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# Polymyalgia rheumatica shows metabolomic alterations that are further altered by glucocorticoid treatment: Identification of metabolic correlates of fatigue

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ABSTRACT

*Objective:* In polymyalgia rheumatica (PMR), glucocorticoids (GCs) relieve pain and stiffness, but fatigue may persist. We aimed to explore the effect of disease, GCs and PMR symptoms in the metabolite signatures of peripheral blood from patients with PMR or the related disease, giant cell arteritis (GCA).

*Methods:* Nuclear magnetic resonance spectroscopy was performed on serum from 40 patients with untreated PMR, 84 with new-onset confirmed GCA, and 53 with suspected GCA who later were clinically confirmed non-GCA, and 39 age-matched controls. Further samples from PMR patients were taken one and six months into glucocorticoid therapy to explore relationship of metabolites to persistent fatigue. 100 metabolites were identified using Chenomx and statistical analysis performed in SIMCA-P to examine the relationship between metabolic profiles and, disease, GC treatment or symptoms.

*Results:* The metabolite signature of patients with PMR and GCA differed from that of age-matched non-inflammatory controls ($R^2 > 0.7$). There was a smaller separation between patients with clinically confirmed GCA and those with suspected GCA who later were clinically confirmed non-GCA ($R^2 = 0.135$). In PMR, metabolite signatures were further altered with glucocorticoid treatment ($R^2 = 0.42$) but did not return to that seen in controls. Metabolites correlated with CRP, pain, stiffness, and fatigue ($R^2 \geq 0.39$). CRP, pain, and stiffness declined with treatment and were associated with 3-hydroxybutyrate and acetoacetate, but fatigue did not. Metabolites differentiated patients with high and low fatigue both before and after treatment ($R^2 > 0.9$). Low serum glutamine was predictive of high fatigue at both time points (0.79-fold change).

*Conclusion:* PMR and GCA alter the metabolite signature. In PMR, this is further altered by glucocorticoid therapy. Treatment-induced metabolite changes were linked to measures of inflammation (CRP, pain and stiffness), but not to fatigue. Furthermore, metabolite signatures distinguished patients with high or low fatigue.

*Abbreviations:* ANCA, anti-neutrophil cytoplasmic antibody-associated vasculitis; CRP, C-reactive protein; GC, glucocorticoids; GCA, giant cell arteritis; NMR, nuclear magnetic resonance; NOESY, Nuclear Overhauser Effect spectroscopy; OPLS-DA, orthogonal partial least squares discriminant analysis; PCA, principal component analysis; PLS-R, partial least squares regression analysis; PMR, polymyalgia rheumatica; RA, rheumatoid arthritis; VIP, variable importance for prediction.

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## 1. Introduction

Polymyalgia rheumatica (PMR) and giant cell arteritis (GCA) are closely-related diseases occurring in older people [1]. Whilst the exact aetiology remains unclear, genetics, environmental factors and age all contribute to onset and disease progression [1]. In PMR, inflammation occurs at extracapsular musculoskeletal sites (synovium, tendons, muscle) [2,3], whereas in GCA inflammation occurs in the arterial wall [1]. Glucocorticoid (GC) treatment remains the mainstay of treatment for both diseases, effectively controlling inflammation but often resulting in adverse effects [4]. Despite this, many patients with PMR and GCA continue to report levels of fatigue that affect their quality of life [5,6] - this is likely due to the complex, interacting impact of inflammatory disease and GC therapy. There is an urgent clinical need to understand the molecular mechanisms responsible for driving fatigue in patients with well-controlled inflammation to improve their clinical management.

Metabolomics is an emerging technology to detect the physiological derangements of diseases and treatments [7]. This technology has identified various derangements of metabolites correlating with disease activity in rheumatoid arthritis (RA) [8,9], Takayasu arteritis [10], and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis [11]. For example, inflammation was correlated with increased lactate and lipid levels [8], whilst fatigue scores correlated with decreased specific metabolites linked to in the urea cycle and fatty acid metabolism in RA patients [12]. Furthermore, GC therapy for rheumatic diseases has been described to cause various metabolomic alterations, including an increase in lysophospholipids in women with RA [13] and a fall in hydroxyacetone and 3-hydroxybutyrate levels in a mixed population of patients with PMR and RA [14]. Yet few studies have investigated the contribution of circulating metabolic profiles to patient symptoms in PMR and GCA and how these are altered upon treatment.

Clinical variables are poor predictors of prognosis in PMR [15], but patient stratification is currently hampered by lack of biomarkers that correlate with outcomes of PMR and its treatment. Metabolomics has been suggested as a possible approach for early detection of GC-related adverse effects [16]. Here, we sought to identify metabolomic correlates of pain, stiffness, and fatigue in a longitudinal dataset of GC-treated patients with PMR. We revealed that PMR has a distinct metabolomic signature from non-inflamed controls.

### 1.1. Methods

A summary of the participants, sample collection and analysis are shown in Supplementary Fig. 1.

*Ethical approval*

All samples were obtained with written, informed consent and approval (UK GCA Consortium 05/Q1108/28, Leeds West Research Ethics Committee, Clinical trial identifier: NCT04102930; ADDRESS-PMR 13/LO/1094, NRES Committee London – Camberwell St Giles, Characterisation of genes/proteins in autoimmune/inflammatory diseases 04/Q1206/107, Leeds East Research Ethics Committee; TABUL 09/H0505/132., Berkshire Research Ethics Committee; Clinical trial identifier NCT00974883), in compliance with the Declaration of Helsinki. Routine clinical and patient outcome measures were also obtained.

#### 1.1.1. Participants

Forty patients with GC-naive PMR were recruited into the ADDRESS-PMR (The Diagnostic Accuracy of Ultrasound in Suspected PMR) study. All PMR patients had a confirmed clinical diagnosis of PMR, with 36/40 fulfilling the ACR/EULAR classification criteria for PMR. Reasons for non-fulfilment were: absence of patient-reported bilateral shoulder aches (n = 2), normal inflammatory markers (n = 1), and insufficient points due to lack of patient-reported early morning stiffness (n = 1). Patients completed pain and stiffness numeric rating scores and the FACIT-F (Functional Assessment of Chronic Illness Therapy – Fatigue subscale) questionnaire [17]. C-reactive protein was measured in the routine diagnostic laboratory. For fatigue analysis to align with the pain and stiffness VAS, FACIT-F scores were reversed (i.e., 52 minus the FACIT-F score) so that those with high fatigue levels had higher fatigue scores. Patients were treated with prednisolone according to clinical guidelines for PMR [18] and attended follow-up at 4 and 26 weeks (visits 2 and 3) at which time points PMR was confirmed clinically. At each of these three visits, serum was stored for later analysis.

As a comparator inflammatory disease, 137 patients with suspected GCA recruited into the multi-centre TABUL (The Temporal Artery Biopsy -v- Ultrasound in diagnosis of Giant Cell Arteritis) study [19] were included in the current study if biological samples had been collected within 7 days of high dose prednisolone treatment. Of these, 84 were later classified as GCA (confirmed GCA) and 53 suspected GCA who were subsequently clinically confirmed with alternative diagnoses (clinically confirmed non-GCA). Patient recruitment pre-dated the ACR/EULAR 2022 classification criteria for GCA, with biopsy negative patients being clinical diagnosed by an expert panel based on review of symptoms and signs. Thirty-nine age-matched controls with no acute inflammatory or infective illness or underlying autoimmune or inflammatory disease were recruited from members of staff at the University of Leeds or at a cataract pre-assessment clinic. All samples were obtained with written, informed consent and approval (UK GCA Consortium 05/Q1108/28, Leeds West Research Ethics Committee, Clinical trial identifier: NCT04102930; ADDRESS-PMR 13/LO/1094, NRES Committee London – Camberwell St Giles, Characterisation of genes/proteins in autoimmune/inflammatory diseases 04/Q1206/107 Leeds East Research Ethics Committee; TABUL 09/H0505/132., Berkshire Research Ethics Committee; Clinical trial identifier NCT00974883), in compliance with the Declaration of Helsinki. Characteristics of patients and age and sex-matched controls are given in Supplementary Table 1.

#### 1.1.2. Sample collection, preparation and metabolomic analysis

Full methodology can be found in the Supplementary Methods Document. Blood was collected and processed for metabolomic analysis as previously described [8,20]. Samples were analysed at 300K using a standard 1D-1H-Nuclear Overhauser Effect spectroscopy (NOESY) pulse sequence with water saturation using pre-sat in a Bruker on Bruker AVANCE II 600 MHz NMR spectrometer (Bruker Corp., USA). Spectra were read and processed with Metabolab software (Version 2018.x; Birmingham, UK) [21] and phased, aligned and binned as previously described [8,22,23]. Metabolites were identified in Chemonx (Version 8.1; Chenomx Inc., Edmonton, Canada) [24].

#### 1.1.3. Statistical analysis

Data were initially subject to principal component analysis (PCA) to access the variability and identify any outliers. Supervised analyses were then performed to assess variation in the data with regards to a Y variable (e.g. case vs control or CRP) using orthogonal partial least squares discriminant analysis (OPLS-DA) or partial least squares regression analysis (PLS-R).

OPLS-DA was used to compare between groups of interest (i.e. case vs control, or PMR patients at different treatment points). This assessed the fold change between the groups of interest and gives each metabolite a variable importance for prediction (VIP) score which indicates how much that metabolite contributes to the model (higher number = higher contribution): a cut-off of 0.9 was used to take metabolites forward in the models.

PLS-R is a form of regression analysis that identifies which metabolites predict a given variable. Here, we used measured levels of C-reactive protein (CRP), pain, stiffness, and fatigue. Similarly, to the OPLS-DA models, metabolites are given a VIP score to indicate their contribution to the model, and 0.9 was used as a cut-off.

In both the OPLS-DA and PLS-R model, qualities were assessed using

$R^2$ (goodness of fit), $Q^2$ (goodness of prediction) and cross-validated ANOVA (CV-ANOVA) to determine significance levels. An $R^2 > 0.25$, with the difference between $R^2$ and $Q^2 < 0.2$, and $P < 0.05$ was deemed to be a well modelled and significant: the higher the $R^2$ the better the fit. All statistical analysis was performed in SIMCA-P, version 16 [25] (Umetrics, Sweden), with the exception of metabolites of interest where Kruskal-Wallis with Dunn's post-test was performed on the metabolite concentrations in GraphPad version 8.0.0.

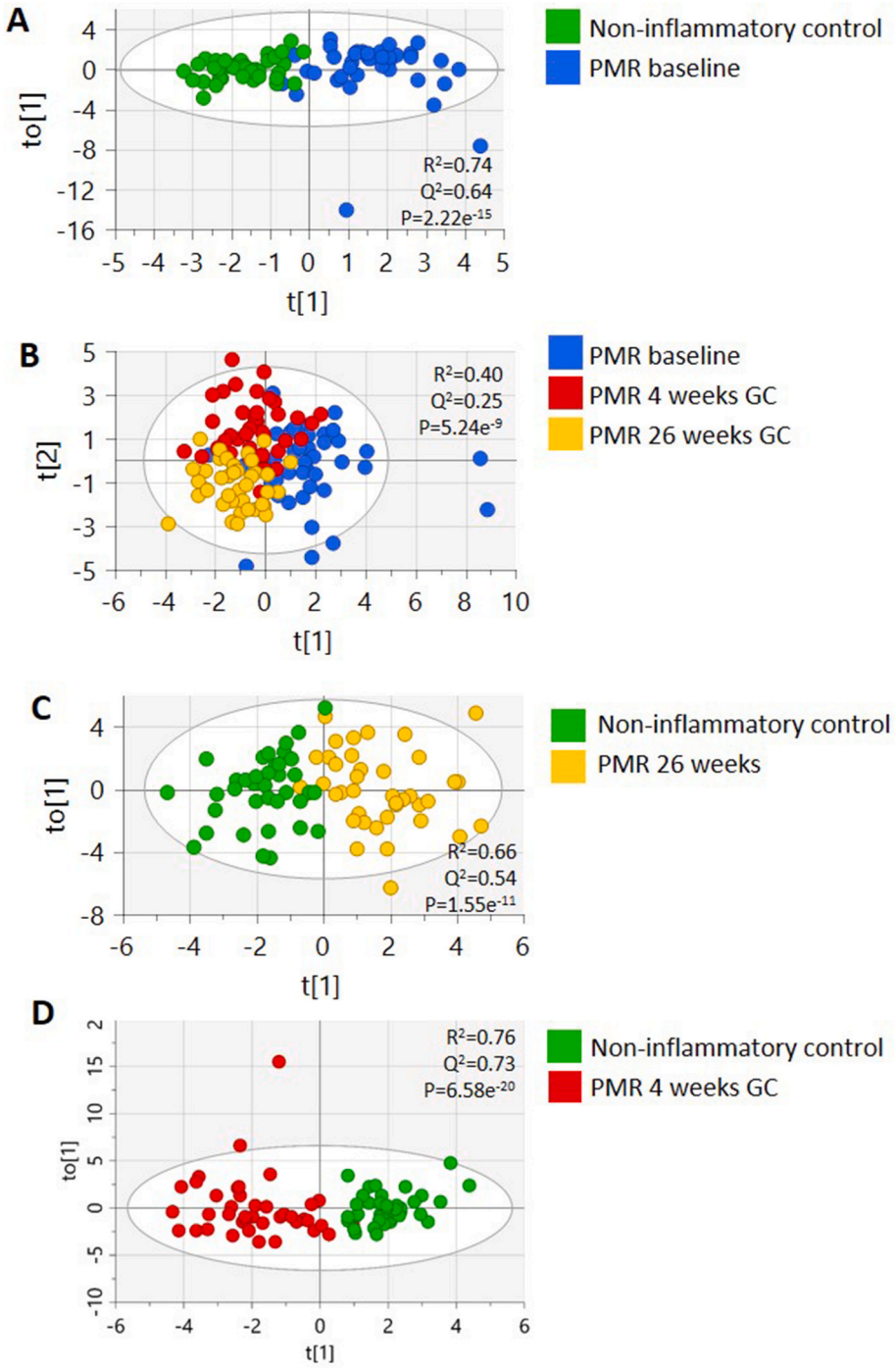


**Fig. 1. Metabolite concentrations reveal distinct metabolite profiles exist in polymyalgia rheumatica patients and are sensitive to glucocorticoid treatment.** One-dimensional 1H nuclear magnetic resonance (NMR) spectra were obtained from the serum of 39 age and sex-matched controls with no infection or underlying autoimmune and inflammatory disease (non-inflammatory control; green) and 40 patients with polymyalgia rheumatica (PMR) at 3 visits: (i) baseline pre-steroids with active inflammation (blue), at (ii) 4 weeks (red) and (iii) 26 weeks (yellow) of glucocorticoid (GC) treatment. Metabolite concentrations were determined from the spectra with Chenomx and analysed in SIMCA. Samples were subjected to orthogonal partial least squares discriminant analysis (OPLS-DA), then metabolites with a variable importance score of >1 were taken forward and subjected to OPLS-DA. (**A**) Comparison of metabolite profile in PMR vs controls, $R^2 = 0.74$, $Q^2 = 0.64$, $P = 2.22e^{-15}$. (**B**) Effect of GC treatment on the metabolomic profile of PMR patients, $R^2 = 0.40$, $Q^2 = 0.25$, $P = 5.24e^{-9}$ (**C-D**) Comparison of metabolite profile in GC treated PMR patients at **(C)** 26 weeks (, $R^2 = 0.66$, $Q^2 = 0.54$, $P = 1.55e^{-11}$) or **(D)** 4 weeks (, $R^2 = 0.76$, $Q^2 = 0.73$, $P = 6.58e^{-20}$) to non-inflammatory controls. Axis expressed as the proportion of variance captured by the latent variables (t and to). P values were determined using cross-validated ANOVA.

#### 1.1.4. Pathway analysis

Metabolites with a variable importance score (VIP) score of >0.9 in the PLS-R for CRP, pain, stiffness and fatigue were selected for pathway analysis in MetaboAnalyst [26]. Importance and relevance of pathways are indicated by the impact factor (X axis) and –log(P value) (Y axis).

#### 1.1.5. Patient and public involvement

Patients were involved in the design of the ADDRESS-PMR study and in the TABUL study. The TABUL study had a patient representative on the steering committee. Patients and the public were not directly involved in the metabolomic laboratory studies.

### 1.2. Results

#### 1.2.1. Circulating metabolome changes with polymyalgia rheumatica and is sensitive to glucocorticoid treatment

Firstly, the baseline metabolome of treatment-naïve PMR patients was compared to the matched non-inflammatory controls using OPLS-DA of NMR spectra (Supplementary Figs. 2A and B) and metabolite concentrations (Fig. 1A), which revealed clear separation between the two groups. Subsequently OPLS-DA was used to compare PMR patient serum spectra (Supplementary Figs. 2C–H) and metabolite concentrations (Fig. 1B) at 3 time points: (i) baseline prior to steroid therapy, and at (ii) 4 weeks and (iii) 26 weeks of GC treatment. We observed a significant difference between the 3 groups (Supplementary Fig. 2C –H and Fig. 1B) where, using metabolite concentrations, separation between treatment-naïve and treated patients was largely dependent on latent variable t[1], whilst latent variable t[2] revealed further separation between the GC treatment duration (4 or 26 weeks). Finally, there was a persistent separation between PMR at 4 and 26 weeks and controls, despite GC therapy (Supplementary Figs. 2I and J, and Fig. 1C and D).

Metabolites that contributed to separation of groups in the OPLS-DAs (Fig. 1) were identified. The fold changes and variable importance of prediction (VIP) scores of these are given in Supplementary Table 2, and concentrations of selected metabolite were plotted (Supplementary Fig. 3). Of these, the concentration of glycerol (Supplementary Fig. 3A) was higher, and methanol (Supplementary Fig. 3B) was lower in PMR patients compared to controls. Pyruvate, lactate and ornithine concentrations tended to be higher in PMR patients compared to controls (albeit not statistically significant). These metabolites were further and significantly increased upon GC treatment (Supplementary Figs. 3C–E). On the other hand, acetone was increased in PMR patients following 4 weeks GC treatment but then subsequently decreased with longer (26 weeks) use (Supplementary Fig. 3F). Taken together, these results demonstrate the serum metabolome is altered by disease (PMR), then further altered by GC treatment, but does not return to the same state as in non-inflamed controls despite 26 weeks of GC treatment.

#### 1.2.2. Separation of metabolome in patients with suspected GCA according to final diagnosis

Given that PMR is closely related to GCA [1], we next evaluated the metabolomic profile in NMR spectra from patients with a confirmed diagnosis of GCA in the TABUL study, compared to two other groups: Firstly, we compared patients with GCA with age-matched controls without infections, autoimmune and inflammatory disease (Fig. 2A, Supplementary Fig. 4A). Secondly, using samples only from the TABUL study, we compared patients with clinically confirmed GCA to those who's suspected GCA diagnosis was clinically confirmed as not GCA (non-GCA) (Fig. 2B, Supplementary Fig. 4B). OPLS-DA revealed a separation between patients with GCA and age-matched controls (Fig. 2A, Supplementary Fig. 4A). Within the TABUL study, however, there was a much smaller separation between the confirmed patients with GCA and those diagnosed as non-GCA (Fig. 2B, Supplementary Fig. 4B). Many of the non-GCA patients had an elevated CRP due to other inflammatory conditions (Supplementary Table 1) and thus it was possible that the changes we observed were correlating with inflammation rather than mechanisms specific to GCA. Removing the inflammation-associated metabolites according to their variable importance for prediction (VIP) scores marginally increased the separation between the confirmed GCA and confirmed non-GCA cases (Supplementary Fig. 5), albeit the $R^2$ was still very low (0.18), suggestive of poor modelling and separation between the groups.

#### 1.2.3. Inflammation and patient reported outcomes are associated with metabolomic profile

Metabolomic studies in RA have demonstrated an association between metabolomic profile and CRP [8,27]. To access this in the PMR and GCA patients, PLS-R was performed using the metabolite concentrations (Fig. 3) and NMR spectral bins (Supplementary Fig. 6). This gave moderate $R^2$ and $Q^2$ values indicating an association between the metabolite concentrations and systemic inflammation. Pathway analysis of metabolites revealed that the *synthesis and degradation of ketone bodies* pathway, in particular 3-hydroxybutyrate and acetoacetate, significantly contributed to the CRP prediction in patients with both PMR and GCA (Fig. 3, Supplementary Figs. 6A–B). Collectively, this suggests that concentrations of multiple metabolites, especially ketone bodies, correlate with systemic inflammation, as reflected by CRP.

Using PLS-R, we then examined whether metabolite concentrations

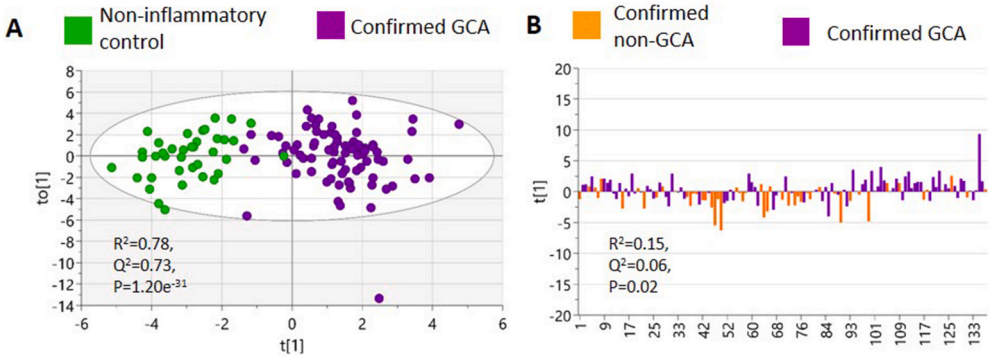


**Fig. 2. Metabolite concentrations in sera distinguishes giant cell arteritis patients from non-inflammatory controls and suspected giant cell arteritis.** One-dimensional $^1$H nuclear magnetic resonance (NMR) spectra were obtained from the serum of 84 giant cell arteritis (confirmed GCA) patients (purple), 39 age and sex-matched controls with no inflammation, control (non-inflammatory control, green) and 53 patients with suspected, but not confirmed giant cell arteritis (confirmed non-GCA) and analysed in SIMCA. In SIMCA, samples were subjected to orthogonal partial least squares discriminant analysis (OPLS-DA). Metabolites with a variable importance score of >1 were then re-subjected OPLS-DA and analysed. Comparison of GCA with (**A**) non-inflammatory control $R^2 = 0.78$, $Q^2 = 0.73$, $P = 1.20e^{-31}$ and (**B**) confirmed non-GCA $R^2 = 0.15$, $Q^2 = 0.06$, $P = 0.02$. Axis values indicate the proportion of variance captured by the latent variables. P values determined with cross-validated ANOVA.

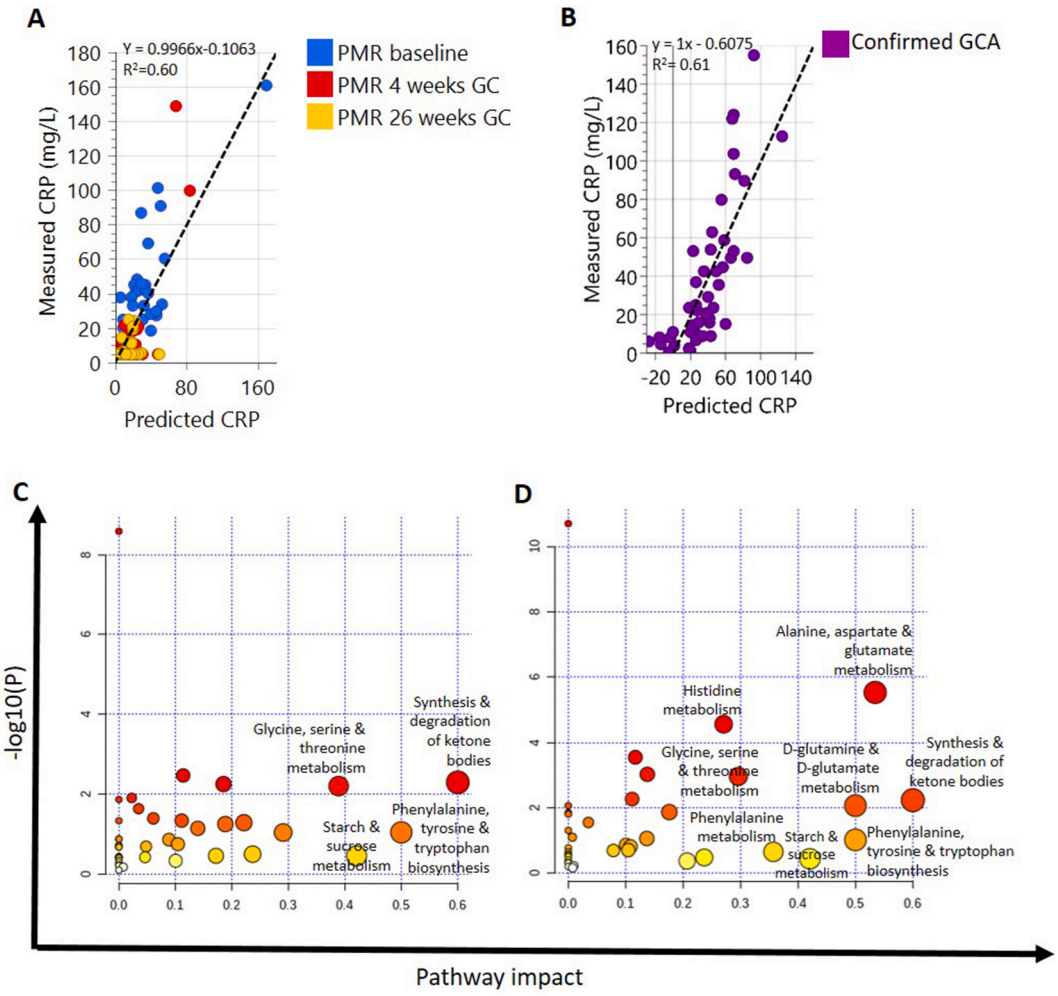


**Fig. 3. Metabolite concentrations in sera correlate with C-reactive protein.** One-dimensional 1H nuclear magnetic resonance (NMR) spectra were obtained from the serum of patients and metabolite concentrations were determined from the spectra with Chenomx. Predicted values of patient reported outcomes were determined from the metabolites using partial least squares regression analysis (PLS-R). Metabolites with variable importance for prediction (VIP) > 1 were taken forward and predicted values from these metabolites were compared to the CRP measured on the day the samples were taken, for (**A**) PMR patients before (blue) and after glucocorticoid treatment at 4 (red) and 26 weeks (yellow), $P = 2.65e^{-13}$, $R^2 = 0.60$, $Q^2 = 0.42$ and (**B**) GCA patients, $P = 2.55e^{-05}$ $R^2 = 0.61$, $Q^2 = 0.38$. (**C-D**) VIP >0.9 for individual metabolites were subsequently assessed for importance in metabolic pathways using Metaboanalyst for (**C**) PMR, (**D**) GCA patients, axis show the –log P values and the impact of metabolites on each pathway (0–1). In C and D, the circle size denotes amount of pathway enrichment (larger = more enriched), whilst colour indicates level of significance (red/darker = more significant) – where the most significantly contributing pathways appear in the top right corner of the plots. Statistical analysis was determined using cross validated ANOVA.

could predict pain, stiffness, and fatigue in PMR patients (*see* Supplementary Fig. 7 *for changes in scores over time*). Baseline median scores were 8 (IQR 7–9) for both pain and stiffness VAS, and 25 (IQR 17–32) for fatigue (52 - FACIT-F score). A significant association was found between metabolites and pain, stiffness, or fatigue with moderate levels of prediction (Fig. 4). Using colour to visualise the samples according to the date of patient visit (0, 4 or 26 weeks treatment) shows that pain and stiffness decreased with treatment (Fig. 4A and B, Supplementary Figs. 6C and D) as expected, whereas some patients had persistent fatigue after 26 weeks of GC treatment (Fig. 4C, Supplementary Fig. 6E). Importantly, these patterns were predicted by the metabolites, with $R^2 \geq 0.39$ for pain, stiffness, and fatigue. Correlation was also observed when PLS-R was performed at each time point separately (Supplementary Fig. 7). Like the CRP prediction, pathway analysis revealed that *synthesis and degradation of ketone bodies* significantly contributed to the pain and stiffness prediction (Fig. 4D and E), whilst no relationship with this metabolic pathway was seen with fatigue. These data indicate that pain and stiffness, but not fatigue, are associated with inflammation in PMR.

To investigate this further we removed the metabolites predictive of CRP (VIP>1) and used the remaining metabolites in PLS-R analysis of fatigue (Fig. 5A). These remaining metabolites significantly correlated with fatigue, suggesting an inflammation-independent distinct metabolite profile underpinning fatigue (Fig. 5A). Using all metabolites, we further divided patients into those with "high" and "low" fatigue scores at baseline and after 26 weeks GC treatment to identify metabolites associated with fatigue. Groups were determined by selecting the patients still suffering from fatigue (FACIT-F < 20 [28]) after 26 weeks GC treatment, of which there were 9, then selecting the 9 patients with lowest levels of fatigue at 26 weeks (Fig. 5B), and then the 9 patients with highest and lowest fatigue at baseline (Fig. 5C). OPLS-DA revealed a separation between the "high" and "low" fatigue groups at both time points, Fig. 5D and E, with the metabolites that drive the separation in low and high fatigue scores described in Supplementary Table 3. Of particular interest was the significantly lower glutamine concentration and higher histidine and 2-hydroxyisobutyrate concentrations at both timepoints. This suggests that circulating metabolite concentrations correlate with fatigue in patients with PMR.

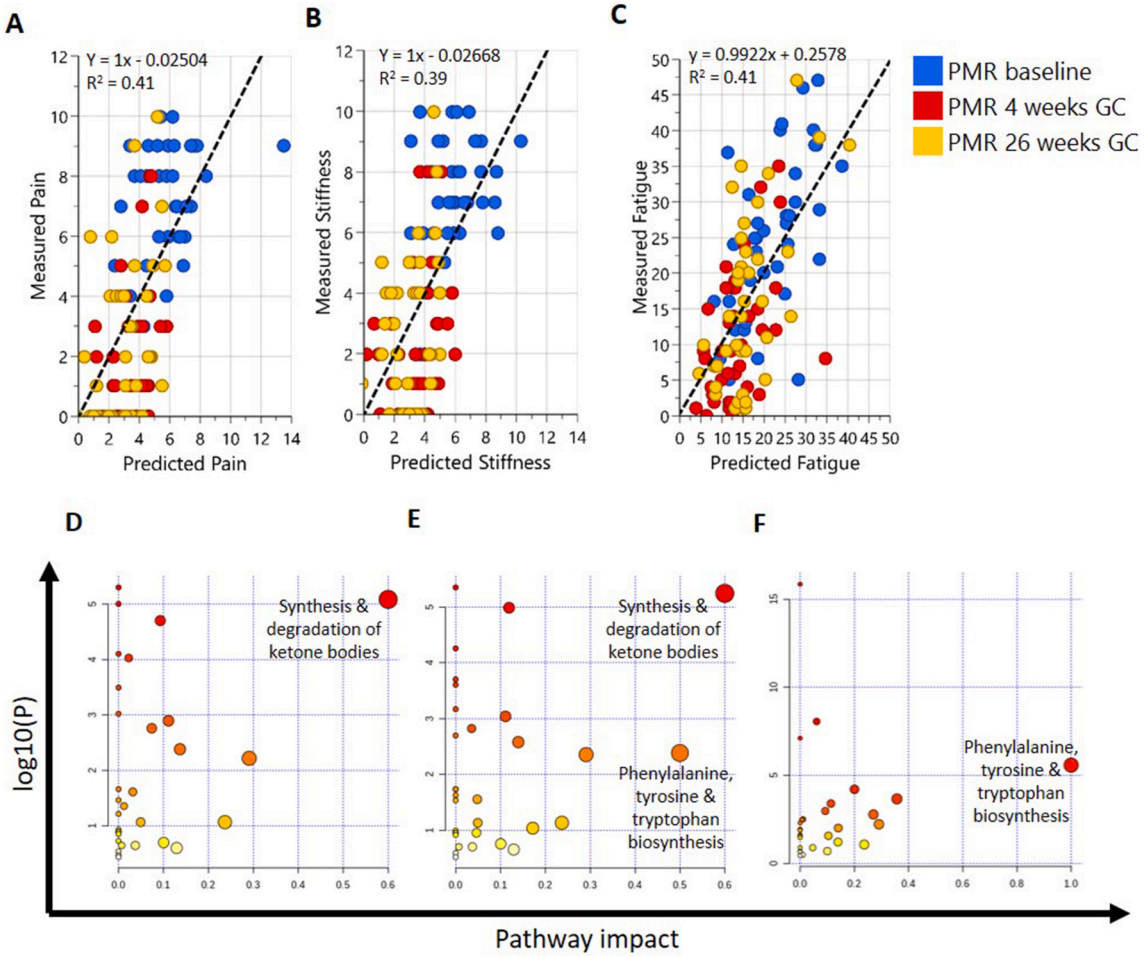


**Fig. 4. Metabolite concentrations in sera correlate with patient reported outcome measures.** One-dimensional $^{1}$H nuclear magnetic resonance (NMR) spectra were obtained from the serum of 40 patients with polymyalgia rheumatica at baseline (blue) and following glucocorticoid (GC) treatment for 4 (red) and 26 weeks (yellow). Metabolite concentrations were determined from the spectra with Chenomx and analysed in SIMCA. Predicted values of patient reported outcomes were determined from the metabolites using partial least squares regression analysis (PLS-R). Metabolites with variable importance for prediction (VIP >1) were taken forward and predicted values from these metabolites were compared to patient reported outcome measures for (**A**) pain, P = $5.05e^{-10}$, $R^2$ = 0.41, $Q^2$ = 0.31, (**B**) stiffness P = $4.36^{-05}$, $R^2$ = 0.39, $Q^2$ = 0.25 and (**C**) fatigue, P = $8.10e^{-05}$, $R^2$ = 0.41, $Q^2$ = 0.19, where P values determined with cross-validated ANOVA. (**D-F**) VIP >0.9 for individual metabolites were subsequently assessed for importance in metabolic pathways using Metaboanalyst for (**D**) pain, (**E**) stiffness and (**F**) fatigue, axis show the –log P values and the impact of metabolites on each pathway (0–1). In D-F, the circle size denotes amount of pathway enrichment (larger = more enriched), whilst colour indicates level of significance (red/darker = more significant) – where the most significantly contributing pathways appear in the top right corner of the plots.

## 2. Discussion

The molecular mechanisms underpinning PMR and GCA, and the effect of GC treatment on these, remains poorly understood. Moreover, it is currently unclear why a subset of PMR patients continue to suffer fatigue despite the control of the pain and stiffness with GC treatment. Here, we have demonstrated that the circulating metabolomic profile of patients with PMR and GCA is distinct from age and sex-matched controls, as seen in other chronic inflammatory diseases [8,10]. In PMR, this disease-specific profile is altered with GC treatment, but does not return to that seen in age-matched controls; these alterations are akin to those observed in other GC-treated inflammatory diseases [29]. Pain and stiffness, but not fatigue, appeared to be coupled with inflammation and shared similar predictive metabolites (acetoacetate, 3-hydroxybutyrate and methanol). Furthermore, metabolite profiles, particularly a low glutamine concentration, distinguished patients reporting high and low fatigue, and were predictive of fatigue both before and after treatment. We have shown for the first time that disease, GC treatment, and importantly fatigue, correlate with significant changes in the circulating metabolic profile of PMR and GCA patients. Our data highlights the possibility of using glutamine as a biomarker to predict and monitor fatigue in these patient groups. Furthermore, investigations into the role of glutamine in fatigue may pave the way to develop novel therapeutics to significantly reduce fatigue across a range of inflammatory diseases.

Elevated levels of ketone bodies, glycerol, lactate, and pyruvate are often reported in patients with inflammatory diseases; we observed similar increases in these metabolites in PMR and GCA. Collectively these data strongly indicate these metabolites are a hallmark of inflammation, rather than being a disease-specific metabolic response [8]. Indeed, the elevated ketone bodies (3-hydroxybutyrate and acetoacetate) and glycerol levels observed here and in RA [8] are consistent with inflammation driving lipolysis [30]. Of note it is common for PMR and GCA to present with weight loss alongside raised laboratory markers of inflammation [31].

Both acetoacetate and 3-hydroxybutyrate were also predictive for pain and stiffness levels in our PMR cohort. Given several reports highlighting the close relationship between pain, stiffness and inflammation in PMR e.g. Ref. [32], it is unsurprising that some of these inflammation-associated metabolites also predicted pain and stiffness. It must be noted, however, that at baseline, patients with PMR had fasted,

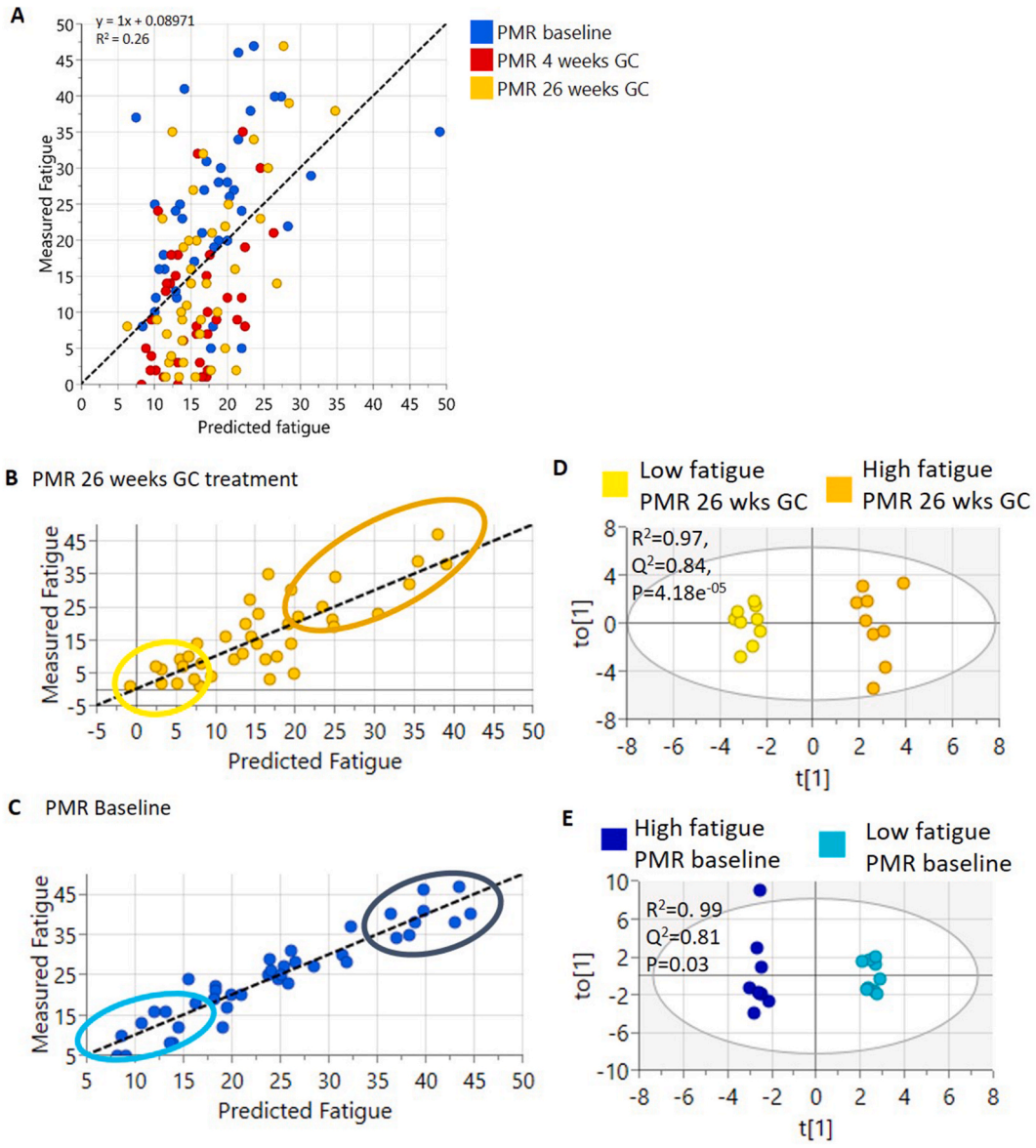


**Fig. 5. Non-inflammatory metabolites correlate with fatigue and metabolites can distinguish between patients with high and low fatigue in polymyalgia rheumatica patients.** One-dimensional 1H nuclear magnetic resonance (NMR spectra were obtained from the serum of 40 patients with polymyalgia rheumatica (PMR) at 3 visits; baseline (blue) and following glucocorticoid (GC treatment for 4 (red) and 26 weeks (yellow). Metabolite concentrations were determined from the spectra with Chenomx then analysed in SIMCA. **(A)** Firstly, partial least squares regression (PLS-R) identified metabolites predictive of CRP (with a variable importance of prediction, VIP>1). These were removed and PLS-R of fatigue performed on the remaining metabolites. Metabolites with a VIP >1 were re-subjected to PLS-R and predicted vs measured fatigue, P = 0, $R^2 = 0.26$, $Q^2 = 0.14$. P value determined with cross-validated ANOVA. (**B-E**) Using all metabolites, predicted values of fatigue were determined with PLS-R separately on patients at (**B**) 26 weeks and (**C**) baseline. The 9 highest and lowest scoring patients (circled) for the predicted and measured fatigue were separated into groups and subjected to orthogonal partial least squares – discriminant analysis (OPLS-DA), for (**D**) PMR patients 26 weeks into GC treatment, P = $4.18e^{-05}$, $R^2 = 0.97$, $Q^2 = 0.84$ and (**E**) Baseline PMR patients, P = 0.03, $R^2 = 0.99$, $Q^2 = 0.81$.

whilst at 4 and 26 weeks they had not. This may have contributed to the observed association as ketosis and the generation of ketone bodies is related to fasting [33]. Furthermore, loss of appetite and reduced food intake are common in inflammatory states and could also have affected metabolite levels.

By contrast, lactate and pyruvate increased with inflammation, but did not predict pain, stiffness, or fatigue, most likely due to the GC-associated changes described below. An inflammation-induced hypoxic environment, combined with increased energy demands of proliferating immune cells and proteolysis, results in a switch to aerobic glycolysis and gluconeogenesis, leading to lactate accumulation and pyruvate synthesis [8]. Other studies have also found elevated lactate levels in PMR [34]. Lactate derivatives can inhibit the motility of T-cells resulting in their accumulation in tissue, as well as inducing Th17 formation and reducing the cytolytic function of $CD8^+$ T-cells [35], raising the question of whether lactate is involved in PMR and GCA pathogenesis via these mechanisms. Therefore, the changes observed in lactate and 3-hydroxybutyrate could indicate not only alterations in energy metabolism, but also of T-cell signalling and thus warrant further investigation to fully dissect their role in the pathogenesis of PMR and GCA.

GC treatment is the gold standard for patients with PMR and GCA, but it had divergent effects on the inflammation-associated metabolites: while some metabolites were reduced following therapy (ketone bodies and glycerol), others were increased (lactate and pyruvate). The decreased ketone body (3-hydroxybutyrate and acetoacetate) levels

suggest a reduction in lipolysis, similar to that observed in healthy males following continuous treatment with 7.5 mg or 30 mg prednisolone over 2 weeks [36]. In contrast, increased lipolysis was reported in healthy males following a single low dose (4 mg) of dexamethasone [16]. Thus, acute and prolonged GC treatments appear to exert differential effects on systemic metabolism [16,37], which may account for reduced levels of ketone bodies seen in PMR following 4 and 6 months of GC therapy. The elevated levels of lactate and pyruvate we report here are a typical response following short-term GC treatment [16]; elevated lactate levels are a feature of insulin resistance [38] and might be potentially an early biomarker of patients at risk of steroid-induced hyperglycaemia, as elevated lactate predicts incident diabetes in the general population [39]. GCs inhibit pyruvate conversion to acetyl-CoA [40]. Pyruvate levels are well-known to increase with adiposity and obesity [41,42]. In PMR, a previous study showed elevated muscle interstitial lactate and pyruvate concentrations, that rose further with GC treatment [43].

Surprisingly, methanol sharply increased in patients after GC treatment indicative of alterations to its synthesis or degradation. Methanol and its oxidised product, formaldehyde, are often present in blood due to exogenous sources (fruit, vegetables, alcoholic beverages, or the artificial sweetener aspartame), but can also be formed endogenously via gut bacteria fermentation or metabolic processes involving S-adenosyl methionine [44]. We did not have data on whether patients with PMR altered their diet following GC therapy and this would be an important consideration for future studies.

Fatigue is a common symptom experienced by patients with chronic inflammatory diseases, especially those treated with GC, but as yet the underlying mechanisms responsible for fatigue have remained elusive. We have identified a fatigue-specific metabolic signature that distinguishes between patients with PMR who have high or low fatigue levels, independent of disease duration or GC treatment timeline. Of particular interest is the possibility that the metabolite glutamine may act as a biomarker for fatigue, with markedly reduced levels of glutamine observed in highly fatigued patients both before and after treatment. Indeed, low sera glutamine has been reported to be associated with active disease in a GCA and PMR patients [45]. Glutamine is a key fuel source for rapidly dividing leukocytes, playing a pivotal role in the immune response, but also is required as a fuel for muscle during intense exercise [46]. Glutamine is normally produced by skeletal muscle; therefore, low serum glutamine concentrations occur in catabolic states such as high altitude-associated muscle wasting [47]. Recently, low glutamine levels were described in association with human obesity and insulin resistance, with animal studies indicating a possible beneficial effect of glutamine supplementation [48]. It should also be noted that glutamine levels were not prominently deranged in a metabolomic study of fatigue in rheumatoid arthritis [12] and our findings in PMR require replication. Low glutamine levels in treated PMR patients might reflect ongoing immune activation, PMR- or GC-related muscle changes, or a combination of both factors. Regardless, glutamine might be an additional biomarker of poor prognosis in patients with PMR.

GC-induced obesity is associated with reduced gut microbiome diversity [49]; this reduced microbiome diversity has been suggested to play a role in the development of GC-related complications such as osteonecrosis [50]. Whether altered gut microbiota might play a role in the metabolomic alterations of GC therapy, and specifically whether interventions to improve gut microbiome diversity might be beneficial in alleviating adverse effects, remains unknown.

Whilst this exploratory study provides a first look at the metabolite changes within PMR and GCA patients, there are limitations. Most significantly, the sample size is relatively small, especially once exploring patient subsets (i.e., those with fatigue following GC treatment), and validation of these findings would be required in separate cohorts. Moreover, the PMR patient group had notably fewer smokers, a known factor that affects the circulating metabolome [51]. Given the limited sample numbers we were unable to control or adjust for smoking within this study, but this would need to be taken into consideration in subsequent studies on larger cohorts. The data comparing GCA and non-GCA patients is hard to interpret since many of the non-GCA patients had elevated CRP and/or were treated with similar doses of glucocorticoids to the GCA patient. This may have contributed to the non-significant result observed between the two patient groups and makes it difficult to identify disease-specific alterations.

## 3. Conclusions

Our data suggest that metabolomic derangement can be detected in PMR and GCA, and that GC therapy for these diseases is associated with further metabolomic changes. However, GC therapy does not return the metabolome back that seen in age-matched controls. CRP, pain, and stiffness all correlate with an “inflammatory” metabolome signature. By contrast, fatigue was associated with a different time course and different metabolomic fingerprint than pain and stiffness, suggesting a multifactorial aetiology for this symptom. Further validation is now urgently required to allow the definition of subsets of PMR at greater risk of fatigue or of GC-related adverse effects that might warrant early intervention to optimise long-term outcome.

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## CRediT authorship contribution statement

**Julia E. Manning:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Emma Harris:** Data curation, Formal analysis, Investigation, Resources, Writing – review & editing. **Hannah Mathieson:** Data curation, Formal analysis, Investigation, Resources, Writing – review & editing. **Louise Sorensen:** Data curation, Formal analysis, Investigation, Resources, Writing – review & editing. **Raashid Luqmani:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision. **Helen M. McGettrick:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Ann W. Morgan:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Stephen P. Young:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Sarah L. Mackie:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

SLM has conducted consultancy on behalf of her institution for Roche/Chugai, Sanofi, AstraZeneca, AbbVie and Pfizer; was supported by Roche to attend EULAR 2019 and by Pfizer to register for virtual attendance at ACR2021. AWM has received research funding from Roche and has conducted consultancy on behalf of her institution for Roche/Chugai, Sanofi, Regeneron, GlaxoSmithKline, AstraZeneca and Vifor. JEM and HMM have received research funding from Novartis. HMM has received funding from Roche and Pfizer. RL has received funding from Celgene, Pfizer, Roche and Vifor. Other authors have no competing interests to declare.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

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## References

[1] C.M. Weyand, J.J. Goronzy, Clinical practice. Giant-cell arteritis and polymyalgia rheumatica, N. Engl. J. Med. 371 (1) (2014) 50–57.
[2] H. Marzo-Ortega, et al., Evidence for a different anatomic basis for joint disease localization in polymyalgia rheumatica in comparison with rheumatoid arthritis, Arthritis Rheum. 56 (10) (2007) 3496–3501.
[3] S.L. Mackie, et al., Whole-body MRI of patients with polymyalgia rheumatica identifies a distinct subset with complete patient-reported response to glucocorticoids, Ann. Rheum. Dis. 74 (12) (2015) 2188.
[4] A. Proven, et al., Glucocorticoid therapy in giant cell arteritis: duration and adverse outcomes, Arthritis Care Res. 49 (5) (2003) 703–708.
[5] J. Liddle, et al., What is the impact of giant cell arteritis on patients' lives? A UK qualitative study, BMJ Open 7 (8) (2017) e017073.
[6] S.L. Mackie, et al., "An impediment to living life": why and how should we measure stiffness in polymyalgia rheumatica? PLoS One 10 (5) (2015) e0126758.
[7] C.B. Clish, Metabolomics: an emerging but powerful tool for precision medicine, Cold Spring Harbor molecular case studies 1 (1) (2015) a000588-a000588.
[8] S.P. Young, et al., The impact of inflammation on metabolomic profiles in patients with arthritis, Arthritis Rheum. 65 (8) (2013) 2015–2023.
[9] C. Sasaki, et al., Metabolomic approach to the exploration of biomarkers associated with disease activity in rheumatoid arthritis, PLoS One 14 (7) (2019) e0219400.
[10] A. Jain, et al., NMR-based serum metabolomics of patients with Takayasu arteritis: relationship with disease activity, J. Proteome Res. 17 (9) (2018) 3317–3324.
[11] B. Al-Ani, et al., Changes in urinary metabolomic profile during relapsing renal vasculitis, Sci. Rep. 6 (2016) 38074.
[12] I. Surowiec, et al., Metabolomics study of fatigue in patients with rheumatoid arthritis naïve to biological treatment, Rheumatol. Int. 36 (5) (2016) 703–711.
[13] J. Fu, et al., Differences between serum polar lipid profiles of male and female rheumatoid arthritis patients in response to glucocorticoid treatment, Inflammopharmacology 24 (6) (2016) 397–402.
[14] F. Cedola, et al., Choline metabolite is associated with inflammation in arthritis in the elderly, in: Arthritis Rheumatology, 2019.
[15] C. Dejaco, et al., Current evidence for therapeutic interventions and prognostic factors in polymyalgia rheumatica: a systematic literature review informing the 2015 European League against Rheumatism/American College of Rheumatology recommendations for the management of polymyalgia rheumatica, Ann. Rheum. Dis. 74 (10) (2015) 1808.
[16] N. Bordag, et al., Glucocorticoid (dexamethasone)-induced metabolome changes in healthy males suggest prediction of response and side effects, Sci. Rep. 5 (1) (2015) 15954.
[17] N.J. Vogelzang, et al., Patient, caregiver, and oncologist perceptions of cancer-related fatigue: results of a tripart assessment survey. The Fatigue Coalition, Semin. Hematol. 34 (3 Suppl 2) (1997) 4–12.
[18] B. Dasgupta, et al., BSR and BHPR guidelines for the management of polymyalgia rheumatica, Rheumatology 49 (1) (2010) 186–190.
[19] R. Luqmani, et al., The role of Ultrasound compared to biopsy of temporal arteries in the diagnosis and treatment of giant cell arteritis (TABUL): a diagnostic accuracy and cost-effectiveness study, Health Technol. Assess. 20 (90) (2016) 1–238.
[20] S. Tiziani, et al., Optimized metabolite extraction from blood serum for 1H nuclear magnetic resonance spectroscopy, Anal. Biochem. 377 (1) (2008) 16–23.
[21] C. Ludwig, U.L. Günther, MetaboLab–advanced NMR data processing and analysis for metabolomics, BMC Bioinf. 12 (2011) 366.
[22] Y. Xi, D.M. Rocke, Baseline correction for NMR spectroscopic metabolomics data analysis, BMC Bioinf. 9 (1) (2008) 324.
[23] F. Dieterle, et al., Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics, Anal. Chem. 78 (13) (2006) 4281–4290.
[24] A.M. Weljie, et al., Targeted profiling: quantitative analysis of 1H NMR metabolomics data, Anal. Chem. 78 (13) (2006) 4430–4442.
[25] Z. Wu, et al., Introduction to SIMCA-P and its application, in: V. Esposito Vinzi, et al. (Eds.), Handbook of Partial Least Squares: Concepts, Methods and Applications, Springer Berlin Heidelberg, Berlin, Heidelberg, 2010, pp. 757–774.
[26] J. Chong, et al., MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis, Nucleic Acids Res. 46 (W1) (2018) W486–W494.
[27] N.R. Sproston, J.J. Ashworth, Role of C-reactive protein at sites of inflammation and infection, Front. Immunol. 9 (2018) 754.
[28] A. Fazaa, et al., Optimal assessment of fatigue in rheumatoid arthritis: visual analog scale versus functional assessment of chronic illness therapy – fatigue, Ann. Rheum. Dis. 80 (Suppl 1) (2021) 1113–1114.
[29] A. Vignoli, et al., A NMR-based metabolomics approach to the assessment of inhaled pharmacotherapy in patients with COPD, Eur. Respir. J. 54 (suppl 63) (2019) OA3585.
[30] N. Rittig, et al., Regulation of lipolysis and adipose tissue signaling during acute endotoxin-induced inflammation: a human randomized crossover trial, PLoS One 11 (9) (2016) e0162167.
[31] H.A. Bird, et al., An evaluation of criteria for polymyalgia rheumatica, Ann. Rheum. Dis. 38 (5) (1979) 434–439.
[32] E.L. Matteson, et al., Patient-reported outcomes in polymyalgia rheumatica, J. Rheumatol. 39 (4) (2012) 795–803.
[33] A. Ahmed, et al., Impact of intermittent fasting on human health: an extended review of metabolic cascades, Int. J. Food Prop. 21 (1) (2018) 2700–2713.
[34] P. Chariot, et al., Impaired redox status and cytochrome coxidase deficiency in patients with polymyalgia rheumatica, Ann. Rheum. Dis. 60 (11) (2001) 1016.
[35] R. Haas, et al., Lactate regulates metabolic and pro-inflammatory circuits in control of T cell migration and effector functions, PLoS Biol. 13 (7) (2015) e1002202.
[36] D.H. van Raalte, et al., Low-dose glucocorticoid treatment affects multiple aspects of intermediary metabolism in healthy humans: a randomised controlled trial, Diabetologia 54 (8) (2011), 2103-2112.
[37] E.B. Geer, J. Islam, C. Buettner, Mechanisms of glucocorticoid-induced insulin resistance: focus on adipose tissue function and lipid metabolism, Endocrinol Metab. Clin. N. Am. 43 (1) (2014) 75–102.
[38] F. Berhane, et al., Plasma lactate levels increase during hyperinsulinemic euglycemic clamp and oral glucose tolerance test, J. Diabetes Res. 2015 (2015) 102054.
[39] S.P. Juraschek, et al., Lactate and risk of incident diabetes in a case-cohort of the atherosclerosis risk in communities (ARIC) study, PLoS One 8 (1) (2013) e55113.
[40] S. Connaughton, et al., Regulation of pyruvate dehydrogenase kinase isoform 4 (PDK4) gene expression by glucocorticoids and insulin, Mol. Cell. Endocrinol. 315 (1–2) (2010) 159–167.
[41] L. Hodson, et al., Metabolic signatures of human adipose tissue hypoxia in obesity, Diabetes 62 (5) (2013) 1417–1425.
[42] J.W.H. Doar, V. Wynn, D.G. Cramp, Blood pyruvate and plasma glucose levels during oral and intravenous glucose tolerance tests in obese and non-obese women, Metabolism 17 (8) (1968) 690–701.
[43] F. Kreiner, H. Galbo, Elevated muscle interstitial levels of pain-inducing substances in symptomatic muscles in patients with polymyalgia rheumatica, Pain 152 (5) (2011) 1127–1132.
[44] Y.L. Dorokhov, et al., Metabolic methanol: molecular pathways and physiological roles, Physiol. Rev. 95 (2) (2015) 603–644.
[45] A. Iliou, et al., NMR-based metabolomics in giant cell arteritis and polymyalgia rheumatica sequential sera differentiates active and inactive disease, Rheumatology (2023). PMID: 37935429.
[46] V. Cruzat, et al., Glutamine: metabolism and immune function, supplementation and clinical translation, Nutrients 10 (11) (2018) 1564.
[47] A.J. Wagenmakers, Amino acid metabolism, muscular fatigue and muscle wasting. Speculations on adaptations at high altitude, Int. J. Sports Med. 13 (Suppl 1) (1992) S110–S113.
[48] L. Dollet, et al., Glutamine regulates skeletal muscle immunometabolism in type 2 diabetes, Diabetes 71 (4) (2022) 624–636.
[49] D. Qiu, et al., Glucorticoid-induced obesity individuals have distinct signatures of the gut microbiome, Biofactors 45 (6) (2019) 892–901.
[50] C.Y. Chen, et al., Glucocorticoid-induced loss of beneficial gut bacterial extracellular vesicles is associated with the pathogenesis of osteonecrosis, Sci. Adv. 8 (15) (2022) eabg8335.
[51] F. Gu, et al., Cigarette smoking behaviour and blood metabolomics, Int. J. Epidemiol. 45 (5) (2016) 1421–1432.