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Systems diagnosis of chronic diseases, explored by metabolomics and ultra-weak photon emission

He, M.

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Author: He, M.

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Chapter 4

Spontaneous ultra-weak photon emission in correlation to inflammatory metabolism and oxidative stress in a mouse model of collagen-induced arthritis

Min He, Eduard van Wijk, Herman van Wietmarschen, Mei Wang, Mengmeng Sun, Slavik Koval, Roeland van Wijk, Thomas Hankemeier and Jan van der Greef

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Abstract

The increasing prevalence of rheumatoid arthritis has driven the development of new approaches and technologies for investigating the pathophysiology of this devastating, chronic disease. From the perspective of systems biology, combining comprehensive personal data such as metabolomics profiling with ultra-weak photon emission (UPE) data may provide key information regarding the complex pathophysiology underlying rheumatoid arthritis. In this article, we integrated UPE with metabolomics-based technologies in order to investigate collagen-induced arthritis, a mouse model of rheumatoid arthritis, at the systems level, and we investigated the biological underpinnings of the complex dataset. Using correlation networks, we found that elevated inflammatory and ROS-mediated plasma metabolites are strongly correlated with a systematic reduction in amine metabolites, which is linked to muscle wasting in rheumatoid arthritis. We also found that increased UPE intensity is strongly linked to metabolic processes (with correlation co-efficiency $|r|$ value >0.7), which may be associated with lipid oxidation that related to inflammatory and/or ROS-mediated processes. Together, these results indicate that UPE is correlated with metabolomics and may serve as a valuable tool for diagnosing chronic disease by integrating inflammatory signals at the systems level. Our correlation network analysis provides important and valuable information regarding the disease process from a system-wide perspective.

1. Introduction

Rheumatoid arthritis (RA) is one of the most prevalent chronic auto-immune diseases, occurring in about approximately 1% of the population in Western countries [1], [2]. RA manifests as a complex inflammatory syndrome that typically includes joint swelling, pain, and hyperthermia, as well as synovial hyperplasia and destruction of cartilage and bones in the joints. RA is considered a systemic disease that is caused by a variety of pathophysiological processes [3]. These processes are accompanied by increased levels of cytokines such as tumor necrosis factor α (TNF- α) and interleukins (IL-1 β and IL-6) in the blood and interstitial fluids, activation of NF- κ B pathways (to inhibit apoptosis in various immune cells), and systemic disruptions in inflammatory metabolite synthesis [4]–[6].

Experimental studies of RA—particularly the pathophysiological mechanisms of therapeutic interventions—are often conducted using animal models. The most commonly used model for RA is the collagen-induced arthritis (CIA) mouse model, which has pathophysiological processes and features similar to patients with RA [7]–[11]. In addition, advances in metabolomics technology, which now enable researchers to measure extremely low concentrations of metabolites in several pathways simultaneously [12], has facilitated the study of RA in considerably more detail, thereby increasing our understanding of the pathological mechanisms that underlie the disease [13]. We previously studied the differences in molecular profiles between CIA mice and control mice by examining differences with respect to inflammation and reactive oxygen species (ROS), analyzed using univariate and multivariate metrics [14]. In addition to the well-characterized inflammatory phenomenon, issues related to muscle wasting and energy expenditure are also present in RA [15]–[18], and this is reflected by the presence of amine metabolites in the plasma of CIA mice [19].

Differences between CIA mice and control mice were also observed with respect to the intensity of ultra-weak photon emission (UPE), which reflects differences in the organization of the system at a biophysical level [20]. UPE is a process that occurs in all living organisms and is the spontaneous emission of light with extremely weak intensity (10^1 – 10^3 photons/sec/cm²) in the UV, visible, and near-IR spectra [21]. Many studies have focused on the relationship between UPE and ROS production during metabolic processes [22]–[26]. Considering that ROS production is closely associated with inflammatory diseases and impaired metabolic processes, it is reasonable to expect that UPE is also associated with inflammatory disease and/or metabolic processes. UPE might therefore be used to help diagnose inflammation and inflammation-related diseases. UPE has been proposed for monitoring lipid peroxidation in cell membranes [27], and applications using UPE in human studies—and their potential relationship with ROS—were summarized by van Wijk [23]. Moreover, the putative relationship between UPE, physiological state, and metabolic processes has been proposed by several research groups [28]–[31]. Here, we performed an integrated analysis of the biochemical and biophysical differences between CIA mice and control mice, based on the hypothesis that a combined analysis would reveal unique insight into the biochemical and biophysical changes that occur during RA.

Network biology is an emerging field in biomedical research, and network biology tools are increasingly used to identify clusters of correlated parameters, to visualize or explore high-dimensional data, and to understand or interpret interactions that reflect part of a complex biological system [32], [33]. Correlation networks have been used in “omics” studies to combine complex data sets, for example combinations of metabolomics, genomics, and/or proteomics data sets. Correlation networks are also used to support the biological interpretation of large data profiles and to differentiate disease phenotypes [34]–[37]. Here, we expanded the systems-based approach of correlation-based analyses in order to examine the relationship between metabolomics profiling and UPE data. Using this correlation network analysis, we visualized systematic perturbations in bio-photons,

inflammatory processes, and ROS-related mediators. This approach may be used to facilitate the diagnosis of disease and/or to discriminate between disease syndromes, particularly with respect to complex chronic diseases such as RA and type 2 diabetes mellitus.

2. Materials and Methods

2.1 Animal study samples, Modelling, and ethics Statement

CIA was induced by the intraperitoneal injection of type II collagen and lipopolysaccharide in adult (6-7 weeks of age) DBA/1J male mice as described previously [38]; the CIA and control (Ctrl) groups contained 10 mice each. The injections were performed on days 0, 14, 28, 42, and 56; After 70 days' modeling, UPE intensity was measured in each paw, and blood was collected into pre-cooled EDTA tubes (BD Vacutainer, Plymouth, UK). The blood samples were centrifuged at 3000×g for 10 minutes, and then stored at -80°C until metabolic measurements were performed [14]. All animal experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Bethesda, MD). All animal care and experiments were approved by the Tohoku Institute of Technology Research Ethics Committee, Sendai, Japan.

2.2 Instruments and data acquisition

2.2.1 UPE instruments and settings

UPE was measured using a 600 series CCD camera system (Spectral Instruments, Inc., Tucson, AZ) equipped with a closed-cycle mechanical cryogenic unit (held at -120°C) as the cooling system. Prior to the UPE measurement, mice were maintained in controlled dark conditions. The detailed settings of the CCD system including figures about the measured location on mice is described in Van Wijk et al [20]. In brief, the CCD camera was mounted on the top of a dark chamber, and the animal was immobilized using isoflurane anesthesia. UPE intensity was recorded at five independent regions on each paw and used for further correlation analysis. The regions were named according to the paw measured, and numbers were added (ranging from 1 to 5, indicating the location closest to the tip of the paw through the location farthest from the tip of the paw) as follows: LFP (left front paw) 1 through LFP5; LHP (left hind paw) 1 through LHP5; RFP (right front paw) 1 through RFP5; and RHP (right hind paw) 1 through RHP5.

2.2.2 Extraction of plasma metabolites and metabolomics analysis

Plasma samples were aliquoted and extracted via different methods in order to obtain separate classes of compounds, including oxylipins, amine metabolites, and oxidative stress-related metabolites. Oxylipins (bioactive lipid mediators derived from polyunsaturated fatty acids) were extracted using solid phase extraction and analyzed using an Agilent 1290 HPLC coupled to an Agilent 6490 triple quadrupole mass spectrometer with electrospray ionization as described previously [14], [39]. Amine metabolites (including free amino acids and their biogenic metabolites) were extracted using AccQ-TagAQC derivatization and analyzed using a Waters ACQUITY UPLC coupled to a Waters Xevo mass spectrometer with electrospray ionization source as described by Noga et al. [40]. Oxidative stress-mediated metabolites—primarily PGs/IsoPGs, NO₂-FAs, lysophosphatidic acids, and sphingosine/sphingosine-related sphingolipids—were extracted using liquid-liquid extraction and analyzed using a validated method with an Agilent 1290 HPLC coupled to an Agilent 6490 triple quadrupole mass spectrometer with electrospray ionization. The peak area of each target compound was corrected using the appropriate internal standard (ISTD), leading to a ratio (target compound/ISTD) that was used for further analysis in the correlation study.

2.3 Data preprocessing and statistical analysis

The metabolomics and UPE data collected from both the CIA and Ctrl groups were included in the correlation analysis. Univariate correlations were performed using the Spearman's rank correlation method using RStudio software (version 3.0.3). Absolute values of the Spearman's rank correlation coefficient ($|r|$) >0.7 were considered to reflect a strong correlation between parameters, and this threshold was used to create highly correlated graphical networks using Cytoscape software (version 3.3.0, <http://www.cytoscape.org>) with the MetScape plug-in for extracting and integrating information and for visualizing the correlation networks

[41], [42]. Positive and negative correlations were indicated by positive and negative values of r , respectively.

3. Results and Discussion

3.1 Collagen-induced arthritis alters the local distribution of UPE

Differences in UPE between CIA and Ctrl mice have been reported previously [20]. A schematic figure was displayed, in order to show the CCD setup of UPE instrument as well as the locations for UPE measurements on mouse front and hind paws (Fig. 1). Here, we used correlation networks to visualize the relationship between individual UPE intensities at the locations measured in both CIA mice and in Ctrl mice (Fig. 2), as visualizing the profile of location-based UPE may provide important information regarding the disease. We then interpreted the differences and similarities between the two groups with respect to their correlation structures.

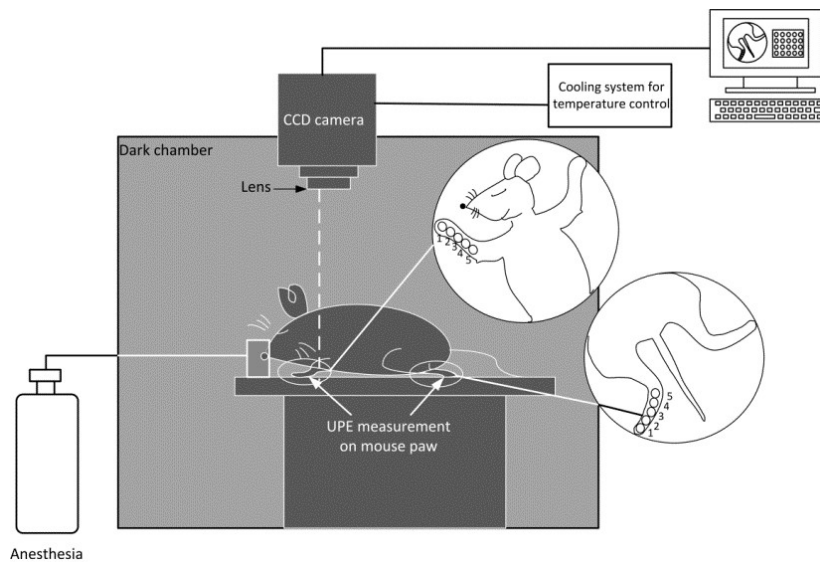


Fig. 1 Schematic figure of CCD set-up as well as locations for UPE measurements on mouse front and hind paws. Adapted from E. van Wijk et al. 2013.

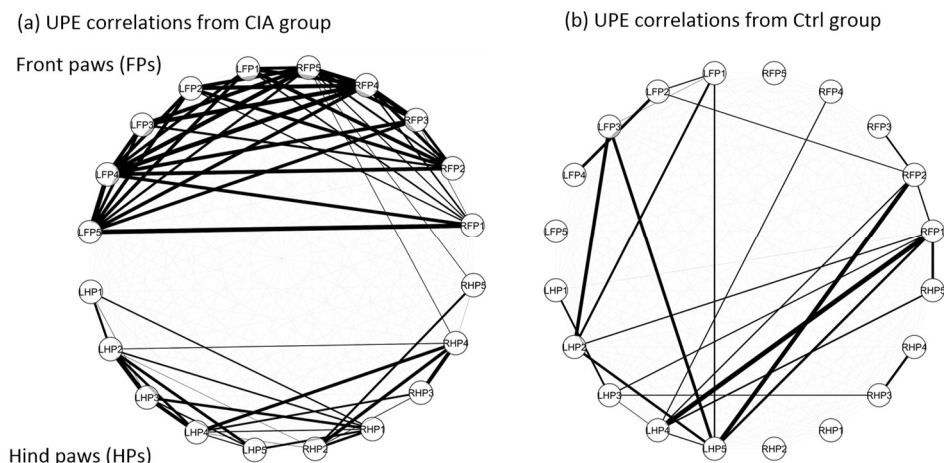


Fig. 2. Bio-photonic variance is revealed by location-based UPE-to-UPE correlation networks. The figure illustrates the differences in correlations between CIA mice (a) and Ctrl mice (b). In CIA mice, the strong correlations also indicate a strong similarity in UPE between the LFP (left front paw) and RFP (right front paw), as well as between the LHP (left hind paw) and RHP (right hind paw). The numbers (1 through 5) indicate the specific locations for the measurements (see Materials and Methods). Thus, the differences between the front paws and hind paws are clearly visible in the CIA group. The networks were established using the Spearman correlation analysis, and the lines represent Spearman correlation coefficients ($|r| > 0.7$).

The correlations were quantified using the parameters (i.e., $|r|$ values and p -values) obtained from the Spearman correlation analysis. In total, 71 and 26 strongly positive UPE-to-UPE correlations were found in the CIA and Ctrl groups, respectively; no strongly negative correlations were found. The difference in the number of strongly positive correlations between the CIA and Ctrl groups can be seen visually in Fig. 2. In the CIA group, UPE intensity was tightly correlated between the two front paws and between the two hind paws (Fig. 2a). In contrast, we found no clear correlation patterns in the Ctrl group (Fig. 2b).

3.2 Differences in metabolite correlations between CIA mice and control mice

Next, we acquired metabolic data from plasma samples using HPLC-MS/MS. The following three groups of metabolites were extracted using three validated

methods and detected using three specific instruments: amine metabolites (including free amino acids and their biogenic metabolites), oxylipins, and oxidative stress-related metabolites. A total of 110 endogenous metabolites were detected in the plasma samples, including 30 oxylipins, 45 amine metabolites, and 35 oxidative stress-related lipids. Univariate and multivariate analyses were then applied to the metabolite sets in order to characterize the differences between CIA mice and Ctrl mice at the metabolomics level. Previously, we reported the differences between CIA mice and Ctrl mice with respect to oxylipins and amine metabolites [14], [19]. Based on the oxidative stress platform, after log transformation and auto-scaling of the data, we also found a number of key metabolites that differed between the CIA the Ctrl groups ($p < 0.05$, Student's t -test). Table 1 summarizes the key metabolites that differed significantly between the CIA and Ctrl groups.

Table 1. Summary of the key metabolites that significantly differed between the CIA and Ctrl groups

Oxylipins		Amine metabolites		Oxidative stress	
Compound	Changes	Compound	Changes	Compound	Changes
9,10-DiHOME	↓	Methionine	↓	PGE3	↓
9-KODE	↓	Homocysteine	↓	8,12-iso-iPF2a	↓
13-HDoHE	↑	Threonine	↓	cyclic-LPA C16:0	↓
14-HDoHE	↑	Proline	↓	cyclic-LPA C18:2	↓
12,13-DiHOME	↓	Alanine	↓		
9,12,13-TriHOME	↓	Valine	↓		
12-HEPE	↑	Cystathionine	↓		
9,10,13-TriHOME	↓	Lysine	↓		
9,10-EpOME	↓	Glycylglycine	↓		
10-HDoHE	↑	Serine	↓		
9-HODE*	↓	Asparagine	↓		
8-HETE*	↑	Cysteine	↓		
13-KODE*	↓	Tryptophan	↓		
12,13-EpOME*	↓	Methionine sulfoxide	↓		
13,14-dihydro-PGF2a*	↑	Homocitrulline	↓		
12-HETE*	↑	Isoleucine	↓		
		Gamma-glutamylalanine	↓		
		Histidine	↓		
		Glutamine	↓		
		Leucine	↓		

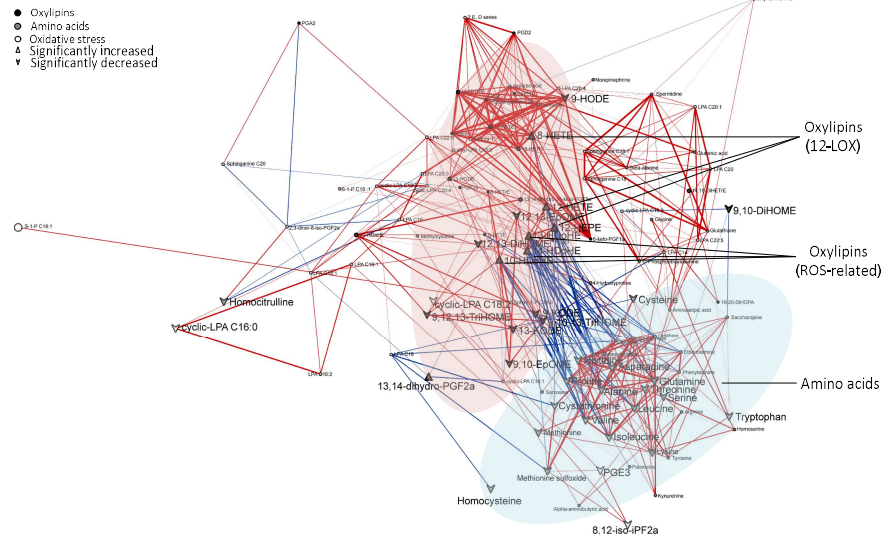
Abbreviations: DiHOME, dihydroxyoctadeca(mono)enoic acid; EpOME, epoxyoctadecamonoenoic acid; HDoHE, hydroxydocosaheptaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; KODE, ketoctadecadienoic acid; PG, prostaglandin; TriHOME, trihydroxyoctadecenoic acid.

↓: Decreased in CIA mice; ↑: Increased in CIA mice;

*: Extra important oxylipins which contributed to the group clustering are based on multivariate analysis (VIP>1).

Differences in metabolites generally do not occur independently, but often change together with other, related metabolites, as metabolic reactions are often part of a dynamic system and have many biological processes in common [35]. Metabolic network analysis is an emerging approach used to diagnose disease, and it has the advantage of integrating “omics” datasets in order to identify links and select useful information from among chaos [34], [43]. We therefore performed a correlation network analysis in order to visualize pair-wise metabolic correlations and to extract novel information regarding dynamic alternatives. A merge between the metabolite-to-metabolite correlation networks measured in the plasma of CIA and Ctrl mice is illustrated in fig. 3a and 3b, respectively. Next, the Spearman correlation coefficient between metabolites (r_m) was calculated, and only strong correlations (either positive or negative) (i.e., with an $|r_m|$ value >0.7) were included in the resulting network. We found a total of 394 positive correlations and 91 negative correlations in the CIA group, and a total of 864 positive correlations and 117 negative correlations in the Ctrl group. In general, metabolites that are in the same chemical class or in the same biochemical pathway tended to correlate with each other; these so-called “chemical class-based” clusters and “pathway-based” clusters were more pronounced in the Ctrl group, leading a highly connected region among oxylipins and another region among amine metabolites. This network analysis revealed certain structural or pathway similarities among those highly connected metabolites with respect to significant positive correlations. Moreover, the associations between oxylipins and amine metabolites were relatively weak in the Ctrl group, possible because oxylipins and amine metabolites are generated via two separate metabolic pathways.

(a) Metabolite-to metabolite correlations from CIA group



(b) Metabolite-to metabolite correlations from Ctrl group

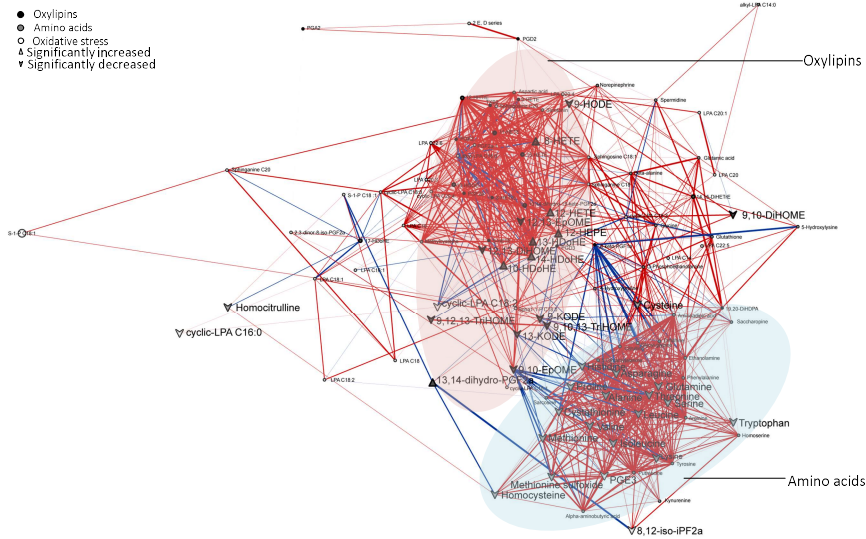


Fig. 3. Metabolic correlation networks in the CIA and Ctrl groups. Depicted are the metabolite-to-metabolite correlation networks for CIA (3a) and Ctrl (3b) mice. All of the metabolites detected in our analysis are included in the networks models.

Nodes with a positive correlation are indicated with solid red lines, and nodes with a negative correlation are indicated by solid blue lines. Shaded ellipses with a light red or light blue background indicate clusters of oxylipins or amine metabolites, respectively. Thickness of lines indicate gradient correlation strength: the thicker the line is, the stronger the correlation is (visible correlation coefficient: $|r|$ ranges from 0.7 to 1). (For better visualization of the detailed figures, please visit the web version of this article online: http://www.sciencedirect.com/cache/MiamiImageURL/1-s2.0-S1011134416307539-gr3_lrg.jpg/0?wchp=dGLzVIV-zSkWl&pii=S1011134416307539)

Interestingly, we found that some of the strong correlations in the Ctrl group—including both “oxylipin-to-oxylipin” and “amine-to-amine” correlations—were weaker in the CIA group. In contrast, the CIA group contained more negative oxylipin-to-amine correlations (Fig. 3a) than the Ctrl group (Fig. 3b). For example, the HETEs and HDoHEs that were elevated in CIA mice were strongly correlated with the branched chain amino acids valine, leucine, and isoleucine, as well as with cystathionine, alanine, glutamine, and asparagine. The use of HETEs and HDoHEs as inflammatory/ROS-related biomarkers has been described previously [14], and we also found that decreases in these amine metabolites may reflect muscle wasting and/or energy expenditure (cachexia) in RA [19]. Therefore, our analysis of metabolic correlation networks suggests that the increased inflammation and ROS levels reflected by oxylipins may also be associated with the onset of muscle wasting and increased energy expenditure in RA.

3.3 UPE is correlated with inflammatory signaling-related metabolites in CIA mice

As discussed in the Introduction, UPE arises as a result of metabolic reactions, particularly oxidation-reduction (redox) reactions; therefore, we hypothesized that UPE emission patterns may be correlated with metabolite patterns. To test this hypothesis, we created a correlation network to visualize potential associations between UPE intensity and peak area ratios of measured metabolites (see Materials and Methods). Therefore, we used UPE-to-metabolite correlations (i.e., between a given UPE value, u , and a given metabolite, m) in the correlation

networks, and the Spearman's correlation coefficient $|r_{um}|$ was calculated for each UPE-metabolite pair in both the CIA group and the Ctrl group.

The heat map in Fig. 4 depicts a general UPE-to-metabolite correlation profile used to compare the differences measured between the CIA group and the Ctrl group. A cluster analysis reveals clear location-based clusters in the CIA mice. The heat map also indicates a systemic change in the CIA group (i.e., the majority of positive correlations, shown in red) compared with the Ctrl group (i.e., the majority of negative correlations, shown in green). After removing relative weaker coefficient from the Spearman correlation analysis ($|r_{um}| < 0.7$), networks were built to reflect the highly correlated entities and to show the most important metabolites (fig.4b). After we removed the relatively weaker correlations from the Spearman correlation analysis (i.e., $|r_{um}|$ values < 0.7), we built a network to reflect the strongly correlated entities and to illustrate the most relevant metabolites (Fig. 4b). Circle-attributed networks were then used to identify the key correlations and to compare the CIA group with the Ctrl group. A total of 27 strongly positive correlations and 79 negative correlations were identified in the Ctrl group, and a total of 146 positive and 9 negative correlations were identified in the CIA group.

The correlation networks revealed that the majority of UPE-to-metabolite correlations in the Ctrl group were negative, whereas the majority of UPE-to-metabolite correlations in the CIA group were strongly positive. The major metabolites that were positively correlated with UPE in the CIA group are the monohydroxyeicosatetraenoic acids (HETEs), prostaglandins (PGs), thromboxane (TBX) synthase products, lysophosphatidic acids (LPAs), sphingolipid signaling molecules, and some amine metabolites (Fig. 4b). UPE intensity measured at various locations was correlated with various metabolites in the CIA group. For example, UPE intensity in the front paws was more strongly correlated with some LPAs, whereas UPE intensity in the hind paws was more strongly correlated with PGs (13,14-dihydro-15-keto-PGF2a, PGE2, PGD2, and 6-keto-PGF2a), TBX synthase products (TBX2 and 12-HHTre), HETEs (8-HETE, 15-HETE, 11-HETE, and 12-HETE), and sphingolipids; see the CIA correlation networks in Fig. 4b.

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networks reflect the inflammation, ROS production, and muscle wasting associated with RA. Each dot indicates an individual parameter that includes a given metabolite and UPE value: yellow dots reflect location-based UPE intensity, and black, gray, and white dots represent oxylipins, biogenic amines, and oxidative stress-related metabolites, respectively. Also shown (between the Ctrl and CIA network models) are enlarged views of the key metabolites that differed significantly based on our univariate and multivariate analyses. The up-triangles and down-triangles indicate the direction of the metabolic change in the CIA mice (i.e., up-regulation or down-regulation, respectively). (For better visualization of the detailed figures, please visit the web version of this article online: http://www.sciencedirect.com/cache/MiamiImageURL/1-s2.0-S1011134416307539-gr4_lrg.jpg/0?wchp=dGLbVBA-zSkzV&pii=S1011134416307539)

Next, the pathways related to these metabolites based on our previous study [14] and the Kyoto Encyclopedia of Genes and Genomes were organized (Fig. 5). LPAs act on G protein-coupled signaling and cellular signaling responses and function as inflammatory mediators [44], [45]. PGs and TBXs, which are synthesized from arachidonic acid via COX-II pathways, have well-established pro-inflammatory functions [46], [47]. The 12/15-LOX products (12-HETE, 15-HETE, and 8-HETE) promote the production of cytokines and activate the NF- κ B pathway to inhibit cellular apoptosis [48], [49]. In addition, 8-HETE, 12-HETE, and 11-HETE can also be peroxidized non-enzymatically by ROS to inhibit apoptosis [50]–[54]; therefore, these three HETEs may be important inflammatory mediators [55], [56]. The sphingomyelin-derived sphingolipids sphingosine and sphingosine-1-phosphate (S1P) are signaling molecules in immune cells that mediate neutrophil activation and apoptosis, and are therefore also considered to be inflammatory mediators [57]–[62]. Based on the correlation networks, it can be seen that these inflammatory mediators participated in the systemic perturbations (measured using both metabolomics and UPE) in the CIA mice, even though some of these mediators were not altered significantly in our univariate analysis. We also conclude that UPE intensity is correlated with systemic inflammatory mediators, ROS mediators, and cellular signaling processes; therefore, measuring UPE intensity may provide a means to diagnose inflammatory disease. In addition, UPE may also be used to monitor lipid peroxidation which relate to inflammation and ROS level in both healthy and diseased individuals (Fig. 6). Thus, a specific phenotype of a disease can be complemented by measuring both “omics” profiles

and UPE patterns, thereby providing a more detailed understanding of the disease and its underlying processes.

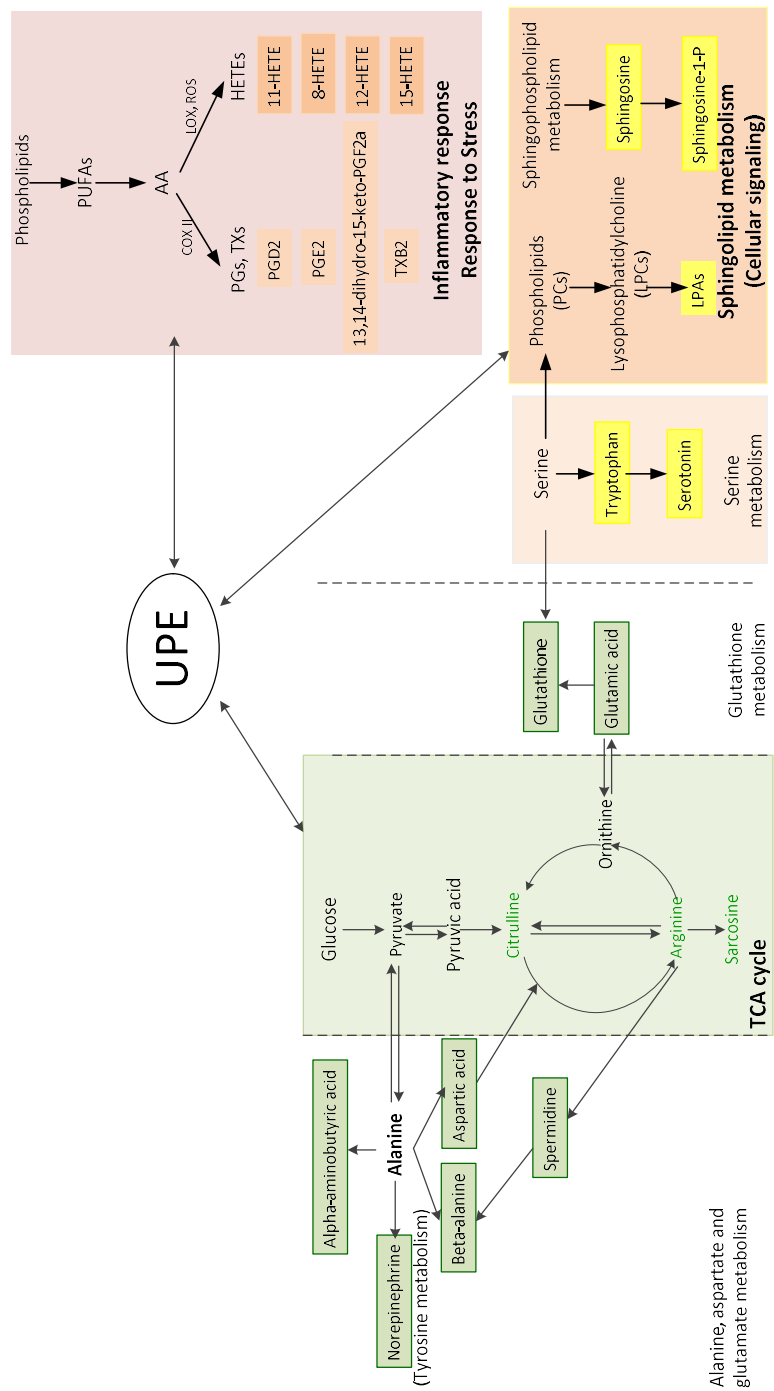


Fig. 5. Putative biochemical pathways with metabolites that can be correlated with UPE. UPE intensity can be linked to inflammatory responses and ROS-related stress in RA, which are reflected by COX-II and 12/15-LOX oxylipins derived from arachidonic acid (AA). During cell signaling, the production of LPAs and sphingosine also associates with the increased UPE intensity. Another pathway that may be linked with UPE intensity is the TCA cycle.

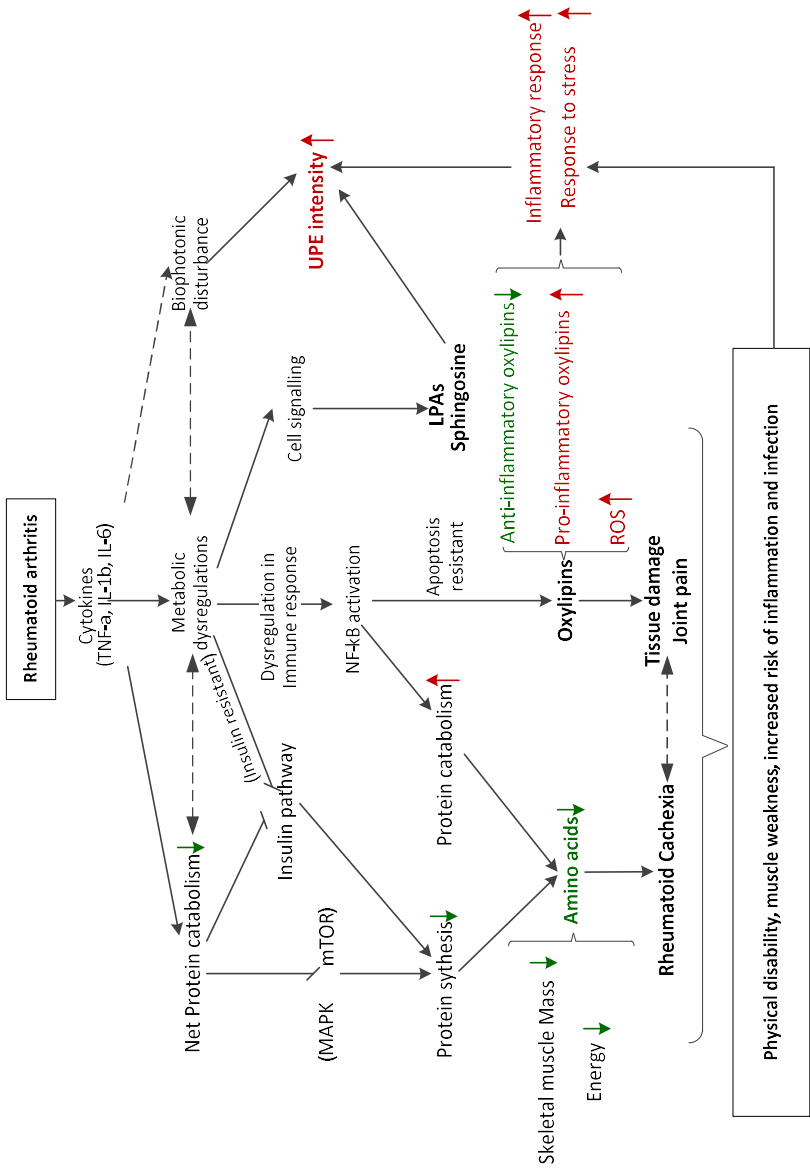


Fig. 6. Systemic and dynamic changes in RA. The production of cytokines dynamically interrupts living systems at both the biochemical level (i.e., the proteomics and metabolomics levels) and the bio-photon level. In addition, the biochemical changes are in correlation to the UPE changes. Elements marked in red/green are those increased/decreased in our CIA mice model.

In the CIA mice, a strongly negative correlation between cystathionine and UPE was measured, whereas several amine metabolites—including serotonin, tryptophan, and aspartic acid—were positively correlated with UPE. Cystathionine is a scavenger of free radicals [63]; therefore, given its significant decrease in CIA mice compared to Ctrl mice, the negative correlation between cystathionine and UPE intensity indicates that the increase in UPE intensity may be due to a decrease in antioxidants in RA. Both tryptophan metabolism and the serotonergic system have been well described as key pathways that can influence signaling in the central nervous system [64]. Thus, UPE may also be correlated with metabolic systems that are associated with neurotransmission. In addition, based upon pathways that regulate amine metabolites listed in the Kyoto Encyclopedia of Genes and Genomes, all of the other amine metabolites that were positively correlated with UPE are associated either directly or indirectly with the TCA cycle (see Fig. 5). The correlations identified between UPE intensity and these metabolites may suggest that during disease, some of the electrons that would otherwise participate in chemical reactions to produce energy (for example, with amine metabolites in the TCA cycle) actually escape and set free the energy which they carry, as photons, whereby the electrons change from high to low energy level states. Simultaneously, free radicals and/or ROS are produced, driving lipid peroxidation to produce inflammatory HETEs and PGs. While such a speculation need more rigorous validation.

The reduction in amine metabolites in the plasma of CIA mice compared to Ctrl mice may be linked to the contribution of muscle wasting in arthritis [19]. Considering that we found strong correlations between amine metabolites and UPE intensity, and given that muscle wasting is a common feature in many disease processes, including some cancers [65], HIV/AIDs [66], type 2 diabetes [67], renal failure, uremia[68], and heart failure [69], UPE may also have potential perspective for the use of monitoring energy wasting and muscle wasting in other diseases. In this respect, future studies should examine the relationship between muscle wasting and UPE.

Interestingly, HETEs, PGs, sphingosine, and S1P—which were strongly correlated with UPE intensity in our study—are also considered to be important inflammatory biomarkers in a variety of diseases, including RA [70], cardiovascular disease and/or atherosclerosis–related inflammation [59], [61], [71], [72], congestive heart failure [60], cancers and other tumors [54], [73], some prostate diseases [55], and nonalcoholic steatohepatitis [56]. Therefore, our finding that UPE is correlated with these inflammatory mediators may shed light on the biological mechanisms that underlie these diseases from a systems biology perspective.

4. Conclusions

Given its complex pathophysiology, RA has been studied using a variety of technologies and approaches. Indeed, integrating various data sets can provide important information regarding the disease process and possible treatment strategies. Generating correlation networks can provide valuable information, and these networks have been used recently within a wide range of “omics” studies, including proteomics, genomics, and metabolomics, thereby helping distinguish specific diseases and/or phenotypes [34], [35], [74]. Here, we performed the first study that integrates UPE with metabolomics in both diseased mice (i.e., mice with collagen-induced arthritis) and healthy control mice; this novel, powerful approach yielded meaningful information regarding RA. Moreover, we found specific correlations between metabolomics and UPE. Lastly, our correlation network analysis shows a systematic way to illustrate the complexity of RA , including dysregulation of both UPE and metabolomics.

Using our correlation networks, we also found that oxylipins were negatively correlated with certain amine metabolites in the CIA group. This may indicate a systematic perturbation under inflammation and ROS response in RA-induced situation. However, further study is needed in order to elucidate whether the inflammation and ROS are the consequence of muscle wasting, or vice versa. We also found that UPE was correlated with certain inflammatory mediators, and we expanded the biological interpretation of RA using correlation networks.

In conclusion, our correlation network analysis provides valuable information regarding the disease process from a system-wide perspective. Understanding the underlying biochemical phenomena that give rise to UPE is of great importance to learn about potential applications of UPE in early disease characterization.

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