

Role of Serum Fatty Acids in Children with Henoch-Schönlein purpura by GC-MS analysis

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Abstract

BACKGROUND: The objectives of this work were to discover the changes of serum Medium- and Long-Chain fatty acids levels and its possible relationship with Henoch-Schönlein Purpura (HSP), also referred to as Immunoglobulin A vasculitis in children. **METHODS:** A total of 58 children with HSP and 28 healthy children were recruited for this study. Serum fatty acids were analyzed by gas chromatography with mass spectrometry (GC-MS). **RESULTS:** 31 species of Fatty acids were discovered to have a significant difference between HSP group and healthy control group (CON group). The contents of all detected 37 fatty acids in the HSP group were higher than the healthy group. Parts of fatty acids were found in our study having significant change according to the treatment. Palmitate (C16:0) and 18 carbon atoms (C18) of fatty acids were abundant in all three groups of HSP. Elaidate (C18:1T), cis-11,14,17-Eicosatrienoic acid ester (C20:1) and cis-15-tetracosenoate (C24:1) were found to have a correlation on renal damage of HSP. **CONCLUSION:** Our study provides clinical evidence to support that fatty acid metabolism is associated with HSP by GC-MS method. Glucocorticoid therapy has a certain relationship with fatty acid metabolism during HSP treatment. Meanwhile, long-chain MUFAs may have an impact on renal damage of HSP. In addition, we speculate that a low BMI may be a kind of manifestation of abnormal fatty acid metabolism in HSP. All in all, further study is needed to explore the specific mechanism of fatty acids and HSP.

Background

Henoch-Schönlein Purpura (HSP), also called Immunoglobulin A vasculitis, is a systemic IgA immune complex-mediated vasculitis [1]. The primary clinical manifestations of HSP include palpable purpuric rashes in the extremities (especially the lower extremities), arthritis, gastrointestinal symptoms and renal damage [2,3]. The disease mainly occurs in children and is more common in the age of 2 to 8 years old. Although its pathogenesis remains unclear, multiple etiologies, including genetic background and environmental factors, have been suggested to contribute to the pathogenesis of HSP. Because the symptoms of HSP are relatively variable and heterogeneous, diagnosing the disease is challenging [4]. Fatty acids, as main elements of biological membranes, are reported to have effects on inflammation, vascular function and thrombosis in the progression of many diseases [5]. So, we test the contents of serum Medium- and Long-Chain fatty acids including saturated fatty acids (SFAs), MUFAs and polyunsaturated fatty acids (PUFAs) in children with HSP, and try to find out some potential associations between HSP and healthy children.

Methods

2.1 Subjects and blood sample collection

Fifty-eight patients with HSP and twenty-eight healthy children were recruited for this study. All the HSP patients were younger than 18 years old and met the American Rheumatology Association and EULAR/PreS recognized diagnostic criteria for HSP, that is palpable purpuric rashes in the extremities

especially the lower extremities (necessary) with any of the following: 1) diffuse abdominal pain; 2) biopsy showing significant IgA deposition; 3) acute arthritis or joint pain in any joint; 4) manifestations of kidney damage [hematuria and (or) proteinuria]. The CON group were recruited with a screening questionnaire from healthy children without any other diseases, such as heart, liver and kidney abnormality, immunodeficiency disease, tuberculosis, hepatitis, as well as any drug-using histories in recent six months, such as prednisone, tacrolimus, cyclosporine, cyclophosphamide, etc. Ethical approval for this study was obtained from Ethics Committee of the Second Xiangya Hospital of Central South University. Written informed consent was obtained from both parents before the study.

We collected basic information and clinical data of those patients. Heparin anticoagulated tubes were used to collect approximately 3 ml of whole blood samples from children with HSP and healthy controls. Centrifuge at 1300g-2000g at 4 ° C for 10 minutes and take the upper plasma (not less than 0.3ml). The plasma samples were quickly frozen in liquid nitrogen, then stored at -80 ° C waiting for testing.

2.2 Standard preparation

40-methyl vitamins mixed standard solution were prepared for nine mixed standard concentration gradients, including 0.5 mg/L, 5 mg/L, 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L, 2500 mg/L. In the fatty acid methyl ester standards, the concentration of each component in the total concentration was 2% and 4%.

2.3 Metabolite extraction

The plasma samples were thawed in ice, and 150 μ L of them were taken into the centrifuge tube. Add 6 mL dichloromethane-methanol solution and vortex for 2 min and shake for 20 min at room temperature. Centrifuge at 2000 rpm for 10 minutes. Transfer the lower chloroform phase to a new glass test tube. 3 ml of n-hexane and 0.25 μ L of internal standard (C19, 10 mg/mL) were added for extraction. Vortex for 2 min, then add 3 mL KOH / methanol (0.4mol/L). After standing and layering, take the supernatant to a new glass test tube, blow dry with nitrogen, add 200 μ L of n-hexane, and vortex for 2min. After standing and layering, take the supernatant to a sample bottle.

2.4 GC-MS Analysis

The samples were separated on an Agilent DB-WAX capillary column (30 m \times 0.25 mm ID \times 0.25 μ m) gas chromatography system. The temperature programming was as follows: the initial temperature was 50 °C and remained as such for 3 min. The temperature increased at 10 °C/min up to 220 °C and remained there for 20 min. The last temperature increased at 15 °C/min up to 250 °C and remained there for 10 min. The carrier gas was helium, and the carrier gas velocity was 1.0 mL/min. A QC sample was used for testing and evaluating the stability and repeatability of the system. An Agilent 6890N/5975B gas chromatography-mass spectrometer was used for analysis. The temperatures of the injection port and transmission line were 280 °C and 250 °C respectively. The electron bombardment ionization (EI) source, SIM scanning mode, and electron energy were 70 eV.

2.5 Statistical analysis

MSD ChemStation software was used to extract peak areas and retention times. Draw a curve and calculate the content of long-chain fatty acids in the sample. Analyses of significant differences in metabolite levels were performed using t-test. A p-value of less than 0.05 was considered statistically significant.

Results

3.1 Clinical characteristics of the patients

A total of 58 children with HSP (28 males, 30 females) and 28 healthy children (14 males, 14 females) were recruited for this study. The mean age of the patients with HSP was 9.17 ± 3.08 years, and that of the CON group was 10.60 ± 3.66 years (Table 1). There was no significant difference in age between the two groups ($p=0.061$), while there was a significant difference in body mass index (BMI) between HSP group and healthy controls ($p=0.03$). The HSP patients were divided into three groups, including the untreated group (diagnosed as HSP for the first time and has not been treated with any immunotherapy drugs, includes glucocorticoids, 25.86%), the regular treated group (single or combined treated with immunotherapy drugs, 51.72%), and the withdrawal group (completely discontinued with any drugs for at least 3 months and currently without any clinical symptoms, 22.41%). The main clinical symptoms in patients with HSP included palpable rashes in the extremities (48.28%), arthritis (20.70%), gastrointestinal symptoms (20.70%) and renal damage (53.45%). 17 of total HSP patients did the renal biopsy (29.31%). And the results of blood routine and renal function in all patients were collected for correlation analysis.

3.2 The compositions of serum fatty acids in children with HSP and the healthy controls

40 fatty-acid methyl ester standards were analyzed using the established fatty acid analysis method, and a total of 37 fatty acids were detected and quantified in this experiment (Table 2), of which 1 fatty acid was not separated due to its isomers, and 2 were not detected. The internal standard was separated from each standard, and the chromatographic separation of each metabolite is good. The peak shape is sharp and symmetrical, which can quantify each metabolite by mass spectrometry. The linearity and correlation coefficient for each component according to the proportion of each component in the total concentration showed that the linearity of each analyte in the linear range is good, and the correlation coefficients are greater than 0.99. The results of our study showed that contents of all detected fatty acids in HSP patients upregulated comparing with the healthy children. Among them, 31 of total fatty acids had significant differences between HSP group and CON group ($p<0.05$), which includes 11 kinds of SFAs, 8 kinds of MUFAs and 12 kinds of PUFAs.

3.3 Correlation analysis with laboratory test results of HSP

The correlation between plasma metabolites and clinical indicators of HSP was shown in Figure 1. Heat map summarizing level fold changes of significantly Fatty acids metabolites in GC-MS Analysis of blood

samples. Red and blue represent positive and negative correlations of metabolite in the HSP group comparing with healthy controls. The results show that many blood cell components are related to different kinds of free fatty acids.

3.4 Compositions of fatty acids in different groups of HSP

The distribution of serum fatty acids in the three groups of HSP (the regular treated group, the untreated group and the withdrawal group) was shown in Figure 2(a) using a barplot picture. It showed that palmitate (C16:0) and C18 were abundant in all three groups. Docosahexaenoic acid (DHA, C22:6) was significantly upregulated in the untreated group contrast with the regular treated group in figure 2(b). Palmitoleate (C16:1) and linolelaidate (C18:2TT) were significantly upregulated in the regular treated group compared with the withdrawal group in figure 2(c). Undecanoate (C11:0), myristoleate (C14:1), cis-11,14-Eicosadienoic acid ester (C20:2), cis-11,14,17-Eicosatrienoic acid ester (C20:1), docosapentaenoate (C22:5N6) and erucate (C22:1) were significantly upregulated in the untreated group compared with withdrawal group in figure 2(d).

3.5 Relationship between serum fatty acids and renal damage of HSP

The patients of HSP were divided into two groups according to whether have proteinuria or (and) hematuria. Wherein, group A represents there was no manifestation of kidney damage; and group B showing there were observed renal damage including proteinuria and hematuria. Figure 3(a) showed the compositions of fatty acids in the two groups. And we found there were significant differences in C18:1T, C20:1 and C24:1 between the two groups showing in Figure 3(b). Moreover, the three fatty acids, which are all MUFAs, were significantly higher in the group with renal impairment than that in the group without renal impairment.

Discussion

GC-MS is a method that combines the characteristics of gas chromatography and mass spectrometry to identify different substances almost in a blood or urine sample when used in medical research [6,7]. Diseases such as systemic lupus erythematosus [8], look for metabolic markers in this way. In our study, the different kinds of free fatty acids were isolated and analyzed by GC-MS [9]. Thirty-one fatty acids were found to be differentially upregulated in the plasma in the HSP group compared to the control group, which contains both saturated fatty acids and unsaturated fatty acids. However, there are few studies having investigated the relationship between fatty acids and the development of HSP. So, we can only speculate on the possible role of fatty acids we have detected in the pathogenesis of allergic purpura through the well-known fatty acid metabolism.

Generally, the common SFAs [10] include caprylic acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, etc. Palmitic acid was found to be able to stimulate the release of extracellular vesicles from proximal tubular epithelial cells, resulting in kidney dysfunction in the context of metabolic disease[11]. MUFAs like oleic acid have the ability to regulate cholesterol metabolism. long-chain MUFAs

have been shown to attenuate atherosclerosis development in mouse models. Yang et. [12] found C20:1 or erucate (C22:1) was equally effective in reducing atherosclerosis in LDLr mice may through activation of the Ppar signaling pathways and favorable alterations in the proteome of lipoproteins. In our study, we found C18:1T, C20:1 and C24:1 were significantly higher in the group with renal damage than that in the group without renal impairment, which shows long-chain MUFAs may have an impact on renal damage. PUFAs can be classified into omega-3 and omega-6 polyunsaturated fatty acids according to the position of the first double bond from the methyl end. The two most important omega-3 PUFAs for the human body are DHA and eicosapentaenoic acid (EPA), which are both demonstrated to ameliorate renal disease [13]. Studies have shown that DHA and EPA have anti-atherosclerotic effects and have a significant effect on reducing blood lipids and inhibiting platelet aggregation. DHA can also effectively reduce the activity of inflammatory factors mainly by inhibiting the 5-lipoxygenase metabolism pathway of neutrophils and monocytes and increase the synthesis of leukotriene B5. Arachidonic acid (AA), a kind of omega-6 PUFA, mainly manifests as the form of phospholipids in the cell membrane, which is released from the phospholipids under stress by phospholipase A2 and phospholipase C [14]. AA has a series of physiological activities such as esterifying cholesterol, increasing vascular elasticity, reducing blood viscosity, and regulating blood cell function. And it synthesizes bioactive substances such as prostaglandins, thromboxanes and leukotrienes, which play a very important role in the human immune system [15]. All the above fatty acids are up-regulated in HSP group, indicating that fatty acid metabolism has played an important role in the development of HSP, but the specific mechanism is still unknown, and further research is needed. Our research group is already studying the role of different types of fatty acids in the pathogenesis of HSP, and will be verified by animal experiments.

So far, there are no specific laboratory indicators detected in the disease of HSP. There are some significant correlations between free fatty acids and clinical indicators, which are considered to be related to the biofilm membrane lipid composition. There were some kinds of fatty acids found in our study having changes according to the treatment, which may suggest that fatty acid metabolism played a significant role in the improvement of HSP. All of the patients in the regular treated group used glucocorticoids (methylprednisolone or prednisone). And parts of the patients used both glucocorticoids and other immune-suppressors such as tacrolimus, cyclophosphamide, Mycophenolate mofetil. Previous reviews have shown glucocorticoids are important determinants of fatty acid metabolism in both animals and humans [16]. A finding of Stephanie Tung, *at al* [17] showed fatty acid oxidation may take part in the mechanism of resistance to glucocorticoid-mediated cytotoxicity, and PPAR α inhibition may improve the therapeutic efficacy of glucocorticoids. However, more detailed investigations are needed to dissect the integrated effects on fatty acid and glucocorticoid metabolism.

Last, we surprisingly found in our study that there was a significant difference in BMI between HSP group and healthy controls of the same age ($p=0.03$). HSP patients tend to have a lower BMI. However, some studies about HSP did not show the difference between HSP group and healthy patients in BMI, or length as well as weight [18]. BMI is an indicator closely related to fatty acid metabolism [19]. In the study of many diseases, such as diabetes, the important role of BMI in fat metabolism has been found [20]. So, a

low BMI may be an indicator of disorders of fatty acid metabolism in HSP. Yet, whether and how HSP is related to BMI remains to be investigated.

Conclusions

Our study provides clinical evidence to support that fatty acid metabolism is associated with HSP by GC-MS method. Glucocorticoid therapy has a certain relationship with fatty acid metabolism during HSP treatment. Meanwhile, long-chain MUFAs may have an impact on renal damage of HSP. In addition, we speculate that a low BMI may be a kind of manifestation of abnormal fatty acid metabolism in HSP. All in all, further study is needed to explore the specific mechanism of fatty acids and HSP.

Abbreviations

HSP: Henoch-Schönlein Purpura

GC-MS: Gas chromatography with mass spectrometry

CON group: Healthy control group

BMI: Body mass index

MUFAs: Monounsaturated fatty acids

SFAs: Saturated fatty acids

PUFAs: Polyunsaturated fatty acids

DHA: Docosahexaenoic acid

EPA: Eicosapentaenoic acid

AA: Arachidonic acid

Declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from Ethics Committee of the Second Xiangya Hospital of Central South University. Written informed consent was obtained from both parents before the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

KS has contributed at all stages, from the design of the study to the final written version of the paper. HS and HW contributed substantially to the conception and design of the study. GK, HS and HW have been involved in the analysis and interpretation of the data. All authors revised the manuscript and approved the final version.

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Tables

Table 1: Clinical characteristics of the patients

Characteristic	Group	
	HSP(n=58)	CON(n=28)
Age (year)	9.17±3.08	10.60±3.66
BMI(kg/m ²)	16.50±2.85	18.57±4.57
Rashes (n)	28(48.28%)	
Abdominal pain (n)	12(20.70%)	
Arthritis or joint pain (n)	12(20.70%)	
Hematuria (n)	20(34.48%)	
Proteinuria (n)	13(22.41%)	
Kidney biopsy (n)	17(29.31%)	
Untreated group (n)	15(25.86%)	
Regular treated group (n)	30(51.72%)	
Withdrawal group (n)	13(22.41%)	
	4.44±1.84	
• Blood urea nitrogen (mmol/l)	40.78±18.87	
• Serum creatinine (umol/l)		
Uric Acid (umol/l)	268.95±67.81	
White Blood Cell (10 ⁹ /l)	8.88±2.69	
Leukocyte (%)	60.3±13.2	
Lymphocyte (%)	32.1±12.5	
Red Blood Cell (10 ¹² /l)	4.70±0.47	
Hemoglobin (g/l)	129±12.3	
Platelet (10 ⁹ /l)	310±86.9	

All values presented as number (%) or mean ± standard deviation. *p* value of comparison for age between HSP and CON group was 0.061; *p* value of comparison for BMI between HSP and CON group was 0.03.

Table 2: The percentage composition of 37 free fatty acids found in plasma from patients with HSP group and CON group

No	Free fatty acid	Fold Change	p value	Mean ± standard deviation	
Chemical name				HSP(n=58)	CON(n=28)
1	C6:0 hexanoate	NA	NA	0	0
2	C8:0 octanoate	1.958	<0.001	0.034±0.002	0.017±0.001
3	C10:0 decanoate	5.610	0.411	0.222±0.271	0.040±0.007
4	C11:0 undecanoate	1.773	<0.001	0.013±0.007	0.007±0.003
5	C12:0 dodecanoate	1.137	0.672	0.737±0.713	0.648±1.212
6	C13:0 tridecanoate	2.111	0.146	0.006±0.011	0.003±0.006
7	C14:1 myristoleate	1.561	0.004	9.450±5.816	6.053±2.747
8	C14:0 myristate	2.890	0.003	6.026±6.669	2.085±1.947
9	C15:1 cis-10-pentadecenoate	1.606	0.004	0.715±0.439	0.445±0.262
10	C15:0 pentadecanoate	3.254	<0.001	0.922±0.897	0.283±0.206
11	C16:1 palmitoleate	2.911	<0.001	2.835±1.800	0.974±0.425
12	C16:0 palmitate	2.341	<0.001	205.706±91.747	87.878±29.930
13	C17:1 cis-10-heptadecenoate	3.718	<0.001	0.825±0.815	0.222±0.138
14	C17:0 thyl heptadecanoate	2.795	<0.001	1.958±1.535	0.701±0.316
15	C18:3N6 γ-linolenate	2.961	<0.001	2.561±1.802	0.865±0.344
16	C18:2TT linolelaidate	2.061	<0.001	203.965±76.713	98.960±30.316
17	C18:1 oleate	2.188	<0.001	108.742±43.763	49.710±16.429
18	C18:2 linoleate	2.687	<0.001	91.650±75.122	34.114±31.357
19	C18:1T elaidate	3.390	0.004	104.847±127.147	30.928±47.252
20	C18:0 stearate	2.060	<0.001	89.292±30.546	43.354±12.192
21	C20:4 arachidonate	2.063	<0.001	53.559±17.360	25.960±8.396
22	C20:5 cis-5,8,11,14,17-Eicosapentaenoic acid ester	2.469	<0.001	3.318±1.895	1.344±0.677
23	C20:3N8 cis-8,11,14-Eicosatrienoic acid ester	2.469	<0.001	13.638±6.174	5.524±2.525
24	C20:2 cis-11,14-Eicosadienoic acid ester	1.890	<0.001	6.748±3.213	3.571±1.182
25	C20:1 cis-11,14,17-Eicosatrienoic acid ester	2.032	<0.001	2.183±1.303	1.074±0.414
26	C20:0 arachidate	2.162	0.001	0.516±0.392	0.239±0.192
27	C21:0 heneicosanoate	2.573	0.009	0.016±0.018	0.006±0.007
28	C22:5N3 docosapentaenoate	1.812	0.023	6.965±6.739	3.845±3.243
29	C22:6 cis-4,7,10,13,16,19-Docosahexaenoic acid ester	1.852	<0.001	14.752±5.598	7.966±2.668
30	C22:4 docosatetraenoate	2.070	0.036	6.538±8.192	3.158±2.456
31	C22:5N6 docosapentaenoate	1.809	<0.001	5.909±3.203	3.266±2.443
32	C22:2 cis-13,16-Docosadienoic acid ester	1.696	0.003	4.709±3.215	2.777±1.221
33	C22:1 erucate	1.507	0.008	23.939±14.932	15.888±6.963
34	C22:0 behenate	4.998	0.025	0.399±0.743	0.080±0.100
35	C23:0 tricosanoate	2.371	0.002	0.022±0.020	0.009±0.011
36	C24:1 cis-15-tetracosenoate	1.832	0.257	0.614±1.255	0.335±0.421
37	C24:0 tetracosanoate	4.698	0.090	0.172±0.415	0.037±0.048

Fold Change: Multiples of change of metabolites in the comparison group, greater than 1 indicates up-regulation, and less than 1 indicates down-regulation;

p-value: Statistical analysis of metabolites in the comparison group, p <0.05 is a significant difference in metabolites.

Figures

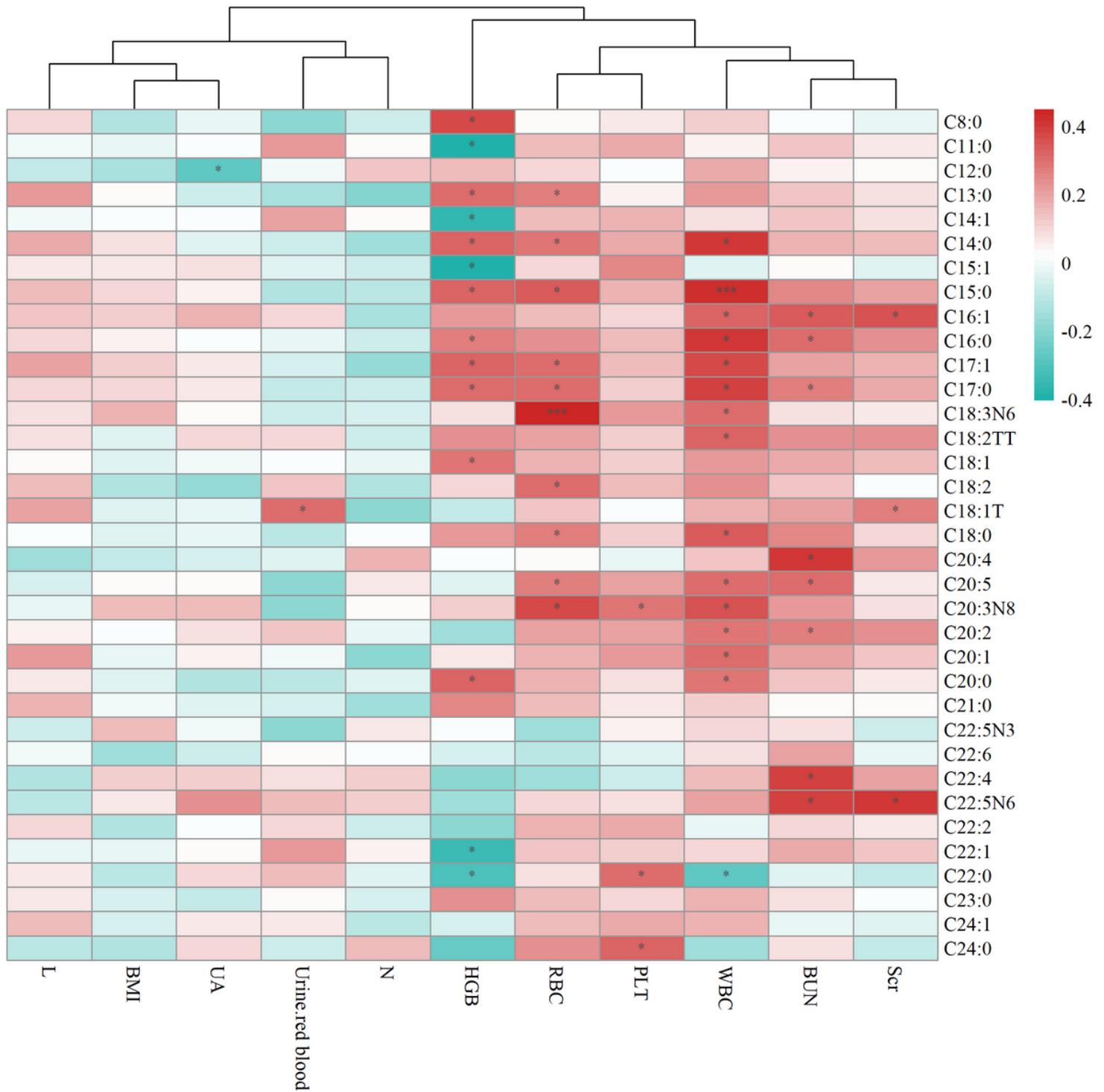


Figure 1

Heat map summarizing the correlation between serum fatty acids and laboratory test results of HSP Scr means Serum creatinine (umol/l), BUN means Blood urea nitrogen (mmol/l), UA means Uric Acid (umol/l), WBC means White Blood Cell ($10^9/l$), RBC means Red Blood Cell ($10^{12}/l$), PLT means Platelet ($10^9/l$), HGB means Hemoglobin (g/l), N means the percent of Leukocyte, L means the percent of Lymphocyte, BMI shows Body Mass Index. * means p<0.05, *** means p<0.001

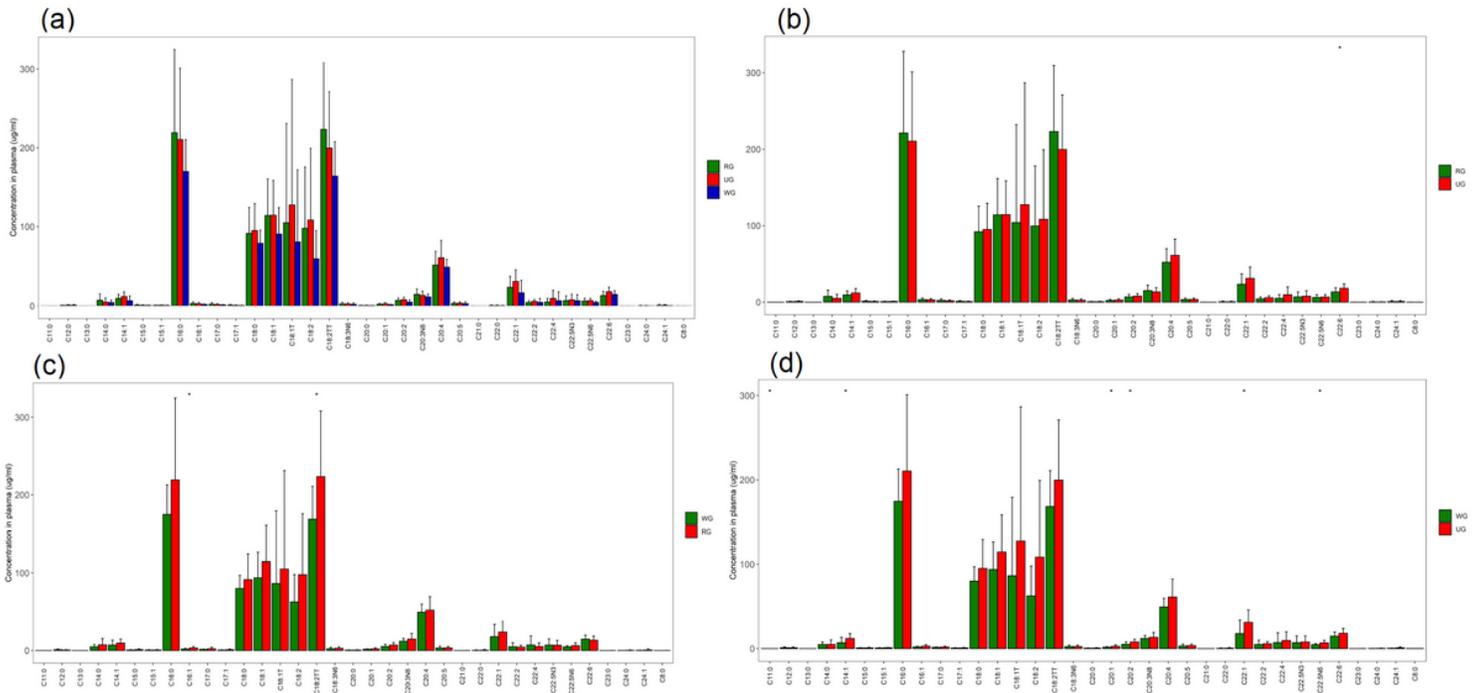


Figure 2

Distribution and correlations of 37 fatty acids among the three group of HSP (a) Distribution of 37 fatty acids in the three group of HSP; (b) Correlations of 37 fatty acids between the untreated group and regular treated group; (c) Correlations of 37 fatty acids between the regular treated group and withdrawal group; (d) Correlations of 37 fatty acids between the untreated group and withdrawal group. RG means regular treated group, UG means the untreated group, WG means the withdrawal group.

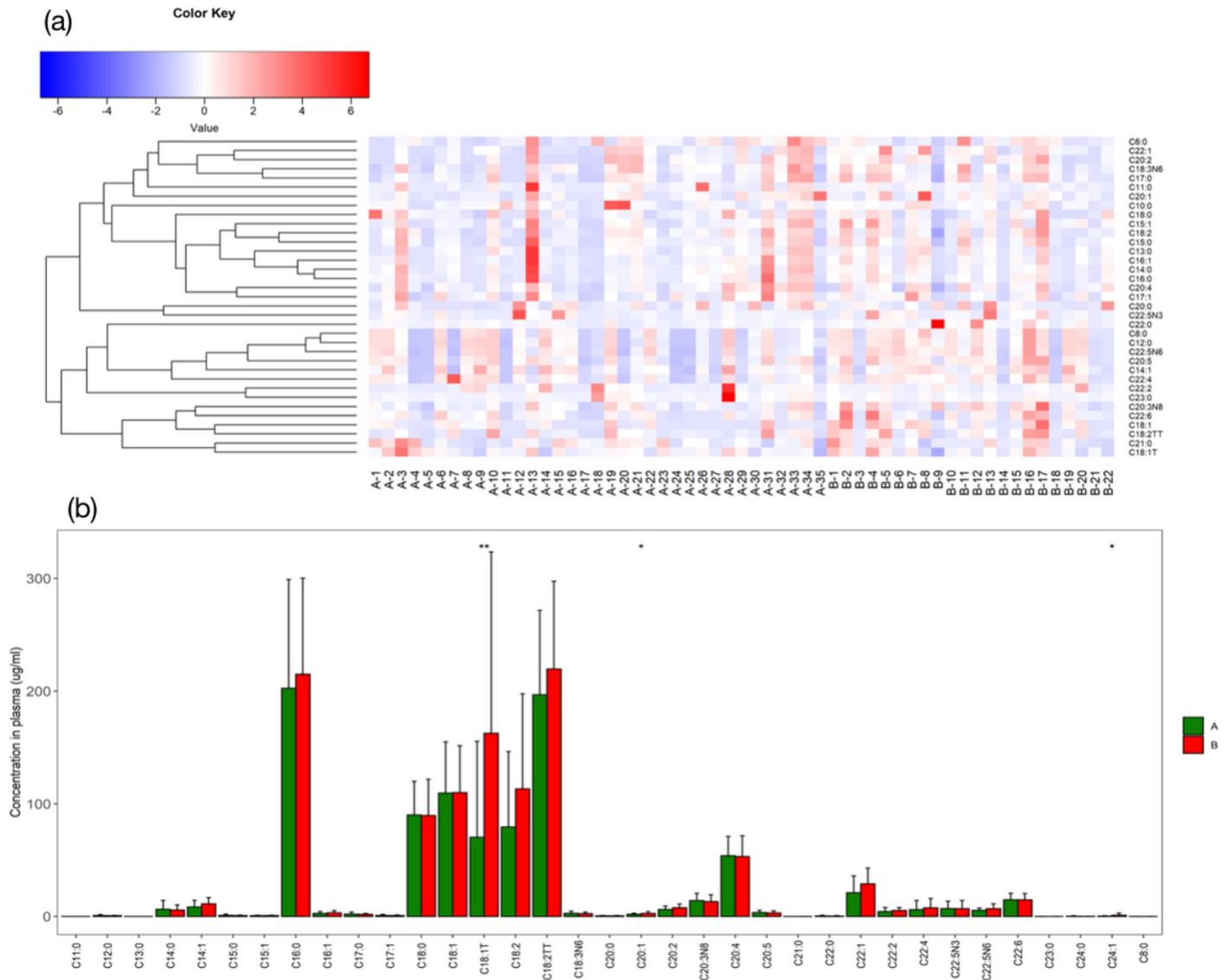


Figure 3

Relationship between serum fatty acids and renal damage. (a) Heat map picture showing the compositions of fatty acids in the group A and group B. (b) Distributions and correlation in the compositions of fatty acids between group A and group B. A means the group without renal damage, B means the group with renal damage including proteinuria or (and) hematuria. * means $p < 0.05$, ** means $p < 0.01$.

Supplementary Files

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