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Physical Activity Associates with Lower Systemic Inflammatory Gene Expression in Rheumatoid Arthritis

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Abstract

Objective: While general population studies have shown inverse associations between physical activity and common inflammatory biomarkers, the effects of physical activity on inflammatory gene expression and signaling pathways in rheumatoid arthritis (RA) remain unknown. We aimed to determine whether physical activity independently associates with expression of inflammatory genes among people with RA.

Methods: This was a prospective observational study of adults with RA. Physical activity was measured by quantitative actigraphy over 7 consecutive days, and peripheral blood collected during the same time period was used for RNA sequencing followed by differential gene expression, pathway, and network analyses.

Results: Actigraphy and RNA sequencing data was evaluated on 35 patients. The cohort mean age was 56±12 years, 91% female, 31% white, 9% African American, 9% Asian, 40% Hispanic. We found 767 genes differentially expressed (padj<0.1) between patients in the greatest versus lowest physical activity tertiles, after adjusting for sex, age, race, and ethnicity. The most active patients exhibited dose-dependent downregulation of several immune signaling pathways implicated in RA pathogenesis. These included CD40, STAT3, TREM-1, IL-17a, IL-8, toll-like receptor and interferon signaling pathways. Upstream cytokine activation state analysis predicted reduced activation of TNF-alpha and interferon in the most active group. In sensitivity analyses we adjusted for RA disease activity and physical function and found consistent results.

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Conclusion: RA patients who were more physically active had lower expression of immune signaling pathways implicated in RA pathogenesis, even after adjusting for disease activity, suggesting that physical activity may confer a protective effect in RA.

Brief Summary:

In patients with rheumatoid arthritis, physical activity independently associates with a dose-dependent reduction in the expression of inflammatory genes implicated in disease pathogenesis.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that affects 1.5 million Americans and is characterized by joint and systemic inflammation, resulting in chronic pain, functional limitations, and premature cardiovascular disease[1]. While existing therapeutics such as biologic disease-modifying antirheumatic drugs (DMARDs) reduce disease severity, they often fail to adequately control symptoms and confer a significant risk of adverse drug events, including increased risk of life-threatening infections. Given these limitations, there is an unmet need for non-pharmacologic strategies to augment existing medical management of RA, improve long-term clinical outcomes, and reduce disease-related symptoms.

While historically it was thought that exercise might exacerbate rheumatic conditions, that precept has since been disproven[2]. Driving this shift were clinical trials of resistance and aerobic exercise programs for people with RA, in which the exercise treatment interventions were found to be safe and resulted in unchanged or improved disease activity scores compared to controls[3,4]. As an example, a recent exercise pilot trial found that older adults with RA randomized to a 10-week high-intensity interval walking intervention had a 38% reduction in disease activity at the end of the intervention[5].

The mechanisms responsible for the therapeutic effect of exercise in rheumatic diseases are incompletely understood. One hypothesis is that skeletal muscle-secreted myokines confer anti-inflammatory effects following episodes of physical activity[6]. This hypothesis is supported by several studies in the general population that demonstrated a clear association between higher levels of physical activity, reductions in pro-inflammatory cytokine signaling, and lower systemic inflammation[7–10]. Though studies in the general population have observed favorable effects of exercise on immune function, how physical activity influences systemic inflammation in the context of immune-mediated inflammatory diseases such as RA remains poorly understood and represents an important question in need of further investigation.

We conducted a prospective observational cohort study to investigate mechanistic relationships between physical activity and systemic inflammatory gene expression in patients with RA. Using quantitative actigraphy, whole blood transcriptomics, and detailed clinical phenotyping, we asked whether physical activity independently associates with expression of inflammatory genes implicated in the pathogenesis of RA.

Methods

Study design, clinical cohort and ethics statement

We studied participants enrolled in an ongoing prospective observational cohort study of patients with RA: "Sleep disturbance in rheumatoid arthritis: phenotypes, causes, and impact" (RAZZ; R01 AR069616). Patients were enrolled in RAZZ between 2018 and 2021 after being recruited from an extensive network of community, academic, and safety net rheumatology clinics in the San Francisco Bay Area, including the University of California San Francisco (UCSF) and Zuckerberg San Francisco General (ZSFG) Hospital rheumatology clinics. Inclusion criteria for the parent cohort included: age greater than or equal to 18 years; a physician's diagnosis of RA; and English or Spanish fluency. All patients in the parent cohort with actigraphy and transcriptomic data were eligible for inclusion in the current analysis. However, due to the unexpected occurrence of the COVID-19 pandemic beginning in early 2020, which precluded in-person non-essential clinical research studies, we were only able to obtain blood samples for transcriptomic data generation on the subset of participants with baseline visits before the pandemic.

During the period of data collection for this analysis, study visits were conducted in-person. At each study visit, we collected detailed clinical information, assessed RA disease activity, and downloaded actigraphy data collected between study visits. In addition, peripheral blood was collected and stored at −80°C for subsequent RNA sequencing (RNA-seq). The UCSF Institutional Review Board approved all procedures (UCSF IRB protocol #17–21790), and all research was performed in accordance with the Declaration of Helsinki and UCSF IRB guidelines and regulations. Written informed consent was obtained from all participants.

Clinical measures

Physical activity.—Physical activity was assessed objectively using the GT9X ActiGraph Link device (ActiGraph, Inc). This ActiGraph device resembles a small watch and contains a validated tri-axial accelerometer and integrated gyroscope and magnetometer, which collectively capture absolute subject movement. Participants wore the ActiGraph on their wrists for 7 consecutive 24-hour periods (one full week) before returning them for data analysis. ActiGraph physical activity data are provided as counts per minute (CPM), which are a result of aggregating post-filtered raw accelerometer data over 1-minute intervals (epochs) and are used to define the time awake spent in sedentary activity (0–99 CPM), light activity (100–1951 CPM), moderate activity (1952–5724 CPM), vigorous activity (5725–9498 CPM), and very vigorous activity (>=9499 CPM) based on established cut points that correspond to metabolic equivalent (MET) levels[11]. Participants were categorized using the highest, middle, and lowest tertiles for percentage of time spent in at least moderately intense physical activity across the entire RA cohort, resulting in three physical activity groups: the least active (labeled "inactive"), the most active (labeled "active"), and an intermediate group.

RA-specific disease factors.—Age of diagnosis was obtained by self-report. Disease activity was assessed with the Rheumatoid Arthritis Disease Activity Index (RADAI), a validated patient-reported instrument[12]. Participants were also queried regarding current

treatment with glucocorticoids—including dosage and frequency—as well as other immunomodulatory medications.

Other variables.—Sociodemographic data was collected, including sex, age, race, ethnicity, and educational attainment (categorized as high school graduate or less, versus those with additional education). Physical function was measured using the Patient-Reported Outcomes Measurement Information System (PROMIS) Physical Function Scale.[13] Height and weight were measured during the baseline in-person visit, and body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters squared (m^2). Participants provided information on health-related behaviors (e.g., smoking) and comorbidities such as cardiovascular disease, diabetes mellitus, asthma, and cancer.

RNA sequencing

Whole blood was collected and stored at −80C. Following RNA extraction (Zymo Pathogen Magbead Kit) and DNAse treatment, human globin and ribosomal RNA was depleted using FastSelect (Qiagen) according to described methods[14]. RNA was then fragmented and underwent library preparation using the NEBNext Ultra II RNAseq Kit (New England Biolabs) as previously described[14]. Libraries underwent 146 nucleotide paired-end Illumina sequencing on an Illumina Novaseq 6000 instrument.

Gene expression data processing and quality control

Following demultiplexing, sequencing reads were aligned against the human genome (NCBI GRC h38) using STAR[1] to extract gene counts. Samples retained in the dataset had 3.0 $\times 10^5$ gene counts, and the median across all samples was 5.8×10^5 gene counts. Data merging and normalization across different activity groups were performed. Gene counts were normalized with the median of ratios method using R package *DEseq2*. For covariates, one-hot encoding was applied for categorical variables and min-max scaling was performed for numeric covariates. As an additional quality control measure, genes expressed in less than 30 percent of the patients in each group were filtered. In total 19,260 genes were kept for the subsequent analysis.

Differential gene expression analysis

Differentially expressed genes were identified using the R package *DEseq2*[15]. Sex, age, race, and ethnicity were included as covariates in the linear model. Next, to address the possibility of confounding from inability to participate in physical activity among patients with more severe disease, we conducted two sensitivity analyses in which we adjusted for disease activity and physical function in addition to the aforementioned demographic variables. Finally, to correct for the potential batch effect, the R package *sva*[16] was used to calculate a surrogate variable (SV), which was also integrated into the differential expression linear model as a covariate. Independent hypothesis weighting (IHW) was used as a multiple testing procedure and the significance of differential expression was defined as an adjusted p-value < 0.1.

Pathway analysis

Ingenuity Pathway Analysis (IPA)[17] as carried out on differentially expressed genes with a P < 0.1 ranked by log2 fold change. Significant IPA results were defined as those with a Z-score absolute value greater than 2 or an overlap P value < 0.05. The top three up- and down-regulated canonical pathways based on Z-score, as well as all pathways related to immunity and inflammation with an |Z| > 1 and overlap P value < 0.05, were included in Figure 1C. Upstream regulating cytokines with an |Z| > 2 and overlap P value < 0.05 were included in Figure 1D. Complete IPA results are provided in supplementary materials (Supplementary Data File 2).

Network analysis

The network analysis was performed using STRING v.11. (https://string-db.org/) The network matrix was exported as a .tsv file and the plot was recreated using Cytoscape (https://cytoscape.org/). Each protein was visualized as a node and each potential protein-protein interaction was visualized as an edge. The size of each node reflects its degree centrality metrics. Figure 2A represents one of multiple network maps. Complete network map data are provided in Supplementary Data File 3.

In silico analysis of cell type proportions

Cell-type proportions were estimated from bulk host transcriptome data using the CIBERSORT X algorithm following previously described methods[14]. The estimated proportions were compared between the three patient groups using a Mann-Whitney-Wilcoxon test (two-sided) with Bonferroni correction.

Data Availability

Genecounts are available under Gene Expression Omnibus accession number GSE179302. All code is available at Github https://github.com/drychkov/PA_in_RA.

Results

There were 35 adults in the RAZZ cohort with complete actigraphy (physical activity), RNA-seq (gene expression), and clinical data. The cohort had a mean age of 56 years (SD 12.1), was 91% female, and self-reported the following racial and ethnic identities: 31% white, 9% African American, 9% Asian, and 40% Hispanic (Table 1). The mean disease duration was 13.1 years (SD 11.3) and 71% of the cohort was seropositive for rheumatoid factor and/or anti-cyclic citrullinated peptide antibody. 54% of participants were taking methotrexate, 51% were treated with a biologic DMARD, and 26% were treated with systemic glucocorticoids. Only 13% were taking oral glucocorticoids greater than 7.5 mg prednisone equivalent per day.

Consistent with prior studies of physical activity behavior among people with RA, the cohort was relatively sedentary[18]; none of the patients engaged in vigorous physical activity during the 7-day period of actigraphy monitoring. The percentage of time spent in moderate physical activity was 18% in the most active group compared to 4.5% in the least active group (Supplementary Table 1). Though the study was not powered to detect statistically

significant demographic or clinical differences between the physical activity groups, we found that patients in the active group were younger (mean age = 50 years) compared to those in the intermediate (mean age = 56 years) and inactive groups (mean age = 63 years) (p = 0.04) (Table 1).

Differential gene expression analysis comparing the most versus least physically active groups (based on activity tertile) identified 767 genes at an adjusted P value (padj) < 0.1 (Figure 1a). Principal Component Analysis (PCA) demonstrated clear separation based on physical activity groups (Figure 1b), and pathway analysis (Methods) revealed that the most physically active patients exhibited downregulation of diverse innate and adaptive immune signaling pathways implicated in the pathogenesis of RA, including CD40, STAT3, TREM-1, IL-17a, IL-8, toll-like receptors, and type I interferon signaling (Figure 1c, Supplementary Data File 1, 3A)[19]. Prediction of upstream cytokine activation states from the transcriptomic data suggested inhibition of type I, II and III interferons, and activation of epoetin, among the most physically active patients (Figure 1d, Supplementary Data File 2B).

Given the role of pathologic inflammation in RA, we next sought to more rigorously evaluate the proinflammatory genes downregulated in the most active group by performing a network connectivity analysis. This revealed relationships between genes related to type 1 interferon signaling (e.g., *MX1*, *IFI44L*, *IFIT1*) and inflammasome signaling (e.g., *IL-1RN*, *NOD2*), as well as other cytokine signaling pathways (e.g. *IL-6R*, *IL17RA*) (Figure 2a, Supplementary Data File 3). To more deeply characterize the relationships between these inflammatory genes and physical activity, we evaluated their expression across all three physical activity groups. Intriguingly, a dose-dependent correlation between physical activity and reduced expression of proinflammatory genes was observed (Figure 2b).

We considered that differences in immune cell populations might underlie the observed differentially expressed genes, and thus performed *in silico* cell type deconvolution. We found no statistically significant differences in predicted proportions between the highest and lowest physical activity groups, but we observed a trend toward decreased monocytes in the most active group (Supplementary Figure 1, Supplementary Data File 4).

Lastly, we considered that differences in ability to engage in exercise among patients with more active disease and/or more joint damage might explain our findings. To test for this possibility, we conducted two sensitivity analyses in which disease activity (sensitivity analysis #1) and physical function (sensitivity analysis #2) were included as covariates. The sensitivity analysis in which we adjusted for disease activity revealed consistent results compared to the main analysis, including 674 differentially expressed genes (padj < 0.1) and similar downregulation of interferon and other proinflammatory cytokine signaling pathways among the most active patients (Supplementary Data File 5). The sensitivity analysis adjusted for physical function yielded 332 differentially expressed genes, including those most statistically significant and biologically relevant from the primary analysis (e.g., *IFI44L*, *MX1*), as well as similar pathway analysis results (Supplementary Data File 6).

Discussion

Randomized controlled trials of exercise interventions for RA have found that exercise improves joint pain, fatigue, and disease activity[3,4,20–23], and decreases the risk of progressive joint damage[20], but the mechanisms underlying these apparent benefits have remained in question. In this study we used quantitative actigraphy and whole blood transcriptomics to show that physical activity moderates inflammatory signaling among people with RA.

Studies in the general population have found that higher levels of physical activity associate with lower inflammatory biomarkers, including C-reactive protein (CRP) and TNF-α, even after adjusting for excess adiposity[7–10]. Our results are consistent with these observations, as we observed an association between physical activity and attenuation of several proinflammatory signaling pathways implicated in RA pathogenesis, including: CD40, STAT3, TREM-1, IL-17a, IL-8, toll-like receptor, and type I interferon[19]. Furthermore, our gene expression data demonstrated lower TNF activation among the most active relative to the least active patients in this RA cohort.

Unexpectedly, we found that interferon signaling was inversely correlated with physical activity. This finding has clinical significance given that interferon gene expression in peripheral blood has been associated with RA autoantibody production, development of chronic RA in patients with early inflammatory arthritis, poorer response to initial therapy, and nonresponse to rituximab[24]. Additionally, recent single cell RNA-seq studies have shown a correlation between peripheral blood mononuclear cell interferon gene expression and infiltration of synovium with plasma cells in people with RA[25]. We also found that physical activity was associated with significantly attenuated toll-like receptor and *IL-17RA* signaling, which have been shown to contribute to unbalanced production of cytokines in RA[26]. We considered that disease severity or lower physical function might represent potential confounders, but in sensitivity analyses that included disease activity and physical function as covariates, we found consistent results.

Intriguingly, the association of physical activity with reduced expression of several proinflammatory genes was dose dependent. The threshold of moderate activity employed in Actigraph scoring is quite modest at ~2.0 METs, which corresponds to home activities such as standing to wash dishes, food shopping, or walking at a 2.0 mph pace. The most active group spent an average of 4.3 hours/day in these moderate intensity activities, compared to the least active group's 1.1 hours/day. The dose-dependent relationship between moderate activity and attenuation of proinflammatory gene expression suggests that greater exposure to physical activity could confer a larger impact on inflammatory signaling—and therefore greater potential therapeutic benefit—in this patient population.

The gene expression differences associated with physical activity in our study were in the range of those observed in studies of RA treatment interventions. For instance, a study by van Baarsen et al. that evaluated gene expression differences before and after treatment with infliximab in 33 RA patients found 1623 differentially expressed genes at a padj < 0.05 with a median log2 fold change of 0.86[27], including some of the same genes

found to be influenced by physical activity in our study (e.g. *IL17R*). This compared to 206 differentially expressed genes at a padj < 0.05 and log2 fold change of 2.1 in our cohort. Another observational study that compared 14 RA patients before and six months post-rituximab treatment identified zero differentially expressed genes at a padj < 0.05 but 124 with at least a 2-fold change in expression[28], including many of the same interferon-related genes that we observed (e.g., *IFI44L*). Further work is needed to understand the comparative effect size of physical activity on inflammatory gene expression with respect to pharmacologic treatment interventions for RA.

This study has limitations. Because this was an observational study, we were able to identify associations between physical activity and inflammatory gene expression, but we cannot prove causation or directionality. Only a subset of patients in the RAZZ cohort had complete data for both actigraphy measures and transcriptomics, which limited the sample size for the current analysis. Additionally, though we included a validated patient-reported assessment of disease activity that has been shown to strongly correlate with the Clinical Disease Activity Index (CDAI)[29], the study would have been enhanced by including a physician evaluation of disease activity. Finally, we were unable to assess the impact of vigorous physical activity because no patient engaged in vigorous physical activity during the study. Despite these limitations, we believe the findings remain noteworthy as this is the first study to assess physical activity in the context of RA using whole blood transcriptomics. The level of physical activity observed in this cohort realistically reflects the activity patterns observed in this patient demographic[18], and if anything, our results may have been more pronounced in a cohort with a broader range of activity levels.

Given the limitations of existing pharmacologic treatments for RA and the need for non-pharmacologic adjunctive strategies, our findings have important clinical implications. Despite major advances in treatment, including the advent of biologic disease modifying antirheumatic drugs (DMARDs), RA remains a chronic, incurable condition, and fewer than half of patients treated with immunosuppressive DMARDs achieve disease remission[30,31]. Furthermore, adverse drug events from RA medications are common, with an estimated incidence of 15 per 100 patient-years[32], leading many people with RA to express a preference for non-pharmacologic treatment approaches[33,34]. Taken together, the prevalence of persistent symptoms despite treatment, risk of side effects from DMARDs, and patient preference for lower-risk treatment approaches emphasize the critical need for adjunctive non-pharmacologic interventions for RA. Our results suggest that physical activity interventions have the potential to not only improve overall health and mitigate the risk of important comorbidities among people with RA, but may also attenuate pathologic inflammatory signaling and disease activity.

This work provides an important foundation for future studies, including additional research to further characterize the biological mechanisms linking physical activity to inflammatory signaling using proteomic, metabolomic, and single cell RNA sequencing approaches. Our study also highlights the need for a randomized clinical trial to assess the direct effect of exercise on blood transcriptomic markers, and to compare gene expression among RA patients across a greater spectrum of physical activity. Lastly, a key outstanding question is

whether our findings extend to other autoimmune rheumatic diseases such as systemic lupus erythematosus (SLE).

In summary, among a representative cohort of RA patients, we found a striking and dose-dependent association between moderate physical activity and attenuated expression of genes involved in both innate and adaptive immune signaling, even after adjusting for disease severity and other covariates. These findings provide the first mechanistic evidence to support a disease-modifying effect of physical activity in RA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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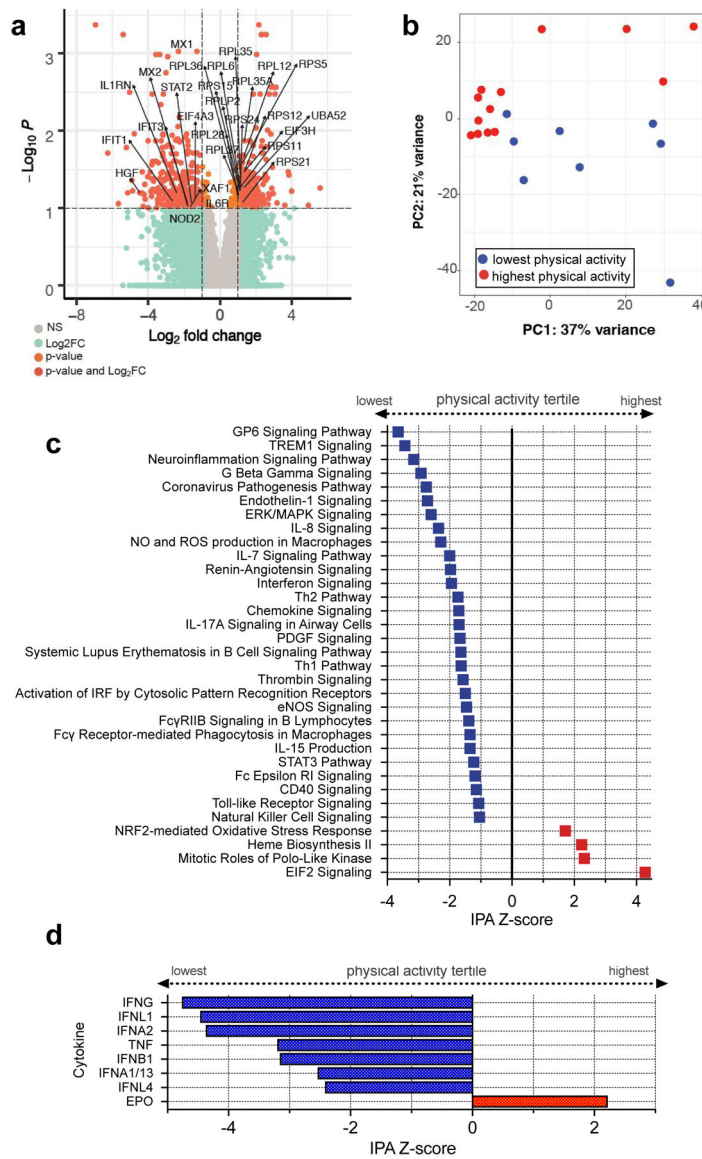
Figure 1. Differentially expressed (DE) genes in peripheral blood between RA patients in the highest and lowest tertiles of physical activity.

a) Volcano plot of DE genes depicting 365 genes up-regulated in the highest activity tertile and 402 down-regulated genes at an adjusted p-value < 0.1 with respect to the highest physical activity tertile. Genes related to immune signaling and translation are highlighted. **b)** Principal Component Analysis based on the DE genes demonstrates separation of patients based on tertile of physical activity. **c)** Ingenuity Pathway Analysis (IPA) based on differential gene expression analyses demonstrating expression of canonical signaling pathways by IPA activation Z-score. Top pathways by |Z| score and pathways related to immunity and inflammation with a |Z| > 1 depicted, with an overlap P value < 0.05. Values and related genes tabulated in (Supplementary Data X). **d)** Predicted activation state of upstream cytokines in the highest versus lowest physical activity tertiles. Cytokines with a |Z| > 2 plotted. Values and related genes tabulated in (Supplementary Data X).
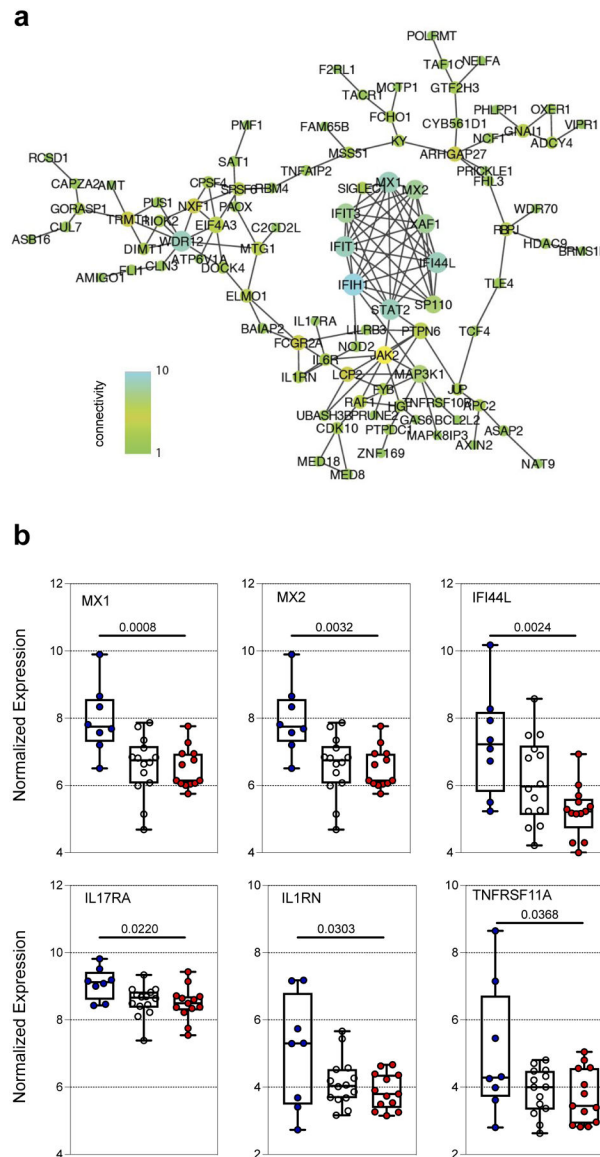
Figure 2. Connectivity and dose-dependent downregulation of proinflammatory genes based on physical activity tertile.

a) Network connectivity map of proinflammatory genes downregulated in the highest physical activity tertile, constructed using STRING v.11. Color bar represents the degree of connectivity based on the number of edges to which each node/gene is connected. Complete network connectivity table of all differentially expressed genes in Supplementary Data File 3. b) Boxplots depicting normalized expression of six representative immune genes correlated with physical activity tertile in a dose-dependent manner.

Table 1.

Characteristics* of Patients with Rheumatoid Arthritis (RA) According to Physical Activity Category

Characteristics	Overall	Physical Activity** Status			P
	(N = 35)	Active (N = 13)	Intermediate (N=14)	Inactive (N=8)	
Sociodemographic Factors:					
Age, mean ± SD	56 ± 12	50 ± 11	56 ± 11	63 ± 12	**0.04**
Female	32 (91%)	13 (100%)	13 (93%)	6 (75%)	0.06
Race					0.21
Asian	3 (9%)	0 (0%)	2 (14%)	1 (13%)	
African American	3 (9%)	0 (0%)	1 (7%)	2 (25%)	
White	11 (31%)	3 (23%)	4 (29%)	4 (50%)	
Unknown	14 (40%)	8 (62%)	5 (36%)	1 (13%)	
Multiple	4 (11%)	2 (15%)	2 (14%)	0 (0%)	
Hispanic ethnicity	14 (40%)	8 (62%)	5 (36%)	1 (13%)	0.08
Education < college degree	18 (51%)	8 (62%)	6 (43%)	5 (50%)	0.62
RA Specific Characteristics:					
RA disease duration, mean years ± SD	13 ± 11	12 ± 11	14 ± 13	15 ± 12	0.82
Disease activity by RADAI, mean ± SD	3.9 + 1.9	3.9 + 1.3	4.5 + 2.1	3.1 + 1.8	0.21
Rheumatoid Factor (RF) Positive	21 (60%)	7 (54%)	10 (71%)	4 (50%)	0.52
Anti-CCP Antibody Positive	20 (57%)	7 (54%)	11 (79%)	2 (22%)	0.05
Seropositive (RF and/or anti-CCP)	25 (71%)	9 (69%)	12 (86%)	4 (50%)	0.20
Treated with methotrexate	19 (54%)	8 (62%)	5 (36%)	6 (75%)	0.17
Treated with TNFi	12 (34%)	3 (23%)	7 (50%)	2 (25%)	0.28
Treated with any conventional DMARD	24 (69%)	9 (69%)	8 (57%)	7 (88%)	0.34
Treated with any biologic DMARD	18 (51%)	6 (46%)	10 (71%)	2 (25%)	0.10
Current systemic glucocorticoid use	9 (26%)	3 (23%)	4 (29%)	2 (25%)	0.95
Comorbidities and Health Status:					
Cardiovascular Disease	2 (6%)	0 (0%)	1 (7%)	1 (13%)	0.47
Diabetes Mellitus	5 (14%)	1 (8%)	3 (21%)	1 (13%)	0.59
Asthma	5 (14%)	2 (15%)	1 (7%)	2 (25%)	0.51
History of malignancy	3 (9%)	0 (0%)	1 (7%)	2 (25%)	0.14
Body Mass Index (kg/m2), mean ± SD	28.8 ± 5.5	29.0 ± 6.8	28.9 ± 5.2	28.6 ± 4.1	0.33
Current nicotine use	2 (6%)	1 (8%)	0 (0%)	1 (13%)	0.44
Physical Function***, mean ± SD	40.7 ± 5.2	41.5 ± 4.3	39.6 ± 5.7	41.2 ± 5.9	0.61

*
Data are n (%) unless otherwise indicated. P-values calculated using chi-squared tests for categorical measures and ANOVA for continuous measures.

**
Physical activity groups were created from the lower (inactive), middle (intermediate), and upper (active) tertiles for percentage of time spent in at least moderately intense physical activity among all study participants in the larger RA cohort.

RADAI = Rheumatoid Arthritis Disease Activity Index, range 0–10.

CCP = cyclic citrullinated peptide.

TNFi = tumor necrosis factor inhibitor.

DMARD = disease modifying antirheumatic drug.

Cadiovascular disease was defined by history of stroke, coronary artery disease, and/or myocardial infarction.

*** Physical Function measured via the Patient-Reported Outcomes Measurement Information System Physical Function Scale