Global metabolomic profiling of human synovial fluid for rheumatoid arthritis biomarkers

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Abstract

Objective

The objective of this study was to analyse the metabolomic profiles of rheumatoid arthritis synovial fluid to test the use of global metabolomics by liquid chromatography-mass spectrometry for clinical analysis of synovial fluid.

Methods

Metabolites were extracted from rheumatoid arthritis (n=3) and healthy (n=5) synovial fluid samples using 50:50 water: acetonitrile. Metabolite extracts were analysed in positive mode by normal phase liquid chromatography-mass spectrometry for global metabolomics. Statistical analyses included hierarchical clustering analysis, principal component analysis, Student's t-test, and volcano plot analysis. Metabolites were matched with known metabolite identities using METLIN and enriched for relevant pathways using IMPaLA.

Results

1018 metabolites were detected by LC-MS analysis in synovial fluid from rheumatoid arthritis and healthy patients, with 162 metabolites identified as significantly different between diseased and control. Pathways upregulated with disease included ibuprofen metabolism, glucocorticoid and mineralocorticoid metabolism, alpha-linolenic acid metabolism, and steroid hormone biosynthesis. Pathways downregulated with disease included purine and pyrimidine metabolism, biological oxidations, arginine and proline metabolism, the citrulline-nitric oxide cycle, and glutathione metabolism. Receiver operating characteristic analysis identified 30 metabolites as putative rheumatoid arthritis biomarkers including various phospholipids, diol and its derivatives, arsonoacetate, oleananoic acid acetate, docosahexaenoic acid methyl ester, and linolenic acid and eicosatrienoic acid derivatives.

Conclusion

This study supports the use of global metabolomic profiling by liquid chromatography-mass spectrometry for synovial fluid analysis to provide insight into the aetiology of disease.

Key words

rheumatoid arthritis, metabolomics, mass spectrometry, biomarkers, synovial fluid

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Introduction

Rheumatoid arthritis (RA) is one of the most common autoimmune disorders affecting 2% of the North American population (1). It is associated with significant disability and is accompanied by a huge economic burden of $46.7 billion (2). RA is a chronic disease characterised by joint damage, synovitis, and systemic inflammation, yet its aetiology remains unknown. Despite recent advances in treatment options for RA, adequate biomarkers with high sensitivity and specificity for early diagnosis of RA are currently unknown (3). Biomarkers of RA are imperative for early intervention to slow and/or prevent progression into the late stages of diseases.

Metabolomics, which measures low-molecular weight molecules, is the most recent of the 'omics' fields to be used for biomarker discovery (4). Metabolites are the physiological end products of gene expression, and a metabolomic profile consists of quantitative measurements of thousands of metabolites. By analysing the metabolite pathways, metabolomic profiling can generate phenotypes of disease states and insight into disease pathogenesis. Metabolomic profiling is performed either in a global or targeted manner, where a global profile seeks all metabolites and a targeted profile examines sets of pre-identified metabolites.

Previous studies have attempted to identify biomarkers of RA with targeted metabolomics using nuclear magnetic resonance spectroscopy (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) (5-8). To our knowledge, only one prior study used global metabolomic profiling for RA biomarkers using GC-MS (7). In contrast to GC-MS, LC-MS is advantageous for complex biological samples because the samples undergo chromatographic separation in addition to mass spectrometry, which provides increased sensitivity and greater coverage of the metabolites present in a sample. To our knowledge, no study to date has used LC-MS analysis for global metabolomic profiling in search of RA biomarkers.

Despite several prior studies identifying biomarkers of RA, only one has searched for biomarkers of RA in human synovial fluid (SF) (7). SF, located in the joint cavity, contains a pool of metabolites produced by many types of joint cells (*i.e.* osteoblasts, osteoclasts, osteocytes, chondrocytes, etc.). Thus, we hypothesised that global metabolomic profiles of RA synovial fluid would both reveal the local pathological changes occurring during RA and provide metabolites as candidate biomarkers. In this pilot study, we used global metabolomic profiling by LC-MS in search of biomarkers of RA in human SF.

Materials and methods

Synovial fluid samples

SF samples were obtained under IRB approval (RA; n=3) or purchased from Articular Engineering (Northbrook, IL) *post mortem* (healthy; n=5). No clinical data were obtained for RA patients, and only partial clinical data including age, gender, race, and cause of death were obtained for healthy patients. Samples were kept at -80˚C until metabolite extraction.

Metabolomics

This study is an expansion of our previously published findings identifying potential biomarkers of OA in human SF, and metabolite extractions, HPLC-MS analysis, and processing of mass spectra were performed as previously described (9). Briefly, samples were thawed, cells and debris were removed by centrifugation, polymers were precipitated with acetone, and metabolites were extracted with 50:50 water: acetonitrile. Metabolite extracts were analysed in positive mode using an Agilent 1290 UPLC system with an Agilent 6538 Q-TOF mass spectrometer (Agilent, Santa Clara, CA) in normal phase using a Cogent Diamond Hydride HILIC 150 x 2.1 mm column (MicroSolv, Eatontown, NJ).

Spectra were processed for retention time and mass-to-charge (m/z) ratio normalisation, noise threshold (intensity: 1000), and peak detection using MZMine 2.14 (10). Metabolites with median intensity values of zero for both RA and healthy datasets were eliminated from analysis. Metabolite m/z values were matched to metabolite identities using METLIN with a mass tolerance

of 15 ppm, with $+1H^+$ or $+1Na^+$ adducts (11). M/z values were also checked against our internal library of metabolite standards to confidently identify metabolite identities by both retention time and m/z value (m/z: ± 0.01, 30 ppm; retention time: ± 0.25 min) (12).

Statistical analyses

All statistical analyses were completed using MATLAB (Mathworks, Inc.). Significant metabolites were identified by Student's *t*-test with false-discovery rate (FDR) corrections (FDR-adjusted *p*-value<0.05). Significantly different metabolites were visualised as a volcano plot by plotting the negative $\log_{10}$ of the *p*-value (y-axis) for each m/z value and the fold change ($\log_2$(RA/healthy)) of the median m/z value intensity of RA and healthy SF (x-axis) against each other to illustrate both significance and magnitude of change. M/z values with zero intensity for either group were excluded from the volcano plot analysis. Fold-change normalised, log-transformed metabolite intensities were analysed by principal component analysis (PCA) to examine the variation between the RA and healthy groups. Metabolites exclusively detected in RA or healthy SF were visualised in a scatterplot of median metabolite intensities plotted against one another (± standard deviation). Global metabolomic profiles were visualised using hierarchical cluster analysis (HCA) and illustrated sample-to-sample variation and clusters of co-regulated metabolites. Co-regulated metabolites were matched to metabolite identities via METLIN and enriched for relevant pathways using IMPaLA (13). Implicated pathways were identified at a significance level of 0.05. Putative biomarkers of RA were identified using receiver operating characteristic (ROC) analysis if they were capable of correctly classifying a sample as RA or healthy. Potential biomarkers were determined if the area under the receiver operating curve (AUC) was greater than 0.9.

Results

Metabolomic profiles of rheumatoid arthritis synovial fluid

1018 metabolites were detected across RA and healthy SF. 162 metabolites were significantly different between RA and healthy SF as determined by a Student's *t*-test (*p*-value<0.05). However, only three remained statistically significant after FDR corrections likely due to the small sample size of the RA dataset (n=3). HCA of the 162 significantly different metabolites revealed 2 clusters of co-regulated metabolites (Fig 1A). Cluster 1.1 contained 55 metabolites downregulated in RA SF. These metabolites mapped to the following pathways: purine and pyrimidine metabolism, biological oxidations, arginine and proline metabolism, nitric oxide metabolism, the citrulline-nitric oxide cycle, and glutathione metabolism (Table I, Supplemental Table I). Cluster 1.2 contained 107 metabolites upregulated in RA SF, mapping to ibuprofen metabolism, glucocorticoid and mineralocorticoid metabolism, alpha-linolenic acid metabolism, and steroid hormone biosynthesis (Table I, Supplementary Table I).

PCA resulted in separate clustering of RA and healthy SF, with two principle components (PC1=33.6%, PC2=17.8%) associated with 51.4% of the overall variation (Fig. 1B). To visualise metabolites found exclusively in RA and healthy SF, we created a scatterplot using median metabolite intensities. 310 metabolites were below the detection limit in healthy SF, while 282 metabolites were below the detection limit in RA SF (Fig. 1C). These metabolites were either not detected in

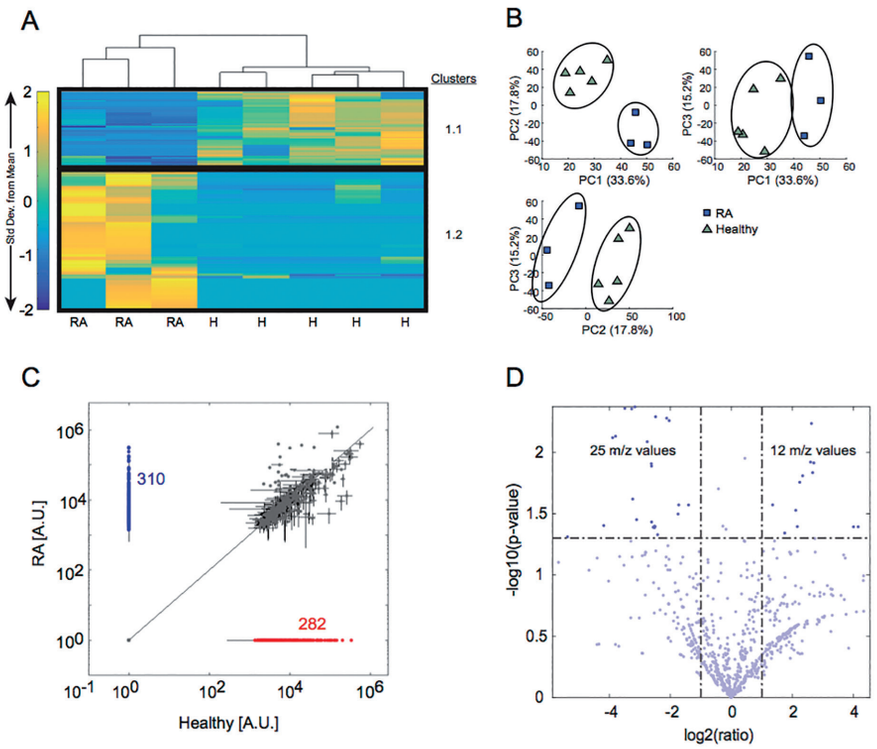

Fig. 1. Global metabolomic profiling finds distinct metabolic phenotypes of RA and healthy SF. **(A)** HCA of RA (n=3) and healthy (H = healthy; n=5) median metabolite intensities from human SF. Of the 1018 metabolites detected in human SF, 162 were significantly different between cohorts (*p*<0.05). Clusters of co-regulated metabolites altered with disease are outlined in black boxes and referenced as 1.1 and 1.2. Cluster 1.1 includes 55 co-metabolites lower in RA and cluster 1.2 includes 107 metabolites higher in RA. **(B)** PCA of the metabolite intensities in RA and healthy SF. Together, PC1, PC2, and PC3 were associated with 62.6% of the variation between healthy and RA SF and illustrate clear separation between cohorts. **(C)** Scatter plot of median metabolite intensities of RA and healthy SF plotted against one another (AU: Arbitrary Units). 310 metabolites were below the noise threshold in healthy SF and appeared to be unique to RA SF (blue). 282 metabolites were below the noise threshold in RA SF and were considered exclusively detected in healthy SF (red). **(D)** Volcano plot of RA and healthy SF, comparing the fold change and *p*-value of individual metabolites. Vertical dashed lines mark the twofold change and horizontal lines mark the *p*-value cut-off of 0.05. The upper right (12 metabolites) and upper left (25 metabolites) quadrants contained significant metabolites up- or downregulated in comparison to healthy SF with a *p*-value<0.05 and greater than two-fold change. Note: Adapted from Carlson *et al*. (2018).

Table I. Pathways altered in RA human synovial fluid.

Pathways	Metabolites Detected	Metabolite Cas ID	Metabolite Identity	Direction	*p*-value
Gene expression	3	554-01-8;74-79-3;4754-39-6	5-methylcytosine; arginine; deoxyadenosine	↓	0.00166
Gamma-glutamyl cycle	2	636-58-8;74-79-3	Glutamyl-cysteine; arginine	↓	0.00871
Biological oxidations	4	122-78-1;636-58-8;501-36-0;74-79-3	Phenylacetaldehyde; glutamyl-cysteine; resveratrol; arginine	↓	0.00873
Agmatine biosynthesis	1	74-79-3	Arginine	↓	0.0104
DNA methylation	1	554-01-8	5-methylcytosine	↓	0.0104
NAD metabolism	1	501-36-0	Resveratrol	↓	0.0104
Ibuprofen metabolism	1	51146-55-5	5-hydroxyibuprofen	↑	0.0125
Glucocorticoid and mineralcorticoid metabolism	1	80-92-2	5β-Pregnane-3α, 20α-diol	↑	0.0135
NO2-dependent IL-12 pathway in NK cells	1	74-79-3	Arginine	↓	0.0138
Pyrimidine metabolism	2	554-01-8;951-78-0	5-methylcytosine; 2'-deoxyuridine	↓	0.0142
Arginine and proline metabolism	2	636-58-8;74-79-3	Glutamyl-cysteine; arginine	↓	0.0152
α-Linoleic acid metabolism	1	6402-36-4	Traumatic acid	↑	0.0166
Nitric oxide metabolism	1	74-79-3	Arginine	↓	0.0173
VEGFR1 specific signals	1	74-79-3	Arginine	↓	0.0207
Putrescine biosynthesis	1	74-79-3	Arginine	↓	0.0207
Cytosine methylation	1	554-01-8	5-methylcytosine	↓	0.0207
Creatine biosynthesis	1	74-79-3	Arginine	↓	0.0241
Arginine and ornithine metabolism	1	74-79-3	Arginine	↓	0.0241
Lipoate biosynthesis	1	4754-39-6	Deoxyadenosine	↓	0.0241
SHP2 signalling	1	74-79-3	Arginine	↓	0.0275
Corticosteroids and cardioprotection	1	74-79-3	Arginine	↓	0.0275
Glutathione biosynthesis	1	636-58-8	Glutamyl-cysteine	↓	0.0275
Metabolism of angiotensinogen	1	74-79-3	Arginine	↓	0.0309
Protein repair	1	74-79-3	Arginine	↓	0.0309
Phenylethylamine degradation	1	122-78-1	Phenylacetaldehyde	↓	0.0309
Amino acid metabolism	3	636-58-8;74-79-3;4754-39-6	Glutamyl-cysteine; arginine; deoxyadenosine	↓	0.037
Vitamin C metabolism	1	122-78-1	Phenylacetaldehyde	↓	0.0377
Endothelin pathways	1	74-79-3	Arginine	↓	0.0377
Ion channels	1	74-79-3	Arginine	↓	0.0377
Urea cycle	2	636-58-8;74-79-3	Glutamyl-cysteine; arginine	↓	0.0378
Wybutosine biosynthesis	1	4754-39-6	Deoxyadenosine	↓	0.0378
Thromboxane A2 receptor signalling	1	74-79-3	Arginine	↓	0.041
Molybdenum cofactor biosynthesis	1	4754-39-6	Deoxyadenosine	↓	0.041
ABC transporters	2	2041-14-7;74-79-3	2-aminoethylphosphonic acid; arginine	↓	0.0417
α9 β1 integrin signalling	1	74-79-3	Arginine	↓	0.0444
Citrulline-nitric oxide cycle	1	74-79-3	Arginine	↓	0.0444
Phenylalanine degradation	1	122-78-1	Phenylacetaldehyde	↓	0.0477
Steroid hormone metabolism	1	80-92-2	5β-Pregnane-3α, 20α-diol	↑	0.0861

Rheumatoid arthritis significantly altered metabolic pathways in human SF. Enrichment of co-regulated metabolites lower in RA in Fig. 1A, cluster 1.1 mapped to significantly downregulated pathways via IMPaLA. Enrichment of co-regulated metabolites upregulated in RA in Fig. 1A, cluster 1.2 mapped to significantly upregulated pathways via IMPaLA. This is a condensed list of implicated pathways, with the full list presented in Supplementary Table 1.

their respective cohorts or were present in very low concentrations that were below the noise threshold. Data in this scatterplot further demonstrate the variation in metabolomic profiles and clear discrimination between RA and healthy SF. Volcano plot analysis of RA and healthy metabolites also showed 12 m/z values significantly upregulated and 25 m/z values significantly downregulated compared to healthy SF, with p-values<0.05 and fold changes greater than 2 (Fig. 1D). Enrichment of these metabolites yielded pathways consistent with clusters 1.1 and 1.2 (Supplementary Table II).

Biomarker candidates for rheumatoid arthritis

ROC analysis identified 60 m/z values with an AUC >0.9 for classification of RA, 30 of which were also identified as potential biomarkers of OA (9). These overlapping biomarkers for both OA and RA were removed from the analysis. Thus, the remaining 30 m/z values were searched in METLIN and 15 of those m/z values mapped to 119 possible metabolite identities (Table II, Supplementary Table III). The other 15 m/z values did not map to any known metabolite identities, thus further analysis is needed to determine if they could be novel metabolites. Potential RA biomarker identities included various phospholipids (PS, PG, PE, and PI-Cer),

Table II. Putative biomarkers of RA in human synovial fluid.

m/z	Adduct	Mass	ppm	Metabolite Identity
184.9431763	[M+Na]	183.9353	3	Arsonoacetate
343.2590332	[M+H]	342.2559	12	Cannabidiol dimethyl ether
	[M+H]	342.2559	12	Docosahexaenoic Acid methyl ester
	[M+H]	342.2559	12	TrHA
	[M+H]	320.2715	4	Dihomo-Linolenic Acid methyl ester
	[M+H]	320.2715	4	3a,20b-Pregnanediol
	[M+H]	320.2715	4	7a,17-dimethyl-5b-Androstane-3a,17b-diol
	[M+H]	320.2715	4	Pregnanediol
	[M+H]	320.2715	4	5-Pregnane-3,20-diol
	[M+H]	320.2715	4	5(Z),8(Z),11(Z)-Eicosatrienoic Acid methyl ester
	[M+H]	320.2715	4	6, 17-Dimethyl-5alpha-androstane-3beta,17beta-diol
	[M+H]	320.2715	4	11,17-Dimethyl-5alpha-androstane-11beta,17beta-diol
	[M+H]	320.2715	4	3-Ethyl-5alpha-androstane-3alpha,17beta-diol
	[M+H]	320.2715	4	21:3(5Z,14Z,17Z)
	[M+H]	320.2715	4	1-Hydroxy-2,12,15-heneicosatrien-4-one
	[M+H]	320.2715	4	(E,E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl hexanoate
	[M+H]	320.2715	4	7E,9E-Heneicosadien-6,11-one
497.3209839	[M+H]	496.3122	3	Postin
	[M+H]	496.3165	5	PG(P-18:0/0:0)
	[M+H]	474.3345	5	18-acetoxy-1,25-dihydroxyvitamin D3 / 18-acetoxy-1,25-dihydroxycholecalciferol
	[M+H]	474.3345	5	11-acetoxy-3beta,6alpha-dihydroxy-24-methyl-27-nor-9,11-seco-5alpha-cholesta-7,22 E-dien-9-one
	[M+H]	474.3345	5	(3beta,17alpha,23S,24S)-17,23-Epoxy-3,24,29-trihydroxy-27-norlanost-8-en-15-one
	[M+H]	474.3345	5	(3beta,17alpha,23S)-17,23-Epoxy-3,28,29-trihydroxy-27-norlanost-8-en-24-one
509.3752747	[M+Na]	508.3705	4	2'-Apo-beta-carotenal
520.3198242	[M+H]	497.3353	8	Tumonoic Acid I
520.3356934	[M+H]	519.3325	7	1-Linoleoylglycerophosphocholine
	[M+H]	519.3325	7	Phosphatidylcholine
	[M+H]	519.3325	7	LysoPC(18:2(9Z,12Z))
520.3833008	[M+Na]	519.3689	13	PC(P-19:1(12Z)/0:0)
523.3704224	[M+H]	522.3709	14	1-hydroxy-18-[m-(1-hydroxy-1-ethylpropyl)-benzyloxy]-23,24,25,26,27-pentanorvitamin
	[M+H]	522.3604	5	Oleic Acid-biotin
	[M+H]	500.3866	10	Oleananoic acid acetate
	[M+H]	500.3866	10	3beta-Hydroxylanostane-7,11-dione acetate
774.5570068	[M+H]	773.5571	9	Phosphatidylserine
798.5493774	[M+H]	797.5418	0	Phosphatidylinositol-ceramide
	[M+H]	775.5516	10	Phosphatidylethanolamine
799.5548706	[M+H]	798.541	8	Phosphatidylglycerol
	[M+H]	776.5567	11	1-Stearoyl-2-Oleoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)]
	[M+H]	776.5567	11	PG(16:0/20:1(11Z))
800.5652466	[M+Na]	777.5672	10	Phosphatidylethanolamine
822.5514265	[M+Na]	799.5727	12	Phosphatidylserine
824.5645142	[M+Na]	823.5479	11	C18-OH Sulfatide
826.5829468	[M+H]	825.5731	3	Phosphatidylinositol-ceramide
	[M+H]	803.5829	13	Phosphatidylethanolamine
	[M+H]	803.604	12	Phosphatidylserine

Global metabolomic profiling of human synovial fluid identified putative biomarkers of RA. 30 m/z values had an AUC >0.9 and were classified as potential RA biomarkers. M/z values were matched to metabolite identities using the metabolite database, METLIN. 15 m/z values did not match to any known metabolite identities and require further experiments to determine if they are novel metabolites. This is a condensed list, with the full list of 119 candidate metabolite identities included in Supplementary Table III.

diol and its derivatives, arsonoacetate, oleananoic acid acetate, docosahexaenoic acid methyl ester, and linolenic acid and eicosatrienoic acid derivatives (Table II, Supplementary Table III).

Discussion

To our knowledge, this is the first study to perform global metabolomic profiling of human RA SF by LC-MS. The global metabolomic profiles contained over 1000 metabolites in human SF, with 162 differing significantly between RA and healthy cohorts. Furthermore, HCA and PCA clearly discriminated RA samples from healthy sam-

ples, illustrating their distinct metabolomic phenotypes. Enrichment analysis of co-regulated metabolites revealed a number of significantly altered biochemical pathways in RA SF including inflammatory and anti-inflammatory pathways, energy metabolism, nitric oxide metabolism, and amino acid metabolism. Importantly, we identified 30 candidate biomarkers of RA in human SF using global metabolomic profiling including various phospholipid species, fatty acids, and acetate derivatives.

Global metabolic profiles of RA patient SF found anti-inflammatory drug pathways, amino acid metabolism, leukotriene biosynthesis, alpha-linolenic acid metabolism, glucocorticoid metabolism, and steroid metabolism to be implicated in RA (Table I, Supplementary Table I). RA is a chronic inflammatory disease and many patients take NSAIDs likely explaining the anti-inflammatory drug pathways detected in RA SF (14). Glucocorticoids are strong anti-inflammatory drugs commonly used to locally treat RA. Other pathways identified that support the inflammation in RA are leukotriene biosynthesis and alpha-linolenic metabolism. Leukotrienes are eicosanoid inflammatory mediators derived from arachidonic acid. Alpha-linolenic acid and arachidonic acid are precursors to prostaglandins, which aid in the generation of the inflammatory response (15).

The results of this study support altered energy metabolism in RA SF, as previously reported. Yang *et al* observed increased glycolytic activity and subsequent increased lactic acid in RA SF (8). Increased lactic acid and pyruvate resulting from heightened glycolytic activity can drive abnormal angiogenesis in diseased joints (8). The results of the present study indirectly support this altered energy metabolism by finding that angiotensinogen and angiotensin metabolisms were significantly altered in RA SF. More directly, however, we identified NAD metabolism as a significantly altered pathway in RA SF. NAD is an important cofactor mediating energy signalling and has recently been shown to play a role in a wide range of diseases (16). Importantly, nicotinamide phosphoribosyltransferase (NAMPT), also known as visfatin, is a key enzyme in NAD biosynthesis and has been proposed as a clinical biomarker of RA (17). The results herein further support NAD metabolism as a key pathway in RA pathogenesis.

Consistent with previous studies, we identified a number of oxidative stress-related pathways associated with RA. RA is characterised by elevated levels of reactive oxygen species (ROS) that induce oxidative stress, leading to damage of DNA and lipids (18). ROS attack other cellular components including residual amino acids, and protein aggregates while also inhibiting the formation of crosslinks (19). Antioxidants work to eliminate ROS, but in RA they are not sufficiently expressed relative to increased levels of ROS (20). Glutathione (gamma-glutamyl cycle) and nitric oxide are examples of antioxidants whose metabolism was found to be downregulated in the RA SF. Arginine (substrate in the NO cycle) metabolism as well as citrulline (product of NO cycle) production were also downregulated in RA SF – providing further support of altered NO metabolism. Citrulline and arginine are also intermediary molecules in the urea cycle, which produces NO as a byproduct via nitric oxide synthase. Taken together, these results support oxidative stress in the pathogenesis of RA.

Creatine metabolism was also downregulated in the RA samples, which is consistent with the disease pathology, as ~ 67% of RA patients involuntarily lose strength – even those with controlled RA (21). This process has been termed rheumatoid cachexia. With an excess of inflammatory cytokines in RA, signalling pathways are disrupted causing a shift from anabolic to catabolic matrix metabolism (22, 23). Given this, creatine is not necessary for muscle hypertrophy and is subsequently downregulated.

Other metabolites detected in this study have been previously identified in the literature as significantly different between RA and healthy SF. These include pipecolic acid, carnitine, tryptophan, citric acid, mannosylglycerate, and valine (7, 8). These metabolites map to known pathways we found to be altered in RA, including energy metabolism, lysine degradation, and tryptophan metabolism. Because these data include established pathways in RA, these results validate the use of global metabolomics profiling for studying RA aetiology.

While this study exemplifies the use of LC-MS-based global metabolomic profiling for RA biomarker discovery, there are important limitations. This study identified potential biomarkers capable of distinguishing RA from healthy SF. However, these studies did not compare RA to SF from patients with other types of arthritis. To validate these results, future studies would need to determine that these potential biomarkers could also distinguish RA SF from other diseased SF such as gout or psoriatic arthritis. Further validation studies are needed with larger sample sizes to attempt to replicate these results; these preliminary results can be used to guide a power analysis for that research. We refrain from extensive interpretation of the candidate RA biomarkers due to the small sample size (n=3). RA is a heterogeneous disease, and a sample size of n=3 is unlikely to cover all phenotypes of the disease (24). Furthermore, RA samples lacked clinical information, making it impossible to age and gender match RA samples to healthy controls. RA samples were also from living patients presenting to a rheumatology clinic in contrast to the *post mortem* healthy samples. However, past studies report no significant differences between *post mortem* SF and SF from living patients (25, 26). Future studies will increase the sample size and obtain sufficient clinical information to age- and gender- match samples in diseased and control cohorts. Future studies will also validate the identities of potential biomarkers with targeted methods, tandem mass spectrometry, and corresponding assays.

This study supports the use of global metabolomic profiling using LC-MS for further understanding of disease pathogenesis and biomarker discovery. The metabolic perturbations exhibited in RA synovial fluid are associated with changes in energy metabolism, chronic inflammation, and oxygen and nutrient

availability (27). Currently, few biomarkers of RA exist and those that do cannot adequately distinguish RA from other inflammatory forms of arthritis. Global metabolomic profiling holds great promise for identifying biomarkers of RA that could be used in a clinical setting for early diagnosis of disease and identifying opportunities for intervention.

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