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REVIEW ARTICLE

Metabolomics in Osteoarthritis Knee: A Systematic Review of Literature

Akhilesh Arjun[1,2] · Girinivasan Chellamuthu[3,4] · Naveen Jeyaraman[5] · Madhan Jeyaraman[4,5] · Manish Khanna[6]

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Abstract

Introduction Osteoarthritis (OA) is a common degenerative disorder of the synovial joints and is usually an age-related disease that occurs due to continuous wear and tear of the cartilage in the joints. Presently, there is no proven medical management to halt the progression of the disease in the early stages. The purpose of our systematic review is to analyze the possible metabolites and metabolic pathways that are specifically involved in OA pathogenesis and early treatment of the disease.

Materials and Methods The articles were collected from PubMed, Cochrane, Google Scholar, Embase, and Scopus databases. "Knee", "Osteoarthritis", "Proteomics", "Lipidomics", "Metabolomics", "Metabolic Methods", and metabolic* were employed for finding the articles. Only original articles with human or animal OA models with healthy controls were included.

Results From the initial screening, a total of 458 articles were identified from the 5 research databases. From these, 297 articles were selected in the end for screening, of which 53 papers were selected for full-text screening. Finally, 50 articles were taken for the review based on body fluid: 6 urine studies, 15 plasma studies, 16 synovial fluid studies, 11 serum studies, 4 joint tissue studies, and 1 fecal study. Many metabolites were found to be elevated in OA. Some of these metabolites can be used to stage the OA Three pathways that were found to be commonly involved are the TCA cycle, the glycolytic pathway, and the lipid metabolism.

Conclusion All these studies showed a vast array of metabolites and metabolic pathways associated with OA. Metabolites like lysophospholipids, phospholipids, arginine, BCCA, and histidine were identified as potential biomarkers of OA but a definite association was not identified, Three pathways (glycolytic pathway, TCA cycle, and lipid metabolic pathways) have been found as highly significant in OA pathogenesis. These metabolic pathways could provide novel therapeutic targets for the prevention and progression of the disease.

Keywords Osteoarthritis · Metabolomics · Knee · Lipidomics

✉ Madhan Jeyaraman
madhanjeyaraman@gmail.com

1 Department of Orthopaedics, KIMS Health Hospital, Kollam, Kerala, India

2 Dr RML National Law University, Lucknow, Uttar Pradesh, India

3 Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu, India

4 Orthopaedic Research Group, Coimbatore, Tamil Nadu, India

5 Department of Orthopaedics, ACS Medical College and Hospital, Dr MGR Educational and Research Institute, Chennai, Tamil Nadu 600077, India

6 Department of Orthopaedics, Dr KNS Mayo Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Introduction

Primary osteoarthritis (OA), a degenerative joint condition, is the most prominent type of arthritis. Patients with OA are typically elderly patients, with almost 80% of them being at the age of 65 years or above [1]. The most affected joint is the knee where OA occurs mostly due to loss of balance between continuous wear and tear and remodeling occurring in the joint. This degenerative process is significantly influenced by inflammation as well. Besides the knee, hip, spine, and small joints are also involved with the slow progressive loss of cartilage. Although there is still a long way to go before total control over OA progression is reached, early detection of this condition can help us to better manage

this condition and prevent morbidity. Currently, clinical and radiological methods are used to classify or grade OA.

Conservatively knee osteoarthritis is treated with NSAIDs, cartilage supplements, and calcium. Injections of hyaluronic acid [2] or intraarticular steroids are tried. Proximal fibular osteotomy (PFO) and high tibial osteotomy [3] are two joint preservation surgeries that would be instrumental in halting the initial phase of the disease. KOA is often diagnosed at an advanced stage when a patient exhibits medial joint line tenderness, joint crepitus, effusion, and deformity, which after initial conservative measures might eventually progress to require total knee arthroplasty (TKR).

A closer look at the molecular metabolites in KOA patients could help us better understand the disease which could potentially lead to the development of newer methods of identifying the disease and their treatment which would ultimately mean better management in KOA. Though many studies have been conducted recently to find and characterize biomarkers, however, none of these biomarkers have received clinical validation. It is possible to analyze thousands of different molecules all at once through a single targeted method using mass spectrometry (LC–MS), gas Chromatography–Mass Spectrometry (GC–MS), and H-NMR (proton nuclear magnetic resonance spectroscopy). Other techniques such as microarrays for deoxyribonucleic acid, ribonucleic acid, or protein can also simultaneously analyze thousands of molecules all at once [4]. Although metabolite signatures were identified, no published literature is available for the treatment of KOA. Proteomic, lipidomic, and metabolomic approaches aim to identify molecular profiles or signatures of different tissues such as cartilage, bone, synovium, meniscus, and tendon. Followed by synovial fluid, serum, and even urine and fecal samples, hoping to find certain predictive molecules or molecular classes responsible for OA development, disease progression, or possible therapeutic targets. The goal of this study is to uncover common molecular signatures that can be adopted in the future for the prognosis and treatment of KOA. Our objective is to provide a review of molecules in the synovial fluid or urine or other samples that can distinguish between KOA and non-KOA patients.

Materials and Methods

The systematic review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) Guidelines [5]. The study protocol has been registered in PROSPERO with registration number CRD42024428345. The studies that met the PICO criteria that were included in the review are mentioned below. The review included studies that fulfilled the PICO criteria as mentioned below.

- *Population:* Human or animal subjects with KOA
- *Intervention:* Identification of KOA-specific metabolites from body fluid and tissue samples.
- *Comparator:* Healthy controls or no comparator
- *Outcome:* Identification of molecules specific to KOA in different fluid and tissue samples in the body. Their correlation with diagnosis, treatment, and prognosis of KOA.

Study Design

Any original studies qualifying the PICO criteria.

Search Strategy

On February 14th, 2023, the PubMed, Cochrane, Google Scholar, Embase, and Scopus databases were searched. Search keywords employed for the review were "Knee", "Osteoarthritis", "Proteomics", "Lipidomics", "Metabolomics", "Metabolic Methods", and metabolic* used in various combinations with the Boolean operators—"AND" "OR" and "NOT". The review included the original studies on animal and human KOA models with established primary and surgically induced OA, respectively. Only English language studies were selected for review. Analytical, observational, and cross-section studies were included in the review. Review articles, conference papers, brief reports, opinions, and editorials were excluded from the review. The screening was done by two reviewers independently on the Rayyan QCRI tool. Any discrepancy between the reviewers was resolved by the involvement of the third reviewer.

Data Extraction

Two reviewers independently retrieved relevant data from articles included for analysis. The following data were extracted:

1. Study characteristics: Year of publication, authors, the animal model used (if applicable), and methodology used for the study,
2. Baseline characteristics: Method of induction of arthritis, number of subjects in both the groups, nature of the control group, names, and the number of metabolites isolated, and
3. Outcomes: Clinical changes in patients, histologic changes in KOA, and metabolite level changes from baseline.

Risk of Bias and Quality Assessment

The risk of bias in the included studies was assessed by the JBI risk of bias tool [6] by two reviewers independently.

A third reviewer was included to resolve any discrepancy between the reviewers.

Results

Search Results

After title screening, 458 articles were retrieved from 5 databases. After eliminating the duplicates, the abstracts of 297 articles were subjected to screening. In the end, a total of 53 papers were selected for full-text screening. While screening all the included papers, one article was excluded because of the use of non-English language, one article did not have details of metabolites, and one article did not have any healthy controls. These three were excluded. To synthesize the data, 50 papers were included. The PRISMA flow diagram of the study selection is shown in Fig. 1.

Quality Assessment

The methodological quality of the included studies was assessed using the JBI tool and is included as supplementary file 1. The included studies did not show a high risk of bias to warrant exclusion.

General Characteristics

Thirteen studies focused exclusively on animal studies and the remaining thirty-seven papers focused on human studies. Based on samples used for metabolic analysis, 6 studies used urine samples which are shown in Table 1, 15 studies used plasma which is presented in Table 2, 16 studies used synovial fluid (Table 3), 1 study used fecal samples (Table 4). Four studies were based on joint tissue (Table 5), and eleven studies used serum samples (Table 6).

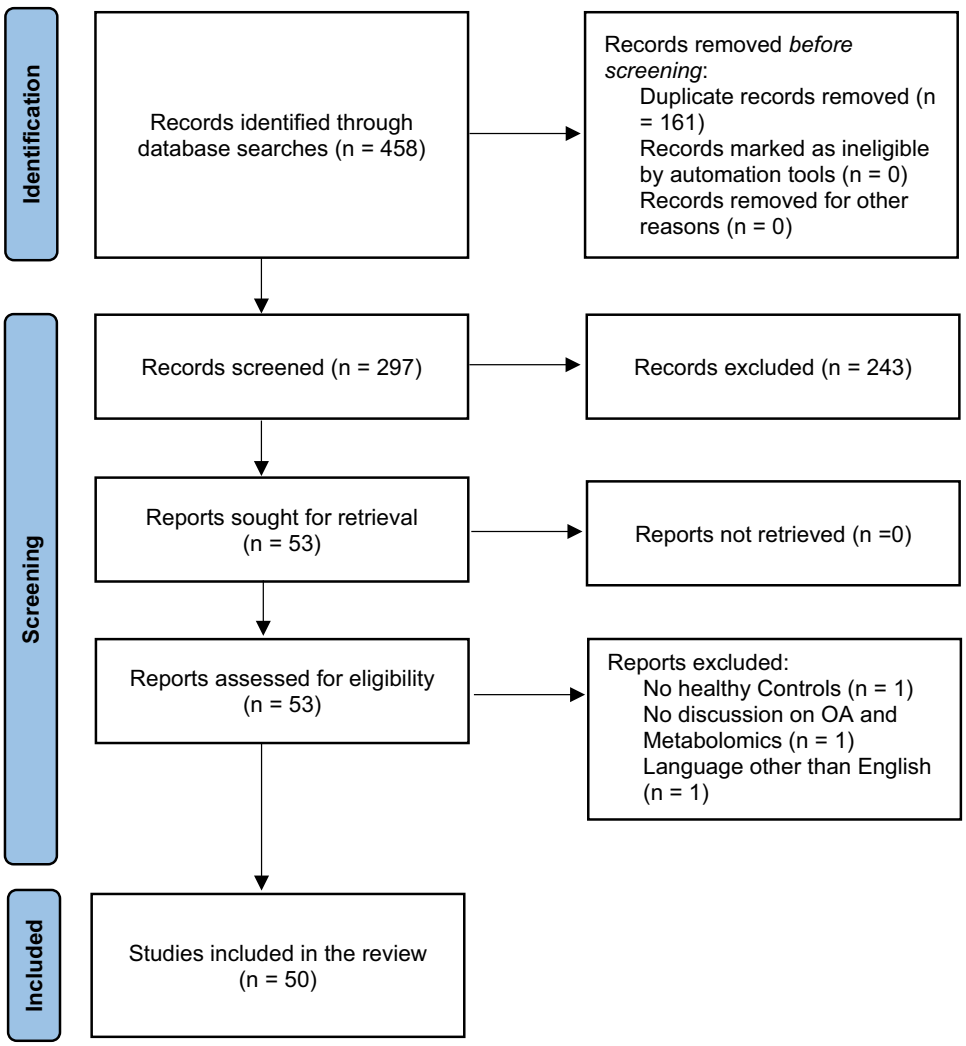

Fig. 1 PRISMA flow diagram

Table 1 Metabolic analysis in urine samples

References	Species	Comparator (controls)	Pathway affected	Metabolites in OA
Abdelrazig et al. [18]	Human	Healthy non-OA controls	Pyruvate and TCA pathway (increase activity) Purine pathway Lysine metabolism Glutamine metabolism	Acyl phosphate, fumarate, and S-lactoylglutathione increased Significant low levels—4-hydroxybutyrate, 3-oxoalanine, and homocysteine sulfinic acid Low levels—tryptophan, pipecolic acid, hypoxanthine, and aminoadipic acid Increased levels of 3-nitrotyrosine are observed Increased urinary excretion of 2-keto-glutamic acid(eightfold)
Li et al. [19]	Human	Healthy controls and two OA phenotypes	TCA pathway activity increased	Increased isocitrate aconitate and histamine levels Reduced histidine and glutamine levels
Jiang et al. [17]	Animal	Healthy rat models without OA	TCA cycle Glycolysis pathway Fatty acid metabolism Inhibition of activation of insulin signaling	Decreased—glutamine, N-carbamyl glutamate, urocanic acid, 5-amino valeric acid, and asparagine Increased alanine content Increased—lactic acid, pyruvic acid, and α-ketoglutaric acid levels 2,8-Hydroxyquinoline levels are decreased Increased diglycerol and D-(glycerol-1-phosphate) Decrease—cytosine levels
Loeser et al. [20]	Human	Age, BMI, and gender matched non-radiographic progressive OA models	Amino acid and collagen metabolism TCA cycle and fatty acid metabolism	Higher—hydroxybutyrate, pyruvate, creatine/creatinine, and glycerol levels Lower—histidine and methylhistidine levels Lower—urine arginine levels
Yin et al. [16]	Animal	Sham-operated rat models	TCA cycle upregulated Phenylalanine metabolism Nucleotide metabolism Lipid metabolism affected	Glycine, hippuric acid, acetoacetic acid, 5 hydroxy indole acetic, alanine, threonine were upregulated Adipic acid, glutamine, phenylacetic acid, azelaic acid, tryptophan, histidine, succinic acid were downregulated
Lamers et al. [52]	Human	Healthy non-OA controls	Glycolysis pathway Histidine metabolism	Lower levels of histidine and methyl histidine Upregulated hydroxybutyrate, pyruvate, creatine/creatinine and glycerol levels

Metabolomics in Synovial Fluid

We analyzed 16 synovial fluid studies, of which 12 studies were human and 4 studies were animal. One study employed both synovium tissue and synovial fluid as their sample, whereas two other studies simultaneously evaluated metabolites in synovial fluid and plasma. The remaining 13 studies were evaluated based on synovial fluid as the sample.

Animal Studies All the animal studies used different animal models. One study used male mice [7], another used 18 skeletally mature female Suffolk-cross sheep [8] and one study was conducted on white rabbits [9]. Two out of four studies demonstrated altered levels of branched-chain amino acids (BCCA). Levels of leucine, isoleucine, and valine were upregulated in OA models compared to healthy controls. All three studies did not show any common metabolites in their results. Mickiewicz et al. [8] indicated a fundamental reduction in the concentration of glucose in OA models. Yiwen et al. [9] revealed a consequential accumulation of arginine and proline levels in ACL transected rabbit knee and noted elevated levels of N1-acetylspermidine. In

Table 2 Metabolic analysis in plasma samples

References	Species	Comparator	Pathway affected	Metabolites in OA
Datta et al. [41]	Animal	Lean fat-fed mice	Lipid metabolism	Increased lysophosphatidylcholine analogs and one phosphatidylcholine analog Increased expression of leptins
Guan et al. [24]	Animal	Healthy non-OA group	Iron metabolism	HIF 1alpha levels are elevated in OA patients
Meessen et al. [43]	Human	Healthy non-OA controls	Lipid metabolism	Ratio of medium and large triglycerides to total lipids is altered and amino acids glutamine and histidine are altered in OA groups
Rockel et al. [56]	Human	No comparator	tRNA aminoacylation Vitamin B9 and B12 metabolism	L-Carnitine, tyrosine, histidine, lysine, leucine and valine levels are increased in late OA LysoPC levels increased in late OA
Rockel et al. [57]	Human	Healthy non-OA controls	Lipid metabolism Arginine cycle	Arginine, lysophosphatidylcholine to phosphatidylcholine analog ratios are altered
Sasaki et al. [58]	Human	No comparator	Cystine–cysteine cycle upregulation. TCA cycle Urea cycle	Increased levels of cystine, uric acid, and tyrosine
Werdyani et al. [59]	Human	Healthy non-OA controls	Lipid metabolism Amino acid metabolism	Increased levels of acylcarnitine C4 Decreased levels of Arginine Elevated levels of LysoPC:PC ratio
Xu et al. [39]	Human	Age- as well as BMI-matched mutual comparison between male and female OA group	Lipid metabolism	Decreased levels of acylcarnitines
Zhang et al. [27]	Human	Healthy non-OA controls	Arginine metabolism	Increased levels of arginine, lysophosphatidylcholine acyl, phosphatidylcholine diacyl, phosphatidylcholine acyl alkyl, and hydroxy sphingomyelin
Zhang et al. [42]	Human	Healthy non-OA controls	Lipid metabolism	Significantly increased lysoPCs to PCs ratio
Pousinis et al. [25]	Animal	Mouse Sham controls	Lipid metabolism Sphingolipid metabolism Arachidonic acid metabolism	Elevated levels of cholesterol derivatives CE (18:2), CE (20:4), and CE (22:6) PC levels upregulated
Costello et al. [26]	Human	No comparator	Lipid metabolism Amino acid metabolism	Significant elevated levels of proline correlate with pain in non-responders post-TKR Taurine, PC are also elevated PC (C36:8) was significant with function in non-responders
Huang et al. [60]	Human	No comparator	Amino acid metabolism	Elevated levels of succinic acid, xanthurenic acid, and L-tryptophan
Weidong et al. [11]	Human	Healthy non-OA controls and OA without diabetes controls	Lipid metabolism Glycolysis Amino acid metabolism	Upregulation of leucine, C34:3, and PC 36:3
Zhai et al. [28]	Human	Non radiographic OA progressor controls	Phenylalanine pathway (tryptophan metabolism)	Higher phenylalanine levels in OA

Table 3 Metabolic analysis in synovial fluid samples

References	Species	Comparator (controls)	Pathway affected	Metabolites in OA
Anderson et al. [14]	Human	RA controls	Taurine metabolism Glycophospholipid Glycolysis pathway Tricarboxylic acid (TCA) cycle	High—citrate, creatinine, glucose, glutamine, glycerol, pyruvate, and taurine
Corina et al. [12]	Human	Early OA with late OA comparison	Amino acid pathway Lipid pathway	Sphingomyelin Inosine 5′-phosphate, Phosphatidylcholine Diadenosine5′,5′-diphosphate Phosphatidylcholine has higher levels in late OA Heme has higher levels in early OA
Piccionello et al. [38]	Animal	Mutual comparison between OA groups (from grade 0–4)	Methionine pathway Mannose pathway	Altered levels of mannose, betaine, choline, 2-hydroxyisobutyrate, isoleucine, and lactate Mannose and betaine levels were elevated in OA grade 1 2-Hydroxyisobutyrate levels decreased with OA progression
Hahn et al. [7]	Animal	Male mice with control fat diet	Lipid metabolism Glucose metabolism pathway Amino acid metabolism	Branched-chain amino acids and short-chain fatty acids are altered
Kim et al. [15]	Human	Mutual comparison between different stages of OA	Glycolysis, the tricarboxylic acid (TCA) cycle, and amino acid and fatty acid metabolism	Malate, ethanolamine, squalene, glycerol, myristic acid, oleic acid, lanosterol, heptadecanoic acid, and capric acid, were significantly changed between the early- and late-stage OA groups. (higher in late OA) Arabitol, glucose, and galactose were higher in KL grade 1; and urate, alanine, pyruvate, and terephthalate were higher in KL grade 2 Levels of citrate, succinate, and fumarate showed trends toward increasing in the late-stage OA (grade 3 and 4)
Mickiewicz et al. [8]	Animal	Ovine sham surgery models	Glycine, serine, and threonine metabolism. Arginine and proline metabolism Alanine, aspartate and glutamate metabolism TCA cycle	Phenylalanine, isoleucine, hypoxanthine, leucine, choline, tyrosine, 2-hydroxybutyrate, methionine, lactate, aspartate, lysine, succinate, formate, valine, glutamate, tryptophan, alanine, serine, arginine, asparagine, hydroxyproline, threonine, acetate, and proline increased in the SF of surgical stifle joints Concentration of glucose, pyruvate, ethanol, 2-oxoglutarate, 3-hydroxybutyrate, and 2-oxovalerate was reduced in the surgical samples
Mickiewicz et al. [53]	Human	Healthy non-OA controls	Fatty acid metabolism and lipid metabolism TCA cycle	Concentrations of O-acetyl carnitine, N-phenylacetyl glycine, methionine, ethanol, creatine, malate, ethanolamine, 3-hydroxybutyrate, and hexanoyl carnitine were reduced
Xu et al. [39]	Human	Age- as well as BMI-matched mutual comparison between male and female OA group	Lipid metabolism	Decreased—acylcarnitines levels

Table 3 (continued)

References	Species	Comparator (controls)	Pathway affected	Metabolites in OA
Zhang et al. [13]	Human	No comparator	Fatty acid metabolism	Increased acylcarnitine: free carnitine levels Increase in levels of glycerophospholipids, sphingolipids, and amino acids
Alyssa et al. [40]	Human	RA patients and healthy non-OA controls	Creatine biosynthesis Fatty acid metabolism Arginine biosynthesis	Increase levels of phospholipids Significant upregulation of L-citrulline observed Decreased levels of Arginine
Hu et al. [9]	Animal	Sham surgery models	Lipid metabolism (linolenic acid pathway) Amino acid metabolism (tryptophan metabolism)	Increased levels of acetyl spermidine, octadecanetriol, phenylalanine, 5 hydroxy-1-tryptophan and tryptophan
Carlson et al. [10]	Human	Healthy non-OA controls	Amino acid metabolism Urea cycle Porphyrin metabolism	Upregulated glycine, serine, alanine, threonine, lysine, arginine, and proline
Marta et al. [54]	Human	Healthy non-OA models and RA controls	Nucleotide pathway Ceramide metabolism	Sphingomyelin levels (2.4-fold early OA and 4.4-fold late OA) Elevated levels of ceramide Decreased levels of cardiolipins
Weidong et al. [11]	Human	Healthy non-OA controls and OA without diabetes controls	Lipid metabolism Glycolysis Amino acid metabolism	Upregulation of leucine, C34:3, and PC 36:3
Vishal et al. [55]	Human	Healthy non-OA controls	Protein biosynthesis Amino acid metabolism Glucose energy pathway	Increased lactate levels, lipoproteins, glucose Increase in creatinine levels
Jessica et al. [22]	Human	No comparator	Amino acid metabolism Glucose metabolism	Glucose most important, others include glycine, dimethyl sulfone, dimethyl alanine, acetate, acetone, and tryptophan

Table 4 Metabolic analysis in fecal samples

References	Species	Comparator (control)	Pathway affected	Metabolites in OA
Rushing et al. [21]	Human	OA controls without hand OA and KL stage 0–1	Leukotriene metabolism Tryptophan metabolism Pyruvate metabolism	Indole-3-acetate, tryptophan, indole pyruvate, indole acetaldehyde are elevated Elevated levels of dipeptides and tripeptides Hippuric acid levels are decreased in OA

Table 5 Metabolic analysis in joint tissue samples

References	Species	Comparator	Pathway affected	Metabolites in OA
Jessica et al. [22]	Human	No comparator	Amino acid metabolism Glucose metabolism	Glucose most significant metabolite differentiating low- and high-grade synovitis, followed by glycine, dimethyl sulfone, dimethyl-alanine, acetate, acetone and tryptophan
Haudenschild et al. [23]	Animal	No comparator	Vitamin D metabolism Amino acid metabolism Pyrimidine pathway	Upregulation of vitamin D3 in OA. Downregulation of deoxycytidine triphosphate, glutamine, glutamate, arginine, and proline
Shiyu et al. [61]	Human	Mutual comparison between grade 3 OA and grade 4 OA patients	CoA biosynthesis pathway Glycerophospholipid pathway Histidine, lysine, glycine pathway Fructose and mannose pathway	Levels of choline, 2-propylpiperidine, rhamnose and monomethyl glutaric acid were higher in grade 4 OA Methylhistamine, sphingomyelin, zeranol, propanol, 5-aminopentanamide, dihydrouracil, 2-hydroxypyridine and 3-amino-2-piperidone levels were lower in grade 4 OA
Welhaven et al. [62]	Human	Healthy cartilage from tissue bank	Lipid pathway (glycerophospholipid pathway) Amino acid pathways. Vitamin K and E metabolism	Elevated levels of arachidonic acid, leukotriene F4, panaxydol were higher in OA cartilage Vitamin E metabolism was upregulated, but vitamin A levels were decreased Decreased levels of histidine but increased levels of tryptophan, methionine, cysteine, aspartate, and asparagine were noted in OA samples

addition, tryptophan and its derivatives were also elevated in samples of OA models compared to sham controls. Hahn et al. [7] for their study used two groups of mice—control fat-fed (CF) and high fat-fed (HF) mice. These two groups were evaluated at the end of the 52 weeks. In CF mice, they revealed downregulation of metabolites like lysine degradation, pyruvate metabolism, fatty acid metabolism, steroid biosynthesis, and tryptophan metabolism; in HC mice on the other hand, there was a positive association with coenzyme A biosynthesis, short-chain fatty acid synthesis, and BCCA synthesis.

Human Studies We analyzed 12 human studies, 6 of which showed an upregulation of the TCA cycle. Amino acid metabolism was altered in 8 out of 12 studies. One study indicated changed nucleotide metabolism, while seven studies revealed altered lipid metabolism. Arginine levels varied in ten studies, eight out of ten studies exhibited depletion of arginine levels in OA, but Carlson et al. [10] laid out an enhancement of glycine, serine, alanine threonine, lysine, proline, and arginine levels in OA. Weidong et al. [11] found that OA patient's leucine levels were considerably higher when compared to healthy controls. Corina et al. [12] found 43 metabolites, among them 9 were important. But out of these nine, four were considered significant for the study, they were phospholipids, phosphatidylcholines, sphingomyelin, and ceramide. This study also revealed that phosphatidylcholine, diadenosine

Table 6 Metabolic analysis in serum samples

References	Species	Comparator (controls)	Pathway affected	Metabolites in OA
Schadler et al. [32]	Human	No comparator	Leptin pathway	Higher FABP4 and leptin levels
Onurol et al. [48]	Human	Compared with Non-obese OA Control group and Healthy non-OA Control group	Glycerolipid, nitrogen, glycerophospholipid, -lipid metabolism	Phosphatidylethanolamine, lysoPC, and PA levels were high Indole acetic acid levels are increased Significantly elevated levels of valine
Tootsi et al. [63]	Human	Healthy non-OA controls	Urea cycle Glycine–serine pathway	Increased levels of leucine, arginine Serine, and spermidine Lower levels of glycine and serotonin Decreased spermine:spermidine ratio
Xie et al. [33]	Human	No comparator	Fatty acid metabolism Beta oxidation of long-chain fatty acids	Altered ratios of C16:1:C14 and C16:1:C12, choline:lysine and choline:C5-OH and Sarcosine: Proline and C3:1:C2
Chen et al. [31]	Human	Healthy non-OA controls	Amino acid metabolism	Elevated levels of ten metabolites (leucine, arginine, valine, isoleucine, tryptophan, alanine, lysine, creatine, tyrosine and 4- hydroxy- L-proline) Downregulated metabolites (glutamine, phenylalanine, serine, proline, GABA, creatinine, dimethylglycine, taurine, asparagine, aminobutyric acid, acetyl carnitine and citrulline)
Tristan et al. [29]	Animal	Healthy control rats	TCA cycle Amino acid metabolism	72-h analysis post-surgery (ACL resection OA models)—Increase concentration of glycine and 9-hexadecenoylcarnitine and decreased carnosine levels 4-week follow-up—Increase levels of dodecenoylcarnitine, acetic acid and 9- decenoylcarnitine 10 weeks—decrease levels of D- mannose and increased levels of hexanoyl carnitine and butyryl carnitine
Anthony et al. [30]	Animal	Sham control sheep models	Amino acid pathway (BCCA pathways)	4 weeks post-ACL resection OA models showed lower levels of BCCA (valine, leucine and isoleucine) 12 weeks postop Increased levels of glutamine, creatine, creatinine and 3 methyl histidine were seen with OA models compared to sham models
Tootsi et al. [49]	Human	Healthy non-OA controls	Lipid metabolism	The levels of C10:1, C10:2, C12, C12:1, C14, C14:2, C14:1-OH, CPT1-ratio and total AC/C0 were found to be significantly lower in the OA group
Qingmeng et al [64]	Human	Healthy non-OA controls	Amino acid metabolism	Significantly decreased levels of glycine and histidine in OA L-Tryptophan levels were elevated with OA compared to healthy controls
Zhai et al. [37]	Human	Healthy non-OA Controls	Amino Acid metabolism	Serum BCAA to histidine ratios are significantly altered
Neus et al. [36]	Animal	No comparator	Amino acid metabolism Lipid metabolism Arachidonic acid pathway	Eight organic acid derivatives, benzenoids, Organoheterocyclic compounds and lipid molecules were altered in all OA-induced samples

5′,5′- diphosphate, and lysophosphatidylcholine levels were heightened in late OA. Zhang et al. [13] detected a total of 86 metabolites, among which they found that glycerophospholipids, sphingolipids, biogenic amine, and acylcarnitine were higher in OA patients. Anderson et al. [14] compared OA with rheumatoid arthritis (RA) controls. Citrate, creatinine, glucose, glutamine, glycerol, pyruvate, and taurine levels were raised in OA patients while 3-hydroxybutyrate, acetate, isoleucine, leucine, sarcosine, and threonine were higher in RA. Kim et al. [15] graded OA using different metabolites. From the synovial fluid of both early and late-stage OA, 114 metabolites were identified, of which 28 were the ones differentiating early OA from late OA. The key metabolites differentiating were higher levels of malate, ethanolamine, squalene, glycerol, myristic acid, oleic acid, lanosterol, heptadecanoic acid, and capric acid in late OA.

Metabolomics in Urine Sample

We analyzed six studies that evaluated urine metabolites, which consisted of two animal studies and four human studies.

Animal Studies Both studies used Sprague–Dawley rats as their medium of analysis. Both studies similarly noted significant upregulation in the TCA cycle and fatty acid metabolism. Yin et al. [16] identified 14 metabolites of which glycine, hippuric acid, acetoacetic acid, 5 -hydroxy indole acetic, alanine, and threonine were upregulated but citric acid, adipic acid, glutamine, phenylacetic acid, azelaic acid, tryptophan, histidine, succinic acid were downregulated. Due to abnormal activity of the TCA cycle, citric acid and succinic acid levels were decreased in the OA rat's urine samples. Jiang et al. [17] identified multiple components as potential biomarkers of OA including, alanine, alpha-ketoglutarate, asparagine, maltose, and glutamine all of which were significantly altered in OA compared to healthy controls.

Human Studies All four studies in common exhibited elevated levels of amino acid metabolism, but only three studies showcased an upregulation of the TCA cycle. Abdelrazig et al. [18] recognized 26 altered metabolites in patients with inflammatory OA. Alterations in amino acid metabolism, pyruvate metabolism, and TCA cycle were also noted by them. According to Li et al. [19], OA patients with joint effusion had lower levels of glutamine and histidine and higher levels of aconite, isocitrate, citrate, and histamine. Loeser et al. [20] recognized that arginine and histidine levels were reduced in OA patients in contrast to healthy controls.

Metabolomics in a Fecal Sample

Only one fecal study which was based on a human model was analyzed in our study. This study revealed alterations in amino acid, tryptophan, and leukotriene metabolism. Rushing et al. [21] analyzed metabolite changes associated with gut microbiota in obese OA patients. More than 100 metabolites have been isolated but the most prominent differentiators between OA cases and controls were dipeptides and tripeptides. In comparison to controls, it was identified that OA patients' fecal samples had lower hippuric acid (phenylalanine derivative).

Metabolomics in Joint Tissue Samples

Four studies analyzed joint tissue sampling for metabolic analysis in OA, of which three were based on human models and one study on an animal model. One human study utilized both synovial fluid and synovial tissue for analysis while the other three studies including the animal study focused only on joint tissue study. All four studies showed altered amino acid metabolism, but no similar metabolites were detected. Jessica et al. [22] showcased that 42 metabolites were isolated from synovial tissue and 29 metabolites from synovial fluid but only 3 metabolites (lactate, dimethylamine, and creatine) positively correlated in both samples. The most crucial metabolite to distinguish between low-grade and high-grade synovitis has been revealed to be glucose. Haudenschild et al. [23] used mice models with ACL resection, which were injected with intraperitoneal flavopiridol (cdk9 inhibitor). Flavopiridol downregulates transcription of early response genes, which were associated with joint damage. The study concluded that flavopiridol prevented the upregulation of vitamin D3, phylloquinone, and acetylcarnitine.

Metabolomics in Plasma Study

A total of 15 studies were analyzed, of which 3 were animal studies and 12 were human studies. In the 12 studies based on human models, 2 used plasma and synovial fluid for analysis.

Animal Studies All the animal studies used mice models for analysis. Only lipid metabolism was commonly altered in all the studies, with elevated levels of Lysophosphatidylcholine and phosphatidylcholine derivatives. One study observed significant alteration in the TCA cycle and its metabolites. Another study analyzed gut metabolites targeting HIF 1alpha to inhibit OA. Zhiyuan et al. [24] noted enhanced levels of iron in OA models causing a deleterious impact on joint homeostasis. The study presented higher serum iron levels in OA patients compared to the control group but transferrin

levels and TIBC were lower in OA models. Pousinis et al. [25] analyzed lipidomics study in mice models and found 24 altered metabolites, of which 6 metabolites were considered significant in OA models, these metabolites were related to steroid biosynthesis, sphingolipid metabolism, linoleic acid, alpha-linolenic acid, glycerophospholipid, and arachidonic acid metabolism.

Human Studies A total of 12 human studies were analyzed, among which lipid metabolism is the only pathway commonly affected in most of the studies, followed by amino acid metabolism. Lysophosphatidylcholine: The phosphatidylcholine ratio was elevated in most of these studies, which proves to be a prominent biomarker for OA. Other significant metabolites identified were arginine, glycine, leucine, and histidine. Costello et al. [26] studied the correlation between metabolites and pain and functional non-responders in post-TKR patients—5 phosphatidylcholines (PC), 4 amino acids (proline was the most significant) 2 acylcarnitine, and 1 biogenic amine were found to be associated with pain and 14 PC (PC aaC36:8 was most significant), 7 amino acids, 1 LysoPCs and carnitine were associated with function non-responders in OA. Zhang et al. [27] studied the relevance of arginine in OA patients, out of six significant metabolites, arginine was the most significant metabolite in all stages of the study. Knee OA patients had on average 69 microM lower plasma arginine levels compared to controls. ROC analysis also showed that arginine had the greatest diagnostic value with an AUC of 0.984. The study also found 2.2 times higher levels of ornithine in OA patients compared to control. Zhai et al. [28] studied the significance of phenylalanine in the radiographic advancement of OA. After age, sex, BMI, and clinical site adjustment, the study revealed phenylalanine concentration was substantially linked to knee OA progression. Moreover, phenylalanine levels were highly linked with knee progression in females and not in men.

Metabolomics in Serum Study

Eleven studies were analyzed with serum samples, three were focused on animal models, and eight on human models. While five studies pointed out altered lipid metabolism and seven studies indicated altered amino acid metabolism. LysoPCs and PC were enhanced in three studies and altered levels of glycine, histidine, arginine, and leucine were exhibited in three studies.

Animal Studies The animal study was based on two distinct models; one was on sheep and the other on mice. Both studies in common indicated enhanced amino acid metabolism. Tristan et al. [29] identified 17 significant metabolites after 72 h post-surgery (ACL resection in mice models), but at 4 weeks follow-up, only 8 metabolites were found significant and at 10 weeks follow-up, only 3 metabolites (decreased D-mannose levels and increased hexanoyl carnitine and butyryl carnitine) were elevated between OA models and sham models. Anthony et al. [30] following meniscal destabilization, at 4 weeks, identified that TMAO, glutamine, and acetate levels were increased, and lactate and glycine levels were decreased whereas at 12 weeks postop only TMAO and tyrosine were elevated.

Human Studies In all the human studies, lipid metabolism and amino acid metabolisms were altered. Chen et al. [31] utilized targeted metabolomics analysis and detected 25 amino acids and 4 biogenic amines. The study found the metabolites with the most significant impact were found to be involved in the metabolism of alanine, aspartate, glutamate, arginine, and proline. Schadler et al. [32] studied the association of FABP4 and leptins with knee OA severity and BMI. Higher levels of FABP4 and leptin levels were found in the obese women population which is a predictor for OA progression. Xie et al. [33] studied the relationship between knee cartilage loss and associated metabolites. The study revealed that four metabolites were associated with patellar cartilage loss, and four metabolites each were associated with lateral and medial cartilage loss in OA patients compared to controls.

Discussion

Metabolomics is the comprehensive analysis of small molecules in a biological system. Metabolites are the ultimate end products of different metabolic pathways that project the genotypic, phenotypic, and environmental characteristics of various biological systems. We have analyzed 50 works of literature to detect metabolic perturbations in the urine, blood (plasma and serum), synovium, feces, and synovial fluid of animal models and human models [34].

Potential Biomarkers of OA

The study has identified various biomarkers that were significant in the detection and treatment of OA. These include various amino acids, lipids, nucleotides, and glucose metabolites.

Proteomics in OA

Arginine is a semi-essential amino acid in humans, which is one of the most important metabolites we identified in OA patients. Arginase stimulates collagen formation and cell proliferation through the urea and L-ornithine pathway which causes fibrosis in OA [35]. According to Zhang et al.

[27], patients with knee OA had a plasma concentration of ornithine that was 2.2 times greater than that of the control. The arginine:proline ratio was lower in OA patients than in controls, thereby suggesting that OA is caused by overactivity related to arginine depletion. Five major metabolites were identified by De Sousa et al. [36] as being related to OA; however, arginine was determined to be the notable metabolic marker. *BCCA* includes valine, leucine, and isoleucine as essential amino acids, which act as a medium for various protein and energy metabolism and precursors to other amino acids. Our study concluded that BCCA were significant OA markers. Anthony et al. [30] identified decreased levels of serum BCCA in OA subjects and the ratio of BCCA to histidine was also determined as a biomarker for knee OA. Zhai et al. [37] showed an association between valine:histidine and leucine:histidine ratios and knee OA was statistically significant. Also, the study showed only the BCCA:histidine ratio was significant but not the serum concentration of histidine alone as a marker for OA. *Phenylalanine and tryptophan* are essential aromatic amino acids involved in protein synthesis. According to Guangju Zhai et al. [28], phenylalanine levels in the plasma of OA progressors were reportedly higher than those of non-progressors. The study proved no association of WOMAC pain scores with phenylalanine levels in OA. In contrast, Chen et al. [31] quantified significantly lower levels of phenylalanine in a serum study of OA patients. Mickiewicz et al. [8] identified elevated levels of phenylalanine synovial fluid in mice models. Rushing et al. [21] showed an association of tryptophan metabolism with the maintenance of intestinal health, through kynurenine and serotonin pathways.

Lipidomics in OA

Phosphatidylcholine and LysoPCs and their ratios are identified as potential biomarkers of OA in our study. Corina et al. [12] in their study had identified phosphatidylcholine, diadenosine 5′,5′-diphosphate, and lysophosphatidylcholine had higher values in late OA. According to Hahn et al. [38], OA patients undergoing joint replacements typically had lower PC levels than controls. Bingyong et al. [39] found abnormal levels of glycerophospholipids in synovial fluid samples from advanced OA patients. Alyssa et al. [40] compared synovial fluid between OA, RA, and healthy controls and found 19 significant metabolites were altered in diseased synovium compared to healthy control of which more than half were phospholipids. Poulami et al. [41] compared the progression of OA between high-fat diet-fed mice and lean-diet mice and found four potential biomarkers of OA in high-fat-fed mice which were 3 lysophosphatidylcholine (LysoPCs) analogs and one phosphatidylcholine (PC) analog. In the analysis of the plasma of 109 candidates, Zhang et al. [42] identified that the LysoPCs:PC ratio has shown great specificity and sensitivity in differentiating between healthy controls and advanced OA. Sphingomyelins, ceramides, and long-chain fatty acids were also identified in many of our studies as significant markers of OA. Meessen et al. [43] studied a significant association between fatty acid chain length and stages of OA, especially end-stage OA, but the study showed no association between fatty acid chain length and OA progression.

Metabolic Pathways Affected in OA

TCA Cycle Most of our studies including blood, synovium, synovial fluid, and urine studies showed that the most affected pathway in OA pathology was the TCA cycle. The oxidation of acetyl coenzyme A (CoA) which is produced from proteins, fatty acids, and carbohydrates is how the TCA cycle harvests energy [44]. Abdelrazig et al. [18] examined urine samples from 74 patients with OA and found elevated levels of fumarate, acetyl phosphate, and S-lactoylglutathione which suggest that the disrupted metabolism in cartilage cells is causing increased activity of the pyruvate pathway and the TCA cycle. After analyzing 27 urine samples from OA patients, Li et al. [19] stated that the TCA cycle was more active in OA patients due to increased expression of aconitic acid, isocitric acid, and citric acid.

Glycolysis/Pyruvate Pathway Glycolysis is commonly affected in the progression of OA. Chondrocytes' main energy source is heavily reliant on glycolysis due to a hypoxic environment [45]. Other studies revealed a strong relationship between the advancement of OA and lactate levels which were found to be markedly raised in synovial fluid of OA patients [46]. Jessica et al. [22] analyzed synovial fluid and synovium in 21 and 37 OA patients, respectively, and found upregulation of the glycolytic cycle with increased levels of glucose, lactate, and pyruvate in synovial samples.

Lipid Metabolism A close association between OA and lipid metabolism has been observed. Lipolysis breaks down triacylglycerol into glycerol and fatty acids, which exert an important influence on the articular microenvironment and cellular function of joints. Lipolysis induces the production of excessive adipokines causing inflammation of local tissue [47]. According to Onurol et al. [48], certain glycerophospholipids were elevated in knee OA and can serve as biomarkers of OA. Altered lipid metabolism also causes elevation of phosphatidylethanolamine, LysoPCs, and PA levels in OA patients compared to the control group. After analyzing data from 70 individuals with OA of the knee and hip, Tootsi et al. [49] concluded that the disruption of lipid metabolism is a key pathophysiological characteristic of OA. Alteration in lipid metabolism causes elevation of lev-

els of potential metabolites like glycerophospholipids (PC and LysoPC) and sphingolipids causing activation of proinflammatory mediators.

Markers of OA Staging

a. *Early and late markers of OA:* Metabolomics can be applied in staging OA by identifying markers for assessing the progression and severity of the disease. Our study has analyzed some markers for early and late OA (Table 7). Corina et al. [12] showed heme was found at higher levels in early OA. Studies have also found elevated levels of phosphatidylcholine, diadenosine 5′,5′-diphosphate, and phosphatidylcholine in late OA.
b. *Obesity-related markers of OA:* Some biomarkers were associated with OA in obese patients. Onurol et al. [48] in their study, additionally identified that obesity in OA patients affected the acidosis and metabolites concerning oxidative stress. Markers related to obesity in OA are mentioned in Table 7.
c. *Markers associated with KL staging of OA:* Epidemiological studies of OA have frequently employed the Kellgren–Lawrence classification of diseases as a research tool that graded OA from 0 to 4 grades with increasing severity of the disease [50]. Kim et al. [15] analyzed the synovial fluid of 15 patients and noted a clear separation of metabolites for each stage, KL 1 showed increased levels of arabitol, galactose, glucose, mannose, and tagatose. KL 2 showed increased levels of urate, beta-alanine, pyruvate, and terephthalate. KL 3 and 4 showed increased levels of fatty acids, proline, phenylalanine, squalene, and trehalose-6-phosphate. Peyton et al. [8] analyzed 36 articles which were based on 4 samples (urine, plasma, synovial fluid, and serum) and they concluded that there are more than 10 metabolic pathways and a vast array of metabolites that are related to OA. They could only identify the tryptophan pathway as the only association with OA pathogenesis. Akhbari et al. [51] reviewed only 17 articles which consisted of only synovial fluid samples, which makes their research inconclusive. They had shortlisted 24 biomarkers that are not specific to OA but also to RA and other inflammatory arthropathies. Haartmans et al. [4] focused solely on the method of analysis using 24 studies in different samples and concluded that mass spectrometry was the best tool for metabolomic analysis; however, no metabolites or metabolic pathways associated with OA were identified. Even though numerous metabolites were shortlisted as significant, no direct correlation could be mapped from their analysis. Through the analysis of 50 articles which focused on samples of 6 different samples (urine, synovial fluid, serum, plasma, feces, and joint tissue), we were able to arrive at a definite conclusion to our review. Our study precisely narrowed down three specific pathways associated with OA pathogenesis which are TCA cycle, glycolysis, and lipid pathways. We were also able to narrow down metabolites that are specific for each stage of OA pathogenesis.

Limitations

Our study had some limitations. Heterogeneity is the foremost limitation in our study because selected populations were different in each study irrespective of age, gender, and ethnicity, and samples were also different in each study with various platforms for analysis differing in terms of coverage, sensitivity, and selectivity among the different studies. Each of these studies selected was a retrospective case–control study. These studies make it difficult to pinpoint the exact temporal sequence in which exposure factors and output occurrence occur which restricts the capability to determine the causality. There were numerous metabolites from

Table 7 Metabolic markers of osteoarthritis

Markers of OA	Metabolites
Early OA markers	Heme has a higher level in eOA compared to lOA
Late OA markers	Sphingomyelin, inosine 5′-phosphate, phosphatidylcholine, diadenosine 5′,5′-diphosphate, and phosphatidylcholine have higher levels in late OA Higher levels of glycerophospholipids
OA markers in obesity	Glycolate, hippurate, and trigonelline were among the important metabolites for distinguishing progressors from non-progressors at baseline Alanine, N-dimethylglycine, glycolate, hippurate, histidine, and trigonelline were elevated in obese OA patients Glycerophospholipids were elevated in obese OA patients—biomarkers of obesity-related OA
Pain-related markers	PC aa C28:1 is the key metabolite associated with sustained knee pain PC aa C28:1 to PC aa C32:0 ratios provided further insights into the potential contributions of PC metabolism to sustained pain Increased accumulation of acylcarnitine causes proinflammatory mediators Sphingomyelinase breaks down sphingomyelin into phosphocholine and ceramide which are involved in inflammation

different studies that were associated with OA, but no common association was identified in all studies. However, three metabolic pathways identified were common in all studies and could be narrowed down to a few significant pathways.

Future Recommendations

As per our current study, the use of metabolites in different samples can be tried for accurate analysis and identification of the progression of OA. Different metabolites were identified with different fluid samples which gave us a core idea about assessing the severity and progression of OA. Especially amino acids like arginine and valine, molecules like CAT, and fatty acid metabolites like LysoPC and PC could become targets for newer treatment development for OA but are not specific. Metabolic pathways like glycolysis, TCA cycle, and lipid pathways can be potential key targets for preventing OA progression, but further research in the field of metabolite identification specific to OA is required which will bridge the gap between current evolving ideas and concepts of metabolomics with early diagnosis, treatment, and disease control.

Conclusion

All these studies showed a vast array of metabolites and metabolic pathways associated with OA. Metabolites like lysophospholipids, phospholipids, arginine, BCCA, and histidine were identified as potential biomarkers of OA but a definite association was not identified. Three pathways (glycolytic pathway, TCA cycle, and lipid metabolic pathways) have been found as highly significant in OA pathogenesis. These metabolic pathways could provide novel therapeutic targets for the prevention and progression of the disease.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Standard Statement This article does not contain any studies with human or animal subjects performed by the any of the authors.

Informed Consent For this type of study informed consent is not required.

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