Osteoarthritis and Cartilage 27 (2019) 560–570

Osteoarthritis and Cartilage

A systematic review of the small molecule studies of osteoarthritis using nuclear magnetic resonance and mass spectroscopy

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ARTICLE INFO

Article history:
Received 4 February 2018
Accepted 28 August 2018

Keywords:
Synovial fluid (SF)
Osteoarthritis (OA)
Metabolic syndrome (MeS)
Metabonomics
Nuclear magnetic resonance (NMR) spectroscopy
Mass spectroscopy (MS)

SUMMARY

Objective: To perform a systematic review of the small molecule metabolism studies of osteoarthritis utilising nuclear magnetic resonance (NMR) or mass spectroscopy (MS) analysis (*viz.*, metabolomics or metabonomics), thereby providing coherent conclusions and reference material for future study.
Method: We applied PRISMA guidelines (PROSPERO 95068) with the following MESH terms: 1. "osteoarthritis" AND ("metabolic" OR "metabonomic" OR "metabolomic" OR "metabolism") 2. ("synovial fluid" OR "cartilage" OR "synovium" OR "serum" OR "plasma" OR "urine") AND ("NMR" or "Mass Spectroscopy"). Databases searched were "Medline" and "Embase". Studies were searched in English and excluded review articles not containing original research. Study outcomes were significant or notable metabolites, species (human or animal) and the Newcastle–Ottawa Score.
Results: In the 27 studies meeting the inclusion criteria, there was a shift towards anaerobic and fatty acid metabolism in OA disease, although whether this represents the inflammatory state remains unclear. Lipid structure and composition was altered within disease subclasses including phosphatidyl choline (PC) and the sphingomyelins. Macromolecular proteoglycan destruction was described, but the correlation to disease factors was not demonstrated. Collated results suggested arachidonate signalling pathways and androgen sex hormones as future metabolic pathways for investigation.
Conclusion: Our meta-analysis demonstrates significant small molecule differences between sample types, between species (such as human and bovine), with potential OA biomarkers and targets for local or systemic therapies. Studies were limited by numbers and a lack of disease correlation. Future studies should use NMR and MS analysis to further investigate large population subgroups including inflammatory arthropathy, OA subclasses, age and joint differences.

Introduction

Osteoarthritis (OA) is the most disabling condition in western populations, affecting 10% of the UK population. It is multi-factorial and influenced by metabolism, genetics and the environment. Diagnosis is limited to radiological features of established disease. A suitable biomarker would aid early diagnosis and disease monitoring. The identification of OA biomarkers has had limited success with COMP (cartilage oligomeric matrix protein) and CTX type II (collagen type II telopeptide).

OA has been shown to associate epidemiologically with metabolic syndrome (MeS); a term denoting cardiovascular disease risk

Abbreviations: SF, synovial fluid; OA, osteoarthritis; MeS, metabolic syndrome; GCMS, gas-chromatography mass spectroscopy; RA, rheumatoid arthritis; BCAA, branch chain amino acids; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; PC, phosphatidylcholine; lysoPC, lysophosphatidylcholine; NMR, nuclear magnetic resonance; MS, mass spectroscopy; HPLC, high performance liquid chromatography; HRMAS, high resolution magic angle spinning; ACL, anterior cruciate ligament; N-Ac, N-acetyl; SH, sulphydryl; $DMSO_2$, Dimethylsulfone; NOS, Newcastle-Ottawa Score.

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https://doi.org/10.1016/j.joca.2018.08.024

factors involving increased insulin resistance, a proinflammatory state, atheropathic dyslipidaemia, hypertension or a combination thereof[1]. The incidences of OA and MeS diagnoses has been shown to associate independent of other factors in many studies[2–7]. Statin therapy in animal models has reduced catabolic OA cytokines and limited osteoarthritis, judged by histological assessment[8]. Furthermore, the inflammatory component of the disease appears more prevalent when associated with MeS[9,10]. Hence metabolism is altered in the disease pathogenesis, but also directly linked to disease propagation. Metabolic biomarkers have the potential to diagnose, predict disease and monitor treatment.

In search of agents to limit OA progression, the major focus has been cytokine action and protein signalling. Whilst many treatments exist to treat the symptoms of osteoarthritis, no clinical agent is capable of limiting or halting the rate of disease progression. However, matrix metalloproteases and protease cathepsin K have shown promise. The success of statins in animal models provides hope for metabolic manipulation in the future treatment of OA[8].

Clinical metabolic profiling is a method which comprehensively examines small molecules (<4 kDa) in biofluids or tissues using analytical techniques like nuclear magnetic resonance (NMR) spectroscopy or mass spectroscopy (MS). Significant patterns within the samples are analysed using univariate and multi-variate statistics. The advantage of the technique is the "top down" representation of disease phenotype encompassing environmental and external influences[11]. It is capable of providing biomarkers to: stage disease, subclassify, diagnose, monitor treatment, map populations and personalise healthcare.

The literature describing metabolic homeostasis in OA disease is often conflicting with variable study strength, analytical power and statistical robustness. We aimed to systematically review the evidence of small molecule metabolic changes in OA to present a clear and accurate summary.

Materials and methods

Search criteria and study evaluation

The systematic review utilised the PRISMA guidelines and registered with PROSPERO (95068). Inclusion criteria were articles studying small molecule metabolism in osteoarthritis as a primary or secondary outcome in any species, body fluid or tissue by comprehensive analytical methods of MS or NMR. Studies were searched in English and review articles or narratives without original research were excluded. The MEDLINE and EMBASE databases used the following Boolean search strings (last performed third June 2017):

1. "osteoarthritis" AND ("metabolic" OR "metabonomic" OR "metabolomic" OR "metabolism")
2. ("synovial fluid" OR "cartilage" OR "synovium" OR "serum" OR "plasma" OR "urine") AND ("NMR" or "Mass Spectroscopy")

8851abstracts were identified with 27 reports suitable for inclusion (see Fig. 1).

Quality assessment

Articles were reviewed by two authors (MJ and PA), evaluating relevance, sample numbers, species (human or animal model), statistical power, analytical validity, quality of evidence and conclusions. A Newcastle–Ottawa score evaluated the study design. Relevant metabolites were highlighted and where statistical testing was performed, significance was quoted.

Metabolite databases

A metabolic map was generated using the KEGG database, thereby linking metabolites by association thereby suggest potential intermediates[12].

Results

Quality assessment (see Table I)

Six articles had minor disparities in evaluation, which were discussed and corrected by consensus.

Global assessment of bias

The experimental and analytical design was assessed using the Cochrane criteria for bias. Studies had similar designs and analytical techniques allowing a global bias assessment. Patient selection was rarely random owing to OA disease or the absence thereof. Blinding is impossible at sample collection, neither for the patient or the researcher. Selective detection of some analytical methods may cause reporting bias. Multi-variate statistical analysis models may

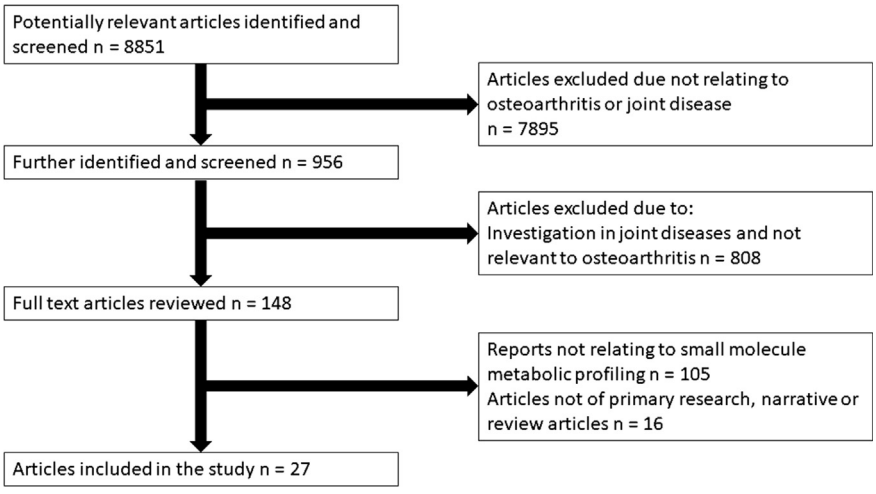

Fig. 1. Flowchart of study selection.

Table I
The evaluation of OA metabolic studies published to date (NOS = Newcastle–Ottawa Score, ACR = American College of Rheumatologists, ESP = End-stage procedure, MCA = Macroscopic cartilage assessment, K-L = Kellgren & Lawrence)

First Author & Year	Country of Origin	Species	Body fluid/Tissue	Joint	Diagnosis	Disease Staging	Controls	Sample size	Validated analysis	Controls	Statistical validity	NOS
Adams, S.B.[55] 2011	USA	Human	Synovium	Knee	Clinical	ESP & Outerbridge grading	Mild disease (Outerbridge grade <2)	8	Not mentioned	Strong	Adequate	7
Adams, S.B.[19] 2012	USA	Human	Synovial	Ankle	Not stated	ESP	No disease (Radiologically)	20	Not mentioned	Strong	Inadequate	5
Borel, M.[49] 2009	France	Guinea pig	Cartilage	Knee	Induced OA	Outerbridge grading	No disease (Outerbridge grade 0)	20	Strong	Weak	Adequate	6
Damyanovich, A.Z.[18] 1999	Canada	Canine	Synovial	Knee	Induced OA	MCA	No disease (MCA)	14	Strong	Strong	Adequate	7
Damyanovich, A.Z.[26] 1999	Canada	Canine	Synovial	Knee	Induced OA	MCA	No disease (MCA)	7	Strong	Strong	Adequate	6
Duffy, J.M.[17] 1993	Ireland	Human	Synovial	Knee	MCA	MCA	No disease (MCA)	16	Weak	None	Adequate	4
Fuchs, B.[27] 2009	Germany	Equine/Canine	Synovial	Not stated	Not stated	Not stated	Disease free animals	6 + 5	Not mentioned	Strong	Inadequate	4
Gierman, L.M.[50] 2013	Holland	Human	Infrapatellar fat pad	Knee	Clinical	ESP	No disease cadaver	13	Strong	Weak	Inadequate	4
Hugle, T[16] 2012	Switzerland	Human	Synovial	Knee	Clinical	None	Non-OA pathology	15	Not mentioned	None	Adequate	4
Jiang, Miao[38] 2013	China	Human	Serum	Not stated	Clinical	Not stated	Healthy volunteers	27	Strong	Strong	Adequate	7
Kim, Sooah[13] 2016	South Korea	Human	Synovial	Knee	Clinical	K-L radiological grading	Mild disease (K-L grading)	15	Not mentioned	Weak	Inadequate	5
Lacitignola, L[22] 2008	Italy	Equine	Synovial	MCPJ	Radiological	None	No radiological disease	14	Not mentioned	Strong	Adequate	7
Lamers, Robert-Jan A. N.[35] 2005	USA	Human	Urine	Knee & Hip	Radiological	K-L radiological grading	No disease (K-L grading)	45	Not mentioned	Weak	Inadequate	5
Lamers, Robert-Jan A. N.[41] 2003	Holland	Guinea pig (Hartley outbred albino model)	Urine	Not stated	None (inevitable disease)	None (inevitable disease)	Vitamin C fed animals	46	Not mentioned	Strong	Inadequate	5
Li, Xin[40] 2010	China	Human	Urine	Knee	Radiological	K-L radiological grading	Healthy volunteers	37	Not mentioned	Weak	Adequate	5
Maher, Anthony D.[42] 2012	Australia	Ovine	Serum	Knee	MCA	MCA	MCA	12	Not mentioned	Strong	Inadequate	5
Marshall, K.W.[20] 2000	Canada	Human	Synovial	Not stated	Clinical	Not stated	Mild disease	10	Not mentioned	Weak	Adequate	5
Meshitsuka, S[21] 1999	Japan	Human	Synovial	Knee	Clinical (ACR criteria)	Not stated	RA patients	16	Not mentioned	None	Adequate	4
Mickiewicz, B.[23] 2015	Canada	Ovine	Synovial	Knee	Surgically induced	Not stated	Sham and non-surgical controls	9	Not mentioned	Strong	Adequate	5
Shet, Keerthi[47] 2011	USA	Human	Cartilage	Knee	ESP	Not stated	No disease cadaver	12	Weak	Weak	Adequate	5
Sitton, N.G.[15] 1986	UK	Human	Synovial	Not stated	Clinical (ACR criteria)	Not stated	Non-OA pathology	29	Strong	None	Adequate	4
Williamson, M.P.[25] 1989	UK	Human	Synovial	Knee	Clinical	Not stated	Non-OA pathology	10	Weak	None	Inadequate	4
Zhai, Guangju[39] 2010	UK	Human	Serum	Knee	Clinical & Radiological	K-L radiological grading	No disease (KL grading	123	Strong	Strong	Adequate	7
Zhang, Q.[56] 2015	China	Human	Serum	Knee	Clinical & Radiological	K-L radiological grading	Mild disease (K-L grading)	40	Not mentioned	Strong	Adequate	6
Zhang, W.[28] 2014	Canada	Human	Synovial	Knee & Hip	ESP	Not stated	Absent metabolic disease	80	Strong	None	Adequate	5
Zhang, W.[36] 2016	Canada	Human	Serum	Knee	ESP	Clinical (ACR criteria)	No disease (ACR criteria)	123	Strong	Strong	Adequate	7
Zhang, W.[37] 2016	Canada	Human	Serum	Knee	ESP	Clinical (ACR criteria)	Absent metabolic disease	144	Strong	Strong	Adequate	7

disproportionately represent the more abundant metabolites irrespective of significance. Supervised statistical methods, by definition, require knowledge of the sample class to perform the analysis, preventing blinding of the data.

Studies inconsistently report ***P*** values and thus a reporting bias may exist towards those that do so. Univariate t-testing assumes normality of the variables. Assaying several hundred metabolites may yield apparently significant ***P*** values unless a false detection rate calculation, permutation testing or validation data sets are used.

A collation of the metabolite findings is presented (Table II). The metabolic studies have been subdivided according to the body fluid or tissue of origin and where an animal study, this is highlighted.

Synovial fluid studies

Synovial fluid (SF) is thought the closest representation of joint metabolism and therefore discussed in detail.

Specific attention is required for Kim *et al.*, metabolically comparing SF in early and late stage disease patients using gas chromatography mass spectroscopy (GCMS)[13]. Fifteen patients were studied and t-testing applied to confidently illustrate no significant difference between early and late stage OA groups assuming normality. However, the groups were ill matched of gender and diabetic status, presenting possible confounding factors. Salicylaldehyde was reported as significant but should be discounted as an aspirin derivative used to relieve OA symptoms. The 28 metabolites reported should be interpreted cautiously due to the uncontrolled confounding factors, limited numbers and lack of metabolite evaluation.

Anaerobic environment

Metabolic homeostasis is disturbed in joint disease with stress causing inflammation and driving an anaerobic environment. This is due to increased metabolic activity, increased oxygen consumption due to energy demand, with those demands not being met. The key question is if the pattern of metabolic disturbance differs between joint disease or stage of OA to allow diagnostic or prognostic markers.

Lactate and pyruvate elevation alongside an anaerobic environment have been observed in SF from septic arthritis and rheumatoid arthritis (RA). RA glucose levels rise in early disease before dropping in later stages[14,15]. It is proposed this may be the case in OA.

Different joint arthropathies were assayed by NMR spectroscopy before being analysed using principal component analysis (PCA): OA results for an anaerobic environment were inconclusive[16]. Other NMR studies showed the lactate levels to be variable[17]. However, groups have reported elevated anaerobic metabolites lactate (2 to 6-fold) and pyruvate (4-fold) in OA SF using both NMR and LCMS techniques[18–20]. A direct comparison of SF from healthy, RA and OA patients revealed elevated lactate levels in diseased joints, using the lactate/alanine ratio to standardise. This effect was seen in the OA group but more extreme in RA[21]. Post-traumatic and end-stage OA SF suggested elevated lactate, although the post-traumatic group also had increased oxidative metabolism with elevation in glutathione, cysteine and cysteine-glutathione[19]. Early and late stage OA SF had subtle (1.2–1.5 fold) elevations of the anaerobic metabolites lactate, citrate and formate in late stage disease but there were only five patients involved[20]. In animal studies, canine OA samples had elevated pyruvate and lactate compared to controls, something mirrored in equine OA SF with a 3-fold increase in both[22].

In anaerobic environments glucose levels are thought to be elevated due to increased demand. Like lactate, the reports of glucose are mixed. Sitton *et al.* found glucose concentrations in OA SF higher than the serum in the same patient, although the reverse was observed in RA[15]. Conversely in advanced OA, Marshall *et al.* noted a lower concentration of sugars and polyols, citing anaerobic metabolism as the cause. Animal studies report an increase in OA glucose concentration in equine (five-fold) and ovine (two-fold) samples[22,23]. Interpreting glucose levels is difficult due to multiple influences including: consumption, altered glucose transport and synovial membrane permeability. The evidence suggests a glucose rise in early disease, most likely from upregulated transport. In late-stage disease a decompensation of glucose consumption vs delivery could cause a drop in levels, correlating with observations.

Fat metabolism

The links between disturbed lipid metabolism in diseases like ischaemic heart disease and inflammatory diseases like OA make lipids a logical target. Whether this is reactionary or causal of OA remains unknown. However, lipid metabolism may provide future biomarkers and insight into the inflammatory component of OA.

NMR spectroscopic analysis allows lipoprotein class information to be characterised by distinguishing between very low density lipid (VLDL), low-density lipoprotein (LDL) and high density lipoprotein (HDL). Lipid classes can be measured in biofluid or tissue extracts including: triglycerides, cholesterol, cholesterol esters and phospholipids. Information is given about the lipid polymer length and the number of double bonds (or saturation) by measuring the ratios of CH_3, CH_2 and CH moieties within the lipid molecule.

Microscopic evaluation of canine knees with transected anterior cruciate ligaments (ACL) revealed chondrocytes storing large amounts of lipid[24]. Healthy human SF has been found to have very low concentrations of the VLDL, HDL and LDL lipoproteins compared to matched serum. In inflammatory joint disease the lipoproteins are elevated; suggested to be secondary to inflammation and increased synovial membrane permeability[14]. However, controls were not age-matched for this finding, being between 25 and 42 years old and thus metabolically different.

An NMR spectroscopy comparison of RA, OA and traumatic SF qualitatively revealed shorter, unsaturated triglyceride chains in OA compared to the other groups[25]. An alternative comparison of human RA and OA SF by NMR reported the CH_3/CH_2 peak area ratio was lower in OA SF, indicating longer fatty acid chains. However, we note the results were variable.

A higher $-CH_3$ triglyceride signal was seen in OA, suggesting an increased concentration of fatty acid chains[17]. Equine OA studies concur with higher levels of unsaturated fatty acids, HDL and choline (3-fold)[22]. This was corroborated in canines with increased terminal CH_3, CH_2 and CH, compared to controls[18].

The ketone bodies 3-$_D$-hydroxybutyrate and 3-$_D$-hydroxyisobutyrate were elevated in late stage OA from studies in human, equine, ovine and canine (2–15-fold), suggesting a switch to fatty acid metabolism[18,20,23,26]. Equine studies noted concurrent 3-fold glycerol liberation[22].

Intra-articular phospholipase A_2 (PLA_2) is upregulated in patients with inflammatory joint disease thereby catalysing the creation of lysophospholipids from phospholipids. An LCMS characterisation of the phospholipid classes in equine and canine SF found lysophosphatidylcholine (lysoPC) content and lysoPC/PC ratio to correlate with RA disease activity, but not OA. The authors acknowledge diet as a strong confounding factor. Furthermore, the sample number was very low at six and five respectively[27].

Zhang *et al.* identified subgroups of osteoarthritis which differed in fat metabolism and acylcarnitine levels. They observed distinctions in the glycophospholipids and the sphingomyelins[28]. No correlation to clinical factors or age matching was performed so it is difficult to draw definitive conclusions.

Table II
A reference of identified metabolites associated with osteoarthritis. Metabolites reported with a $P < 0.05$ are highlighted and the relevant specimen type indicated (*)

Metabolite	Change	Species, Tissue/Fluid type and Reference
1-arachidonoylglycerophosphoethanolamine	Increased	Human SF[19]
1-arachidonoylglycerophosphoinositol	Increased	Human SF[19]
1-monostearin	Increased	*Human SF[13]
1-oleoylglycerophosphoethanolamine	Increased	Human SF[19]
1-stearoylglycerophosphoethanolamine	Increased	Human SF[19]
1-stearoylglycerophosphoinositol	Increased	Human SF[19]
2-hydroxypalmitate	Increased	Human SF[19]
2-hydroxystearate	Increased	Human SF[19]
3-Carboxy-4-methyl-2-oxypentanoate	Increased	*Human serum[56]
3-D-hydroxybutyrate	Increased	*Human SF[19,20], *canine SF[18,26], human urine[35]
3-Hydroxypropionate	Increased	*Human SF[13]
3-indoxyl-sulfate	Increased	Human SF[19]
3-Methyl histidine	Increased	Ovine serum[42]
4-androsten-3beta-17beta-diol-disulfate-1	Increased	Human SF[19]
4-hydroxy-hippurate	Increased	*Human urine[40]
4-Methyl-phenol	Decreased	*Human urine[40]
4-methyl-2-oxopentanoate	Decreased	*Human synovium[55]
4-methylene proline	Increased	*Human urine[40]
4-Oxoproline	Decreased	*Human serum[56]
5-dodecenoate (12:1n7)	Increased	Human SF[19],
5-oxoproline	Decreased	*Human synovium[55]
Acetamide	Increased	*Canine SF[18]
Acetate	Increased	*Canine SF[18,26], *equine SF[22], *human urine[40]
Acetylcarnitine	Increased	*Human synovium[55]
Aconitic Acid	Increased	*Human urine[40]
Adipate	Increased	*Human SF[13]
Alanine	Increased	*Canine SF[18], Canine SF[26], *equine SF[22], guinea pig urine[41]
	Decreased	*Human joint cartilage[47]
Aleic Acid	Increased	*Human SF[13]
Alpha-tocopherol	Increased	*Human SF[13], Human SF[19]
Androsterone sulfate	Increased	Human SF[19]
Arachidonate (20:4n6)	Increased	*Human SF[13], Human SF[19]
Arginine	Decreased	*Human plasma
Asparagine	Increased	*Human SF[13]
	Decreased	*Ovine SF[23]
Aspartic Acid	Increased	*Human serum[38]
Benzoate	Increased	*Human SF[13]
Bilirubin*E-E	Increased	Human SF[19]
Capric Acid	Increased	*Human SF[13]
Carnitine	Increased	Human SF[19]
Choline	Decreased	*Human joint cartilage[47]
Cis-4-decenoyl-carnitine	Increased	Human SF[19]
Citrate	Increased	*Equine SF[22], Canine SF[26] *human SF[20], *human urine[40]
Cortisol	Increased	Human SF[19]
Creatine/Creatinine	Increased	Human urine[35], *canine SF[26], *equine SF[22]
Creatine	Increased	Ovine serum[42]
Creatinine	Increased	Ovine serum[42]
Cystine	Increased	Human SF[19]
Dihomo-linoleate-20-2n6	Increased	Human SF[19]
Dihydroxyfumaric acid	Increased	*Human serum[38]
Dimethylnonanoyl carnitine	Decreased	*Human serum[38]
Ethanolamine	Increased	*Human SF[13]
Fatty Acid CH2 NMR peak	Increased	*Canine SF[18,26], *human SF[25], *guinea pig joint cartilage[49]
Fatty Acid CH2/CH **NMR peak** ratio (chain saturation)	Decreased	Human SF[25]
Fatty Acid CH3 NMR peak	Increased	*Canine SF[18], Canine SF[26]
Fatty Acid CH_3/CH_2 **NMR peak** ratio (FA chain length)	Decreased	Human SF[25]
Formate	Increased	*Human SF[20]
Gamma-glutamylleucine	Decreased	*Human synovium[55]
Glucose	Increased	*Ovine SF[23], *equine SF[22],
	Decreased	Canine SF[26]
Glutamine	Increased	Canine SF[26], *human synovium[55], ovine serum[42], *human urine[40]
Glutamine/Glutamate	Increased	*Canine SF[26],
Glyceraldehyde	Increased	*Human Serum[38]
Glycerol	Increased	*Canine SF[18,26], *equine SF[22], *human SF[13], human urine[35]
Glycine	Decreased	*Human joint cartilage[47], *human serum[56], *human urine[40]
HDL-Choline	Increased	*Canine SF[18], *equine SF[22]
Heptadecanoic Acid	Increased	*Human SF[13]
Hippuric Acid	Decreased	*Human urine[40]

Table II (*continued*)

Metabolite	Change	Species, Tissue/Fluid type and Reference
Histamine	Decreased	*Human urine[40]
Histidine	Decreased	*Human serum[56], *human urine[40], human urine[35]
Homocysteine	Increased	*Human serum[56]
Homoserine	Increased	*Human serum[38]
Homovanillic Acid	Increased	*Human urine[40]
Hydroxyproline	Increased	*guinea pig joint cartilage[49], *human synovium[55]
	Decreased	*Ovine SF[23]
Hypoxanthine	Increased	*Human serum[56], guinea pig urine[41]
Isobutyrate	Increased	*Ovine SF[23]
Isocitric Acid	Increased	*Human urine[40]
Isoleucine	Increased	*Canine SF[18], Canine SF[26] *guinea pig joint cartilage[49]
Kynurenine	Increased	Human SF[19]
Lactate, Lactate/Alanine ratio	Increased	*Human SF[19–21], *Canine SF[18], Canine SF[26],*equine SF[22], *human serum[56], human urine[35], guinea pig urine[41]
	Decreased	Human joint cartilage[47]
Lanosterol	Increased	*Human SF[13]
Leucine	Increased	*Guinea pig joint cartilage[49], *human serum[39], *human plasma[36]
Leucine/Histidine ratio	Increased	*Human serum[39]
Lignoceric Acid	Increased	*Human SF[13]
Linolenic Acid	Increased	*Human SF[13]
Lipoprotein associated fatty acid	Decreased	*Human serum[56],
LysoPC-C28:1	Decreased	*Human plasma[36]
Malic Acid	Increased	*Human SF[13], guinea pig urine[41]
Mannose	Increased	Human SF[19]
Methyl-hippuric acid	Increased	*Human serum[56]
Methylhistidine	Decreased	human urine[35]
Myo-inositol	Increased	*Human synovium[55]
Myristic Acid	Increased	*Human SF[13]
N-acetyl glycoprotein	Increased	*Human SF[17,30], *canine SF[18,26], *equine SF[22], *guinea pig joint cartilage[49]
	Decreased	*Human joint cartilage[47]
N-acetylornithine	Increased	*Human synovium[55]
N-carbamoylaspartate	Increased	*Human SF[13]
Palmitic Acid	Increased	*Human SF[13]
Palmitoleic Acid	Increased	*Human SF[13]
Palmitoyl-sphingomyelin	Increased	Human SF[19]
Phosphatidylcholine acyl-alkyl C34:3	Decreased	*Human plasma[37]
Phosphatidylcholine acyl-alkyl C36:2	Decreased	*Human plasma[36]
Phosphatidylcholine acyl-alkyl C36:3	Decreased	*Human plasma[37]
Phosphatidylcholine diacyl C36:6	Decreased	*Human plasma[36]
Phosphatidylcholine acyl-alkyl C38:0	Decreased	*Human plasma[36]
Pelargonic Acid	Increased	*Human SF[13]
Pentadecanoic Acid	Increased	*Human SF[13]
Phenylacetylglycine	Decreased	*Human synovium[55]
Phenylalanine	Increased	*Human SF[13]
Proline	Increased	*Guinea pig joint cartilage[49]
Propionyl-carnitine	Decreased	*Human serum[56]
Pseudouridine	Increased	Human SF[19]
Putrescine	Increased	*Human SF[13]
Pyridoxine	Decreased	*Human serum[56]
Pyruvate	Increased	Canine SF[26], *equine SF[22], *human serum[56], human urine[35]
Salicylaldehyde	Increased	*Human SF[13]
Serine	Decreased	*Ovine SF[23]
Sphingomyelin	Decreased	*Human serum[56], *human plasma[36]
Squalene	Increased	*Human SF[13]
Stearic Acid	Increased	*Human SF[13]
Succinate	Increased	*Human synovium[55]
Sugars, Polyols	Decreased	*Canine SF[18], *human SF[20]
Threonine	Decreased	*Human SF[20]
Threose	Increased	*Human SF[13]
Tryptophan	Increased	Human SF[19], *human serum[56]
Tyrosine	Increased	Canine SF[26]
Urate	Increased	Human SF[19], *human serum[56]
Urea	Increased	*Human synovium[55]
Uridine	Increased	*Human SF[19],
	Decreased	Ovine SF[23]
Valine	Increased	*Human serum[39]
Valine/arginine ratio	Increased	*Human serum[39]
Valine/glycine ratio	Increased	*Human serum[39]
Valine/histidine ratio	Increased	*Human serum[39]
Valine/tryptophan	Increased	*Human serum[39]

It is apparent that lipid metabolism has osteoarthritis subgroups. The lipid species differ in the presence of the inflammatory joint diseases; with higher triglycerides and more unsaturated fatty acids. The significance of these specific changes, and if a link exists to the spectrum of MeS e.g., diabetes and ischaemic heart disease, is not clear. Further investigation opens the possibility for metabolic treatments and targets in OA disease modification.

Free amino acids

In OA SF, it is proposed that the majority of increased amino acid levels derive from hyaline cartilage collagen and proteoglycan destruction. As such their elevation in SF provides a potential biomarker to early joint destruction; something lacking in current clinical practice.

Sitton *et al.* compared serum and SF in the same patients suffering with OA or RA. Concentrations of selected amino acids, sugars and immunoglobulins were measured using standardised clinical biochemistry techniques. A higher histidine concentration was seen in RA SF compared to serum, an observation not seen with OA despite other studies demonstrating that histamine is produced by OA chondrocytes[15].

The comparisons of advanced and early OA identified a 20% reduction threonine[20]. Whilst MS observations of SF from ankle OA found elevated proline and hydroxyproline, both constituent amino acids of collagen α-helices[19]. Animal studies do conflict with Ovine OA showing a 0.75-fold change in hydroxyproline levels, although sampling was done after ACL reconstruction[23]. Canine OA contained double the measured isoleucine compared to non-diseased controls[18]. Both canine (1.5-fold) and equine (3-fold) OA SF have higher alanine concentrations[18,22,26].

There is speculation that increased concentrations of these amino acids is due to increased synovial permeability (and thus passage from the serum). However, hyaline cartilage components, proteoglycan and type II collagen, are both rich in alanine and the amino acids discussed above. Therefore, a reasonable hypothesis is that the volume of hyaline cartilage destruction is directly relates to the elevations of amino acids.

Hyaluronic acid metabolism

Hyaluronan is a key SF target in metabolic and OA research. Hyaluronan contributes to the SF viscoelastic properties by virtue of its polymer aggregates. Therefore, hyaluronan depolymerisation in OA furthers joint destruction by loss of the viscoelastic properties and hence low friction environment[29]. Hyaluronan supplementation has proven clinically effective via a number of mechanisms but the preservation of hyaluronan appears protective in selected cohorts of OA patients.

The monomeric components of hyaluronan: N-acetylglucosamine and glucuronic acid, are identifiable by NMR spectroscopy. Hyaluronan cleavage in SF is observed by an increase in the terminal N-acetyl (N-Ac) group peak, verified by treating SF with hyaluronidase enzyme[30]. Concurrent formation of lactate, citrate and acetate, (known products of N-acetylglucosamine) is also reported[31,32]. Unfortunately, NMR cannot distinguish between the N-Ac liberation of hyaluronic acid and other proteoglycans, although both are present in joint destruction.

Human OA and RA SF comparison demonstrated N-Ac levels to be similar, albeit with significant variation. The authors concluded the N-Ac elevation correlated with inflammatory samples, although no explanation was proposed[17]. Williamson's study of SF identified varying N-Ac levels with RA and OA. They postulated glycoprotein liberation, but failed to associate with hyaluronic acid depolymerisation. RA and reactive arthropathy patients showed significant degrees of hyaluronan depolymerisation, with OA results variable[25]. The limitation of this study was the assumption of cadaveric knee aspirates as controls.

Animal studies with tighter controls show N-Ac group liberation to double in canine and increase 8-fold in equine OA[18,22].

The link between hyaluronan depolymerisation and SF viscoelasticity is well documented[33]. Thus, a pathway of inflammation, hyaluronan depolymerisation and diminished SF mechanical properties is likely. N-Ac seems a suitable clinical target for the detection of early arthropathy, although it may not distinguish between diagnoses. The extent of this reaction in human OA SF appears more variable than other inflammatory arthropathies. This possibly explains why results of hyaluronan supplementation are variable but occasionally very effective, potentially providing a rationale for personalised healthcare and targeted treatment for patients.

Plasma, serum and urine studies

The majority of OA metabolic profiling has concentrated upon plasma, serum and urine perturbations due to ease of collection. Whilst information may be limited about OA joint metabolism or the disease aetiology, they may provide diagnostic or prognostic biomarkers. Studies dating back to the 1980's indicated metabolites allowing a distinction between joint arthropathies.

Serum histidine levels were lower in OA patients compared to controls in two studies: sulphydryl levels also decreased concurrently[34,35]. The source of sulphydryl remains unclear but likely derived from liberated cysteine.

Zhang studied serum in 72 OA patients and 76 controls, finding an OA association with a two-fold arginine reduction and reduction in selected lipid species by between 15% and 30% (including PC's and sphingomyelin)[36]. A separate analysis of the same dataset investigated associations between OA and diabetes. Metabolic differences were present but in addition leucine and PC's were influenced by both diseases[37]. Studies were robust with appropriate populations, patient matching and suitably powered statistical analysis.

A Chinese group made a serum and urine comparison of control subjects alongside arthropathies: RA, OA, ankylosing spondylitis and gout. The study used GCMS, high-performance liquid chromatography mass spectroscopy (HPLC-MS) and multi-variate statistical analysis. Several age and gender differences were acknowledged by the authors, raising the possibility of confounding factors. All disease groups indicated elevated anaerobic metabolism: elevated lactate, aspartate, homoserine, dihydroxyfumerate and decreased 4,8-dimethylnonanoyl carnitine. A smaller change was present in the OA group compared to other diseases with authors suggesting diagnostic distinction[38]. The inconsistent metabolic disturbance in OA means responsible metabolites are unlikely to be good biomarkers.

Lamers *et al.* assessed 92 OA urinary metabolic profiles by NMR spectroscopy and concluded a possible switch to fat as a preferential energy substrate and an increase in histidine, argued to be indicative of inflammation. Results were observational and without statistical significance[35].

A case–control study, comparing normal and OA subjects, found elevations in valine and leucine, increasing in significance when applied to a histidine ratio[39]. A human urine study, comparing inflammatory and non-inflammatory OA symptoms, revealed a 1.3-fold increase in histamine levels; perhaps expected with inflammatory mast cell release[40]. Anaerobic metabolism has been suggested as a mechanism to distinguish between joint arthropathies, presumably by virtue of a heightened systemic inflammatory state or an indirect reflection of the anaerobic metabolism within the

joint. The clinical reliability upon their results is questionable, but larger studies may demonstrate this capability.

The animal studies have concentrated upon models with induced or inevitable OA. Albino guinea pigs bred with an inevitable early onset OA phenotype have been studied. Serial urine sampling showed lactate, malate, hypoxanthine and alanine were associated with OA onset[41]. Maher *et al.* harvested ovine serum samples pre and post-surgical ligament transection (and hence OA induction), meniscal destabilisation or a sham procedure. NMR and multi-variate data analysis was used to identify metabolic differences between the sham surgery and surgically induced OA. At 4 weeks post-ligament transection they reported reductions in the branch chain amino acids (BCAA) valine, leucine and isoleucine. In addition, 3-methylhistidine was increased. At 12 weeks, glutamine, 3-methylhistidine, creatinine and creatine concentrations increased alongside decreased BCAA. Destabilising the meniscus showed none of the above features except a reduction in creatine and elevation in dimethylsulfone ($DMSO_2$)[42]. Interestingly $DMSO_2$ is a metabolite of gastrointestinal bacteria, suggesting a link between the gut microbiome and OA[43]. Proteoglycan destruction at the cleavage sites could liberate glutamine, suggesting a mechanism to increase levels.

The blood and urine studies correlate with SF findings in many areas, reflecting their close relationship and the derivation of SF from serum. The BCAA may have potential in early diagnostics and serial monitoring where the biological process of structural protein maintenance may be active and upregulated. However, the animal models may differ from human disease because structural maintenance (and hence BCAA consumption) is intact and OA is caused by overwhelming mechanical forces, contrary to most human OA where a biological failure occurs. Fat metabolism appears significantly influenced by OA in SF and plasma. Fatty acid metabolism increases with elevated metabolites involved in fatty acid chain oxidation. Characterising the lipids suggests triglyceride levels rise with unsaturated, longer chains contributing to higher concentrations of HDL.

Cartilage tissue

Metabolic studies of cartilage tissue have used high resolution magic angle spinning (HRMAS) NMR spectroscopy. Early studies suggested a switch to fatty acid oxidation in aerobic glycolysis of cartilage, although the growth plate was studied and not the joint[44]. Proteoglycan depolymerisation and structural cartilage loss was studied by measuring the N-Ac group in the same manner as SF, both free in solution in the cartilage and constituent of the cartilage polymers[45].

HPLC-MS studies of cartilage from OA knees demonstrated metabolites associating with osteophyte production. These included PC's, various amino acids and some associated products of fatty acid metabolism[46]. Comparing OA knee cartilage (sampled at arthroplasty) to non-OA cadaveric tissue revealed lower levels of N-Ac and lactate (1.3-fold), alanine, glycine and choline (2-fold)[47]. Cadaveric tissue is a poor control with the assumption of normal metabolism and samples were not age-matched. Lower alanine and glycine levels suggest proteoglycan depolymerisation and hence cartilage destruction. The authors claimed proteoglycan destruction with a lower N-Ac signal contrary to SF, arguing the reverse in cartilage due to washout into the SF. No evidence to support this theory was presented.

Guinea pig and bovine nasal cartilage (treated with collagenase) both demonstrated the 1.5-fold N-Ac increase alongside proteoglycan breakdown. The collagen amino acids proline, hydroxyproline, leucine and isoleucine are elevated in guinea pig, whilst lysine, glutamate, proline, hydroxyproline and glycine are elevated in bovine-collagenase-treated cartilage[48]. Alterations in lipid metabolism with 2-fold increases CH_2 NMR signals suggest longer lipid chains, with unclear significance[49]. Treatment of cartilage (and hence proteoglycan) with collagenase did not liberate carbohydrates in the same way as the amino acids. This anomaly suggests that the free amino acids are retained within the collagen/proteoglycan matrix whereas carbohydrate moves into the SF[48]. This has implications interpreting SF carbohydrate in the presence of proteoglycan destruction.

In general the changes metabolic observations in cartilage mirror those seen in SF by their close association. However, where products may be retained or washed out from cartilage this may not be so. Cartilage may serve as a more sensitive indicator of structural joint destruction. However, its inaccessibility and need for invasive procedures means any biomarkers are unpractical in clinical practice.

Infrapatellar fat pad

Targeting the fat pad is borne from the concept that it serves as a hub of inflammatory tissue in the knee and thus representative of the inflammatory metabolites. One human study examined the oxylipid and fatty acid composition from OA infrapatellar fat pads by HPLC-MS using port-mortem tissue as controls. The group suggested thromboxane A2, thromboxane B2 and lipoxin A2 distinguished between the disease and control samples. There findings lacked significance, controls were weak and the fitting of the model was poor[50].

Discussion

Metabolic mapping

Collation of the results illustrates how OA metabolic changes are interlinked, whilst postulating metabolic pathways which may be affected (see Fig. 2). This can serve to generate hypotheses, predict unobserved metabolic patterns, link to other OA research fields, and propose future metabolic targets for indepth study. The major areas identified not discussed are the representation by the arachidonate signalling precursors which may propose a link to inflammation from fat metabolism. The presence of the androgen hormones may influence the difference of incidence between the sexes.

The potential for high-throughput studies to further disease understanding and generate biomarkers is illustrated by recent success in both genomic and proteomic studies[51–53]. The integration of studies: genomic, proteomic and metabonomic upon a clinical osteoarthritis cohort provides the greatest hope for future advances in biomarker discovery.

Limitations

Animal studies provided valuable information with strong controls, but are obviously species specific. The largest limitation to any human study is the confounding factors. Most studies have too few samples, controls can be unsuitable and comparisons made to dissimilar diseases. Matching of groups is often inadequate, making confounding factors likely. Statistical significance has been quoted by many but potentially without false discovery rate correction; essential when studying thousands of variables. Hence results need to be treated with caution.

A large scale epidemiological metabonomic study would overcome these challenges but should incorporate clinical factors to address the links between metabolism, inflammation and the clinical features of disease.

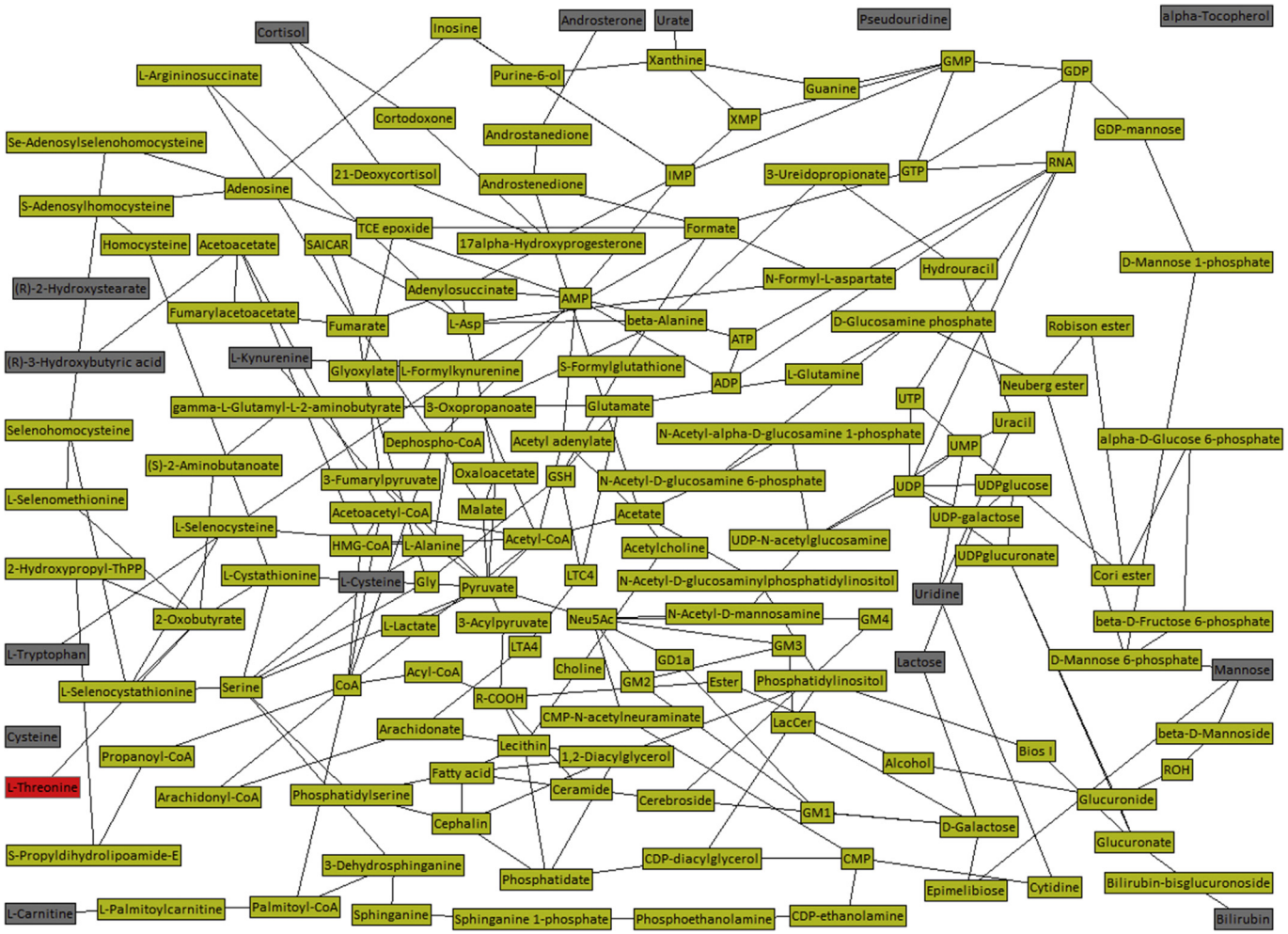

Fig. 2. Metabolic network pathway for osteoarthritic human SF generated using the KEGG database. Reported elevated metabolites are displayed in grey. Those reported to be supressed are highlighted in red. Hypothesised intermediate and potentially affected metabolites are illustrated in yellow.

Conclusions

The products of cartilage, collagen and proteoglycan destruction are detectable. No clinical correlation has been done to assay the significance of the macromolecule destruction, relating to disease progression, symptoms or function.

The wealth of evidence suggests an anaerobic environment with an associated switch to fatty acid metabolism, albeit not diagnostically specific for OA. Disease subgroups differ in lipid metabolism with alteration of lipid composition. However, direct associations of MeS to OA remain unproven and should be a research target. OA has a variable inflammatory component and no study to date has correlated to the metabolic profile. This study has been performed with some success in RA with findings of altered phospholipid metabolism and elevated choline, LDL lipids, cholesterol and fatty acids[54]. A similar study would benefit OA by improving treatments and outcomes in the inflammatory disease cohort.

A strong potential exists for clinical biomarkers and development in the understanding of lipid metabolism influences upon inflammatory OA. Lipid metabolism remains the clear potential influence for OA understanding and treatment. However, a suitably large clinical study is lacking to investigate conclusively whilst controlling for confounding factors.

Author contributions

All authors had significant involvement in the conception, interpretation, drafting/revision and final approval of the manuscript.

Conflict of interest

Professor John Lindon declares a shareholding in the company “Metabometrix Ltd” which is contracted to perform small molecule studies. No other authors disclose any competing interests.

Acknowledgements

The authors thank Professor Keith Jaggard and Professor Justin Cobb for their helpful discussions and support. The authors were supported by the National Institute for Health Research, Imperial Biomedical Research Centre (BRC) and the Department of Orthopaedics at Imperial College Healthcare NHS Trust.

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