

Clinical Efficacy of Acupuncture for Knee Osteoarthritis and Associated Changes in Serum Lipidomics: A Prospective Observational Cohort Study

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Purpose: Knee osteoarthritis (KOA) is a chronic degenerative joint disease causing pain, stiffness, and dysfunction. Acupuncture is widely used in the management of KOA, but its metabolic mechanisms remain unclear. In this study, we evaluated the clinical efficacy of acupuncture in KOA and explored its potential mechanisms via serum lipidomics.

Patients and Methods: Fifty-eight KOA patients received standardized acupuncture at Dubi (ST35), Neixiyan (EX-LE4), Zusanli (ST36), Yanglingquan (GB34), Xuehai (SP10), Liangqiu (ST34), contralateral Quchi (LI11), Taixi (KI3), and Sanyinjiao (SP6), six sessions per week for four consecutive weeks; 22 healthy subjects served as controls. We assessed clinical efficacy using WOMAC and VAS scores, analyzed serum lipid profiles before and after treatment with LC-MS/MS and performed KEGG pathway enrichment.

Results: After treatment, WOMAC and VAS scores significantly decreased ($P < 0.05$). Lipidomics identified 538 differential metabolites between KOA patients and controls, primarily involved *autophagy* and *glycerolipid metabolism*. 218 metabolites changed after acupuncture, including PE (20:1_18:1), LPC (16:0/0:0), and LPE (0:0/18:0), related to *neuroactive ligand-receptor interaction* and *glycerophospholipid metabolism*. 68 lipids showed reversed trends post-treatment.

Conclusion: Acupuncture significantly improved pain and function in KOA and modulated serum lipid metabolism. Regulation of phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and triglycerides (TG), together with enrichment of related pathways, suggests that acupuncture restores lipid homeostasis and alleviates inflammation, supporting its metabolic therapeutic role in KOA.

Keywords: knee osteoarthritis, acupuncture, lipid metabolism, lipidomics

Introduction

Knee osteoarthritis (KOA) is one of the most common chronic degenerative joint diseases, characterized pathologically by degenerative changes in the articular cartilage, narrowing of the joint space, synovial inflammatory hyperplasia, and osteophyte formation at the joint margins.¹ Epidemiological studies have shown that more than 360 million people worldwide are affected by KOA, and its incidence increases markedly with advancing age.² For a long time, KOA was primarily considered a consequence of mechanical wear and tear of the articular cartilage. However, recent studies have increasingly revealed that inflammatory responses, immune dysregulation, and metabolic imbalances play crucial roles in

the onset and progression of the disease. This emerging understanding has shifted the focus from a purely structural injury model to a systemic pathological mechanism, in which alterations in lipid metabolism and related inflammatory processes have become prominent research hotspots.^{3,4}

Among these mechanisms, abnormalities in lipid metabolism have attracted increasing attention.^{5,6} Lipids are not only fundamental components of energy storage and cell membranes but also act as signaling molecules that regulate inflammation, oxidative stress, mitochondrial autophagy, and apoptosis, playing a vital role in maintaining joint homeostasis.^{7,8} Metabolomic studies have revealed that disruptions in various lipids, such as phospholipids (PL), sphingolipids (SL), and triglycerides (TG), are closely associated with cartilage degeneration, inflammatory responses, and pain. These lipids can serve as potential biomarkers for the early detection of osteoarthritis.⁹ Elevated levels of TG and total cholesterol (TC) in serum can accelerate cartilage loss,¹⁰ while dynamic alterations in different lipid subclasses may reflect the severity and phenotypic characteristics of the disease.^{11,12} Therefore, exploring the pathological mechanisms of KOA from the perspective of lipid metabolism holds significant research value.

As a safe and reproducible non-pharmacological therapy, acupuncture has been demonstrated in multiple high-quality randomized controlled trials to effectively relieve pain, improve knee joint function, and cause no serious adverse effects in patients with KOA.^{13–15} Related physical therapy modalities have also shown significant analgesic effects. For example, a study demonstrated that applying low-level laser therapy to acupuncture points significantly reduced pain and improved function in patients with knee osteoarthritis.¹⁶ Furthermore, extracorporeal shock wave and pulse electromagnetic field therapies have proven effective in alleviating pain associated with musculoskeletal disorders.¹⁷ Notably, compared to these physical therapies, the effects of acupuncture are not limited to local analgesia and anti-inflammatory actions, but may also involve systemic metabolic regulation, particularly through the modulation of lipid metabolic pathways.¹⁸ An animal study demonstrated that acupuncture can ameliorate lipid dysregulation and attenuate degenerative cartilage damage.¹⁹ However, whether and how acupuncture improves clinical symptoms in KOA patients through the modulation of lipid metabolism remains insufficiently understood, and mechanistic evidence is still limited.

In this study, a lipidomics approach was employed in combination with clinical efficacy assessments using the WOMAC and VAS scores to systematically investigate the effects of acupuncture on serum lipid metabolism in patients with KOA and its potential underlying mechanisms. The aim was to elucidate the relationship between acupuncture intervention and metabolic pathways, thereby providing new evidence-based insights for the treatment of KOA.

Materials and Methods

Clinical Data

General Information

The study was approved by the Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine (Jiangsu Provincial Hospital of Traditional Chinese Medicine) (Approval No. 2025NL-166-02) and registered in the US Clinical Trials Registry (Registration No. NCT06675981). A total of 58 patients with KOA were recruited from the Affiliated Hospital of Nanjing University of Chinese Medicine. In addition, 22 healthy subjects were enrolled as a normal control group.

Diagnostic Criteria

The diagnostic criteria for knee osteoarthritis were based on the *Guidelines for the Diagnosis and Treatment of Osteoarthritis* (2018 edition)²⁰ issued by the Orthopedic Branch of the Chinese Medical Association: (1) Recurrent knee pain within the past month; (2) Radiographic findings (standing or weight-bearing position) showing joint space narrowing, subchondral bone sclerosis or cystic changes, and osteophyte formation at the joint margins; (3) Middle-aged or elderly patients (≥ 50 years old); (4) Morning stiffness lasting ≤ 30 minutes; (5) Presence of crepitus during joint movement. A diagnosis of knee osteoarthritis can be established when criterion (1) is met in combination with any two of criteria (2), (3), (4), or (5).

Inclusion, Exclusion and Withdrawal Criteria

Inclusion, exclusion, and withdrawal criteria are summarized in [Table 1](#).

Table 1 Inclusion, Exclusion, and Withdrawal Criteria

Inclusion Criteria	
KOA Patients:	
(1)	Meeting the diagnostic criteria for knee osteoarthritis, with either unilateral or bilateral involvement;
(2)	Aged between 40 and 80 years;
(3)	Willing to participate in the study and have signed informed consent.
Healthy subjects:	
(1)	In good general health, with no history of knee osteoarthritis;
(2)	Aged between 40 and 80 years;
(3)	Willing to participate in the study and have signed informed consent.
Exclusion Criteria	
(1)	Patients with other bone diseases such as tuberculous arthritis of the knee, bone tumors, rheumatoid arthritis, or other types of arthritis beyond primary osteoarthritis;
(2)	Patients with sprains, contusions, or other injuries of the lower limb joints;
(3)	Patients with foot deformities or pain, local knee trauma, or other lesions affecting normal gait;
(4)	Patients with mental disorders or cognitive impairments who are unable to complete questionnaire assessments;
(5)	Patients with severe cardiovascular diseases, hepatic or renal dysfunction, immunodeficiency, diabetes, gout, hematologic disorders, or dermatologic diseases;
(6)	Pregnant or lactating women, or women planning to become pregnant within the next six months;
(7)	Patients currently receiving topical plaster therapy or physiotherapy for knee osteoarthritis;
(8)	Patients who have undergone intra-articular injection of corticosteroids or viscosupplementation therapy (eg sodium hyaluronate) within the past six months, or those who have had knee joint replacement surgery;
(9)	Patients with advanced clinical-stage knee osteoarthritis or radiographic grade IV disease, accompanied by severe joint deformity or extensive cartilage destruction;
(10)	Patients with knee joint swelling or a positive patellar floating (ballottement) test;
(11)	Patients currently participating in other clinical trials.
Withdrawal Criteria	
(1)	If a serious adverse event occurs and the investigator determines that the study should be discontinued, the case will be withdrawn from the clinical trial and treated as an invalid case;
(2)	If a participant chooses to discontinue during the study, they may request withdrawal, and the case will be excluded from the clinical trial.

Methodology

Treatment Method

Acupoint selection: Dubi (ST35), Neixiyan (EX-LE4), Zusanli (ST36), Yanglingquan (GB34), Xuehai (SP10), Liangqiu (ST34), Quchi (LI11, contralateral), Taixi (KI3), and Sanyinjiao (SP6).

Procedure: Participants were placed in a supine position, and routine skin disinfection was performed with 75% alcohol. Sterile disposable acupuncture needles (0.25 mm × 40 mm or 0.30 mm × 40 mm) were inserted perpendicularly at each acupoint to a depth of 0.5–1.2 cun, adjusted according to the anatomical characteristics of each point. The lifting–thrusting and twirling manipulation was then applied (lifting–thrusting amplitude: 3–5 fen; frequency: 120–160 times/min; bidirectional rotation angle: >180°; frequency: 120–160 times/min) until the arrival of “Deqi” sensation was achieved.

Needles were retained for 30 minutes, during which manual stimulation was performed every 15 minutes (two times in total) to maintain the “Deqi” sensation. Acupuncture treatment was administered six times per week for four consecutive weeks, totaling 24 sessions. All acupuncture procedures were performed by licensed attending physicians qualified in Traditional Chinese Medicine.

During the study, participants were prohibited from using other treatment methods. Considering medical ethics, if the patient’s condition reaches an intolerable level, the use of non-steroidal anti-inflammatory analgesics (NSAIDs) is allowed.

Observation Indicators

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to assess clinical efficacy. The scale includes five items for pain, two items for stiffness, and seventeen items for physical function, evaluating the degree of difficulty in performing daily activities. The WOMAC scoring ranges are as follows: pain (0–20 points), stiffness (0–8 points), and physical function (0–68 points), with a total possible score of 0–96 points. Higher scores indicate poorer knee joint function and greater impact on daily life. The WOMAC scores of patients in the acupuncture group were evaluated before and after treatment.²¹

Visual Analogue Scale (VAS) was used to evaluate the subjective intensity of pain, with scores interpreted as follows: 0–2, comfortable; 2–4, mild discomfort; 4–6, moderate discomfort; 6–8, severe discomfort; and 8–10, extreme discomfort. For patients with bilateral KOA, the VAS score was recorded based on the more painful side. Assessments were performed before and after acupuncture treatment.²²

Lipid Metabolites: for all participants, fasting venous blood samples (2 mL) were collected at 8:00 a.m. on the day of enrollment. Serum was separated and stored at –80 °C for later analysis. In the acupuncture group, an additional blood sample was collected after two weeks of treatment.

If the patient takes non-steroidal anti-inflammatory analgesics, all treatments received by the patient during the study, including the treatment name or method, dosage, and duration, must be recorded in detail. Efficacy evaluation and observation will be conducted by the research assistant. The patient must suspend the use of any medication for treating knee osteoarthritis for 48 hours prior to the efficacy evaluation.

Lipidomics Analysis

Frozen serum samples were thawed at 4 °C and vortexed for 10s to mix thoroughly. A 50 µL aliquot of each sample was transferred into an Eppendorf tube, followed by the addition of 1 mL of extraction solvent containing internal standards (methyl tert-butyl ether: methanol = 3:1, V/V). The mixture was vortexed for 15 min, then 200 µL of water was added and vortexed for 1 min. Samples were centrifuged at 12,000 rpm for 10 min, and 200 µL of the supernatant was transferred to a new tube. After drying under vacuum, residues were reconstituted with 200 µL of solvent (acetonitrile: isopropanol = 1:1, V/V), vortexed for 3 min, and centrifuged at 12,000 rpm for 3 min. The supernatant was then collected for liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis.

The data acquisition was performed using Ultra-Performance Liquid Chromatography (UPLC)(ExionLC AD, Sciex LLC, Framingham, MA, USA) and MS/MS (QTRAP®, Sciex LLC). Chromatographic separation was achieved on a Thermo Accucore™ C30 column (2.1 × 100 mm, 2.6 µm). The mobile phases were: A: acetonitrile/water (60/40, V/V) with 0.1% formic acid and 10 mmol/L ammonium formate (NH₄HCOO); B: acetonitrile/isopropanol (10/90, V/V) with 0.1% formic acid and 10 mmol/L ammonium formate. The gradient elution program was as follows: 0 min of A/B (80:20, V/V), 2 min of A/B (70:30, V/V), 4 min of A/B (40:60, V/V), 9min of A/B (15:85, V/V), 14 min of A/B (10:90, V/V), 15.5 min of A/B (5:95, V/V), 17.3 min of A/B (5:95, V/V), 17.5 min of A/B (80:20, V/V), 20 min of A/B (80:20, V/V). The flow rate was 0.35 mL/min, the temperature was 45 °C, and the injection volume was 2 µL.

The eluate was introduced into a triple quadrupole linear ion trap mass spectrometer (QTRAP-MS) equipped with an electrospray ionization (ESI) source. The MS parameters were as follows: ion source temperature, 500 °C; ion spray voltage, 5500 V (positive mode) and –4500 V (negative mode); curtain gas (CUR) 35 psi; ion source gas 1 (GS1) 45 psi; gas 2 (GS2) 55 psi; and collision-activated dissociation (CAD) set to medium. In the triple quadrupole system, ion pairs were scanned and detected according to their declustering potential (DP) and collision energy (CE).

Lipid qualitative analysis was performed using the self-built Metware database (MWDB) based on the retention time (RT) and parent–daughter ion pair information. Lipid quantitative analysis was conducted in the multiple reaction monitoring (MRM) mode of a triple quadrupole mass spectrometer.

Statistical Analysis

The data were analyzed using SPSS version 27.0. Measurement data conforming to a normal distribution were expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). The paired-sample t-tests were used for within-group comparisons, and the independent-sample t-tests for between-group comparisons. Non-normally distributed data were expressed as median (interquartile range) [M (P₂₅, P₇₅)], with within-group comparisons performed using the Wilcoxon signed-rank test. The categorical data were expressed as frequencies, and between-group comparisons were analyzed using the χ^2 -test. $P < 0.05$ was considered statistically significant.

Serum lipid metabolite data were processed using Analyst 1.6.3 software. Orthogonal partial least squares-discriminant analysis (OPLS-DA) and model construction were performed using the MetaboAnalyst package in R. Variable Importance in Projection (VIP) scores and P values were calculated, and differential metabolites were identified based on VIP > 1 and $P < 0.05$ (Student's *t*-test). Volcano plots were generated using the EnhancedVolcano package, heatmaps were created with the pheatmap package, Spearman correlation analysis was performed using the psych package, and network diagrams were visualized with Gephi. Identified metabolites were annotated for metabolic pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) compound database (<http://www.kegg.jp/kegg/compound/>).

Results

General Characteristics Comparison Between Groups

This study included 58 patients with KOA and 22 age- and sex-matched healthy controls. No significant differences were observed between the two groups in terms of sex ratio, age, body mass index (BMI), or total cholesterol levels. Compared with the healthy control group, the KOA group exhibited higher levels of white blood cell count (WBC), triglycerides, and low-density lipoprotein (LDL), and lower levels of high-density lipoprotein (HDL), as shown in Table 2.

Comparison of VAS and WOMAC Scores Before and After Treatment

In the KOA group, VAS score decreased from 7.00 (6.00, 7.00) before treatment to 5.00 (3.00, 5.00) after treatment ($P < 0.05$), as shown in Table 3. WOMAC score decreased significantly from 42.09 ± 4.62 before treatment to 28.00 ± 5.57 after treatment ($P < 0.01$). Moreover, scores across all WOMAC subscales were significantly lower after treatment compared with baseline ($P < 0.05$), as detailed in Table 4.

Table 2 Comparison of General Characteristics Between Patients with KOA and Healthy Controls

Variable	KOA (n=58)	Healthy Controls (n=22)	P-value
Male/Female (n)	24/34	8/14	0.683
Age(years)	62 $\pm$ 11	61 $\pm$ 10	0.862
BMI (kg/m ²)	24.03 $\pm$ 1.31	23.45 $\pm$ 1.20	0.073
WBC (10 ⁹ /L)	6.04 $\pm$ 0.50	5.54 $\pm$ 0.34	0.001
Total cholesterol (mmol/L)	4.59 $\pm$ 0.32	4.45 $\pm$ 0.25	0.064
Triglycerides (mmol/L)	1.52 $\pm$ 0.21	1.39 $\pm$ 0.12	0.006
LDL-cholesterol (mmol/L)	2.51 $\pm$ 0.23	2.31 $\pm$ 0.15	0.001
HDL-cholesterol (mmol/L)	1.22 $\pm$ 0.11	1.40 $\pm$ 0.11	0.001

Abbreviations: BMI, body mass index; WBC, white blood cell count; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 3 Comparison of VAS Scores Before and After Treatment in Patients with KOA

Parameter	KOA (n=58)	
	Before Treatment	After Treatment
VAS	7.00 (6.00, 7.00)	5.00 (3.00, 5.00) **

Notes: compared with before treatment, $P < 0.01$ is denoted by "**".

Abbreviation: VAS, Visual Analogue Scale.

Table 4 Comparison of WOMAC Scores Before and After Treatment in Patients with KOA

Parameter	KOA (n=58)	
	Before Treatment	After Treatment
WOMAC (total score)	42.09±4.62	28.00±5.57 **
Pain	9.00 (8.00, 10.00)	6.00 (4.00, 6.00) *
Stiffness	3.00 (2.00, 3.00)	2.00 (1.00, 2.00) *
Physical function	30.62±3.52	21.00±4.18 *

Notes: compared with before treatment, $P < 0.05$ is denoted by "*" and $P < 0.01$ by "**".

Abbreviation: WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Lipid Metabolism Comparison: Patients Before and After Treatment vs Healthy Controls

Compared with healthy controls, the serum lipid profiles of KOA patients showed distinct differences in multivariate analysis. The OPLS-DA model effectively distinguished between the two groups and demonstrated satisfactory explanatory and predictive performance (Figure 1A). By combining univariate and multivariate analyses, differential lipids were identified based on the criteria of $VIP > 1$ and $P < 0.05$. A total of 538 differential lipid metabolites were detected between the two groups, of which 521 were upregulated and 17 were downregulated (Figure 1B). Among these, metabolites such as PC (12:2/22:4), PE (20:1_18:1), PE (P-22:1_20:4), and HexCer (t15:0/16:2(2OH)) were markedly upregulated (Figure 1C). Furthermore, KEGG pathway enrichment analysis revealed that these differential lipid metabolites were mainly involved in pathways such as *Pathogenic Escherichia coli infection*, *Autophagy-animal*, *Glycerolipid metabolism*, and *Phosphatidylinositol signaling system* (Figure 1D).

Changes in Lipid Metabolism After Acupuncture Treatment in KOA Patients

OPLS-DA model constructed in this study indicated a certain degree of separation between the serum lipid profiles of KOA patients before and after acupuncture treatment. However, the Q^2 value of the model was close to zero, suggesting limited predictive ability (Figure 2A). Differential lipid metabolites were identified based on the criteria of $VIP > 1$ and $P < 0.05$. Compared with pre-treatment levels, seven metabolites were upregulated and 211 were downregulated after acupuncture treatment (Figure 2B). Among these, metabolites such as PE (20:1_18:1), LPC (0:0/16:0), LPE (0:0/18:0), and TG (16:0_16:0_18:0) were markedly downregulated (Figure 2C). KEGG pathway enrichment analysis of these differential lipid metabolites revealed that they were mainly involved in *glycerophospholipid metabolism* and *neuroactive ligand–receptor interaction pathways* (Figure 2D). These findings suggest that acupuncture intervention may exert its therapeutic effects by modulating these metabolic pathways.

Impact of Acupuncture on Lipid Metabolism

To explore the impact of acupuncture on lipid metabolism, we compared the differential lipid metabolites identified before and after acupuncture treatment with those differing between KOA patients (before treatment) and healthy controls (HC)

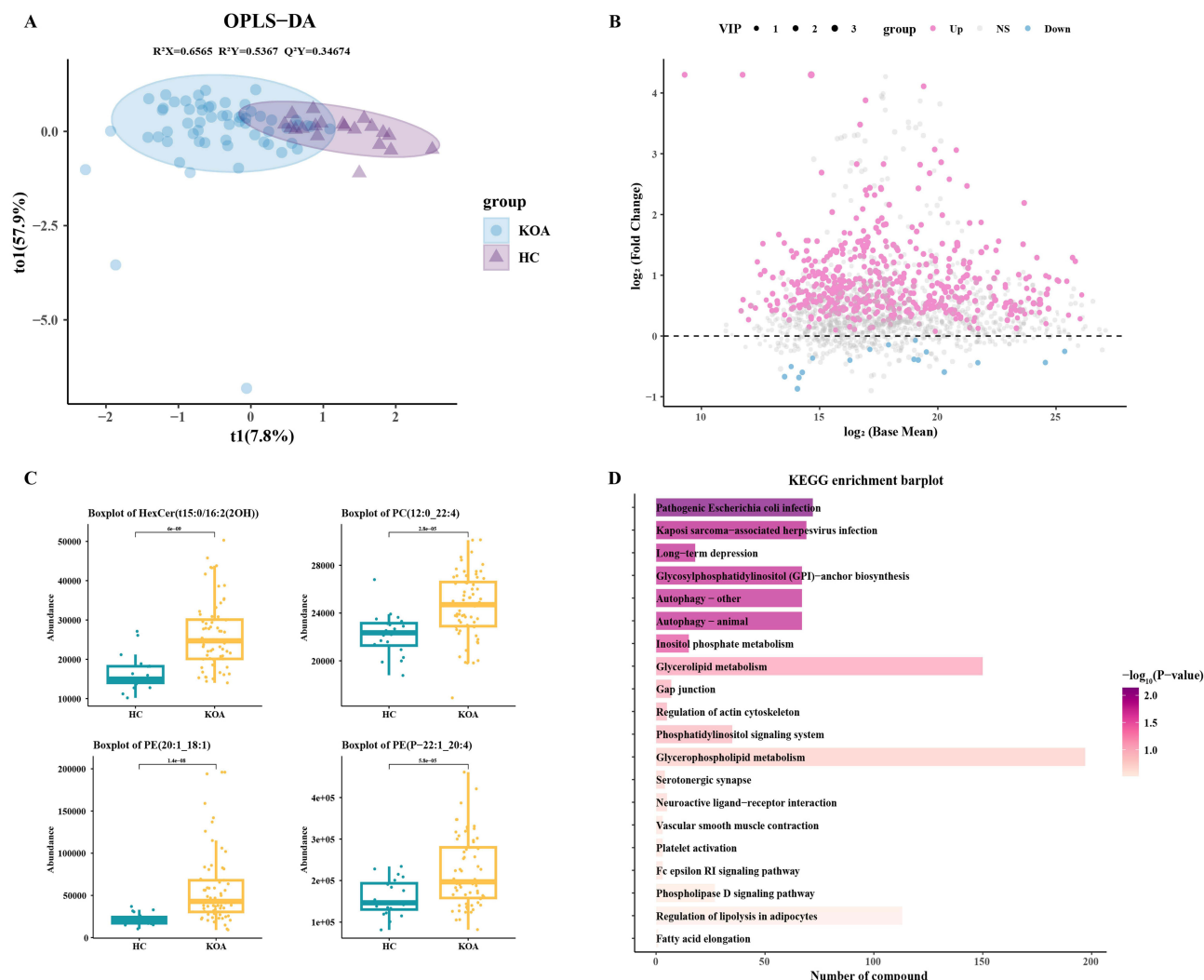


Figure 1 Comparison of Lipid Metabolites Between Patients with KOA Before Treatment and Healthy Controls (HC). **(A)** OPLS-DA model discriminates the serum lipid profiles between KOA patients and the HC group. Blue circles represent the KOA group, and purple triangles represent the HC group. The ellipse indicates the 95% confidence interval. Model parameters: $R^2X = 0.6565$, $R^2Y = 0.5367$, $Q^2 = 0.3467$. **(B)** Volcano plot illustrates the differential metabolites between the KOA and HC groups. Each dot represents a lipid metabolite: pink (Up) indicates upregulated lipids, blue (Down) indicates downregulated lipids, and gray (NS) represents metabolites without significant difference. The x-axis represents the logarithmic value of the relative abundance ($\log_2$ Base Mean) of lipids between groups, and the y-axis represents the logarithmic fold change ($\log_2$ Fold Change). The size of each dot corresponds to the variable importance in projection (VIP) score. **(C)** Box plots show the relative abundance of differential lipid metabolites between the KOA and HC groups. **(D)** KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis. The x-axis represents the number of significantly enriched compounds within each pathway, and the y-axis lists the pathway names. The color of each bar indicates the P-value of enrichment, with darker colors representing higher significance.

group. A total of 68 lipid metabolites showing opposite trends were identified (Figure 3). A heatmap was constructed to visualize the expression levels of these 68 metabolites, which were all decreased after acupuncture treatment, suggesting that the therapeutic effects of acupuncture may be mediated through the modulation of these lipid metabolites (Figure 4). Pearson correlation analysis was performed to examine the relationships among the identified lipid metabolites (Figure 5). The results revealed significant synergistic changes among several key lipids. Specifically, LPC (16:0/0:0) was positively correlated with LPA (0:0/16:0) ($r = 0.95$, $P < 0.01$) and LPG (18:1/0:0) ($r = 0.84$, $P < 0.01$), but negatively correlated with Carnitine C12:1-2OH ($r = -0.54$, $P < 0.01$) and PS (17:0_18:1) ($r = -0.17$, $P < 0.05$). Additionally, PS (17:0_18:1) was negatively correlated with LPA (0:0/22:6) ($r = -0.24$, $P < 0.01$). Spearman correlation analysis was also conducted, and lipid pairs with $|r| \geq 0.8$ and $P \leq 0.05$ were selected to construct a correlation network (Figure 6). Moreover, correlation analyses between these lipids and clinical indices (VAS and total as well as subscale WOMAC scores) were performed. The results showed that serum levels of

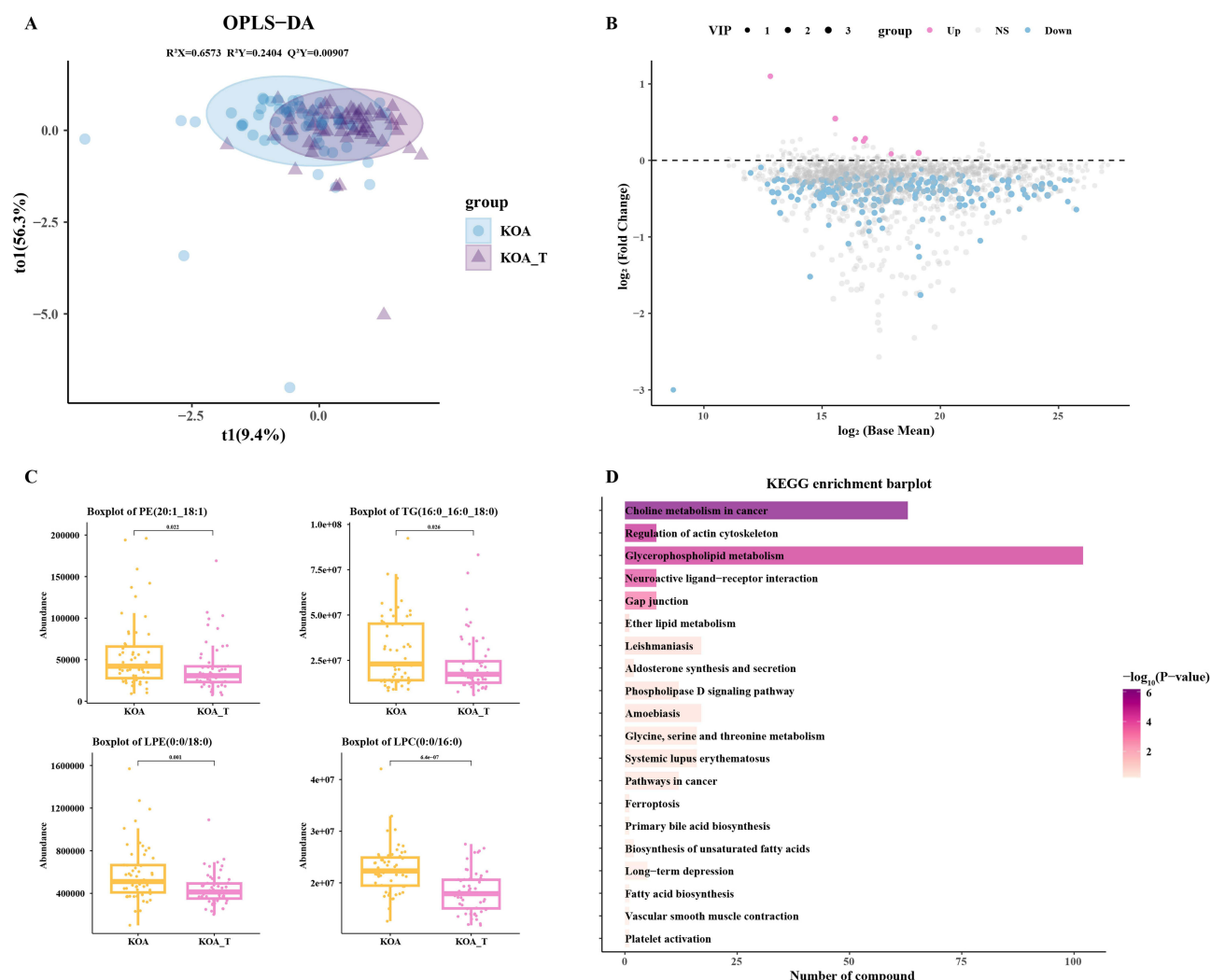


Figure 2 Comparison of Lipid Metabolites Before and After Acupuncture Treatment in Patients with KOA. (A) OPLS-DA model distinguishing the serum lipid profiles of KOA patients before and after acupuncture treatment. Blue circles represent pre-treatment samples (KOA group), and purple triangles represent post-treatment samples (KOA_T group). The ellipse indicates the 95% confidence interval. Model parameters: $R^2X = 0.6573$, $R^2Y = 0.2404$, and $Q^2 = 0.00907$. (B) Volcano plot showing differential lipid metabolites between the KOA and KOA_T groups. Each dot represents a lipid metabolite: pink (Up) indicates upregulated metabolites, blue (Down) indicates downregulated metabolites, and gray (NS) represents non-significant metabolites. The x-axis displays the $\log_2$ -transformed mean relative abundance ($\log_2$ Base Mean), while the y-axis displays the $\log_2$ fold change between the two groups. The size of each dot corresponds to the variable importance in projection (VIP) score. (C) Box plots comparing the relative abundance of differential lipid metabolites between the KOA and the KOA_T groups. (D) KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis. The x-axis represents the number of significantly enriched compounds in each pathway, and the y-axis lists the pathway names. The color gradient of the bars indicates the P value of enrichment, with darker colors representing greater statistical significance.

LPC (16:0/0:0), SPH (d18:0), and TG (16:0_16:0_18:0) were positively correlated with both VAS and WOMAC total scores ($r = 0.33$, $P < 0.01$; $r = 0.56$, $P < 0.01$; $r = 0.25$, $P < 0.05$; $r = 0.40$, $P < 0.01$; $r = 0.57$, $P < 0.01$; $r = 0.28$, $P < 0.05$, respectively). In contrast, Carnitine C12:1-2OH and PS (17:0_18:1) were negatively correlated with WOMAC scores ($r = -0.24$, $P < 0.01$; $r = -0.21$, $P < 0.05$), and Carnitine C12:1-2OH also showed a significant negative correlation with VAS scores ($r = -0.19$, $P < 0.05$) (Figure 7).

Discussion

In this prospective observational study, clinical efficacy evaluation combined with lipidomics analysis revealed that acupuncture significantly alleviated pain and improved joint function in patients with KOA, accompanied by notable alterations in serum lipid profiles. In particular, key lipid species such as PE, LPC, LPE, and TG were markedly downregulated after acupuncture treatment. Pathway enrichment analysis further indicated that acupuncture primarily

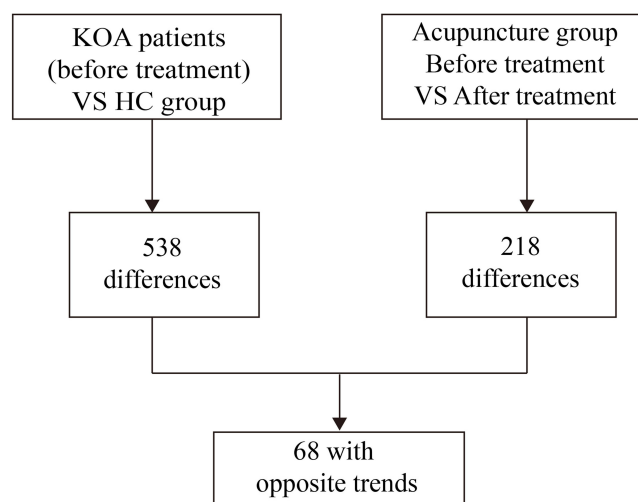


Figure 3 Flowchart of the screening process for lipid metabolites affected by acupuncture treatment in patients with KOA.

modulates *glycerophospholipid metabolism* and *neuroactive ligand–receptor interaction* pathways. These findings suggest that the therapeutic effects of acupuncture are closely associated with the regulation of lipid metabolism, providing metabolomic evidence to support its efficacy in the treatment of KOA.

This study revealed that abnormalities in *glycerophospholipid metabolism* play a critical role in the progression of KOA. Previous studies have demonstrated that, within the osteoarthritic microenvironment, phosphatidylcholine (PC) undergoes excessive conversion to LPC under the actions of lecithin–cholesterol acyltransferase (LCAT) and phospholipase A₂ (PLA₂). This process results in an increased LPC/PC ratio, which has been shown to positively correlate with the percentage of cartilage volume loss.²³ Therefore, the LPC/PC ratio may serve as a potential biomarker reflecting the degree of inflammation and metabolic imbalance in KOA.^{24,25}

LPC, a major phospholipid component of oxidized low-density lipoprotein, plays a pivotal role in inflammatory signaling. LPC can mediate the phospholipid hydrolysis process catalyzed by PLA₂, leading to the release of multiple pro-inflammatory mediators.²⁶ In addition, LPC activates the TLR2/4-NF-κB and MAPK signaling pathways, thereby promoting the secretion of inflammatory cytokines such as IL-1β and TNF-α. These events collectively exacerbate oxidative stress and chondrocyte apoptosis.^{27,28} Similarly, abnormally elevated levels of LPE can disrupt cell membrane stability and trigger inflammatory responses.^{29,30} Our results showed a significant decrease in LPC and LPE levels after acupuncture treatment, suggesting that acupuncture may alleviate inflammation and maintain cartilage homeostasis by modulating PLA₂-related pathways.

Lipid metabolism is closely associated with mitochondrial function, and its dysregulation is an important factor in cartilage degeneration in osteoarthritis.^{31,32} Imbalances in membrane phospholipids such as PC and PE can alter mitochondrial membrane fluidity and electron transport efficiency, thereby disrupting energy metabolism.^{33,34} Excessive lipid accumulation can further suppress the activity of electron transport chain (ETC) complexes, reduce the efficiency of oxidative phosphorylation (OXPHOS), and induce abnormal accumulation of mitochondrial reactive oxygen species (ROS).³⁵ Excessive ROS not only directly damage cellular membranes and proteins but also promote the expression of matrix metalloproteinases (MMPs), leading to extracellular matrix (ECM) degradation and enhanced chondrocyte apoptosis.^{36,37} This study observed a significant decrease in the levels of PE, LPC, and LPE following acupuncture intervention, suggesting that acupuncture may alleviate tissue damage and inflammatory responses by improving mitochondrial homeostasis. In addition, the upregulation of acylcarnitine (carnitine) levels was found to be negatively correlated with symptom scores, indicating that acupuncture may enhance mitochondrial fatty acid (FA) transport and β-oxidation (FAO), thereby improving energy metabolic homeostasis and promoting mitochondrial functional recovery.^{38,39} Experimental evidence has shown that reducing FA transport efficiency can decrease ROS levels, restore mitochondrial membrane potential and ATP production, thereby improving mitochondrial function in

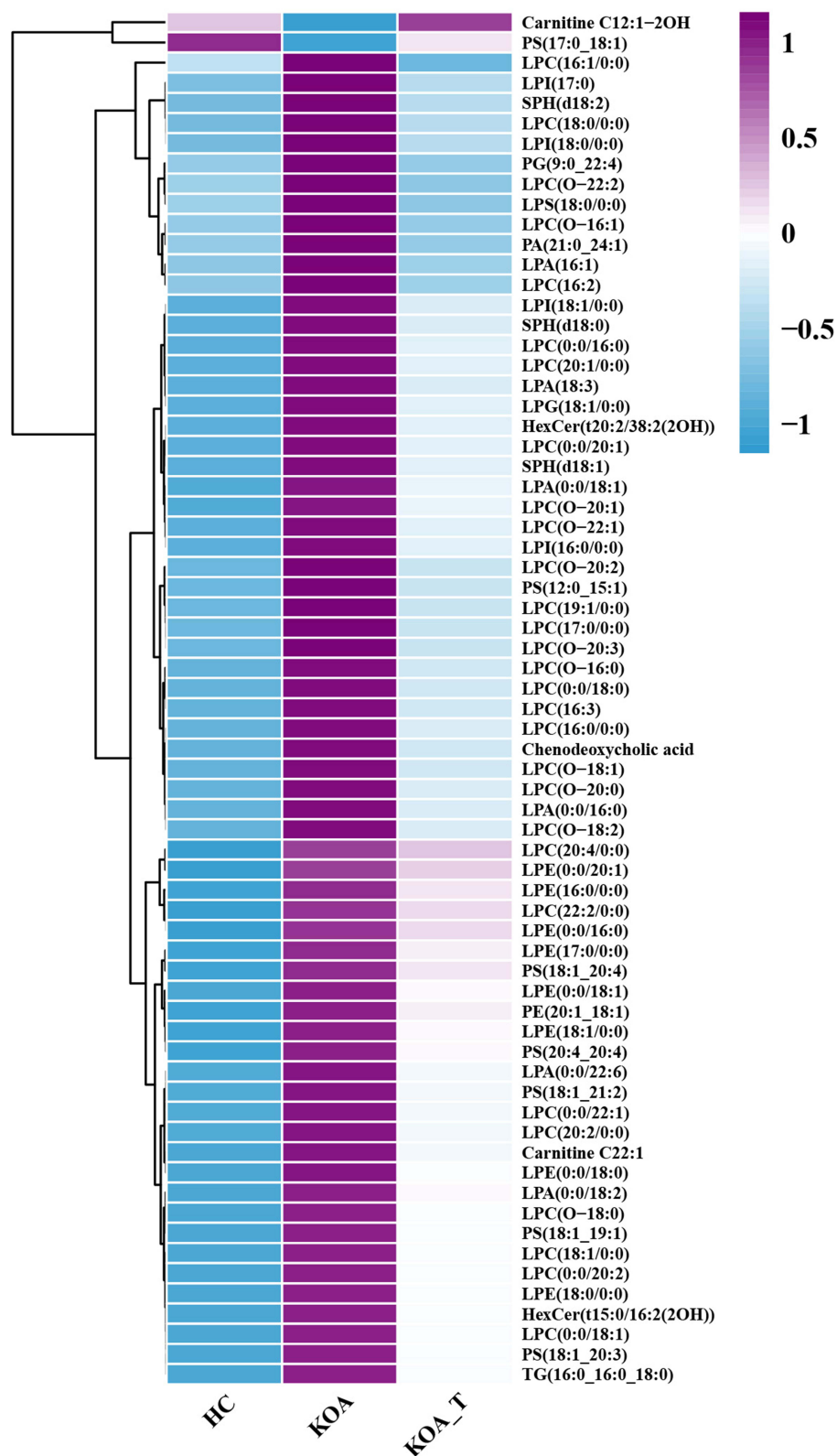


Figure 4 Hierarchical clustering heatmap of 68 differential lipid metabolites. Each column represents a group, and each row represents a lipid metabolite. The color scale indicates the relative abundance of each metabolite, with darker purple representing higher abundance levels.

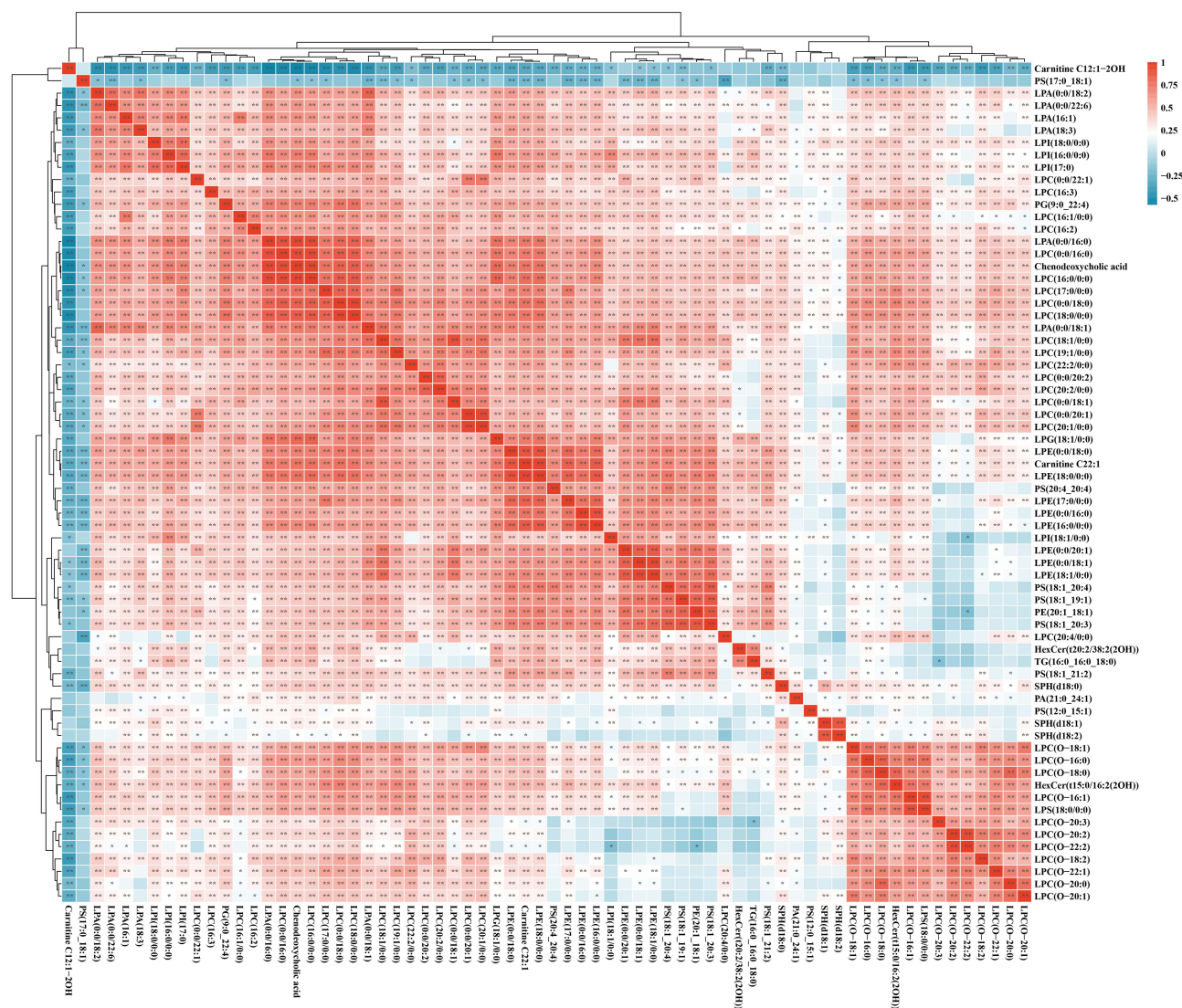
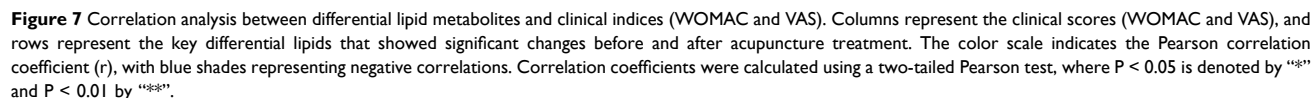
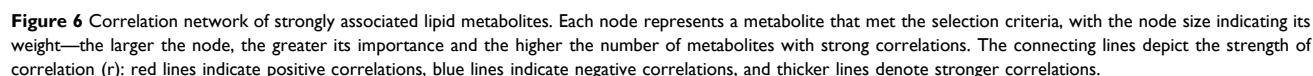


Figure 5 Correlation analysis of differential lipid metabolites. Significantly altered lipids were analyzed for pairwise correlations, with different colors representing the magnitude and direction of Pearson correlation coefficients. $P < 0.05$ is denoted by * and $P < 0.01$ by **.

chondrocytes under oxidative stress.³⁷ Previous studies have found that medium- and long-chain acylcarnitine levels are decreased in patients with OA and are closely associated with radiographic severity, suggesting that dysregulated FAO may accelerate disease progression.⁴⁰

In addition to phospholipids, abnormalities in TG and sphingolipids are also closely associated with the clinical symptoms of KOA. Elevated levels of TC and TG have been shown to increase the risk of bone marrow lesions (BMLs) and cartilage damage,⁴¹ whereas acupuncture has been reported to reduce serum TC and TG levels, thereby alleviating lipotoxicity and improving intra-articular microcirculation.¹⁹ Meanwhile, sphingosine (SPH) and its metabolite sphingosine-1-phosphate (S1P) can activate the S1P/S1P₂ signaling pathway, leading to upregulation of MMPs in chondrocytes and promoting inflammatory responses, whereas inhibition of this pathway can effectively attenuate cartilage degradation.^{42,43} The present study suggests that acupuncture may indirectly modulate inflammation-related signaling by downregulating SPH levels; however, the precise underlying mechanisms remain to be elucidated.

Notably, we observed that several lipid species exhibited consistent trends with clinical scores. The levels of LPC (16:0/0:0), SPH (d18:0), and TG (16:0_16:0_18:0) were significantly positively correlated with VAS and WOMAC scores, indicating that higher levels of these lipids are associated with greater pain intensity and functional impairment. In



This study explored the preliminary mechanisms of acupuncture in the treatment of KOA from both clinical and lipidomic perspectives; however, several limitations should be acknowledged. First, this study adopted a prospective observational design

aimed at exploring metabolic patterns and their dynamic changes rather than establishing direct causal relationships. Future randomized controlled trials are warranted to further validate the lipid metabolic characteristics revealed in this study. Second, although the sample size was relatively small, it was sufficient to perform an initial metabolic profiling analysis; expanding the cohort in future studies would enhance the reliability of the findings. Third, as this study was based on serum lipidomics data, it may not fully reflect the metabolic status within the synovial fluid or cartilage tissue. Integrating tissue-level lipidomic analyses with functional experiments in future work would provide a more comprehensive understanding of the molecular pathways involved. Overall, this study is the first to propose, from a metabolomic perspective, that acupuncture may alleviate KOA symptoms by modulating lipid metabolism, thereby offering new insights into the molecular mechanisms and clinical relevance of acupuncture therapy.

Conclusion

By integrating clinical efficacy evaluation with lipidomic analysis, this study demonstrated that acupuncture significantly alleviates pain and functional impairment in patients with KOA, accompanied by a marked downregulation of multiple serum lipid metabolites, including PE, LPC, LPE, and TG. KEGG pathway analysis indicated that these differential lipids are primarily enriched in key metabolic pathways such as *glycerophospholipid metabolism* and *neuroactive ligand–receptor interaction*. Dysregulated lipid metabolism is closely associated with inflammatory responses, cartilage degeneration, and impaired autophagy in KOA. Acupuncture may exert therapeutic effects by reducing pro-inflammatory lysophospholipids, correcting triglyceride overload, and inhibiting related signaling pathways, thereby mitigating inflammation and matrix degradation while improving the intra-articular microenvironment. Collectively, this study provides new metabolomic evidence and potential mechanistic insights into acupuncture therapy for KOA, highlighting lipid metabolism as a promising therapeutic target for future acupuncture-based interventions in osteoarthritis.

Abbreviations

ATX, autotaxin; BMI, body mass index; BMLs, bone marrow lesions; ECM, extracellular matrix; ETC, electron transport chain; FA, fatty acid; HC, Healthy Controls; HDL, high-density lipoprotein; KEGG, Kyoto Encyclopedia of Genes and Genomes; KOA, knee osteoarthritis; LCAT, lecithin–cholesterol acyltransferase; LDL, low-density lipoprotein; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPG, Lysophosphatidylglycerol; LPS, lysophosphatidylserine; MMPs, matrix metalloproteinases; OPLS-DA, Orthogonal partial least squares-discriminant analysis; OXPHOS, oxidative phosphorylation; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipids; PLA₂, phospholipase A₂; PS, phosphatidylserine; ROS, reactive oxygen species; SL, sphingolipids; SPH, sphingosine; TC, total cholesterol; TG, triglycerides; VAS, Visual Analogue Scale; WBC, white blood cell; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

Data Sharing Statement

The data used to support the findings of this study are available upon reasonable request from either of the two corresponding authors.

Ethics and Consent Statements

The study was registered with the US Clinical Trials Registry (Registration No. NCT06675981). It followed the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine (Jiangsu Provincial Hospital of Traditional Chinese Medicine) (Approval No. 2025NL-166-02). All the patients have been informed and signed informed consent before the experiments.

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Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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