.89	0.106

3.3. Trace element data in subjects before and after treatment

Trace element patient data before and after treatment are shown (Table 4). After 5 days of treatment, Mn and Cu decreased in different extent, whereas Zn and Se increased in the groups. For both Zn and Cu, we observed significant differences compared to before treatment. We also compared trace element data in both groups before and after treatment (Table 5). After treatment, Mn and Cu decreased in both two groups, whereas levels of Zn and Se increased. Zn levels in Group B increased significantly when compared with Group A. Cu in Group B significantly decreased when compared with Group A.

Table 4
Trace element data in 40 subjects before and after treatment (mg/L)

Testing index	T0	T5	p
Mn (mean ± SD)	0.044 ± 0.028	0.035 ± 0.053	0.303
Zn (median (IQR))	1.028 (1.050)	1.052 (1.101)	0.013
Cu (mean ± SD)	1.700 ± 0.601	1.387 ± 0.549	0.000
Se (median (IQR))	0.086 (0.039)	0.095 (0.050)	0.089

Table 5
*Subjects data in both groups before and after treatment

Testing index	A (n = 18)			P	B (n = 22)			P
	T0	T5	Difference value		T0	T5	Difference value	
Mn (mean ± SD)	0.046 ± 0.031	0.029 ± 0.042	-0.017 ± 0.036	0.055	0.042 ± 0.026	0.041 ± 0.062	-0.001 ± 0.062	0.930
Zn (median (IQR))	0.856 (1.292)	0.908 (1.312)	0.170 ± 0.479	0.025	1.027 (0.808)	1.231 (1.017)	0.193 ± 0.900	0.223
Cu (mean ± SD)	1.690 ± 0.631	1.450 ± 0.537	-0.240 ± 0.382	0.016	1.709 ± 0.590	1.336 ± 0.566	-0.373 ± 0.465	0.001
Se (mean ± SD)	0.091 ± 0.036	0.148 ± 0.221	0.057 ± 0.232	0.311	0.083 ± 0.025	0.095 ± 0.029	0.012 ± 0.029	0.065
Note:* Difference = post-treatment value - pre-treatment value								

3.4. Differences of Patient metabolomics before and after treatment

Qualified metabonomic samples were obtained from 37 subjects in both groups; 17 from Group A and 20 from Group B. We used ^{1}H-NMR metabolic fingerprinting of patient plasma to distinguish patient metabolomics before (T0) and after treatment (T5) (Fig. 1). The VIP of chemical shift values of metabolites at T0 and T5 are shown (Fig. 5), of which those with VIP ≥ 1 and $P < 0.05$ were taken as characteristic metabolites (Tables 6 and 7). According to Table 6 and Table 7, after the 5 days treatment, valine, leucine, isoleucine (α-ketoisovaleric acid), taurine, hypotaurine (hypotaurine), arginine, proline (phosphocreatine), ketone (acetoacetic acid and acetone) and other metabolic processes significantly decreased.

Table 6
Small metabolite differences during patient treatment in Group A (P < 0.05)

Metabolic pathway	Chemical shift (PPM)[1]	Metabolite	HMDB[2]	Trend
Valine, leucine and isoleucine degradation	1.12	α-ketoisovaleric acid	HMDB0000019	↓
Taurine and hypotaurine metabolism	3.36	Hypotaurine	HMDB0000965	↓
Arginine and proline metabolism	3.40	Phosphocreatine	HMDB0001511	↓
	3.76	Glycocyamine	HMDB0000128	↓
Ketone body metabolism	3.44	Acetoacetic acid	HMDB0000060	↓
N/A[3]	3.80	Acetyl phosphate	HMDB0031419	↓
[1] parts per million;				
[2] HMDB: Human Metabolome Database;				
[3] N/A: not applicable				

Table 7
Small metabolite differences during patient treatments in Group B (P □ 0.05)

Metabolic pathway	Chemicalshift (PPM)	Metabolite	HMDB	Trend
Valine, leucine and isoleucine degradation	1.12	α-ketoisovaleric acid	HMDB0000019	↓
Ketone body metabolism	2.16	Acetone	HMDB0001659	↓
Pentose phosphate pathway	2.20	D-ribose	HMDB0000283	↓
N/A	2.32	Dimethylglyoxal	HMDB0003407	↓
Caffeine metabolism	4.03	7-dimethylxanthine	HMDB0001860	↓

Table 8
Small metabolite differences at T5 treatment in Groups A and B

Metabolic pathway	Chemicalshift (PPM)	Metabolite	HMDB	Trend
β-oxidation of very long chain fatty acids	0.88	Hexacosanoic acid	HMDB0002356	↓
Arginine and proline metabolism	1.08	Phosphocreatine	HMDB0001511	↓
Pentose phosphate metabolism	2.20	D-ribose	HMDB0000283	↓
Ketone body metabolism	2.24	Acetone	HMDB0001659	↓
N/A	2.32	Dimethylglyoxal	HMDB0003407	↓
Citric acid cycle	2.40	Succinic acid	HMDB0000254	↓
Purine metabolism	3.20	Adenine	HMDB0000034	↓
Caffeine metabolism	4.08	Dimethylxanthine	HMDB0001860	↓
N/A	4.40	Dihydroxy acetone	HMDB0001882	↓
Pyruvate metabolism	4.44	Acetyl phosphate	HMDB0001494	↓

Figure 3 shows PLS-DA analyses based on patient metabolic fingerprint spectra. From these data, we observed metabolic differences between subjects in both groups. VIP metabolite data are shown (Fig. 4), for which the characteristic metabolites with VIP > 1 and P < 0.05 are shown (Table 8). For Group B, β-oxidation of very long chain fatty acids (hexacosanoic acid), arginine and proline metabolism (phosphocreatine), pentose phosphate metabolism (D-ribose), ketone body metabolism (acetone), citric acid cycle (succinic acid), purine metabolism (adenine), caffeine metabolism (dimethylxanthine), and pyruvate metabolism (acetyl phosphate) were all decreased when compared with Group A after T5.

4. Discussion

Although trace element levels in human tissue account for < 0.01% of total organism mass, these components are vital for human growth and development[4]. For enteral feeding, subjects derive adequate trace elements through diversified diets, enteral nutrition products, and oral drug products. For PN, due to chemical molecule stability, a variety of complex drug products containing multi-trace elements are required to meet clinical needs[5]. MTEI-(I) is a complex drug product containing multi-trace elements specially developed for children. It supplements six trace elements such as Zn, Cu, Mn, Se, fluorine (Fl), and iodine (I), but not Fe or Cr, to meet guideline requirements for the addition of trace elements during PN[2, 3].

Inflammatory mechanisms generated by inflammation and oxidative stress responses from free radical accumulation often cause normal proteins, lipids, and nucleic acids to attack, undermine, and destroy normal physiological functions. Trace elements are required for the regulation of substance metabolism, enzyme catalytic activity, etc., and thus affect inflammation and oxidative stress mediators. For example, during oxidative stress and inflammation, trace element distribution will be altered, thus a reasonable intake of these elements will exert positive effects towards inflammation control, and slow or reduce oxidative stress responses[6]. In previous studies, it was shown that appropriate Cu, Zn and Se levels reduced free radicals, enhanced antioxidant capacity, and regulated inflammatory reactions [7–9], while Cu was positively correlated with bacterial levels and inflammatory markers[10, 11] ,and Zn and Se were negatively correlated with inflammation and oxidative stress[12, 13]. Supplementation with Zn improved high Cu-Zn ratios in blood, reduced oxidative stress, improved inflammatory conditions, and maintained immune functions[14]. These data were consistent with our findings suggesting that Cu was decreased, and Zn and Se were increased after PN treatment, with trace element differences in Group B more significant. Equally, we observed that WBC levels in both groups were decreased after PN treatment, with levels in Group B significantly decreased after PN treatment ($p = 0.011$). This observation suggested that the appropriate high-dose administration of I was effectively controlling inflammation and antioxidation.

Hexacosanoic acid is a very long chain fatty acid, and is an important component of phospholipid molecules. In a previous study[15], these molecules were shown to play important roles in cellular biochemical reactions, nutrient storage, and intercellular communications. Due to homeostatic imbalances between molecular transport and utilization, excessive fatty acid accumulation may cause toxicity in some tissues, which becomes manifested as oxidative stress and inflammation, potentially culminating in cell apoptosis[16]. Several studies reported that very long chain fatty acids induced the production of reactive oxygen species in the SK-N-BE neuroblastoma cell line, and enhanced oxidative stress[17]. Gursev *et al.*[18] observed these molecules activated nicotinamide adenine dinucleotide phosphate oxidase activity, and enhanced superoxide anion-mediated lipid peroxidation in skin fibroblasts. In this study, we observed that the β-oxidation of very long chain fatty acids (hexacosanoic acid) was significantly reduced in Group B ($p < 0.05$), indicating subjects were less prone to oxidative damage caused by lipid peroxidation. Therefore, appropriate high-dose administration of I exerted positive antioxidation effects in this group[19].

Stress has an important impact on various metabolic pathways. Under stress conditions, the following metabolic characteristics are often observed; high metabolic rate, increased catabolism, and reduced anabolic metabolism, resulting in a negative balance in overall metabolism. In this study, 37 children were under acute stress after surgery or disease. Chen Weiqiang *et al.*[20] observed that stress induced the loss of Zn from the body, whereas Zn supplementation exerted protective effects. In this study, after supplementing I, we observed that valine, leucine, isoleucine degradation, taurine and hypotaurine metabolism, arginine and proline metabolism, and other amino acid metabolism were all reduced, suggesting a benefit to disease recovery. Equally, ketone metabolism was also reduced, suggesting the high metabolic rate had been relieved. Of these components, β-oxidation of very long chain fatty acids,

pentose phosphate metabolism, ketone body metabolism, citric acid cycle and pyruvate metabolism were all significantly reduced in Group B. These factors were related to energy metabolism[21, 22], indicating that appropriate high-dose administration of I was helpful in relieving stress induced elevated metabolism.

Hypoxia is a basic pathological process implicated in several diseases[23]. Severe hypoxia induces considerable cellular harm, and often leads to death. Kim *et al.*[24]observed that Zn ameliorated hypoxic neuronal death induced by deferoxamine (DFX) and sodium azide (NaN_3). Xinge *et al.*[25] reported that Zn chelating agents had protective effects towards hypoxic ischemic brain damage in zebrafish. Kun *et al.* [26] proposed that exogenous Zn had protective effects towards hypoxic neurons. Hypoxanthine is a naturally occurring purine derivative and is the major catabolite of adenosine triphosphate (ATP) in hypoxic or ischemic tissue[27]. In general terms, a large increase in hypoxanthine levels in bodily fluids indicates ATP depletion [28]. In a trial of subjects with critical illness, burns and burn-induced sepsis [28], the evidence suggested that elevated ATP associated degradation products i.e., adenosine, inosine, hypoxanthine, and xanthine were associated with tissue hypo-perfusion and hypoxia levels. Therefore, it was suggested that purine metabolites such as xanthine and hypoxanthine are potential markers of tissue hypoxia[29]. In our study, the administration of I in Group B significantly increased plasma Zn levels. In our metabolomics study, we observed that purine metabolism in Group B was significantly reduced, and related metabolites were similarly reduced, indicating that appropriate high-dose administration of MTEI-(I) improved hypoxic conditions in these subjects.

5. Conclusion

In summary, the appropriate high-dose administration of MTEI-(I) is beneficial for pediatric patients. Such administration does not increase the burden on visceral organs, and appears to exert protective effects on liver and kidney functions. Several studies have shown that Se protects the kidney from oxidative damage, and reduces oxidative damage to the kidney [30–32]. The metabolite also reduces serum glutamic pyruvic transaminase, total and direct bilirubin, and reduces ultra-structure liver cell damage in rats[33]. Supplementation with Zn also delays the progression of chronic kidney disease damage, and relieves its complications [34, 35]. In this study, Zn and Se plasma levels were increased by I administration. Also, liver and kidney functional analyses of our subjects indicated that Cr, TB, DB and ALB levels decreased after supplementing MTEI-(I), and Cr was significantly decreased in Group B, suggesting that appropriate high-dose supplementation of MTEI-(I) was beneficial in improving renal function.

Additionally, E`conomic aspects and duration in hospital were also considered in this study. Administration at high dose didn’t enhance expenditure and duration dramatically.

Declarations

Ethics approval and consent to participate: The research protocol is approved by the ethical committee of the hospital (No.2017□21□). Informed consent was granted by all the participants.

Consent for publication: Informed consent was obtained from all subjects involved in the study.

Availability of data and materials: The data presented in this study are available on request from the corresponding author on reasonable request.

Competing interests: The authors declare no conflict of interest.

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Authors' Contributions: WC, HJ and GYZ had full access to all of the data in the study and take responsibility for the integrity of the data. YW and QTT took responsibility for the accuracy of the data analysis and prepared the figures. YW, QTT and HW wrote the paper. WC and HJ designed the study and revised the manuscript critically for important intellectual content. BL, TW, JZ, TT, LL, NZ and XLL took responsibility for patient recruitment. All Authors contributed to the final version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the ethics committee of Chengdu Women's and children's Central Hospital. The clinical registration number is ChiCTR-IPR-17013037.

Abbreviations

MTEI-(□), multi-trace element injection □; PN, parenteral nutrition; WBC, white blood counts; N, nitrogen; Cr, chromium; TB, total bilirubin; DB, direct bilirubin; ALB, albumin; Mn, manganese; Cu, copper; Zn, zinc; Se, selenium; ^{1}H-NMR, proton nuclear magnetic resonance; D_2O, deuterated heavy water; FID, free induction decay; ppm, parts per million; PLS-DA, Partial Least Squares-Discriminant Analysis; VIP, variable importance in the projection; Fl, fluorine; I, iodine.

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Figures

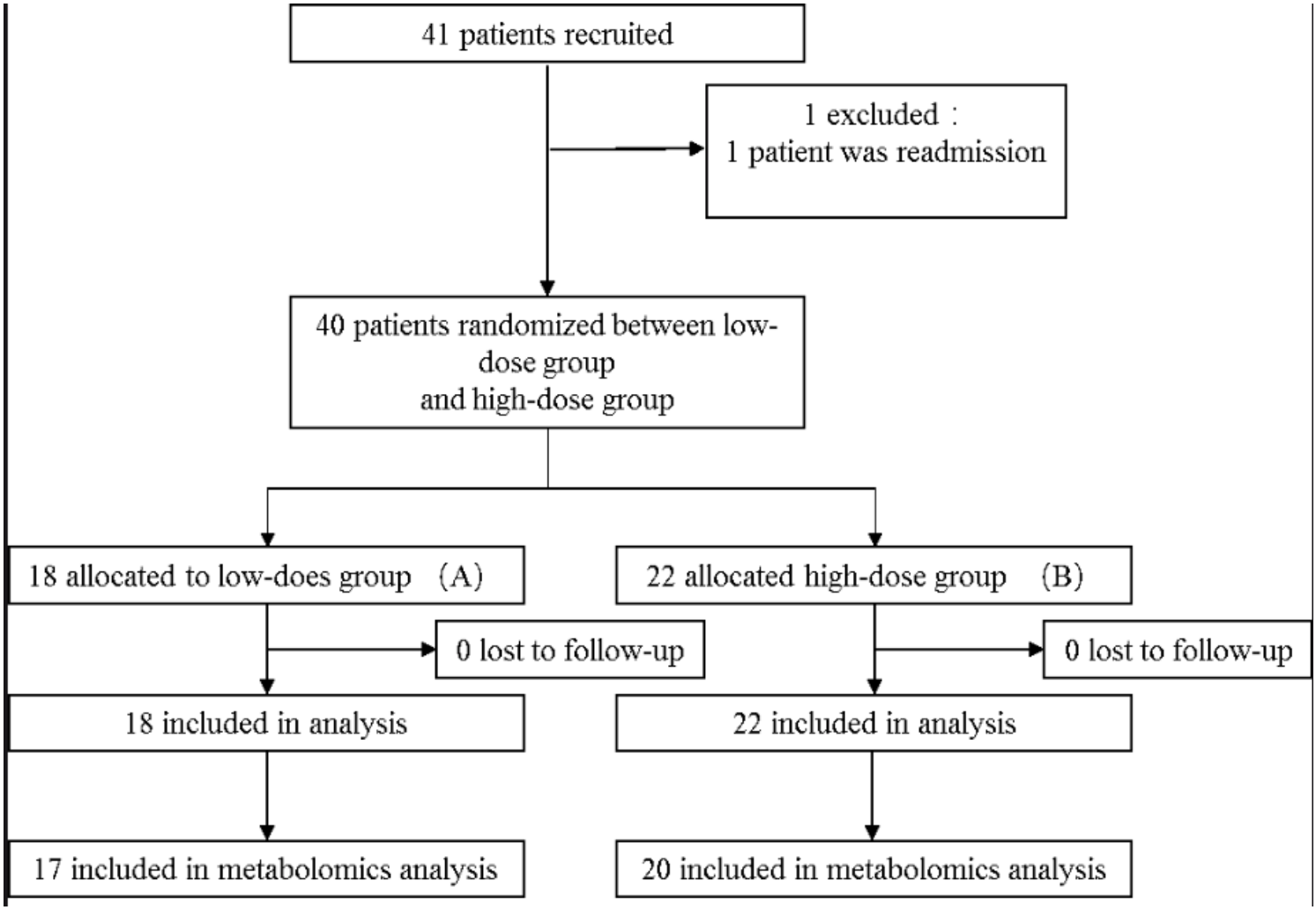

Figure 1

Patient recruitment into groups

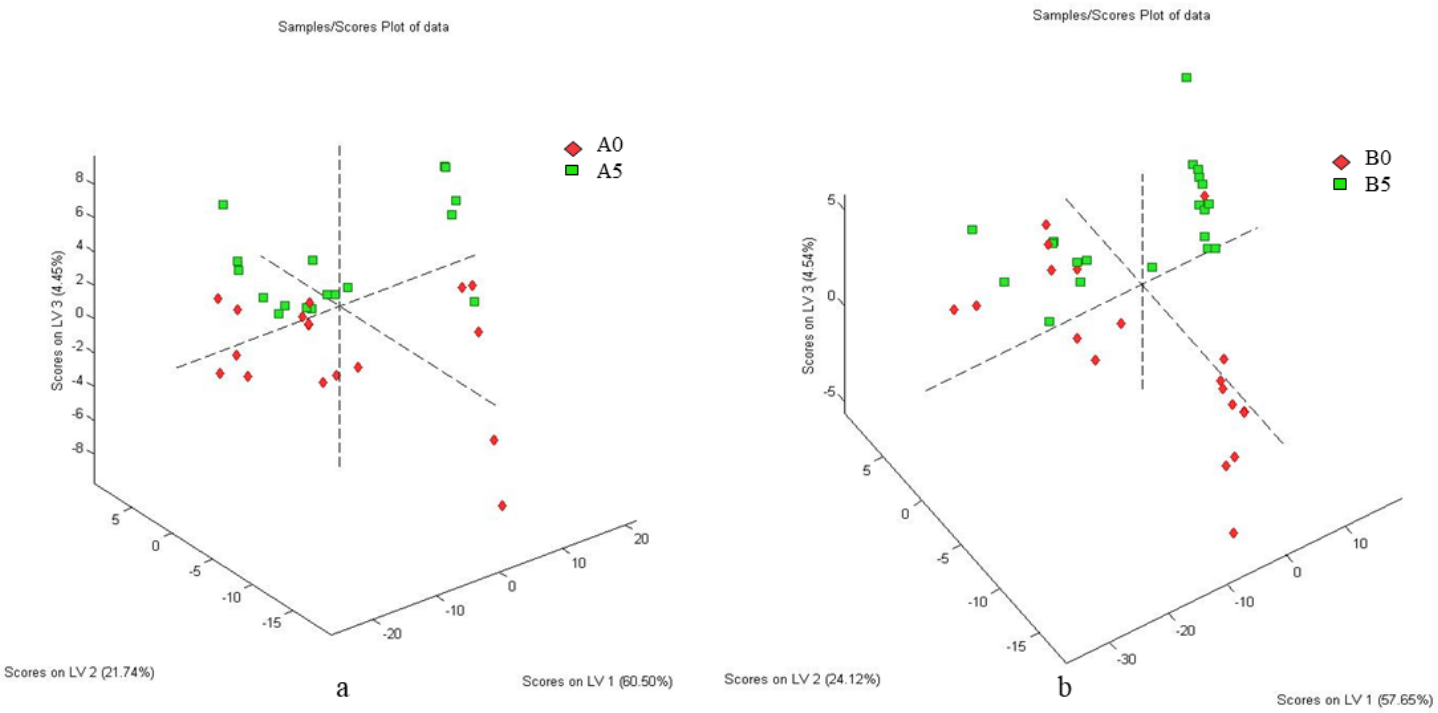

Figure 2

Partial least squares-discriminant analysis (PLA-DA) of subjects at T0 and T5 (a:1 mL/kg; b:2 mL/kg)

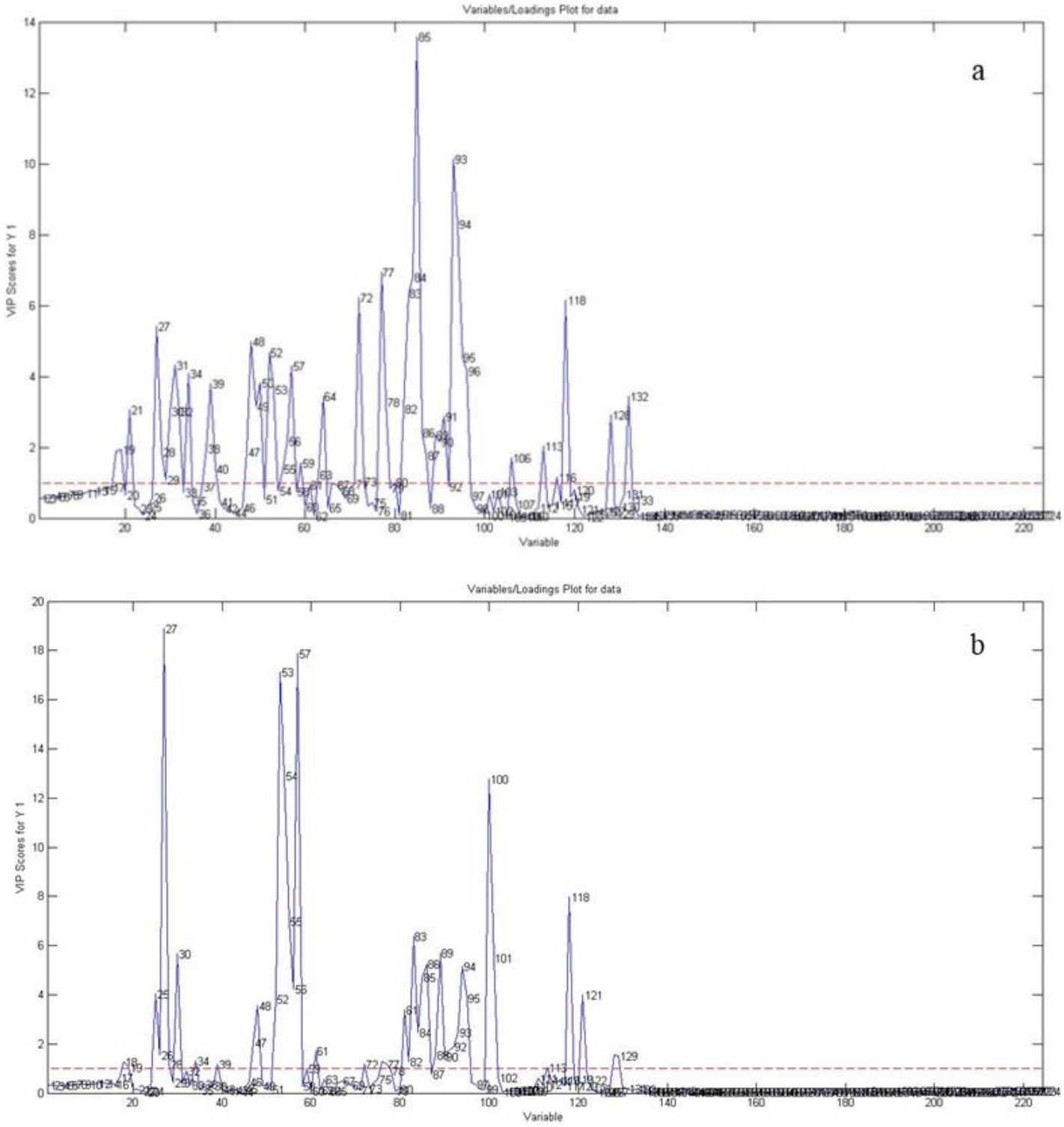

Figure 3

PLS –AD variable importance in the projection (VIP) values of chemical shifts between T0 and T5 (a:1 mL/kg; b:2 mL/kg)

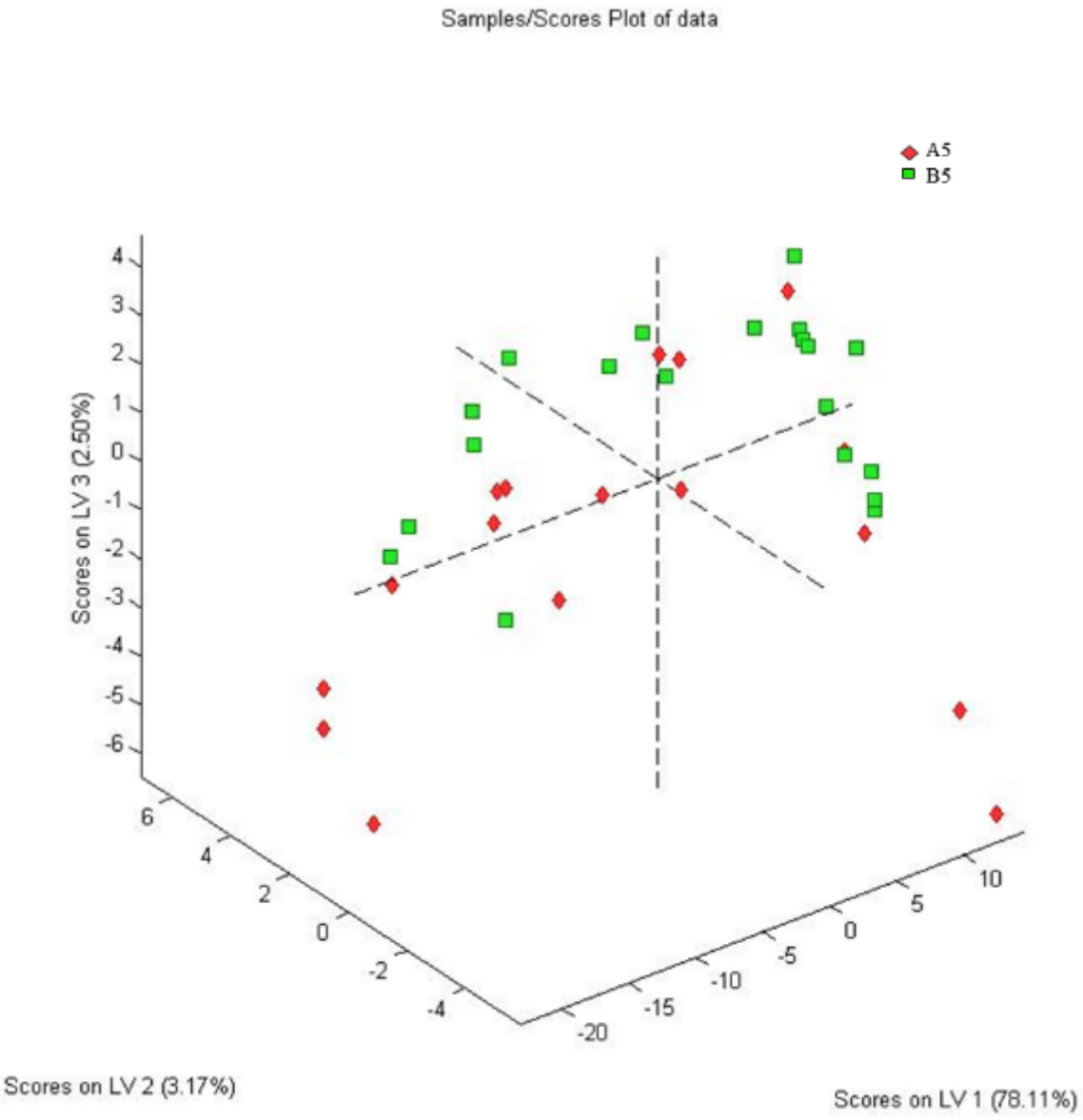

Figure 4

Partial least squares-discriminant analysis (PLS-DA) for subjects at T5 in Group A and Group B

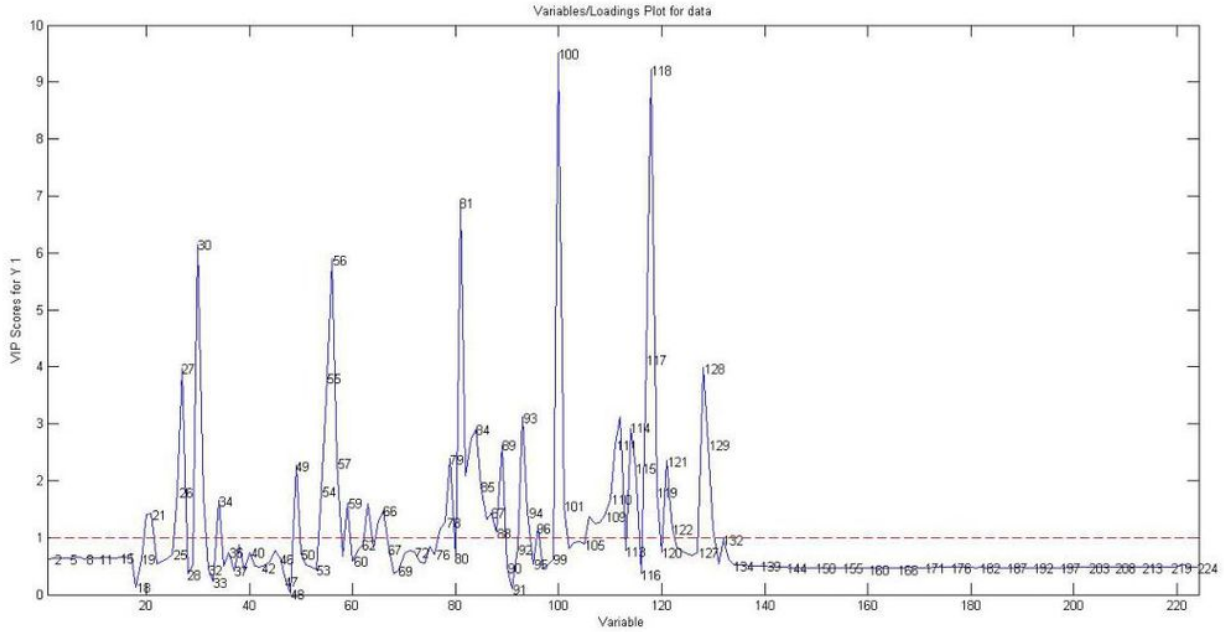

Figure 5

VIP chemical shift values between T5 metabolic differences in Group A and Group B