

Non-targeted metabolomics analysis reveals changes in plasma and skin metabolic profiles after capsaicin gavage in mice

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Research Article

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Abstract

Background

As an essential spice, chili pepper has essential physiological functions for humans. Capsaicin is the main compound found in chili pepper and has complex pharmacologic effects. Capsaicin has been observed to play an important role in obesity, cardiovascular and gastrointestinal diseases, various cancers and dermatologic conditions. Although possessing a broad spectrum of bioactivities, changes in the metabolic profile of the organism are inadequately unknown after oral gavage with capsaicin.

Methods

Therefore, an untargeted metabolomics approach coupled with multivariate statistical analyses was first applied to study the plasma and skin metabolic differences between mouse groups with and without capsaicin treatment by gavage. And we discussed these differential metabolites in the context of the physiological functions of capsaicin.

Results

The results showed that 38 plasma metabolites and 7 skin metabolites were associated with capsaicin intake, mainly associated with energy metabolism, lipid metabolism, and oxidative stress. KEGG pathway enrichment analysis revealed that these differential metabolites are primarily involved in some crucial pathways, such as Central carbon metabolism in cancer, Pyruvate metabolism, and ABC transporters in plasma; sphingolipid metabolism in the skin.

Conclusions

Plasma and skin metabolome showed significant alterations in response to the capsaicin supplementation, and these findings contribute to a better understanding of the potential effects of a capsaicin-rich diet on organisms.

Background

Peppers are one of the most widely consumed vegetables and spices worldwide. In recent years, peppers have become an important part of culinary cultures worldwide and have a long history of use for flavoring, coloring, and preserving food, as well as for medical purposes[1]. The increased use of peppers in food is a major trend around the world[2]. With the enhancement of people's health awareness and the development of medical science, the effect of consuming peppers on human health has become a significant concern. Chili peppers and capsaicin(the main pungent component of chili peppers) harbors many benefits extensively documented in many studies. Dietary and supplementation with capsaicin are

beneficial effects for glucose and insulin levels in humans[3–5] and may also improve body mass index in obese patients[6, 7]. There is evidence that capsaicin has a potentially beneficial effect on the cardiovascular system by activating transient receptor potential vanilloid subfamily member 1 (TRPV1)[8, 9]. The gastroprotective effect of capsaicin through modulation of sensory neurons has also been described[10, 11]. Capsaicin has been shown to possess chemopreventive and chemotherapeutic effects[12, 13], and in vivo studies support the antitumor activity of capsaicin[13, 14]. Capsaicin also seems to have a protective effect on bladder disorders. An animal study showed that pretreatment with capsaicin could prevent spinal cord injury-induced detrusor hyperreflexia in rats[15]. In addition, studies have shown that topical application of capsaicin has an inhibitory effect on pain[16], itch[17], and psoriatic epidermal proliferation[18]. Furthermore, population studies also suggested the beneficial effects of capsaicin on human health. One prospective study found that habitual consumption of spicy foods was inversely associated with death due to cancer, ischemic heart disease, and respiratory diseases[19]. The study also noted that those who ate spicy foods almost every day had a 14% lower risk of death than subjects who ate spicy foods less than once a week[19]. Epidemiologic data also showed that consuming foods containing capsaicin is associated with a lower prevalence of obesity, type 2 diabetes, and cardiovascular diseases[1, 20]. On the other hand, it is observed that the consumption of chili peppers often worsens individual human physiological states such as sore throat, mouth ulcer, constipation, acne, etc., all of which were summarized as "getting inflamed"[21]. Additionally, some reports have also shown the "bad aspects" of capsaicin, such as the increased rate of carcinogenesis with the use of capsaicin[22–24], the detrimental effects on GI tract on prolonged exposure of high doses of capsaicin[25], and the injection of capsaicin into rat pups results in chronically relapsing pruritic dermatitis[26]. Many studies on the mechanism of capsaicin based on TRPV1 have been reported previously[27–29], but TRPV1 alone still cannot fully elucidate the physiological functions of capsaicin. The exact mechanism of action of capsaicin is not yet fully understood, and its effects on the health and disease of the organism remain partially controversial. Therefore, it is of great significance to further explore the impact of the capsaicin-rich diet.

Metabolomics, the emerging "omics" technique, can be used to reflect the comprehensive, unbiased metabolic profile of biological samples, including urine, feces, plasma, and tissue samples, providing details of the metabolic responses of the living organism to external stimuli (including pathological stimuli and drug treatments)[30]. Compared to genomics, transcriptomics and proteomics, metabolomics reflects most directly the physiological state of a biological system, as metabolites are most closely associated with the phenotype of an organism[31, 32]. By analyzing all the metabolites in biological samples, the results can tell us what happened in the organisms[33]. Recently, ultra-high-performance liquid chromatography and Q-TOF mass spectrometry (UHPLC/Q-TOF MS) adds a new dimension to metabolism studies due to its better reproducibility, detection limits and increased chromatographic resolution[34–36]. In this study, we used metabolomics analyses based on UHPLC-Q-TOF MS to gain a broader understanding of metabolic changes in mice orally administered capsaicin. As far as we know, this is the first study to explore metabolic changes in plasma and skin of mice after oral gavage with

capsaicin. The analysis of changes in metabolites and metabolic pathways allows us to understand the physiological functions of capsaicin better.

Materials And Methods

Experimental Animals and Chemicals

Twenty healthy C57BL/6 female mice (20 ± 2 g) aged 6 weeks were purchased from Hunan SJA Laboratory Animal Co., Ltd (Hunan, China). All mice were allowed to acclimatize in cages for 1 week before treatment. Mice had free access to standard diet and water and were maintained under the normal laboratory conditions (temperature of 22–23°C, relative humidity of 45%–50, and 12 h/12 h light/dark cycle). Synthesized capsaicin (97% capsaicin) and soybean oil were obtained from Sinopharm, China. The synthetic capsaicin was dissolved in soybean oil to form a 0.5 mg/ml capsaicin-soybean oil solution. Ammonium acetate (NH₄AC) and ammonium hydroxide (NH₄OH) were purchased from Sigma Aldrich. Acetonitrile was purchased from Merck.

Animal Treatment and Sample Collection

After 1 week of adaptation, the mice were randomly assigned to either control (n = 10) or treatment group (n = 10). In the treatment group, the mice were termed Cap and treated with capsaicin-soybean oil solution (capsaicin of 5 mg/kg mice body weight) on Monday, Wednesday, and Friday (16:00–17:00) of each week for a total of six weeks. The mice in the control group were termed Con and fed with the corresponding volume of soybean oil simultaneously. Before each gavage, their body weights were determined and recorded. Regular monitoring of animal health and well-being was done three times weekly, which included the overall health of the mice and their motor activity, eating, drinking, and weight records. The animals were also frequently monitored for evidence of adverse reactions to capsaicin (vomiting, diarrhea, poor grooming, frantic appearance, poor coat condition, and aversion to handling). No signs of adverse reactions to capsaicin and distress were observed in animals in this study. In addition, no mice died prematurely during the course of the study. After 6 weeks, mice were anesthetized with an intraperitoneal injection of sodium pentobarbital and euthanized by cervical dislocation, followed by heart puncture and blood collection. Plasma samples were extracted from the collected blood by centrifugation (1500g, 4°C, 15 min) and stored at -80°C until UHPLC-Q-TOF/MS analysis. The skin samples of approximately 2x2cm² in size were then collected from the center of the mice's back with tissue scissors (hair and subcutaneous fat were removed, leaving the epidermis and dermis). The skin samples were then also stored at -80°C until UHPLC-Q-TOF/MS analysis. All animal studies were approved by the Research Ethical Committee of The Second Affiliated Hospital of Nanchang University (Nanchang, China).

Sample Preparation for UHPLC-Q-TOF MS analysis

Plasma samples stored at -80°C were gradually thawed at 4°C, and 100 µL aliquots were mixed with 400 µL of cold methanol/acetonitrile (1:1, v/v). The mixture was centrifuged for 20 min (14000g, 4°C). The

supernatant was dried in a vacuum centrifuge. For the UHPLC-Q-TOF/MS analysis, plasma samples were re-dissolved in 100 μ L acetonitrile/water (1:1, v/v) solvent. After thawing, the skin samples with 200 μ l of H₂O and five ceramic beads were homogenized using the homogenizer. 800 μ L methanol/acetonitrile (1:1, v/v) were added to homogenized solution for metabolite extraction. The mixture was centrifuged for 15 min (14000g, 4°C). The supernatant was dried in a vacuum centrifuge. For the UHPLC-Q-TOF/MS analysis, skin samples were re-dissolved in 100 μ L acetonitrile/water (1:1, v/v) solvent.

UHPLC-Q-TOF MS analysis

In this study, twenty plasma samples and twenty skin samples were detected by UHPLC-Q-TOF MS. The UHPLC-Q-TOF MS approaches were described in detail in S1 File. Briefly, analyses were performed using a UHPLC (1290 Infinity LC, Agilent Technologies) coupled to a quadrupole time-of-flight (AB Sciex TripleTOF 6600) in Shanghai Applied Protein Technology Co., Ltd. The flow rate was 0.5 ml/min, and the column temperature was 25°C. The quality control(QC) samples were inserted regularly and analyzed in every 5 samples to evaluate the stability of the system and the reliability of the experimental data.

Data processing

The raw MS data (wiff. scan files) were converted to MzXML files using ProteoWizard MSConvert before importing into XCMS software. For peak picking, the following parameters were used: centWave m/z = 25 ppm, peak width = c (10, 60), prefilter = c (10, 100). For peak grouping, bw = 5, mzwid = 0.025, minfrac = 0.5 were used. CAMERA (Collection of Algorithms of MEtabolite pRofile Annotation) was used for annotation of isotopes and adducts. In the extracted ion features, only the variables having more than 50% of the nonzero measurement values in at least one group were kept. Compound identification of metabolites was performed by comparing of accuracy m/z value (< 25 ppm), and MS/MS spectra with an in-house database established with available authentic standards.

Statistical analysis

After normalized to total peak intensity, the processed data were analyzed by R package(ropis), where it was subjected to multivariate data analysis, including Pareto-scaled principal component analysis (PCA) and orthogonal partial least-squares discriminant analysis (OPLS-DA). The 7-fold cross-validation and response permutation testing were used to evaluate the robustness of the model. The variable importance in the projection (VIP) value of each variable in the OPLS-DA model was calculated to indicate its contribution to the classification. Metabolites with the VIP value > 1 were further applied to Student's t-test at univariate level to measure the significance of each metabolite, the p values less than 0.05 were considered statistically significant. In addition, the body weight of mice was compared by the Student's t-test.

Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis

KEGG pathway analysis enrichment of differential metabolites was performed. We used Fisher's exact test to analyze and calculate the significance level of metabolite enrichment of each pathway to

determine the metabolic pathways that were significantly affected. The smaller the P-value, the more significant the difference in the metabolic pathways.

Results

Effect of capsaicin on body weight in mice

As shown in Table 1, we compared the body weight of Cap and Con before and after the experiment. The results showed that although there was no significant change in the body weight of the Cap after the experiment, the body weight of the Con increased significantly ($P < 0.05$). In addition, Cap's body weight was significantly lower than Con's after the experiment ($P < 0.05$).

Table 1
Comparison of Cap and Con's body weight before and after the experiment.

	Body weight(unit: g)		p-value
	Before intervention	After intervention	
Capsaicin group	16.68 ± 0.40	17.51 ± 1.99	p = 0.225
Control group	16.66 ± 0.33	19.33 ± 0.56	p = 0.000
p-value	p = 0.929	p = 0.019	

Analytical method assessment

In order to monitor the stability and repeatability of the instrument analysis, QC samples were prepared during sample processing. PCA analysis was performed on all extraction peaks of all experimental and QC samples after Pareto-scaling. The relatively tight clustering of the QC samples at the center of all samples (S1 Fig, S2 Fig) suggests the experiment's good repeatability and stability.

Analysis of significant difference metabolite in plasma

In plasma, two hundred and seventy-two metabolite peaks were detected by UHPLC-Q-TOF MS, among which 38 metabolites were significantly different (S1 Table). Thereinto, most differential metabolites are lipids and lipid-like molecules or organic acids and derivatives. Table 2 shows the 11 lipids and lipid-like molecules and 13 organic acids and derivatives affected by capsaicin in our study. After that, OPLS-DA was conducted for each sample to explore the metabolic effect of the murine model after capsaicin gavage. As it can be seen from the score plot (Fig. 1a), there was evident clustering and separation between Cap and Con. The OPLS-DA model was assessed using a 7-fold cross-validation method. The values of R^2Y and Q^2 were 0.993 and 0.569, suggesting good fitness and predictive ability of the OPLS-DA model. Moreover, to avoid overfitting, 200-time permutation tests were performed to validate the model and revealed that the model had high predictability without overfitting (S3 Fig). To further illustrate the alterations in plasma metabolites induced by capsaicin, heatmap analyses were conducted, and the results also showed distinct segregation between Cap and Con. (Fig. 1b)

Table 2(This table is derived from line 207 of this document)

11 lipids and lipid-like molecules and 13 organic acids and derivatives among the differential metabolites of plasma.

Category	Metabolites	VIP	Fold change	p-value
Lipids and lipid-like molecules	Pentadecanoic Acid	2.54	0.539	0.000
	Linoleic acid	2.56	0.595	0.001
	Lecithin	3.72	0.675	0.001
	Heptadecanoic acid	1.75	0.634	0.002
	Docosahexaenoic acid	8.17	0.694	0.004
	Dodecanoic acid	2.31	1.307	0.018
	Eicosapentaenoic Acid(EPA)	3.45	1.258	0.025
	2E-Eicosenoic acid	3.94	1.273	0.030
	Cholic acid	6.62	0.084	0.037
	Diacylglycerol	3.14	1.790	0.045
	Deoxycholic acid	2.53	0.428	0.047
Organic acids and derivatives	Urea	1.73	0.80	0.000
	Carnosine	1.13	0.46	0.000
	L-Norleucine	2.20	1.52	0.000
	Valine	1.15	1.31	0.001
	Indoxyl sulfate	3.17	0.53	0.003
	Sarcosine	1.26	1.74	0.004
	Pyroglutamic acid	2.01	0.75	0.012
	Gamma-Glutamylcysteine	5.92	0.16	0.016
	Pipecolic acid	4.34	1.35	0.018
	L-Malate	1.57	0.31	0.022
	Phenylacetylglycine	1.59	0.60	0.022
	DL-lactate	2.91	1.61	0.026
	Taurine	3.73	0.84	0.041

Analysis of difference metabolite in skin

Three hundred and ninety-two metabolite peaks were detected in the skin, among which 7 metabolites were significantly different (S2 Table). Both OPLS-DA analysis and Heatmap analyses demonstrated clustering and separation of Cap and Con(Fig. 2a, Fig. 2b). Seven-fold cross-validations R^2Y and Q^2 were 0.902 and 0.384. The 200-time permutation tests suggested no overfitting in OPLS-DA models(S4 Figure).

Metabolic pathways impacted by capsaicin

We conducted KEGG pathway enrichment analysis of differentially expressed metabolites by Fisher's exact test to identify detailed information on the key biological pathways relevant to capsaicin. As shown in Fig. 3a and Fig. 3, the results revealed that significant changes have occurred in some crucial pathways, such as Pyruvate metabolism, Central carbon metabolism in cancer, ABC transporters, and Sphingolipid metabolism.

Discussion

Capsaicin and the plasma of mice

Over the past decades, abundant evidence supporting the beneficial role of capsaicin in weight management[37–39]. In the present study, the body weight of Cap was significantly lower compared to Con at the end of the 6-week intervention($P < 0.05$). That means capsaicin slowed weight gain in mice, similar to the previous view that capsaicin has a weight-reducing effect. Capsaicin has been demonstrated to have anti-obesity properties by affecting energy and lipid metabolism[40]. In our study, there are indeed some differential metabolites and metabolic pathways in plasma associated with energy metabolisms, such as DL-lactate(D-lactate and L-lactate), L-Malate, ABC transporters, and Pyruvate metabolism. Both D-lactate and L-lactate can contribute to energy metabolism[41]; L-Malate is an intermediate metabolite of three shuttle acid cycle, directly involved in mitochondrial energy metabolism[42]; In addition, DL-lactate and L-malate are involved in the pathway of Pyruvate metabolism, which plays a pivotal role in cellular bioenergetics and energy production[43, 44]. Previous studies have reported that ABC transporters play an important role in the development of energy metabolism[45]. In short, these changes suggest that the energy metabolism of mice may be altered following capsaicin intake, with consequent effects on their body weight. Similarly, 11 lipids and lipid-like molecules account for a large proportion of differential plasma metabolites, also suggesting altered lipid metabolism in Cap compared with Con. Dysregulated lipid metabolism has been reported to affect body fat mass, adiposity, fatty acid metabolism, and basal metabolic rate[46]. Combined with the results of our study where Cap's weight was lower, we speculate that capsaicin may also inhibit weight gain in mice by modulating changes in lipid metabolism. Furthermore, among the lipids and lipid-like molecules, elevated Diacylglycerol may play an important physiological role which has been demonstrated to have the potential in decreasing body weight gain and body fat accumulation[47].

Capsaicin has been experimentally shown to control cellular oxidative stress due to its antioxidant properties[48–50]. In the current study, many differential metabolites associated with oxidative stress are also present in plasma, such as Cholic acid, Deoxycholic acid, Pipecolic acid, Carnosine, Taurine, Pyroglutamic acid, Gamma-Glutamylcysteines, and 3-Indolepropionic acid. Pipecolic acid, a metabolite of lysine, is known to induce oxidative stress in vitro in the cerebral cortex of experimental rats[51]. Carnosine and Taurine have antioxidant activities[52–54], whose deficiencies might not be conducive to the recovery of oxidative stress damage in the disease. Gamma-glutamylcysteine, Pyroglutamic acid and taurine also play a role in regulating oxidative stress due to their connection with glutathione[55–58]. Our results suggest that capsaicin can exert some physiological effects by modulating oxidative stress through changes in these metabolites. However, among these metabolites, there are both those that cause and those that reduce oxidative stress; it is difficult to determine whether capsaicin attenuated or enhanced oxidative stress overall; in any case, oxidative stress was altered in mice after capsaicin gavage.

The anticancer activity of capsaicin has been extensively studied for a variety of cancer types[59]. In contrast, there is conflicting evidence that capsaicin may also serve as carcinogenic or co-carcinogenic[22], thus, capsaicin may play a role in either preventing or causing cancer. In the present study, in addition to the alteration of energy metabolism, lipid metabolism, and oxidative stress impacted by capsaicin, KEGG pathway enrichment analysis also showed that metabolic pathways associated with cancer were significantly enriched in the plasma of Cap, such as Central carbon metabolism in cancer, Choline metabolism in cancer and Renal cell carcinoma. The central carbon metabolism, namely energy metabolism, is the main source of energy for living organisms. Due to the increased demand for cellular biomass in cancer, Central carbon metabolism has been studied for years for monitoring cancer progression and therapy response[60]. The imbalance in the regulation of carbon metabolism will lead to carcinogenesis[61]. The tumor-associated choline metabolism plays a key role in cell malignant transformation, tumor migration and metastasis[62, 63]. Previous study has reported that treatment of capsaicin reduced proliferation of renal carcinoma cells[64]. In short, our results suggest that capsaicin may have an effect on cancer through changes in these pathways. Furthermore, the cAMP signaling pathway and HIF-1 signaling pathway were also enriched in the plasma of Cap. The cAMP signaling pathway is one of the oldest signaling able to regulate substantial biological behaviors containing cellular growth and differentiation, gene expression and neuronal function[65]. It is now recognized that aberrant activation of cAMP signaling may activate multiple effector proteins at distinct intracellular regions and then contribute to tumorigenesis[66]. HIF-1 is an important signaling pathway that modulates cancer dormancy and metabolism, increases stemness activity, and leads to cancer initiation and progression[67]. All these results demonstrated the metabolic pathways associated with cancer might be disturbed in the mice after being gavaged with capsaicin. However, the specific effects of capsaicin on cancer still need to be further explored.

Capsaicin has also been found to play a crucial part in controlling anxiety and other emotional responses[68]. Studies have also found that oral administration of CAP alleviated depressive behavior in mice[69]. However, its mechanism of antidepressant effects is not yet fully understood. In our study, the

elevation of the differential metabolite Sarcosine in plasma following oral gavage of capsaicin in mice may have a role in the alleviation of depression. Sarcosine, an N-methyl-d-aspartate receptor enhancer, can improve depression-like behavior in rodent models and depression in humans[70]. Our results, therefore, suggest that the antidepressant effect of capsaicin may be related to Sarcosine.

Capsaicin and the skin of mice

There were also some differences in metabolites in the skin of Cap and Con, although the differential results were less. Including the rise of Sphingosine, Hypoxanthine, Creatinine, Thioetheramide-PC and the decline of Erucamide, N-Docosanoyl-4-sphingenyl-1-O-phosphorylcholine, Glycerol monolinoleate. KEGG pathway enrichment analysis shows Sphingolipid metabolism, Sphingolipid signaling pathway, Apoptosis, and Necroptosis significantly enriched in Cap. Sphingosine, one of the metabolites of sphingolipids[71], is involved in these four enriched pathways. Sphingosine with one fatty acid attached is called ceramide[72]. Ceramides based on phytosphingosine, Sphingosine and dihydrosphingosine are essential constituents of the skin lipid barrier that protects the body from excessive water loss[73]. The rise in Sphingosine in the skin of Cap suggests that capsaicin may have an effect on skin lipid barrier function in mice; Of course, this requires further research to confirm. Moreover, Sphingolipids and their metabolites are also involved in signal transduction, such as cell growth, differentiation, senescence, and programmed cell death[74]. In short, capsaicin might affect skin physiological functions in mice through changes in these metabolites and metabolic pathways.

Limitations of the study

This study had some potential limitations, including a small sample and a lack of targeted metabolomics studies. The physiological effects of chili peppers are a complex process that requires further research and exploration.

Conclusion

In summary, there were apparent systemic metabolomics changes in mice after oral gavage with capsaicin, mainly including energy and lipids metabolism, oxidative stress-related metabolism. The main metabolic pathways affected by capsaicin are Pyruvate metabolism, Central carbon metabolism in cancer, ABC transporters, and Sphingolipid metabolism. These findings provide metabolomic insights to assess the physiological functions of capsaicin and contribute a better understanding of the potential effects of a capsaicin-rich diet on organisms.

Abbreviations

TRPV1

Transient receptor potential vanilloid subfamily member 1

UHPLC/Q-TOF MS

Ultra-high-performance liquid chromatography and Q-TOF mass spectrometry

NH4AC

Ammonium acetate
NH₄OH
Ammonium hydroxide
QC
Quality control
PCA
Pareto-scaled principal component analysis
OPLS-DA
Orthogonal partial least-squares discriminant analysis
VIP
Variable importance in the projection
KEGG
Kyoto encyclopedia of genes and genomes.

Declarations

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Authors' contributions

CML, ZX, and SMY designed the study; CML, ZX, SMY and XLOY performed the experiments; CML, SMY, ZX, XPW, and DZ did sample analysis and data analysis; ZX, SMY, and YWZ wrote and prepared and edited the manuscript; CML revised the paper. ZX and SMY contributed equally to this work. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All animal experiments were performed according to the regulation of institutional guidelines for the care and approved by the Research Ethical Committee of The Second Affiliated Hospital of Nanchang University.

Consent for publication

Not applicable.

Competing interest

The authors declare no competing interests.

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Figures

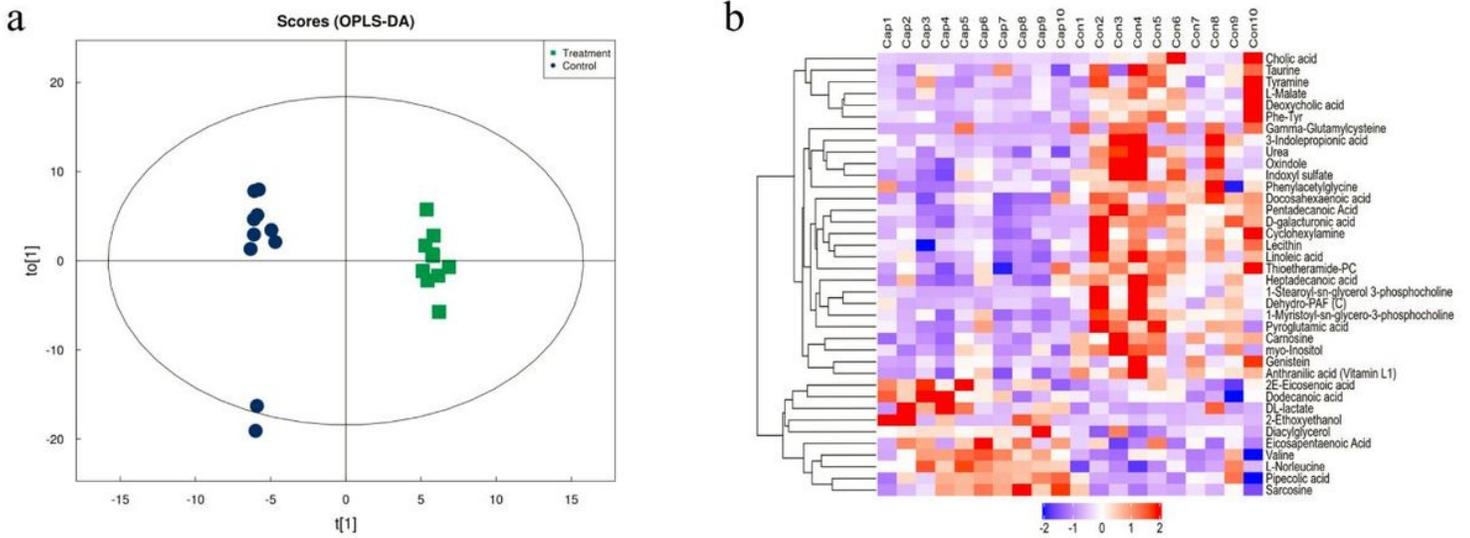


Figure 1

a The OPLS-DA score of each plasma sample. **b** Heatmaps of top 20 plasma differential metabolites.

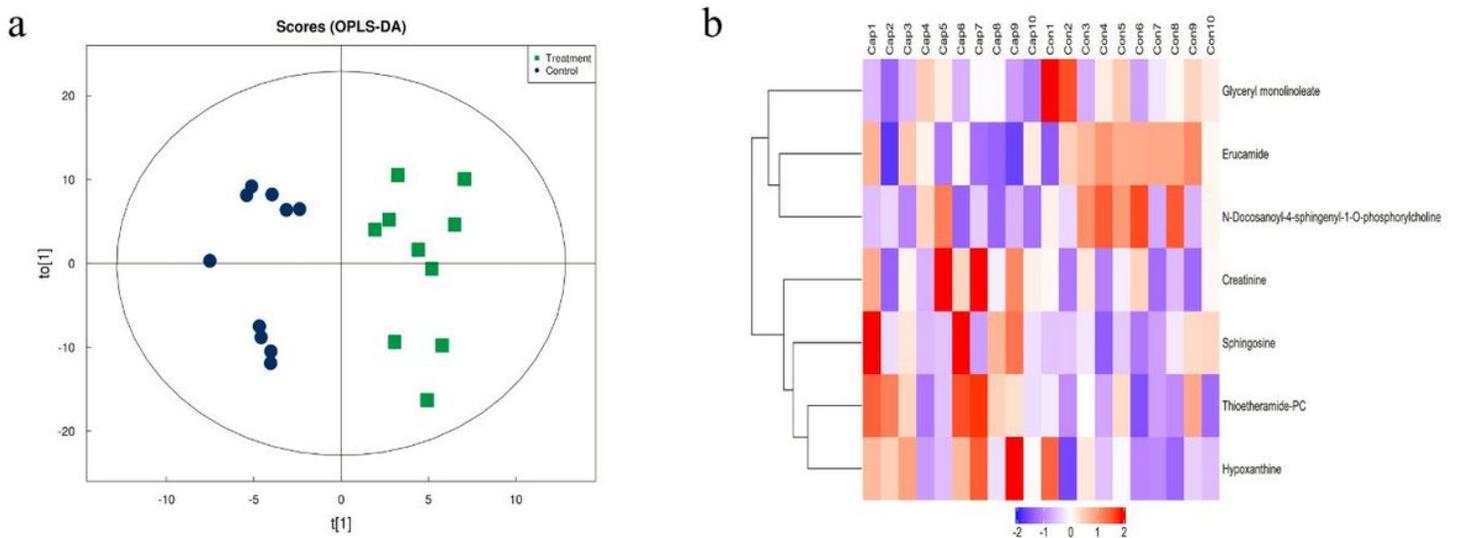


Figure 2

a The OPLS-DA score of each skin sample. **b** Heatmaps of skin differential metabolites.

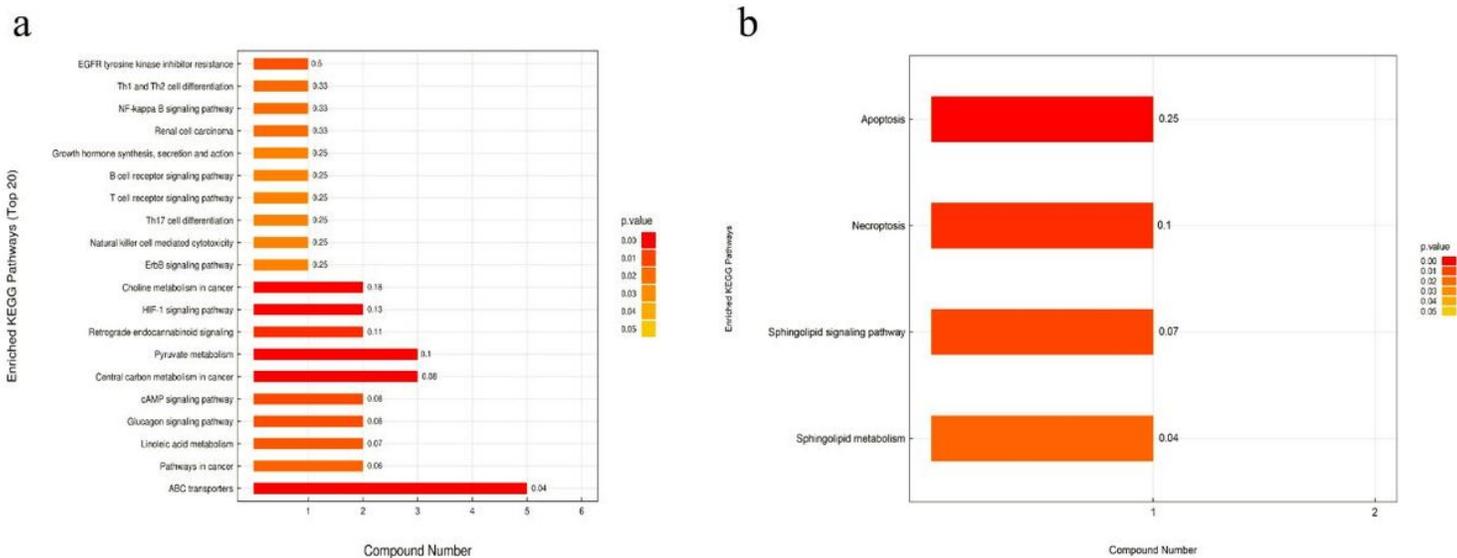


Figure 3

a KEGG pathway enrichment analysis of top 20 plasma differential metabolites. **b** KEGG pathway enrichment analysis of skin differential metabolites.

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