Aims
Metabolic profiling is a top-down method of analysis looking at metabolites, which are the intermediate or end products of various cellular pathways. Our primary objective was to perform a systematic review of the published literature to identify metabolites in human synovial fluid (HSF), which have been categorized by metabolic profiling techniques. A secondary objective was to identify any metabolites that may represent potential biomarkers of orthopaedic disease processes.

Methods
A systematic review was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines using the MEDLINE, Embase, PubMed, and Cochrane databases. Studies included were case series, case control series, and cohort studies looking specifically at HSF.

Results
The primary analysis, which pooled the results from 17 published studies and four meeting abstracts, identified over 200 metabolites. Seven of these studies (six published studies, one meeting abstract) had asymptomatic control groups and collectively suggested 26 putative biomarkers in osteoarthritis, inflammatory arthropathies, and trauma. These can broadly be categorized into amino acids plus related metabolites, fatty acids, ketones, and sugars.

Conclusion
The role of metabolic profiling in orthopaedics is fast evolving with many metabolites already identified in a variety of pathologies. However, these results need to be interpreted with caution due to the presence of multiple confounding factors in many of the studies. Future research should include largescale epidemiological metabolic profiling studies incorporating various confounding factors with appropriate statistical analysis to account for multiple testing of the data.

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Article focus
- To identify all metabolites in human synovial fluid (HSF), which have been categorized by metabolic profiling techniques.
- To recognize any metabolites that may represent potential biomarkers of orthopaedic disease processes.

Key messages
- Over 200 metabolites have been identified in HSF from the published literature.
- A total of 26 putative biomarkers have been demonstrated in osteoarthritis, inflammatory arthropathies, and trauma.
The results should be interpreted with caution due to the presence of multiple confounding factors.

Strengths and limitations
- The study methodology was robust.
- The search criteria were broad to ensure all relevant articles were captured.
- There was notable heterogeneity between studies.

Introduction
Osteoarthritis (OA) is one of the most disabling conditions in the western world, affecting approximately 10% of the UK population and presenting a major healthcare burden. It is a heterogenous disease, which manifests in a number of different phenotypes due to various pathogenic factors, ultimately leading to an alteration of the whole joint structure. It results in progressive degradation of ligaments, cartilage and menisci, synovial inflammation, and changes to the subchondral bone with common clinical and radiological manifestations.

The risk factors for OA are multifactorial and involve a complex interplay between biochemical, cellular, and mechanical factors that ultimately lead to the same endpoint. Consequently, the risk factors for OA can vary among individuals.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by autoantibodies, systemic inflammation, and synovitis leading to damage of the affected joints. Early diagnosis is important to delay disease progression by starting early intervention. A well-known biomarker of RA is rheumatoid factor (RF). However, this is non-specific and detected in other rheumatic and non-rheumatic conditions such as malignancy, infection, and even in some normal individuals. Anticitrullinated protein antibodies (ACPAs) are other biomarkers that have been suggested as a useful tool to differentiate RA from other types of arthritis in the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria. However, as not all RA patients are seropositive for ACPA more reliable diagnostic biomarkers are still required.

Various ‘omics’ technologies including proteomics, transcriptomics, and genomics have been increasingly utilized for the identification of disease biomarkers including those for RA. Transcriptomics has helped discover defence-related and immunity genes in RA patients and to predict the effectiveness of infliximab, the anti-tumour necrosis factor-α (TNF-α) biological agent, in RA patients. Furthermore, genomics has demonstrated differences between ACPA-positive and ACPA-negative diseases.

Metabolic profiling (also known as metabolic phenotyping, metabolomics, and metabonomics) is an increasingly used approach, which studies the low-molecular-weight metabolites within a cell, tissue, or biofluid. These terms have been used interchangeably, leading to some confusion. Therefore, in this article, the term ‘metabolic profiling’ will be used, which is defined as “an individual’s metabolic pattern that would be reflected in the constituents of their biological fluids.”

Metabolic profiling is a top-down method of analysis as it is looking at the metabolites, which are the intermediate or end products of various cellular pathways. Analyzing their concentrations provides a useful avenue to understanding the relationship of their cellular processes and biological reactions. As well as genetic factors, this process accounts for various environmental factors such as diet, medication, smoking, and disease. Typically, it is conducted with biofluids, the most common of which are blood serum/plasma and urine. It can lead to the formation of a ‘metabolic fingerprint’, which is unique to a particular biochemical perturbation, characteristic of a particular disease process, or toxic stimulus among other things.

Metabolic profiling has the ability to detect and potentially quantify hundreds or even thousands of small molecules simultaneously. The most common techniques employed are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). NMR spectroscopy is based on the same physical principles as MRI. It uses the magnetic property of the nuclei called spin to study the interaction of nuclei of the different atoms in a molecule, being therefore useful to determine the structures of molecules. The most commonly used nuclei is the proton (1H) due to its natural abundance in nature (close to 100%). NMR spectroscopy is fast and non-destructive, allowing multiple samples to be measured daily, and the same sample can be analyzed multiple times. MS is more sensitive with greater metabolite coverage than NMR spectroscopy, but it often requires prior separation of the different types of compounds using chromatography. Liquid chromatography (LC), particularly ultra-high-performance liquid chromatography (UPLC) is being more frequently used due to its increased compound resolution and higher throughput. Other techniques include gas chromatography-mass spectrometry (GC-MS), which is more useful for volatile compounds. Regarding biofluids, LC-MS is typically employed, usually with both positive and negative ion detection modes using standard protocols. However, MS does involve sample consumption, thus preventing multiple testing of the same sample. It has been used successfully in clinical medicine, toxicology, environmental science, and plant science. It has also been employed in a number of conditions to influence clinical practice. The various metabolic profiling techniques are often used together to provide a wider coverage of the metabolic space.

Metabolic profiling may be well suited for the purposes of orthopaedic research due to the great heterogeneity of the different disease processes including OA and inflammatory arthropathies such as RA, with recognition that no single biomarker is capable of explaining the breadth of pathological and temporal processes associated with these conditions. Combining several biomarkers would
also increase its discriminatory capacity. Furthermore, as metabolic perturbations occur in real time, they indicate the current disease state, thus providing a distinct advantage over other disease monitoring and diagnostic techniques such as radiography.

More recently, metabolic profiling has been used to identify metabolites within the urine, blood, and synovial fluid (SF) of both animal models and patients with OA. Changes in joint metabolism may be a contributing factor to the pathogenesis of OA. Previous metabolic analysis of SF has led to a better understanding of the metabolic processes associated with OA and to the identification of some of the biomarkers of OA.

The aim of this systematic review was to identify metabolites in human SF (HSF), which have been categorized by metabolic profiling techniques. The secondary aim was to identify any metabolites that may represent potential biomarkers of orthopaedic disease processes.

The scope of this systematic review is to look at the role of metabolic profiling in identifying the small molecule metabolites in HSF and identify any that may represent putative biomarkers, specifically using the techniques associated with metabolic profiling including MS and NMR spectroscopy. Therefore, studies looking at macromolecules including cytokines and interleukins (ILs), plus studies utilizing the techniques of genomics, proteomics, and transcriptomics, were considered outside the scope of this article.

Methods

A systematic review was undertaken in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.

Eligibility criteria. Inclusion criteria consisted of published articles and abstracts in English looking at small molecule metabolism of HSF in any disease state using metabolic profiling techniques. The exclusion criteria were articles not written in English, patients less than 18 years old, expert opinions, review articles, and studies using the same cohort of patients.

Identification of studies. A systematic literature review was conducted of the MEDLINE (Medical Literature Analysis and Retrieval System Online), Embase (Excerpta Medica Database, Amsterdam, The Netherlands), PubMed, and Cochrane databases without date restrictions on 1 August 2018. The search terms used are detailed in Supplementary Table i.

Screening and assessment of eligibility. Two independent reviewers (PA and UK) looked at the titles of the articles identified in the preliminary literature search. Any disagreement resulted in the article proceeding to the next stage of review. The same authors then read the abstracts of the remaining articles. Any disagreement resulted in the articles proceeding to full-text review. The full-text articles were then reviewed by the same authors and any conflict was discussed to achieve consensus.

Risk of bias (quality) assessment. The articles were evaluated for relevance, sample numbers, the underlying disease process, statistical power, analytical validity, quality of evidence, and conclusions. The Newcastle-Ottawa Scale was used to evaluate the study design. Relevant metabolites were highlighted and where statistical testing was performed, significance was quoted. The metabolites themselves were identified using various commercial software packages such as Chenomx NMR Suite (Chenomx, Edmonton, Canada), as well as by identifying them from published databases including the Human Metabolome Database, the Biological Magnetic Resonance Bank, and various in-house databases.

Results

Literature search. The electronic database searches identified 4,477 articles. Following exclusion of any duplicates and reviewing the titles, 4,391 articles were excluded. The abstracts of the remaining 86 articles were reviewed and a further 14 were excluded as they contained duplicate data. Of the remaining 72 articles, 25 were excluded for not meeting the entry criteria, two were removed as they contained data from the same cohort, three were excluded because they did not look at HSF, and two were removed as they looked specifically at synovial membranes and not SF. Of the remaining 40 articles, one article was excluded as it had duplicate data, one was excluded as it only looked at serum and not SF, two were excluded for not using metabolic profiling techniques, 11 were removed because the metabolites were not clearly identified or only a portion of them were presented, and four were excluded as it was unclear in these articles which cohort the metabolites were found in greater quantities. As a result, 21 studies were eventually used (17 articles and four abstracts) (Figure 1).

Study characteristics and quality. The methodology of the published studies was assessed using the Cochrane criteria for bias and the Newcastle-Ottawa Scale. The studies included had similar designs and metabolic profiling techniques. Patient selection was not random, as all studies were looking at specific disease processes. Furthermore, blinding was not possible at sample collection for either the researcher or the patient. Multivariate analysis was performed to detect patterns of changes in the metabolites detected, which did not necessarily involve significance testing. These types of analyses were performed mostly using supervised methods, which required information about the sample class, and therefore the data could not be blinded. As p-values were not consistently reported in all the studies, reporting bias may exist towards those that do so. Furthermore, studies that involve assaying hundreds of metabolites may have overestimated the significance of the p-values, unless false discovery rate (FDR) or validation datasets were utilized. All the identified studies are listed in Table 1.
All the identified metabolites have been listed in Supplementary Table ii. The identified studies have been subdivided into those with healthy controls that have identified putative biomarkers and those looking at specific disease processes.

**Studies with a healthy control group.** Adams et al.\(^3^\) examined the cytokine and metabolic differences between healthy and end-stage post-traumatic arthritic ankle (PTAA) joint SF. They identified 29 metabolites in significantly different concentrations between the PTAA and control groups, the most important of which is glutamate. Their findings suggest a mainly oxidative and pro-inflammatory environment with an imbalance in amino acid (AA) and lipid metabolism among other factors. However, there are no p-values stated in the paper and no FDR or other analysis was performed to account for multiple testing.

The metabolic changes in the physiological responses of early knee OA were performed by Chen et al.\(^4^3\). They identified 22 significant metabolic differences between the two groups. Most serum AA levels were found to be altered in the OA group, suggesting that OA is accompanied and precipitated by changes in AA metabolism. They identified three potential biomarkers: alanine, \(\gamma\)-aminobutyric acid (GABA), and 4-hydroxy-L-proline (Hyp). Alanine and Hyp were increased in the OA group and GABA was reduced in the OA group.

Dubey et al.\(^4^6\) explored whether metabolic profiling would identify a distinctive metabolic signature of seronegative spondyloarthropathy (SSA) that is not influenced
| First author/year | Study design | Country of origin | Joint | Diagnosis | Disease staging | Sample size | Type of analysis | Validated analysis | Controls | Statistical validity | NOS |
|-------------------|--------------|-------------------|-------|-----------|----------------|-------------|-----------------|-------------------|----------|---------------------|-----|
| Adams et al, 2014  | Case control | USA | Ankle | Radiological | Takakura grading | n = 20; c = 20 | UHPLC-MS/MS | Weak | Healthy asymptomatic patients | Adequate | 7 |
| Ahn et al, 2015   | Case series  | South Korea | N/A | Clinical | N/A | n = 24 | GC-TOF-MS | Strong | None | Adequate | 3 |
| Anderson et al, 2014 | Cohort study | UK | Knee | N/A | N/A | n = 10 (OA); n = 14 (RA) | 1H-NMR | N/A | None | Adequate | 0 |
| Carlson et al, 2018 | Case control | USA | N/A | N/A | N/A | n = 5 (OA); n = 3 (RA); c = 5 | LC-MS | Weak | Post-mortem samples | Adequate | 3 |
| Chen et al, 2018  | Case control | China | Knee | Clinical/ radiological | KL | n = 32; c = 35 | UHPLC-TQ-MS | Weak | Healthy asymptomatic patients | Adequate | 8 |
| Dubey et al, 2019  | Case series  | India | Knee | N/A | N/A | n = 8 | 1H-NMR | N/A | None | Adequate | 0 |
| Dubey et al, 2019  | Cohort study | India | Knee | Clinical | N/A | n = 19 (ReA); n = 13 (USPA) | 1H-NMR | N/A | None | Adequate | 0 |
| Dubey et al, 2019  | Case control | India | Knee | Clinical | Braun’s, ASAS, and ACR criteria | n = 52 (SSA); n = 29 (RA); c = 82 | 1H-NMR | Weak | Healthy asymptomatic patients | Adequate | 6 |
| Furman et al, 2017 | Case control | USA | Knee | Clinical/ radiological | Not applicable | n = 8; c = 8 | UHPLC-MS/MS | N/A | Contralateral non-injured knee | Adequate | 7 |
| Hwang et al, 2013 | Cohort study | South Korea | N/A | N/A | N/A | n = 18 (RA); n = 11 (OA) | GC-TOF-MS | N/A | None | Adequate | 6 |
| Kang et al, 2015 | Case series | South Korea | Knee | Clinical/ radiological | KL (OA); ACR (RA) | n = 10 (OA); n = 10 (RA) | UPLC-QTOF-MS | Weak | None | Adequate | 5 |
| Khatib et al, 2018 | Case series | UK | Knee | N/A | N/A | n = 13 | 1H-NMR | N/A | None | Adequate | 3 |
| Kim et al, 2017   | Case series  | South Korea | Knee | Clinical/ radiological | KL | n = 8 (KL1 to 2); n = 7 (KL3 to 4) | GC-TOF-MS | Strong | None | Adequate | 4 |
| Kim et al, 2014   | Case series  | South Korea | N/A | Clinical/ radiological | ACR for RA; ASAS for AS; criteria of the 1990 ISG for BD; MSU crystals in joint fluid for gout. | n = 13 (RA); n = 7 (AS); n = 5 (BD); n = 13 (gout) | GC-TOF-MS | Adequate | None | Adequate | 4 |
| Leimer et al, 2017 | Cohort study | USA | Ankle | Radiological | N/A | n = 19; c = 19 | UHPLC-MS/MS | Adequate | Contralateral non-injured ankle | Adequate | 8 |
| Mekhitsuoka et al, 1999 | Case series | Japan | Knee | Clinical/ radiological | ACR | n = 14 (RA); n = 16 (OA) | 1H-NMR | Adequate | None | Adequate | 2 |
| Mickevicz et al, 2015 | Cohort study | Canada | Knee | Clinical/ radiological | Clinical/ radiological | n = 8; c = 13 | 1H-NMR; GC-MS | Strong | Cadaveric controls | Adequate | 6 |
| Naughton et al, 1995 | Cohort study | UK | Knee | N/A | N/A | n = 22 (RA); c = 6 | 1H-NMR | Adequate | Healthy asymptomatic patients | Adequate | 5 |
| Yang et al, 2015  | Case control study | China | Knee | ACR | N/A | n = 25 (RA); c = 10 | GC-TOF-MS | Adequate | Above knee amputated patients | Adequate | 6 |
| Zhang et al, 2014 | Case series | Canada | Hip/ knee | ACR | ESOA | n = 80 | LC-MS | Adequate | None | Adequate | 5 |
| Zhang et al, 2015 | Case series | Canada | Knee | N/A | ESOA | n = 69 | LC-MS | Adequate | None | Adequate | 5 |
| Zhang et al, 2016 | Case control study | Canada | Knee | ACR criteria and clinical judgement | ESOA | n = 97 | LC-MS | Adequate | No SF sample controls (only serum) | Adequate | 6 |
| Zheng et al, 2017 | Cohort study | China | Knee | KL | KL2 and KL4 | n = 49; c = 21 | GC-TOF-MS and LC-MS | Adequate | Asymptomatic patients | Adequate | 7 |

1H-NMR, nuclear magnetic resonance spectroscopy; ACR, American College of Rheumatology; AS, ankylosing spondylitis; ASAS, Assessment of SpondyloArthritis international Society; BD, Behçet’s disease; C, control group; ESOA, end-stage osteoarthritis; GC-MS, gas chromatograph-mass spectrometry; GC-TOF-MS, gas chromatography/time-of-flight mass spectrometry; ISG, International Study Group; KL, Kellgren and Lawrence; LC-MS, liquid-chromatography mass spectrometry; MSU, monosodium urate; N/A, not available; NOS, Newcastle-Ottawa Scale; OA, osteoarthritis; RA, rheumatoid arthritis; ReA, reactive arthritis; SSA, seronegative spondyloarthropathy; TQ MS, triple quadrupole mass spectrometry; UHPLC-MS/MS, ultra-high performance liquid chromatography/tandem mass spectrometry; UPLC-QTOF-MS, ultraperformance liquid chromatography quadruple time-of-flight mass spectrometer; UHPLC-TQ-MS, ultra-high performance liquid chromatography triple quadrupole mass spectrometry; USPA, undifferentiated spondyloarthropathy.
by age and sex. Their control group consisted of two subgroups of healthy patients, who were stratified by age, creating a young and older control group. There were a number of patient cohorts consisting of those with reactive arthritis (Rea), SSA, and RA. They suggested low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), leucine, lysine/arginine, acetone, glycine, glucose, creatine, polyunsaturated fatty acids (PUFAs), and phenylalanine as putative biomarkers for Rea when compared to an age-matched control group. Conversely, leucine, lysine/arginine, phenylalanine, and valine were suggested as putative biomarkers for discriminating between Rea and RA.

An abstract published by Furman et al.47 analyzed healthy and injured knees to identify any metabolic pathways affected by the knee injury and identify any discriminatory SF biomarkers. They demonstrated significantly increased sphingomyelin (SPM) and 2-hydroxy-fatty acids in the injury group. They suggested that these may be potential SF biomarkers of knee injury and may be prognostic indicators of the risk of post-traumatic arthritis.

Leimer et al.53 attempted to characterize the global metabolic profile of SF after intra-articular ankle fractures with an emphasis on changes in the lipid profile. They identified 16 lipid-based metabolites found in significantly greater quantities following an intra-articular ankle fracture, which subsequently decreased six months post-surgery. Most long-chain fatty acids (FAs) and PUFAs were acutely elevated in the fractured ankles at baseline compared to the control group. However, none of these were suggested as potential biomarkers. They suggest the distinctive lipid signature identified is reflective of injury, fracture, and early changes associated with OA.

The metabolic status of normal and RA SF was assessed qualitatively by Naughton et al.56 They demonstrated increased levels of LDL in RA compared to the control group and suggested this to be secondary to inflammation and increased synovial membrane permeability. However, the controls were not age-matched (25 to 42 years old) and were younger than the RA group (40 to 67 years old), which may result in important metabolic differences.

Zheng et al.61 explored the metabolites of OA. Six metabolites were significantly different between the two groups. Three were found in significantly greater concentrations in the OA group and three in the control group. Gluconic lactone, threonine, and 1,5-Anhydroglucitol (1,5-AG) were in significantly greater concentrations in OA SF compared to the control group. Glutamine, tyramine, and 8-aminoacaprylic acid were in significantly lower concentrations in OA SF compared to the control group. The authors concluded that a new diagnostic model combining two metabolites provides greater sensitivity in diagnosing OA than a single metabolite alone. Furthermore, gluconic lactone may prove to be a novel benchmark for the differential diagnosis of OA from RA due to the significant differences in the concentration of this metabolite between these conditions with a high level of sensitivity and specificity between them.

None of these studies performed FDR or other analysis to account for multiple testing of the data. Consequently, the results must be reviewed with caution. The metabolites identified in this section, which have been proposed to serve as putative biomarkers, are listed in Table II.43,46,47,56,61

### Osteoarthritis studies

Mickiewicz et al.62 identified two metabolites found in significantly greater concentrations in OA SF (fructose and citrate) and nine metabolites (O-acetylcarnitine, hexanoylcarnitine, N-phenylacetylglutine, ethanol, ethanolamine, methionine, malate, creatine, and 3-hydroxybutyrate) found in lower concentrations in OA SF compared to cadaveric controls.

Kim et al.51 characterized the metabolite differences between early- and late-stage OA. They identified 28 metabolites as being significantly different between the groups, with all 28 increased significantly in late-stage OA.

Zhang et al.58 examined the metabolic markers in SF that can be used to classify patients with OA into distinct

| Underlying pathway | Metabolite | Change | Multivariate analysis |
|--------------------|------------|--------|-----------------------|
| OA                 | Alanine43  | Increased in OA | VIP 3.31, p < 0.001 |
|                    | Hyp43      |         | VIP 1.75, p < 0.001   |
|                    | Glucone lactone43 |         | FC 1.54, p < 0.05 |
|                    | Threonine43 |         | FC 2.71, p < 0.05 |
|                    | 1,5-AG43   |         | FC 1.67, p < 0.05 |
|                    | GABA43     | Decreased in OA | VIP 2.61, p < 0.001 |
|                    | Glutamine61 |         | FC 0.28, p < 0.05 |
|                    | Tyramine61 |         | FC 0.30, p < 0.05 |
|                    | 8-Aminocaproic acid61 |         | FC 0.27, p < 0.05 |
| Inflammatory arthropathies | Acetone61 | Increased in ReA | FC 1.54, p < 0.006 |
|                    | Creatine46  |         | FC 0.63, p < 0.001 |
|                    | VLDL46     |         | N/A                   |
|                    | Glucose46  |         | FC 1.12, p < 0.367 |
|                    | Glycine46  |         | FC 1.03, p < 0.02   |
|                    | LDL46      |         | N/A                   |
|                    | Leucine46  |         | FC 0.83, p < 0.051 |
|                    | Lysine/arginine46 |         | FC 0.78/1.21, p < 0.002/p < 0.46 |
|                    | Phenylalanine61 | Increased in ReA vs RA | FC 1.33, p < 0.12 |
|                    | PUFA46     |         | N/A                   |
|                    | Leucine46  | Increased in ReA vs RA | FC 1.88, p < 0.001 |
|                    | Lysine/arginine46 |         | FC 1.46/2.07, p < 0.005/p < 0.001 |
|                    | Phenylalanine61 |         | FC 2.56, p < 0.001 |
|                    | Valine46   |         | FC 1.57, p < 0.001 |
| RA                 | LDL46      | Increased in RA | N/A                   |
| Knee injury        | SPM47      | Increased in knee trauma | p < 0.0065 following FDR |
|                    | 2-hydroxy-fatty acids47 |         | p < 0.0065 following FDR |

1 1-AG, 1,5-Anhydroglucitol; FC, fold change; FDR, false discovery rate; GABA, γ-aminobutyric acid; Hyp, 4-hydroxy-4-proline; LDL, low-density lipoprotein; N/A, not available; OA, osteoarthritis; PUFA, polyunsaturated fatty acid; RA, rheumatoid arthritis; Rea, reactive arthritis; SPM, sphingomyelin; VIP, variable importance on projection score; VLDL, very low-density lipoprotein.
subgroups. Broadly speaking, they identified numerous metabolites included 40 acylcarnitines (one free carnitine), 20 AAs, nine biogenic amines, 87 glycerophospholipids, 11 sphingolipids, and one hexose (> 90% was glucose). Following multivariate analysis, they identified subgroups of OA, which differed in acylcarnitine levels and fat metabolism. They observed distinctions in the glycerophospholipids and SPMs. However, as no age-matching and no correlation to clinical factors took place, it is difficult to draw definitive conclusions.

Using a similar methodology, Zhang et al. later investigated the differences between OA and type II diabetes mellitus. Of note, leucine and phosphatidylcholine (PC) metabolism were influenced by both diabetes mellitus and OA. Phosphatidylcholine is involved in many membrane-related phenomena including forming the essential lipid bilayer of all biological membranes, regulation of membrane trafficking, and signal transduction.

An abstract by Khatib et al. investigated whether the mechanical loading of the joint during pivot shift will reveal a profile of mechanically regulated metabolic biomarkers in patients with ACL deficient knees. Using nuclear magnetic resonance spectroscopy (1H-NMR), they identified a significant difference in alanine and choline between pre- and post-pivot shift testing of ACL deficient knees. These metabolites remained significant when accounting for multiple testing and the authors suggest they might be useful for rehabilitation or surgical intervention in patients with knee injuries who may be at risk of post-traumatic OA.

**Inflammatory arthropathy studies.** A recent study explored RA-related biochemical abnormalities by analyzing the metabolic profile of knee SF from RA patients and a control group. These controls were patients who had a ‘high-level’ amputation. However, the paper does not state the reason for the amputation nor whether the sample was taken before or after amputation, which may have important metabolic consequences. Following multivariate analysis and using a variable projection of importance score (VIP) > 1 plus p < 0.05, 13 of these metabolites were significant between the two groups. Glucose was decreased and lactic acid was increased in RA SF. Levels of glucose-1-phosphate and D-mannose were also decreased.

Ahn et al. evaluated the metabolomic profile of SF in patients with Behçet’s disease (BD) with arthritis compared to those with seronegative arthritis (SNA). They identified 11 metabolites as being significantly increased in BD with arthritis compared to SNA. These include branched-chain AAs (BCAA: valine, leucine, and isoleucine), citramalate, glutamate, and methionine sulfoxide.

In an earlier study, Kim et al. also evaluated potential biomarkers for RA. Their study consisted of patients with RA (n = 13), ankylosing spondylitis (AS) (n = 7), BD (n = 5), and gout (n = 13). These patients were then combined into two groups, which were RA and non-RA. They identified 20 metabolites that remained significantly different between the two groups following robust statistical analysis, which they proposed could be putative biomarkers. Of these, 14 were in significantly greater concentrations in the RA group and six were in greater concentrations in the non-RA group.

**Osteoarthritis versus rheumatoid arthritis studies.** Carlson et al. evaluated global liquid chromatography coupled to mass spectrometry (LC-MS) based metabolic profiles as a tool for quantifying biomarkers within SF. Their control group consisted of five purchased post-mortem samples. It is unclear how long after death these samples were harvested and the death to post-mortem interval is likely to be a major confounding variable, so this uncertainty might have important metabolic consequences. They identified five metabolites (citrnic acid, D-lactic acid methyl ester, hydroxyl-L-proline, L-isoleucine, and L-methionine) found in significantly lower concentrations in OA and RA SF, compared to controls and one metabolite (L-citrulline) found in greater concentrations in OA compared to RA and controls. The authors also performed FDR analysis to account for multiple testing.

A recently published abstract explored the role of NMR spectroscopy in producing analyzable spectra from a low volume of SF taken in a clinical environment. They identified 11 metabolites found in significantly different concentrations between OA and RA SF. Seven were more abundant in OA and six were more abundant in RA SF. Their analysis suggested the metabolic pathways most impacted were: aminoacyl-transfer RNA (tRNA); biosynthesis; nitrogen metabolism; valine, leucine, and isoleucine biosynthesis; glycine, serine, and threonine metabolism; and taurine and hypotaurine metabolism. The authors allude to their methodology being useful for analyzing low-volume SF. However, in their methodology they state that each sample consists of approximately 100 ml. Furthermore, although the authors mention the term “FDR < 0.05”, it is unclear exactly what analysis took place.

Another abstract investigated the metabolites of SF in patients with RA and OA to identify the characteristic metabolites differentiating the two diseases. Using gas chromatography/time-of-flight mass spectrometry (GC/TOF MS) and following multivariate analysis, they identified 17 metabolites as being found in significantly different concentrations between the two groups. Six were upregulated in RA (maltose, lignoceric acid, uracil, mannitol, pyrophosphate, and phosphoric acid) and 11 in OA (lysine, tyrosine, valine, glycic acid, alanine, asparagine, hydroxylamine, tryptophan, glycerol, glutamine, and citrulline).

Kang et al. identified 21 metabolites as being in significantly different concentrations between RA and OA SF. Concentrations of lipid metabolites were typically higher in RA than OA SF, which has previously been demonstrated. Concentrations of tryptophan metabolites also differed significantly between the two groups.
The metabolic homeostasis within a joint is often disturbed in the disease state leading to an anaerobic state secondary to stress and inflammation. Whether these changes differ between the diseased joint and the normal joint is an important question when considering the importance of diagnostic or prognostic putative biomarkers.

**Role of the identified putative biomarkers.** Broadly speaking, the putative biomarkers in OA can be classified into two main groups: AAs plus related metabolites (alanine, Hyp, threonine, GABA, glutamine, tyramine); and sugars plus related metabolites (gluconic lactone, 1,5-AG). Alanine, Hyp, and threonine are found in articular cartilage. Their increase in OA SF could be associated with increased catabolism of the articular cartilage. This may also represent increased energy consumption to account for the increased bone turnover and subchondral sclerosis seen in OA. 1,5-AG is a monosaccharide occasionally used as a short-term marker of glycaemia. Elevation of this metabolite in SF is consistent with the reduced glucose concentration in OA SF, secondary to increased energy expenditure. Increased glucronic lactone in OA SF may be due to auto-oxidation induced by increased levels of reactive oxygen species (ROS). ROS are able to directly induce cartilage degradation by cleaving aggregan and collagen plus activating matrix metalloproteinases (MMPs).

GABA arises from glutamic acid, which regulates glucose, also suggesting increased energy consumption in the diseased joint due to less residual glucose. Glutamine has a role in oxidative metabolism, and reduced levels suggest altered oxidative metabolism in diseased joints secondary to increased energy expenditure. Glutamine has been shown to suppres inflammatory cytokines and protect chondrocytes from heat stress and nitrous oxide (NO)-induced apoptosis. These effects may protect chondrocytes from various types of stress and prevent progressive cartilage degeneration in OA. Tyramine is derived from the AA tyrosine, which is thought to have a role in promoting osteophyte formation. Increased levels have been seen in subchondral bone.

The putative biomarkers increased in inflammatory arthropathies can be classified into four main groups: AAs and related metabolites (creatinine, glycine, leucine, lysine, arginine, phenylalanine, valine); lipids and lipoproteins (LDL, VLDL, PUFAs); sugars (glucose); and ketone bodies (acetone). They identified that AAs are all constituents of articular cartilage with leucine, proline, glutamic acid, and glycine specifically being constituents of proteoglycans. Their increase suggests breakdown of the articular cartilage, likely related to the underlying inflammatory process. Low concentrations of FAs have been demonstrated in HSF. The increased levels of LDL, VLDL, and PUFA identified here are secondary to increased synovial membrane permeability and inflammation associated with underlying inflammatory arthropathies.

**Metabolic changes seen in osteoarthritis.** Fructose elevation suggests a hypoxic condition of the disease and inflamed knee joint. Hypoxia has been shown to result in the upregulation of glucose phosphate isomerase, which catalyzes the conversion of glucose-6-phosphate (G6P) into fructose-6-phosphate (F6P) in inflammatory arthritis. Lower concentrations of O-acetylcarnitine, hexanoylcarnitine, N-phenylacetylglycine, and ethanolamine indicate protracted FA and lipid metabolism in the SF of OA patients compared to controls. Decreased methionine concentrations indicate its use, where it is likely converted to S-adenosylmethionine (SAM), a proposed factor for cartilage damage repair and inflammatory reduction.

Kim et al identified three unique pathways in their study, which corresponded to the metabolic differences they identified. These were FA metabolism, glycolipid metabolism, and the tricarboxylic acid (TCA) cycle. These pathways may be associated with an increasing degree in the severity of OA. Glycerol and various FA concentrations were more prominent in the late-stage OA group. Their findings suggest that FA biosynthesis is predominantly responsible for energy generation in late-stage OA. Furthermore, increased concentrations of malate in the late-stage OA group compared to the early-stage group suggest a possible difference in the energy level between the two groups.

Furthermore, alterations in the concentration and composition of phospholipids covering articular cartilage has been shown to be associated with the development of OA.

**Metabolic changes seen in inflammatory arthropathies.** Glucose was decreased and lactic acid was increased in RA SF. Levels of glucose-1-phosphate and D-mannose...
Metabolic network analysis of all the putative biomarkers identified in this systematic review demonstrating the associated metabolic pathways. All metabolites with a red outline were putative biomarkers. a) Putative biomarkers identified in osteoarthritic synovial fluid (SF). Those in blue were raised and those in orange were reduced in osteoarthritic SF compared to an asymptomatic control group. b) Putative biomarkers identified in inflammatory arthropathies. Those in green and blue were raised in reactive arthritis (rea) compared to an asymptomatic control group; those in blue were also raised in rea compared to rheumatoid arthritis (ra). Valine (in yellow) was raised in rea compared to ra. ADP, adenosine 5’-diphosphate; AMP, adenosine 5’-monophosphate; CoA, coenzyme A; GD1a, N-acetylneuraminyl-D-galactosyl-N-acetyl-D-galactosaminyl-N-acetylneuraminic acid-D-galactosyl-D-galactosylceramide; Gly, glycine; GM1, D-galactosyl-N-acetyl-D-galactosaminyl-N-acetylneuraminic acid-D-galactosyl-D-galactosylceramide; GM2, N-acetyl-D-galactosaminyl-N-acetylneuraminic acid-D-galactosyl-D-glucosylceramide; GM3, N-acetylneuraminyl-D-galactosyl-D-galactosylceramide; GM4, N-acetylneuraminyl-galactosylceramide; GSH, reduced glutathione; LacCer, lactosylceramide; L-Asp, L-aspartic acid; Neu5Ac, N-acetylneuraminic acid; NH3, ammonia; PRPP, 5-phosphoribosyl 1-pyrophosphate; R-COOH, carboxylic acid; ThPP, thiamin pyrophosphate; TPP, thiamin pyrophosphate.
were also decreased. These decreases may be explained
by the increased energy demands caused by inflammation
in RA. Furthermore, the increased consumption of
glucose can lead to increased lactic acid production.
Levels of citric acid were decreased in RA SF. Citric acid
is an important component of the TCA cycle, which
provides the complete oxidation of acetyl-coenzyme A (CoA)
derived from AAs, carbohydrates, and fats. Consequently,
this leads to decreased adenosine triphosphate (ATP) pro-
duction from the aerobic oxidation process. Yang et al
suggest that low glucose and high lactic acid concentra-
tions in RA SF may represent potential biomarkers of RA.

The increased levels of BCAAs, identified in the study
by Ahn et al, result in increased production of IL-1 and/or
TNF-α, which are typically increased in RA and SNA. Elevated expression of citramalate has been suggested to
indicate disturbed metabolism of glutamate in the setting
of active inflammation. Elevated expression of citrulline
and methionine sulfoxide were also identified in this
study. This may reflect neutrophil hyperactivity docu-
mented in BD. Ahn et al suggest that these metabolites
may act as potential biomarkers for discriminating BD
with arthritis from SNA. However, there are no normal
controls in their study.

The metabolites identified in a study by Kim et al are
major intermediates of FA and AA metabolism, the TCA
cycle, and the urea cycle. The authors suggest that AA
metabolism, the TCA cycle, and the urea cycle were more
activated in the RA group compared with those in the
non-RA group. Although the authors suggest these metabolites may be potential biomarkers, there are no
normal controls and several different disease processes
were compared. Consequently, it is difficult to say with
any certainty whether any of these metabolites are indeed
potential biomarkers.

**Metabolic changes seen between osteoarthritis and rheu-
matoid arthritis.** Kang et al identified 21 metabolites as
being in significantly different concentrations between RA
and OA SF. Concentrations of lipid metabolites were typi-
cally higher in RA than in OA SF, which has previously been
demonstrated. Furthermore, regulation of inflammation
includes the roles of lipid mediators and prostaglandins
(PGs). Leukotrienes and PGs are crucial in the develop-
ment of arthritic diseases. Concentrations of tryptophan
metabolites also differed significantly between the OA and
RA groups. This is an exogenous AA that must be provided
in the diet. Tryptophan and its metabolites are involved
in inflammation. One of the metabolites, kynurenine, has
well known anti-inflammatory effects that are toxic to T
cells and which cause apoptosis.

Although this systematic review has identified many
metabolites present in different disease states, including
some putative biomarkers, there are some important
limitations. There were only seven studies identified in
the literature with healthy controls. Furthermore, only two studies performed an analysis to account
for multiple testing of their dataset and neither of these
studies had healthy controls. The presence of multi-
ple confounding factors in many of the studies was
another important limitation. Not all studies accounted
for age or sex and certainly very few considered medi-
comorbidities. Consequently, the results must be
viewed with caution. The solution to these limitations
would be to conduct a largescale epidemiological met-
abolic profiling study incorporating multiple confound-
ing factors such as age, sex, medical comorbidities, and
medications with a view to addressing the correlations
between clinical features of disease, inflammation, and
metabolism. It should be noted that the majority of
the reported works are untargeted metabolic profiling
studies, where the identity of any putative biomarker is
unknown at the outset. In those cases where metabo-
lites were identified or annotated, no parameter of iden-
tification certainty, such as the Metabolomics Standards
Initiative (MSI) level of identification, was reported. Therefore, the occurrence of incorrect identifications is
possible hence affecting further metabolic interpreta-
tion and biomarker validation. Another important limi-
tation of some of the studies is that they do not provide
quantitative percentage or fold change, but only the
direction of change.

In conclusion, metabolic profiling is proving to be an
invaluable method of identifying putative biomarkers in
the field of orthopaedics unique to different patholo-
gies. Although numerous studies have been performed
using these techniques in human SF, larger studies are
required with healthy controls accounting for multiple
confounding factors and using robust statistical analysis
to identify putative biomarkers. This may lead to the
development of new diagnostic techniques and possible
treatment strategies. Recent advances in both proteomic
and genetic studies have demonstrated the importance
of these techniques to improve disease understanding
and identify biomarkers. Future studies integrating
genomic, proteomic, and metabolic profiling techniques
may provide the greatest hope for the advancement of
biomarker discovery.

**Supplementary Material**

Tables showing search terms used for this systematic
review on the role of metabolic profiling in human
synovial fluid (HSF) (Supplementary Table i), and a list of
all identified metabolites by article in HSF (Supplementary
Table ii).
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