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REVIEW ARTICLE

# A review of applications of metabolomics in osteoarthritis

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## Abstract

Osteoarthritis (OA) represents the most prevalent and disabling arthritis worldwide due to its heterogeneous and progressive articular degradation. However, effective and timely diagnosis and fundamental treatment for this disorder are lacking. Metabolomics, a growing field in life science research in recent years, has the potential to detect many metabolites and thus explains the underlying pathophysiological processes. Hence, new specific metabolic markers and related metabolic pathways can be identified for OA. In this review, we aimed to provide an overview of studies related to the metabolomics of OA in animal models and humans to describe the metabolic changes and related pathways for OA. The present metabolomics studies reveal that the pathogenesis of OA may be significantly related to perturbations of amino acid metabolism. These altered amino acids (e.g., branched-chain amino acids, arginine, and alanine), as well as phospholipids, were identified as potential biomarkers to distinguish patients with OA from healthy individuals.

**Keywords** Biomarkers · Metabolic pathways · Metabolomics · Osteoarthritis

## Introduction

Osteoarthritis (OA) represents the most prevalent and disabling arthritis worldwide, affecting several diarthrodial joints, but primarily the knees and hips. Its worldwide incidence rate is approximately 1/10 in male and 1/5 in female over 60 years of age [1]. Our understanding concerning the etiology of OA continues to grow. Many factors, including age, obesity, gender, genetics, and joint injury, have shown to contribute to the development of OA, among which increasing age and obesity are the principal factors [2]. OA is now considered a heterogeneous chronic disease involving multiple joint tissues. It is mainly characterized by the degradation of articular cartilage, subchondral bone sclerosis, osteophyte formation, variable degrees of synovitis, and ligament degeneration, eventually leading to disability in the end state of the disease [3]. The high rates of morbidity and disability associated with OA have led to a reduced quality of life and a great economic burden on society. The economic burden of OA has been estimated to be between 1.0 and 2.5% of the gross domestic product for Western countries [4]. Despite this challenge, there are no effective early diagnostics or main therapeutics for this disease [5]. These statistics could be improved if the understanding of the diagnostic biomarkers and metabolic alterations in OA were clearer [6].

As an emerging field in life science research and a member of the "-omics" family of sciences, metabolomics provides a powerful approach to identify small molecules for several disorders [7]. By measuring and mathematically modeling changes in the levels of products of metabolism, both diagnostic and prognostic biomarkers can be detected and identified for a variety of diseases [8]. Furthermore, metabolomics might provide an opportunity to explain the underlying pathophysiological processes associated with diseases [9, 10]. Metabolite research in OA has had an increase in interest and a relatively rapid development, as the majority of articles related to OA were published in recent years. In this review, we aimed to provide an overview of the studies related to the metabolomics of OA in animal models and humans to describe the metabolic changes and related pathways in the pathogenesis of OA.

## Metabolomics

Recently, the metabolomics approach has been used to identify and quantify small molecules in systemic biology for measuring chemical intermediates or products and providing

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a biochemical "snapshot" of an organism's metabolic state [11]. The types of samples are diverse, including biological fluids (e.g., plasma, urine, and saliva), cells, and tissue extracts. Metabolomics is an ideal tool to discover biomarkers and understand the systems-level effects of metabolites owing to its inherent sensitivity [12]. As a part of the biological system-based approach, metabolomics belongs to the family of -omics sciences, which also encompasses genomics, transcriptomics, and proteomics. In general, omics studies can define networks in which genetic variation (genomics) leads to changes in gene expression (transcriptomics) to affect protein expression (proteomics). Quite differently, metabolomics is a means for measuring chemical intermediates, or metabolites, in a variety of biological samples. In other words, metabolomics tells us what happened instead of what may have happened. With the aid of metabolomics to overcome the limitations of other -omics sciences, the family could provide valuable information on endogenous and exogenous factors [13]. Consequently, metabolomics brings significant benefits in the diagnosis, therapy assessment, and an understanding of the pathogenesis for many disorders, such as cancer [14] and cardiovascular disease [15]. Furthermore, it may also aid in monitoring the effects of medical interventions [16] and studying autophagy [17]. Metabolomics generally involves three steps: metabolism, spectroscopy, and multivariate statistical analysis [11]. Among these, spectroscopy has proven the most useful in providing data that gives us a more comprehensive understanding of metabolism [18].

Most analytical platforms for metabolic profiling are based on spectroscopic techniques. Currently, there are three leading methods in metabolomics studies: nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography–mass spectrometry (LC–MS), and gas chromatography–mass spectrometry (GC–MS). These have similar abilities in providing comprehensive coverage of an organism's metabolic state [19]. NMR spectroscopy has important advantages that include being non-invasive, non-destructive, not requiring elaborate sample preparations, and rich in quantitative information over a wide dynamic range. Therefore, it has played a fundamental role in the development of metabolomics research [20]. Furthermore, it is a powerful structural tool to detect information on isomers and molecular structure, of which similar molecular fragmentation may be difficult to acquire in both GC–MS and LC–MS. Contrarily, however, NMR spectroscopy also has some limitations. For example, it is not well suited for low concentrations. Besides, the low sensitivity and limited resolution of individual metabolites from complex samples are challenging [21].

Mass spectrometry-based metabolomics is superior in sensitivity to NMR. GC–MS is generally considered a versatile and reproducible analytical platform for low-molecular-weight and volatile analytes because of its robust, selective, and sensitive nature [22]. Currently, LC–MS is widely used in metabolomics for a broad range of metabolites, including not only metabolites from high- to low-molecular-weight but also from hydrophilic to hydrophobic molecules, due to its high sensitivity and reliable quantitation [19]. However, in contrast to the NMR approach which handles samples quickly and tends to be more reproducible, mass spectrometry-based techniques require complicated sample preparation steps and chemical derivation that may lead to both metabolite loss and differential adduct formation [23]. Overall, these analytical methods are complementary techniques and work for different analytes depending on the experimental objective and the sample type being investigated (Table 1).

## The metabolomics of OA

As an ideal tool to discover biomarkers and understand the systems-level effects of several disorders, metabolomics can also help elucidate OA. Figure 1 illustrates the overall flowchart of common metabolomics for OA, including the major steps from collecting samples to obtaining the most relevant pathways involved with OA.

OA is best considered a disease of the whole "joint organ." Hence, the metabolic changes of OA could arise in various periarticular tissues, including articular cartilage [22], subchondral bone [24], and the synovium [25]. Meanwhile, it is well-established that the types of metabolic samples are wide ranging and include biological fluids, cells, and tissue extracts. Evidently, those studies on pathogenic mechanisms of diseases are particularly focused on animal models [26]. However, several population-based OA studies can be found in the literature [27]. In 1989, Williamson et al. first used 1H NMR to investigate synovial fluid (SF) components in patients with joint diseases [28]. Nevertheless, the variations seen in humans are much greater than that of experimental animals, which could pose both problems and advantages for modeling. Thus, the analytical platforms used in animals may be more appropriate or affordable than in patients [29]. Significant metabolic changes in OA in various sample types are discussed below.

### Metabolomics of tissues in OA

Tissue analysis is a powerful tool to study the localized responses of diseases and provide relevant metabolic information. A previous trial had investigated cartilage from patients with OA who underwent total knee arthroplasties and from non-OA healthy individuals using high-resolution magic angle spinning NMR spectroscopy. This trial found that the metabolite levels of alanine, N-acetylcholine, and glycine could accurately classify OA and healthy [30]. Another study investigated the synovium from human OA joints by GC–MS and LC–MS and found alterations in 11 metabolites. Among

**Table 1** Comparisons between the analytical techniques in metabolomics

| Techniques | NMR | GC–MS | LC–MS |
|---|---|---|---|
| Detection range | Nanomolar | Sub-picomolar concentrations | Sub-femtomolar concentrations |
| Sample preparation | Minimum sample preparation, fast, and non-invasive | Complicated sample preparation steps that may lead to a metabolite loss | |
| Analysis | Reproducible due to its non-destructive nature | Less reproducible due to the chemical derivations necessary | |
| Isomer identification | Powerful tool | Difficult to acquire | Difficult to acquire |
| Isotope-selective detection | Powerful tool | Difficult to acquire | Difficult to acquire |
| Utilized in vivo | Yes | No | No |
| Major limitation | Lower sensitivity and limited resolution of individual metabolites from complex samples | Thermal degradation of analytes | Matrix effects, ion suppression, and differential adduct formation |

these, seven were present at higher levels in the end-stage of the OA group compared to the control, while four were relatively lower, which specifically indicated altered activities of collagen metabolism, branched-chain amino acids (BCAA) metabolism, energy metabolism, and tryptophan metabolism [31]. Now subchondral bone has received increasing attention in OA due to the pathological changes that of subchondral bone may play an important role in the initiation and progression of OA. The sclerotic subchondral bone from OA patients compared with the non-sclerotic subchondral bone was analyzed by Yang et al. and demonstrated that alteration in taurine and beta-alanine metabolism was clearly found to be associated with the sclerosis of subchondral bone [32].

## Metabolomics of biological fluids in OA

Biological fluids are convenient for metabolic research as they are relatively easy to obtain from animal and human subjects

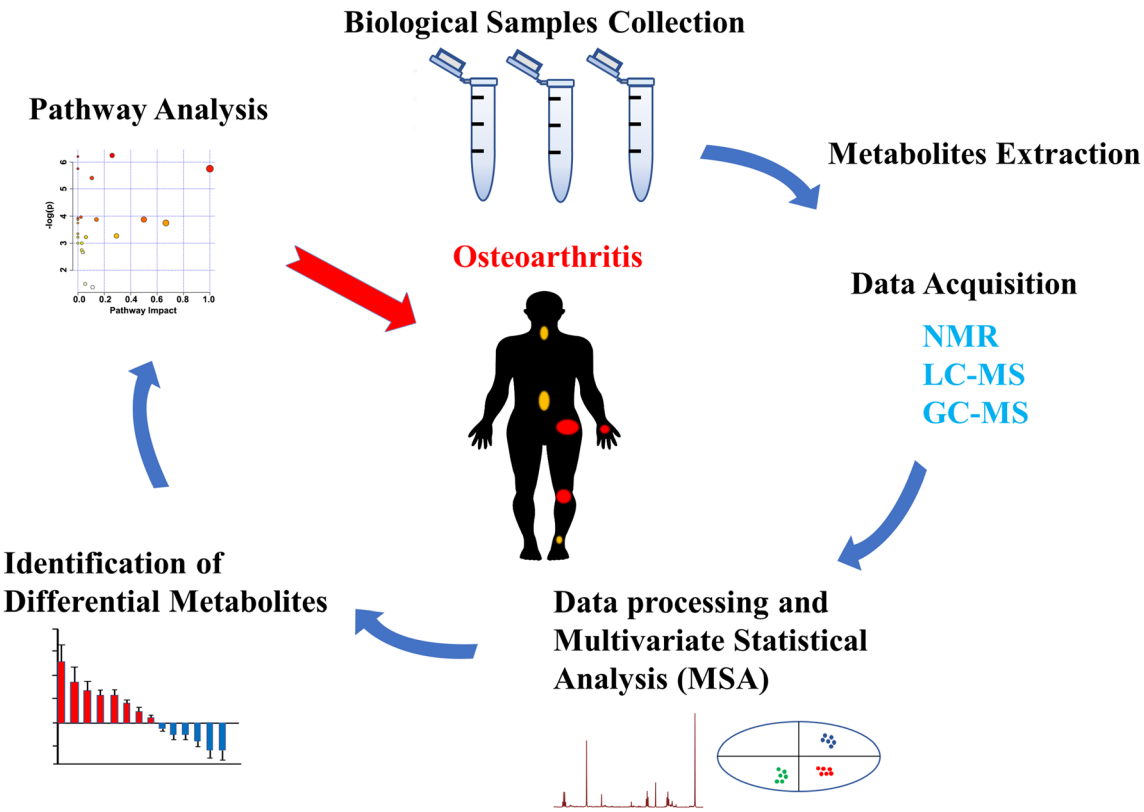


**Fig. 1** A scheme of the overall flowchart of metabolomics for OA. First, OA samples are collected and extracted for spectroscopy examinations by NMR, LC–MS, and GC–MS. Before Fourier transformations, raw spectral data need to be processed, which includes phase corrections, baseline corrections, and resonance alignment. Subsequently, sample data is normalized and subjected to multivariate statistical analysis, including principle component analysis, a partial least squares discriminant analysis, and orthogonal signal correction partial least squares discriminant analysis. Together with univariate data analysis, characteristic metabolites can be determined. Finally, the metabolic pathway analysis is applied to determine the most relevant pathways involved with OA

[33]. These fluids include urine, saliva, cerebrospinal fluid, SF, semen, plasma, serum, and whole blood. These biofluids provide potential advantages to monitor the state of biological organisms. Therefore, in the future, the metabolomics of biological fluids could play a key role in public health [34].

### Blood

Plasma and serum are excellent sources due to the large number of metabolites that can be detected from them, thus contributing to the novel markers associated with the OA risk [34, 35]. Zhang et al. [36] collected plasma from patients with OA and healthy donors. They found that six biochemicals are associated with OA, but arginine played the leading role in discriminating OA from controls. This study suggested that an arginine depletion in OA would result in an imbalance between cartilage repair and damage due to the overactivity of the arginine-to-ornithine pathway. Zhai et al. [37] studied serum-based metabolomics of OA in humans. They compared 123 female knee OA cases and 299 controls in the discovery sample and then designed an independent replication to verify their results. They found that 14 metabolite concentration ratios were significantly correlated to knee OA, especially the ratio of the BCAA to histidine, as well as valine to tryptophan, arginine, and glycine. These findings were later supported by Zhang et al. (2016a) and extended to the male population. Maher et al. [34] studied the serum of sheep that had undergone meniscal destabilization (MD), anterior cruciate ligament transaction (ACLT), or sham operations after 4 weeks and 12 weeks using 1H NMR spectroscopy. Their results suggested that metabolic changes in ACLT were more extensive compared to MD. They documented increased concentrations of dimethyl sulfone in MD after 4 weeks. An increase was also evident in the concentration of 3-methylhistidine and an obvious decreased level in BCAA in ACLT-induced OA over both time points. Furthermore, the increased concentrations of glutamine, creatine, and creatinine after 12 weeks are associated with altered muscle metabolism.

### Urine

Urine samples serve as excellent tools compared to other biofluids since they are easy to sample and are non-invasive. Furthermore, urine needs minimum sample preparation due to its lower protein content. These advantages have ensured the widespread use of urine as an analytical tool in clinical practice [38].

A study had examined urine samples by NMR spectroscopy from 22 patients with OA and 22 controls who both underwent the Intensive Diet and Exercise for Arthritis and suggested that differences in metabolism could be useful in the progression of OA [39]. The Intensive Diet and Exercise for Arthritis study showed that a loss of body weight and a reduction of pain were associated with the levels of interleukin-6 and C-reactive protein in overweight and obese adults with knee OA [40]. This group documented increased concentrations of hydroxybutyrate, pyruvate, and glycerol. In addition, they reported an increased ratio of creatine-to-creatinine in patients with OA and elevated levels of methylhistamine and histidine [39]. Another metabolomic study based on GC–MS using urine was able to distinguish patients with OA from healthy controls. This study described significantly elevated levels of aconitate, isocitrate, citrate, and histamine in patients with OA compared with the healthy. In contrast, it also showed lower levels of histidine and glutamine in the urine from patients with OA [41].

### Synovial fluid

SF could provide a real-time and joint-specific metabolic profile of OA because it is a bathing solution that is in direct contact with all the tissues of the entire joint. Therefore, SF could allow for comprehensive detection of the diseased joint [42].

Carlson et al. [43] found that 35 metabolites were statistically important in identifying the separation between human OA and healthy SF via LC–MS. These metabolites included phosphatidylcholine (PC), lysophosphatidylcholine (lysoPC), ceramides, myristate derivatives, and carnitine derivatives. Enrichment analyses of these significant metabolites suggest chondroitin sulfate degradation with arginine, proline, and nitric oxide (NO) metabolism upregulated in OA. Another study conducted by Mickiewicz et al. [44] utilized NMR compared SF collected from patients with OA and post mortem samples. Remarkable decreases of methionine, N-phenylacetyl glycine, ethanol, creatine, O-acetyl-carnitine, and 3-hydroxybutyrate concentrations were observed in the OA. Alternatively, fructose and citrate concentrations were higher in OA than in non-OA controls. In an ovine model, the NMR assessed significantly dysregulated in an anterior cruciate ligament reconstruction injury through SF. They measured 65 metabolites, and 6 of them, that were isobutyrate, glucose, hydroxyproline, asparagine, serine, and uridine, were relevant to early post-injury degenerative. Moreover, a large percent of them were associated with the hypoxic and acidotic conditions result from injured and inflamed joint [45].

In summary, several types of biological fluids and tissue extracts of OA have been examined and have shown significant metabolic changes between OA and non-OA (Table 2).

## Metabolic pathways likely contributing to OA

During the initiation and development of OA, the majority of metabolomic profiling approaches reveal changes associated with energy metabolism (e.g., glucose metabolism,

**Table 2** Summary of the metabolites altered in OA samples

| Experimental model | Specimen | Techniques | Upregulated metabolites | Downregulated metabolites | References |
|---|---|---|---|---|---|
| Human | Cartilage | NMR | – | N-acetyl, alanine | [30] |
| Human | Synovium | GC–MS/LC–MS | Prolylhydroxyproline, glutamine, acetylcarnitine | 4-methyl-2-oxopentanoate | [31] |
| Human | Plasma | UPLC–MS | – | Arginine, PC, lysoPC | [36] |
| Human | Serum | LC–MS | BCAA | – | [37] |
| Human | Plasma | LC–MS | BCAA, lysoPC | PC | [46] |
| Human | Urine | NMR | Hydroxybutyrate, pyruvate glycerol | Methylhistamine, histidine | [39] |
| Human | Urine | GC–MS | Aconitate, isocitrate, citrate, histamine | Histidine, glutamine | [41] |
| Human | Synovial fluid | NMR | Fructose, citrate | Methionine, N-phenylacetyl glycine, ethanol, creatine, 3-hydroxybutyrate | [44] |
| Ovine | Synovial fluid | NMR | Isobutyrate, glucose | Hydroxyproline | [45] |

*UPLC-MS* ultra-performance liquid chromatography-tandem mass spectrometry

tricarboxylic acid cycle (TCA), and ß-oxidation pathway), lipid metabolism, the eicosanoid pathway, amino acid metabolism, and other metabolic factors [5]. The present studies reveal that the pathogenesis of OA may be significantly related to perturbations of amino acid metabolism. These altered amino acids (e.g., BCAA, arginine, and alanine), as well as phospholipids, were identified as potential biomarkers to distinguish patients with OA from healthy counterparts.

## Branched-chain amino acids

Leucine, isoleucine, and valine are essential amino acids, meaning they are not produced by our bodies and must be taken in as part of our diet. They are also known as BCAA due to their non-linear aliphatic side chains. They make up approximately one-third of skeletal muscle essential amino acids and are essential fuels for body energy metabolism. A number of studies have indicated that significantly dysregulated BCAA concentrations are linked to OA [47, 48]. Recent studies have rekindled BCAA as biomarkers associated with obesity since weight reduction improves the BCAA profile. Moreover, BCAA have been suggested as a major predictor of insulin resistance and cardiovascular diseases [49]. Meanwhile, obesity has long been recognized as a strong risk factor for knee OA, type 2 diabetes, and cardiovascular diseases, and cardiovascular diseases could lead to metabolic OA [50, 51]. BCAA are activators of the mammalian target of rapamycin (mTOR) signaling pathway that regulates a variety of biological functions, such as autophagy [52]. The increased BCAA levels could lead to reduced autophagy and change cell survival and overall tissue homeostasis. BCAA promote mononuclear cell migrations via an activation of mTOR1, which suggests a potential for increased inflammation in OA. BCAA supplements are associated with the increased production of the main proinflammatory cytokines involved in the pathophysiology of OA, including IL-1 and 2, tumor necrosis factor–alpha, and interferon-gamma [53], which are implicated in the degeneration of the articular cartilage matrix [54]. Figure 2 shows a schematic overview of the BCAA metabolic pathway in relation to OA.

## Arginine

Arginine is classified as a semi-essential amino acid in humans and serves as the precursor for the synthesis of many molecules, including urea, NO, proline, glutamate, creatine, and agmatine [55]. Arginine may lead to inflammation-associated diseases, including OA, due to the functions of anti-inflammation and anti-oxidation [56]. Furthermore, recent evidence suggests that arginine concentrations are decreased in patients with OA, which has likely contributed to the progression of OA [57, 58]. Furthermore, there are competing metabolic pathways for arginine as a substrate by arginase (ARG) and NO synthase (NOS). ARG catalyzes L-arginine into L-ornithine in the liver to facilitate the generation of urea. Ornithine can form a metabolic precursor for proline, a key amino acid especially enriched in collagen that contributes to collagen and polyamine synthesis and cell proliferation resulting in fibrosis [59, 60]. Meanwhile, arginine is catabolized to NO and citrulline by NOS [61]. However, the role of NO in the development of OA remains inconclusive. Some studies have suggested that NO and its redox derivatives may play protective roles in a joint [62]. However, other studies have shown a destructive role in mediating the inflammatory response and apoptosis, inhibiting the synthesis of both collagen and proteoglycan, and activating matrix metalloproteinases [43, 63]. A schematic overview of the arginine metabolic pathway in relation to OA is shown in Fig. 3.

## Phospholipids

Phospholipids are important components of the SF that contribute to articular joint lubrication [43]. Kosinski et al. found that SF contains several phospholipid classes including PC,

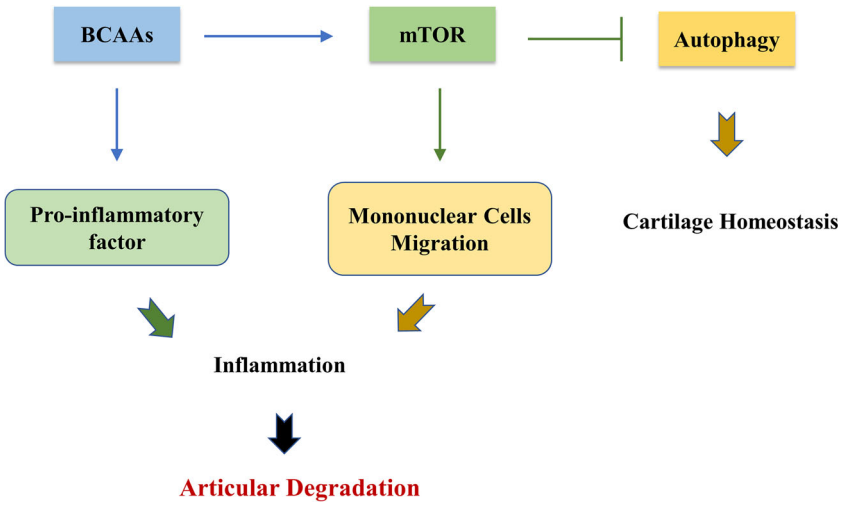


**Fig. 2** Schematic overview of the BCAA metabolic pathway in relation to OA. BCAA are activators of the mTOR signaling pathway that regulates autophagy. The increased BCAA levels would result in reduced autophagy to change tissue homeostasis through this pathway. BCAA promote mononuclear cell migration via an activation of mTOR1 that suggests the potential for increased inflammation in OA

lysoPC, and phosphatidylethanolamine. Moreover, PC is the key phospholipid class in SF. Compared with the controls, SF from patients with OA had higher levels of phospholipids. Therefore, phospholipids, such as PC and lysoPC, may be associated, at least in part, with the pathogenesis of OA [64, 65]. PCs are converted into lysoPCs by phospholipase A2 (PLA2) [66]. The increased lysoPC-to-PC ratio [46] suggests an increased activity of PLA2 to convert PCs to lysoPCs, which reveals either an increased lysoPC concentration or a decreased PC concentration. Furthermore, the deficiency in PCs, especially unsaturated PCs, could lead to articular cartilage damage [67]. PLA2 acts as a chemoattractant at the sites of inflammation and promotes the inflammatory reaction [36], which suggests that PLA2 may be an important effector contributing to OA development. Subsequently, lysophosphatidic acid (LPA) is generated by autotaxin from lysoPCs [68]. An increased level of autotaxin has been detected in patients with OA compared to normal controls [69], and LPA is involved in the development of neuropathic pain and contributes to inflammation [70, 71]. In addition, the increased activity of PC to lysoPC could release free fatty acids, such as arachidonic acid, which may lead to OA joint symptoms, such as cartilage degradation [72]. A schematic overview of the phospholipid metabolic pathway in relation to OA is shown in Fig. 4.

## Alanine

Alanine, one of the 20 amino acids that constitute the proteins of the human body, is synthesized in the liver and obtained from diet. The synthesis of carnosine in skeletal muscle is limited by the availability of beta-alanine. In addition, as a functional amino acid, alanine plays an important role in cell growth and physiological metabolism [73]. It is generally believed that alanine is dysregulated in OA [74, 75]. Through the alanine-glucose cycle, alanine is converted into glutamate and pyruvic acid. Subsequently pyruvic acid participates in the Cori cycle, and then both of them enter the TCA cycle to regulate energy metabolism [76]. Evidently, a decreased level of alanine concentration in cartilage was found that may be associated with degradation of the collagen framework with OA progression [30]. Adenosine triphosphate (ATP), besides being the elementary source of energy in humans, has also been shown to play a vital role in regulating chondrocyte function and repairing damaged cartilage [77], for the reason that the decrease of alanine may be attributed to the depletion of ATP in chondrocytes derived from cartilage. On the

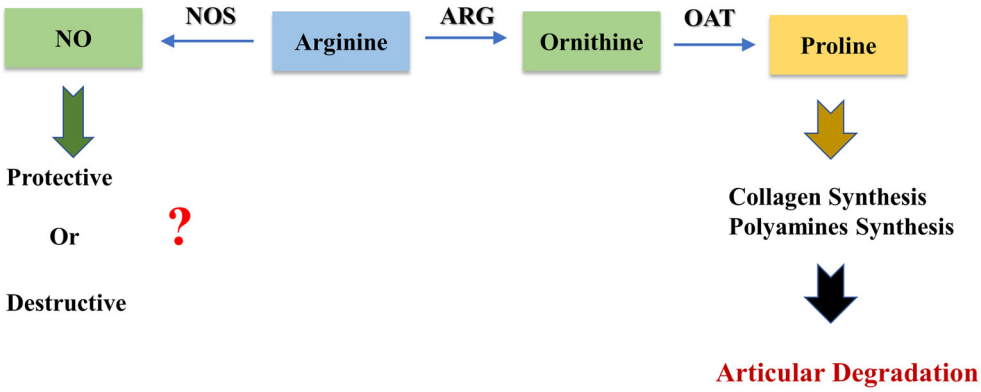


**Fig. 3** Schematic overview of the arginine metabolic pathway in relation to OA. NOS and ARG catalyze the conversion of arginine to NO and ornithine, respectively. Subsequently, ornithine is converted by OAT into proline, a contributor to collagen and polyamine synthesis, resulting in articular degradation. However, the role of NO in the development of OA is still inconclusive

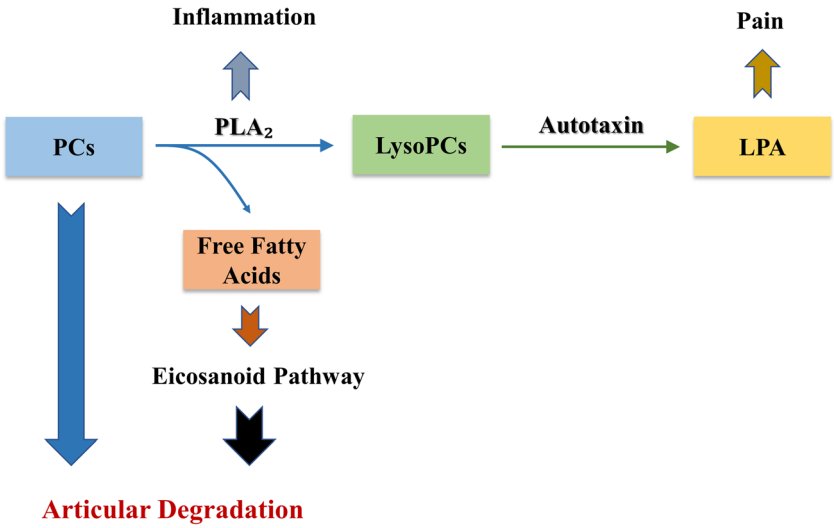


**Fig. 4** Schematic overview of phospholipid metabolic pathway in relation to OA. PCs are converted into lysoPCs by PLA2, and arachidonic acid, belonging to free fatty acid, is produced by the conversion of PCs to lysoPCs. Deficiencies in PCs and the eicosanoid pathway may lead to articular degradation. Subsequent metabolism of lysoPCs via autotaxin generates LPA, which is involved in the development of neuropathic pain and contributes to inflammation

contrary, an increased level of alanine concentration related to the sclerosis of subchondral bone was also found [32]. We know that alanine can provide energy to increase work performance and power output in the muscle cell [78]. Energy consumption was increased in bone cells due to the increased activity of the osteoblast and osteoclast in the sclerotic subchondral bone. Therefore, it is supposed that alanine might have a similar role to increase energy consumption in both muscle cell and bone cells. This points out the significant effect of alanine in the pathogenesis, suggesting an abnormal alanine metabolism occurring in OA. Further, carefully designed studies are required to identify the different performances of alanine in different lesions of OA joint. In this way, we can use alanine as an indicator to fix the lesion to implement symptomatic treatment in the earlier stages of disease. Figure 5 shows a schematic overview of the alanine metabolic pathway in relation to OA.

## Conclusions and future direction

Metabolomics has been applied to several disorders. Here, we have described the evidence in both animals and humans showing the potential of metabolomics as a promising tool for investigating OA. Many metabolic samples have been evaluated, such as blood, urine, SF, and tissue extracts. Table 2 summarizes the metabolic changes of OA in different sample types. Considering that OA is recognized as a tremendous heterogenous and multifactorial disease, multiple markers are essential. Hopefully, useful biomarkers can be provided for OA, therefore, allowing both diagnosis and prognosis. The current literature data suggest that the metabolic pathogenesis of OA may be significantly related to perturbations of amino acid metabolism. These altered amino acids (e.g., BCAA, arginine, and alanine), as well as

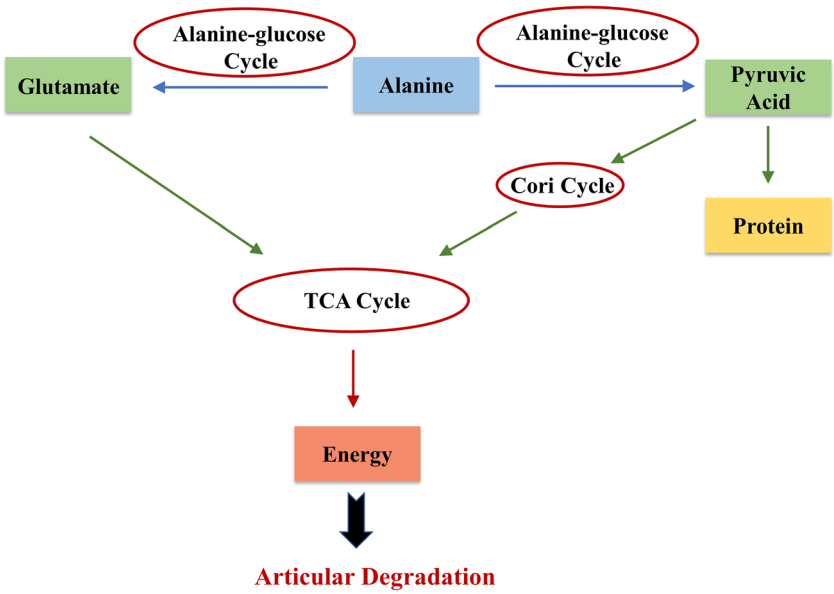


**Fig. 5** Schematic overview of alanine metabolic pathway in relation to OA. Through the alanine-glucose cycle, alanine is converted into glutamate and pyruvic acid. Subsequently pyruvic acid participates in the Cori cycle, and then both of them enter the TCA cycle to regulate energy metabolism

phospholipids, were identified as potential biomarkers to distinguish OA from healthy individuals.

Recently, the ingestion of amino acids has actively been investigated in nutritional science, and BCAA, as well as arginine, could be used as novel nutraceuticals for OA as mentioned above. A present clinical study has shown that exercise therapy combined with BCAA supplements could lead to a significant improvement in contralateral hip abductor muscle strength in women with hip OA [79]. The elderly are more susceptible to OA, and the BCAA uptake response is reduced with aging [80]. Therefore, BCAA supplements may be effective for OA. Unlike BCAA, the body can synthesize L-arginine, but exogenous supplements of L-arginine may also be necessary. Oral supplementation with L-arginine is a benefit to cardiovascular functions and may help treat certain medical conditions [81, 82]. Meanwhile, L-arginine supplementation is used to enhance both tissue growth and general performance, potentiate the ergogenic potential and muscle tolerance to high intensive work and the gas exchange threshold, decrease the recovery performance period, and improve wound healing [83]. Arginine is also a potentially novel anti-obesity amino acid and may be beneficial for the treatment of obesity, which is a risk factor of OA [84]. Moreover, PCs are converted into lysoPCs by PLA2, which also produce LPA and free fatty acids. Thus, PLA2 may be a novel target to develop new drugs to improve OA. In parallel, LPA could be an attractive therapeutic target for both pain and OA pathogenesis. Alanine is identified as a potential biomarker of OA. As mentioned, the performance of alanine varies at different lesions of the OA joint, such as cartilage and subchondral bone. Therefore, it is likely used as an indicator to fix the lesion region to implement symptomatic treatment in the earlier stages of the disease. While carefully designed studies are required to confirm these findings, metabolomics is an exciting field in systems biology that may broadly apply to clinical applications in the foreseeable future and minimize the negative effects of OA in society.

**Author's contributions** All authors discuss the concept of the manuscript. JTL wrote the manuscript. GXN conceived the study; ZN, ZPY, and TL prepared some materials. All authors approved the final version of the manuscript.

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## Compliance with ethical standards

**Disclosures** None.

**Abbreviations** ACLT, anterior cruciate ligament transaction; ARG, arginase; ATP, adenosine triphosphate; BCAA, branched-chain amino acids; GC–MS, gas chromatography–mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; LysoPC, lysophosphatidylcholine; LPA, lysophosphatidic acid; MD, meniscal destabilization; mTOR, mammalian target of rapamycin; NMR, nuclear magnetic resonance; NO, nitric oxide; NOS, NO synthase; OA, osteoarthritis; OAT, ornithine aminotransferase; PC, phosphatidylcholine; PLA2, phospholipase A2; SF, synovial fluid; TCA, tricarboxylic acid; UPLC-MS, ultra-performance liquid chromatography-tandem mass spectrometry

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