Effects of acute aerobic exercise on leukocyte inflammatory gene expression in systemic lupus erythematosus

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ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease with a persistent systemic inflammation. Exercise-induced inflammatory response in SLE remains to be fully elucidated. The aim of this study was to assess the effects of acute exercise on leukocyte gene expression in active (SLE_{ACTIVE}) and inactive SLE ($SLE_{INACTIVE}$) patients and healthy controls (HC). ***Methods****: All subjects (n = 4 per group) performed a 30-min single bout of acute aerobic exercise (~70% of VO_2 peak) on a treadmill, and blood samples were collected for RNA extraction from circulating leukocyte at baseline, at the end of exercise, and after three hours of recovery. The expression of a panel of immune-related genes was evaluated by a quantitative PCR array assay. Moreover, network-based analyses were performed to interpret transcriptional changes occurring after the exercise challenge.* ***Results:*** *In all groups, a single bout of acute exercise led to the down-regulation of the gene expression of innate and adaptive immunity at the end of exercise (e.g., TLR3, IFNG, GATA3, FOXP3, STAT4) with a subsequent up-regulation occurring upon recovery. Exercise regulated the expression of inflammatory genes in the blood leukocytes of the SLE patients and HC, although the SLE groups exhibited fewer modulated genes and less densely connected networks (number of nodes: 29, 40 and 58; number of edges: 29, 60 and 195; network density: 0.07, 0.08 and 0.12, for SLE_{ACTIVE}, $SLE_{INACTIVE}$ and HC, respectively).* ***Conclusion:*** *The leukocytes from the SLE patients, irrespective of disease activity, showed a down-regulated inflammatory gene expression immediately after acute aerobic exercise, followed by an up-regulation at recovery. Furthermore, less organized gene networks were observed in the SLE patients, suggesting that they may be deficient in triggering a normal exercise-induced immune transcriptional response.*

Key words: physical activity, inflammation, autoimmunity, gene array, exercise immunology, network analysis, hubs.

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Abbreviations:
SLE, systemic lupus erythematosus
$SLE_{INACTIVE}$, inactive systemic lupus erythematosus patients
SLE_{ACTIVE}, active systemic lupus erythematosus patients
BMI, body mass index
SLEDAI, systemic lupus erythematosus disease activity index
HC, healthy controls
VAT, ventilatory anaerobic threshold
RCP, respiratory compensation point
VE, ventilation
VCO_2, carbon dioxide output
Baseline, prior to exercise
End-ex, end of exercise
Recovery, three hours of recovery
HR, heart rate
VO_2, oxygen uptake
DE, differentially expressed

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune rheumatic disease characterized by an immune-mediated inflammation of multiple organs (65). Although mechanisms underlying SLE pathogenesis have not yet been fully elucidated, patients with active (SLE_{ACTIVE}) and inactive ($SLE_{INACTIVE}$) disease exhibit up-regulation of the expression of genes involved in both innate and adaptive immunity when compared with their healthy counterparts (18, 23). This molecular dysfunction may result in auto-antibody production, immune complex deposition, and complement system activation (11), ultimately leading to chronic inflammation (11, 33). The consequent exacerbated inflammatory response has been implicated in some SLE-related comorbidities, such as atherosclerosis and endothelial dysfunction (26), which have been thought to be the main causes of mortality in SLE patients (59).

Physical exercise has emerged as a potential tool for counteracting SLE comorbidities (45), endothelial dysfunction (19), aerobic deconditioning (12), cardiac dysautonomia (29), and inflammation (46). In fact, exercise has been shown to exert anti-inflammatory effects in a number of chronic diseases, including inflammatory rheumatic diseases. It has been specu-

lated that such a role of exercise may be due to an anti-inflammatory environment induced by successive acute exercise bouts. In response to a single bout of exercise, skeletal muscle releases interleukin-6 (IL-6) in an exponential fashion according to the duration and intensity of exercise (41, 42). Because IL-6 has classically been considered a pro-inflammatory cytokine primarily secreted by stimulated immune cells (*e.g.*, monocytes and macrophages) (2), its increase in response to a single bout of exercise was initially regarded as a pro-inflammatory response. However, the increase in IL-6 is not preceded by an increase in tumor necrosis factor alpha (TNF-α) and, most importantly, is followed by increased levels of anti-inflammatory cytokines, namely IL-1 receptor antagonist (IL-1ra) and IL-10 (41, 63). Therefore, rather than pro-inflammatory, the acute exercise-induced increase in IL-6 may actually lead to an anti-inflammatory environment (9). Studies with recombinant IL-6 (rhIL-6) infusion further support the anti-inflammatory consequences of the exercise-induced increase in IL-6. When healthy subjects are exposed to a low-dose of *E. Coli* endotoxin in a "low-grade inflammation model", the infusion of rhIL-6 (mimicking the exercise-induced IL-6 response) blunts the otherwise expected increase in TNF-α (62). This anti-inflammatory role of IL-6 has been associated with the transitory increase of this cytokine secreted by the muscle in response to a single bout of exercise (43), while persistent high serum levels of IL-6 have been associated with a persistent inflammatory response and chronic disabilities (20).

Even though consecutive bouts of exercise may potentially exert anti-inflammatory effects, there is limited evidence showing that regular exercise training may attenuate inflammation in chronic diseases. In this regard, Adamopoulos et al. (1) observed a reduction in resting TNF-α, IL-6, soluble TNF receptors 1 and 2 (sTNFR1 and sTNFR2, respectively) in patients with congestive heart failure after 12 weeks of aerobic training [5 sessions per week, 30 min per session, 80% of maximal heart rate (HR_{max})]. Conversely, Niebauer et al. (37) failed to observe any changes in cytokines in patients with the same disease after a similar training program. In patients with coronary heart disease, 12 weeks of aerobic training (3 sessions per week, 45 min per session, 70-80% HR_{max}) resulted in reductions in IL-1, IL-6, interferon-gamma (IFN-γ) and C-reactive protein, and increases in IL-10 (22). Likewise, patients with fibromyalgia showed a reduction in C-reactive protein serum levels and pro-inflammatory cytokines production (IL-1β and TNF-α) in *ex vivo* stimulated monocytes, after 8 months of aquatic aerobic exercises (2 sessions per week, 60 min per session, 40-75% HR_{max}) (39, 40). In contrast, patients with chronic obstructive pulmonary disease did not experience any change in IL-6, TNF-α, sTNFR1 and sTNFR2 after an 8-week aerobic training (5 sessions per week, 60 min per session, 90% peak power) (51). Studies involving patients with autoimmune diseases are scarce. The three studies assessing the effects of aerobic exercise (2 to 5 sessions per week, 30 to 60 minutes per session, 3 to 8 weeks at either 60% VO_{2peak} or 75% HR_{max}) in patients with multiple sclerosis did not show any changes in pro- or anti-inflammatory cytokines (6, 13, 58).

The effects of exercise training upon inflammation in autoimmune inflammatory rheumatic diseases are also controversial. Baslund et al. (7) showed that 8 weeks of an interval aerobic training (4 to 5 sessions per week, 30 min per session, 80% VO_{2max}) did not elicit any changes in a number of immune parameters, namely natural-killer cell activity, monocyte count, lymphocyte proliferative responses or circulating levels of IL-6, IL-1α and IL-1β in patients with rheumatoid arthritis with low disease activity. Likewise, Rall et al. (56) did not observe changes in peripheral blood mononuclear cells production of TNF-α, IL-6, IL-2, IL-1β or lymphocyte proliferation after 12 weeks of a progressive resistance exercise program (2 sessions per week, 3 sets of 8 repetitions, 80% of 1RM) in patients with rheumatoid arthritis. Bearne et al. (8) examined the acute and chronic effects a 5-week lower-body strength exercise program (2 sessions per week, 4 sets of 6 isometric contractions at maximal voluntary contraction) on the cytokine response in patients with rheumatoid arthritis. Before the exercise program, a single bout of strength exercise did not elicit any significant changes on IL-1, IL-6 and TNF-α levels, which supports the safety of exercise in these patients. Similarly, after the training program, no significant changes were observed in resting levels of these cytokines. However, IL-6 level was decreased after an acute single exercise session at the end of the intervention. TNF-α response was also acutely decreased, although this did not reach statistical significance. Although the training program did not change baseline cytokine levels in these patients, it is conceivable to speculate that the chronic exercise may have affected, in a positive way, the inflammatory response to a single bout of exercise. Interestingly, Nader et al. (34) reported decreased expression of pro-inflammatory and pro-fibrotic gene networks (using microarray technique) after 7 weeks of resistance training [3 sessions per week, 45 min per session (3 sets of 10RM in 5 muscle groups)] in patients with dermatomyositis and polymyositis, indicating an important local anti-inflammatory effect of exercise in these patients, which might be associated with improvements in clinical symptoms.

Recently, our group investigated the effects a 12-week aerobic training program [2 sessions per week, 30 to 50 minutes per session, from ventilatory anaerobic threshold (VAT) to 10% below respiratory compensation point (RCP)] in patients with SLE in remission (47). Prior to and after the regular training program, cytokines and sTNFRs were assessed at rest and in response to single bouts of acute moderate and intense exercises. Exercise training led to a decrease in resting sTNFR2 and a trend towards reduction in IL-10. In response to a bout of moderate exercise, area under the curve of IL-10 was reduced and area under the curve of IL-10, IL-6, TNF-α, and sTNFR1 approached comparable values to those of the healthy control group after the exercise training program in SLE group. In response to a bout of intense exercise, area under the curve of IL-10 was also reduced in SLE; additionally, area under the curve of sTNFR2 tended to decrease after training. Altogether, these findings indicate that an exercise training program did not exacerbate inflammation in women with SLE in remission at rest or in response to single bouts of acute exercise, irrespective of exercise intensity. Moreover, exercise training attenuated the exacerbated inflammatory milieu, suggesting that this intervention may promote a homeostatic immunomodulatory effect in SLE.

Mechanisms underlying exercise-induced changes in cytokine pattern are not yet fully elucidated in healthy subjects and patients with an inflammatory disease; however, it has been postulated that modulation in gene expression of leukocyte cells could explain alterations in inflammatory mediators in response to exercise (60). This concept has been supported by gene array studies (10, 15, 16, 53, 54). A growing number of studies have demonstrated that a single bout of exercise can modulate gene expression in circulating leukocytes in healthy subjects (60). Notably, these studies have revealed that exercise can simultaneously stimulate anti- and pro-inflammatory genes as well as growth and repair genes, suggesting a coordinated counterbalanced response to the stress imposed by exercise (53).

Connolly et al. (16) showed that a 30-minute cycling exercise (80% VO_{2peak}) led to an up-regulation of genes related to stress and inflammation in peripheral blood mononuclear cells in healthy subjects. Importantly, altered expression levels of pro-inflammatory genes returned to baseline 60 minutes into recovery, whereas *IL-1RA* gene, which is thought to be an anti-inflammatory mediator, increased during this stage. This response was paralleled by an increase in IL-1ra and IL-6 circulating levels. Heat shock proteins *(HSP)* genes, which have been shown to inhibit NF-κB (27), were also upregulated during the exercise. Based on these findings, the authors suggested that changes in *IL-1RA* and *HSP* gene expression might be related to the anti-inflammatory effect of exercise. Büttner et al. (10) also showed a significant up-regulation of leukocytes genes involved in energy metabolism, extracellular matrix, heat-shock response and inflammation in response to a higher- and lower-intensity exercise (80% and 60% VO_{2max}) in healthy subjects. Amongst the most up-regulated genes in response to exercise were *HSP, IL-1RA*, and membrane metalloendopeptidase *(MME),* which is thought to control the bioavailability of pro-inflammatory peptides. On the other hand, amongst the most down-regulated genes was the natural killer receptor gene *BY55/CD160*, which is an important co-stimulator of the T-cell receptor possibly able to affect T-cell expansion and cytotoxicity (38). The authors suggested that these gene responses might be associated with the anti-inflammatory response to exercise.

In a series of studies, Radom-Aizik et al. (52, 54, 55) assessed gene expression in different leukocyte subpopulations (*i.e.*, monocytes, natural killer cells, and neutrophils) in response to acute exercise in adult healthy subjects (55). These authors observed that ten 2-min bouts of exercise (85% of VO_{2peak}) interspersed by 1-min intervals were able to modulate monocytes gene expression in healthy subjects (55). The main up-regulated genes in the monocytes following exercise were heparin-binding EGF-like growth factor *(HBEGF)*, amphiregulin *(AREG)*, and epiregulin *(EREG)*, which have been associated with vascular remodeling (70). Additionally, the main down-regulated genes in response to exercise were *TNF-α, TLR4*, and *CD36*, which have been associated with the anti-inflammatory effects of exercise (21, 61). Radom-Aizik et al. (52) also showed that an acute exercise protocol (2-min exercise at 77% of VO_{2peak}, interspersed by 1-min intervals) modulated pathways related to cancer, cell communication, and inflammation in circulating NK cells. This conclusion was based on the modulation of TNF family genes, with an increase in the gene expression of TNF-induced protein 3 *(TNFAIP3)*, and decreases in the gene expression of TNF ligand superfamily member 13b *(TNFSF13B)* and TNF ligand superfamily member 4 *(TNFSF4)*. Finally, because neutrophils remain elevated or continue to increase after exercise, unlike peripheral blood mononuclear cells, Radom-Aizik et al. (54) investigated the effects of a 30-min exercise bout (VO_{2peak}) on neutrophil gene expression in healthy young men. The authors showed that many of the altered genes were related to regulation of cell physiology, immune response, stress response, apoptosis, and signal transduction. A number of genes involved in growth and tissue repair were up-regulated (*e.g., ARE*, a member of the epidermal growth factor), as well as genes potentially involved in angiogenesis [*e.g.*, platelet-derived factor D *(PDGFD)* and fibroblast growth factor receptor-2 *(FGFR2)*]. Moreover, up-regulation of pro- and anti-apoptotic genes was also observed. Finally, as observed in peripheral blood mononuclear cells, both pro- and anti-inflammatory genes were up-regulated. For instance, granzyme A *(GZMA)* and perforin *(PRF1)* genes, which are known to control protein that promote cell lysing of pathogens, thereby allowing neutrophils to kill or damage these cells, were up-regulated. In addition, annexin 1 *(ANXA1)*, which is thought to inhibit the release of free radicals from activated neutrophils (48), and *HSP* genes were also up-regulated. The apparently "paradoxical response" to exercise led the authors to suggest that "the genomic response in neutrophils immediately following the perturbation of exercise might be characterized as a cellular 'wake up' call". These findings were further supported by another study (36), which observed an activation of the innate immunity characterized by an increase in gene expression of toll-like receptors in neutrophils after a single bout of exercise.

Therefore, in healthy individuals, a global gene expression analysis of circulating peripheral blood mononuclear cells and neutrophils shows that exercise stimulates an orchestrated up-regulation of both pro- and anti-inflammatory genes, which are normalized after at least one hour of recovery (16, 54). However, a concern remains that exercise may trigger an immunological "danger" type of stress and inflammatory response characterized by "pre-activated" circulating leukocytes (*e.g.*, asthma, musculoskeletal injury, and anaphylaxis), which could lead, at least in theory, to detrimental effects in inflammatory diseases (17). In contrast to this hypothesis, we did not observe exacerbated inflammation following both acute and chronic exercise in SLE patients (40, 41). Nonetheless, the influence of exercise on inflammation-related gene expression in blood cells of SLE patients has not been addressed. Therefore, this study aimed to investigate the effects of acute aerobic exercise on the modulation of immune-related gene expression in circulating leukocytes from $SLE_{INACTIVE}$ and SLE_{ACTIVE} patients and healthy individuals. Based on our previous findings (40, 41), we hypothesized that a single bout of exercise would similarly modulate leukocyte gene expression in SLE patients and controls, without inducing an exacerbated gene inflammatory response.

Table 1. Demographic, clinical and therapy data of patients ($SLE_{INACTIVE}$ and SLE_{ACTIVE}) and healthy controls (HC).

	SLE_{ACTIVE} (n = 4)	$SLE_{INACTIVE}$ (n = 4)	HC (n = 4)
Age (years)	32.5 ± 3.4	34.5 ± 3.4	29.3 ± 4.8
Body mass (kg)	69.8 ± 8.8	62.0 ± 5.3	58.0 ± 4.9
Height (cm)	164.8 ± 2.9	158.1 ± 3.6	157.8 ± 5.4
BMI (kg/m²)	25.7 ± 3.0	24.9 ± 3.1	23.3 ± 1.6
SLEDAI	6.3 ± 1.1*	1.3 ± 0.8	-
Disease duration (years)	6.8 ± 2.8	6.8 ± 1.8	-
Drugs [nº(%)]			
Glucocorticoid	4 (100%)	0 (0%)	-
Antimalarial	3 (75%)	3 (75%)	-
Azathioprine	1 (25%)	1 (25%)	-
Methotrexate	1 (25%)	0 (0%)	-
Mycophenolate mofetil	2 (50%)	1 (25%)	-

Data are presented as the mean ± standard deviation or n (%). BMI = body mass index; SLEDAI = systemic lupus erythematosus disease activity index; SLE: systemic lupus erythematosus; $SLE_{INACTIVE}$: women with inactive SLE; SLE_{ACTIVE}: women with active SLE. * denotes significant differences between SLE_{ACTIVE} versus $SLE_{INACTIVE}$ ($P < 0.05$).

MATERIAL AND METHODS

Ethical approval

This study was approved by the Local Ethical Committee (School of Medicine of University of Sao Paulo – nº 0185/11 and the subjects signed an informed consent. This study was registered at clinicaltrials.gov as NCT01515163. All of the procedures were in accordance with the Helsinki Declaration revised in 2008.

Participants and Experimental design

Four $SLE_{INACTIVE}$ and four SLE_{ACTIVE} women were consecutively selected and were regularly followed at the Outpatient Lupus Clinic of the Rheumatology Division of the School of Medicine at the University of Sao Paulo, Brazil. All patients fulfilled the American College of Rheumatology criteria for SLE diagnosis (25). Disease activity was determined by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores. The inclusion criteria for both SLE groups were an age range of 20 to 40 years and physical inactivity for at least six months prior to the selection process. Exclusion criteria were secondary rheumatic disease (*e.g.*, Sjögren syndrome, antiphospholipid syndrome), body mass index (BMI) ≥ 30 kg/m², acute renal failure, cardiac and pulmonary involvement, fibromyalgia, and musculoskeletal and joint disorders precluding the exercise testing. For the $SLE_{INACTIVE}$ group, inclusion criteria were SLEDAI scores < 4 and the absence of glucocorticoid therapy for at least six months prior to entry. All patients in the SLE_{ACTIVE} group had SLEDAI scores of between 4 and 8 and received a daily glucocorticoid dose of ≤ 20 mg. Disease manifestations were defined as follows: cutaneous disease, articular involvement, neuropsychiatric disease, renal disease, cardiopulmonary disease, and hematologic complications. Four age- and BMI-matched healthy women were selected for this study as the healthy control (HC) group. The participants' demographic and laboratory parameters are demonstrated in Tables 1 and 2. Patients and controls were randomly selected from a larger group of participants described elsewhere (46).

All subjects (*i.e.*, $SLE_{INACTIVE}$, SLE_{ACTIVE}, and HC) performed a maximal graded treadmill cardiopulmonary exercise test to

Table 2. Laboratory parameters of patients ($SLE_{INACTIVE}$ and SLE_{ACTIVE}) and healthy controls (HC).

	SLE_{ACTIVE} (n = 4)	$SLE_{INACTIVE}$ (n = 4)	HC (n = 4)
C3 (90-180 mg/dL)	70.8 ± 14.4#	100.7 ± 9.0	130.8 ± 17.4
C4 (10-40 mg/dL)	6.3 ± 1.3#	13.1 ± 4.9	27.0 ± 7.6
CPK (26-192 U/L)	83.0 ± 15.0	111.7 ± 38.0	103.5 ± 22.0
Creatinine (0.50-0.90 mg/dL)	0.83 ± 0.18	0.77 ± 0.12	0.78 ± 0.08
Erythrocytes (4.0-5.4 million/mm³)	4.2 ± 0.4	4.1 ± 0.2	4.5 ± 0.3
Hematocrit (35-47%)	39.3 ± 2.9	38.9 ± 0.5	38.2 ± 1.0
Leukocytes (4.0-11.0 mil/mm³)	5.6 ± 0.5	4.3 ± 1.2	8.0 ± 3.6
Platelets (140-450 mil/mm³)	202.5 ± 45.2	259.5 ± 66.5	255.8 ± 50.4
CRP (< 5 mg/L)	3.2 ± 4.6	2.2 ± 2.5	3.3 ± 2.3
ESR (5.6-11.0 mm)	7.5 ± 9.0	12.0 ± 6.4	7.0 ± 5.0

Data are presented as the mean ± standard deviation. CPK: creatine phosphokinase; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate. # denotes significant differences between SLE_{ACTIVE} versus HC ($P < 0.05$).

determine their ventilatory anaerobic threshold (VAT) and RCP. Thereafter, the subjects in the $SLE_{INACTIVE}$, SLE_{ACTIVE}, and HC groups underwent a 30-min single bout of acute aerobic exercise, and a leukocyte gene expression analysis was performed prior to exercise (Baseline), immediately after exercise (End-ex), and after three hours of recovery (Recovery).

Cardiopulmonary exercise test

A maximal graded exercise test was performed on a treadmill (Centurion 200, Micromed, Brazil) with increments in velocity and grade each minute until volitional exhaustion. Oxygen consumption (VO_2) and carbon dioxide output were obtained through breath-by-breath sampling and expressed as a 30-s average using an indirect calorimetric system (Cortex - model Metalyzer IIIB, Leipzig, Germany). HR was continuously recorded at rest, during exercise and at recovery using a 12-lead electrocardiogram (Ergo PC Elite, Inc. Micromed, Brazil). Cardiopulmonary exercise test results were considered to be maximal when one of the following criteria was met: VO_2 plateau (*i.e.*, < 150 mL/min increase between two consecutive stages), HR of no less than 10 beats below age-predicted maximal HR and respiratory exchange ratio value of above 1.10 (49). The VO_2peak was calculated as the average value for the final 30 s of the test. The VAT was identified following previously described procedures (68). In brief, this value was determined when the ventilatory equivalent for VO_2 (VE/VO_2) increased without a concomitant increase in the ventilatory equivalent for carbon dioxide (VE/VCO_2). The RCP was determined when the VE/VO_2 and VE/VCO_2 increased simultaneously.

Single bout of acute aerobic exercise

At least 72 hours after the cardiopulmonary exercise test, a single bout of acute aerobic exercise was performed on a treadmill for the assessment of gene expression in the leukocytes.

The acute aerobic exercise bout was set at an intensity corresponding to 50% of the delta difference (Δ) between the VAT and the RCP ($SLE_{INACTIVE}$: 69.0 ± 11.3% of VO_{2peak}; SLE_{ACTIVE}: 67.6 ± 5.1% of VO_{2peak}; HC: 63.2 ± 6.6% of VO_{2peak}). The exercise bout was comprised of a 5-min warm-up and 30 min of exercise at the pre-determined exercise intensity.

Blood sampling and RNA isolation

Prior to exercise, the antecubital vein was cannulated for blood sampling. Approximately 10 mL of blood were collected with the patients in the seated position immediately before the acute exercise (Baseline), immediately after the acute exercise (End-ex), and 3 hours after the end of the acute exercise (Recovery). Blood was drawn into tubes containing anticoagulant (EDTA) for subsequent analysis. White blood cells were isolated using an erythrocyte lysis buffer (17 mM tris-HCl and 0.144 M ammonium chloride, pH 7.2) followed by two washing steps in PBS containing 2 mmol/L EDTA. The buffers were used at 4°C, and the tubes were placed on ice. Total RNA was isolated from the resulting cell pellet using the Trizol reagent (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's instructions. The RNA concentrations were determined using the NanoDrop spectrophotometer (NanoDrop, Thermo Scientific, Wilmington, DE). RNA quality was assessed using the Agilent Bioanalyzer 2100 System (Agilent Technologies, Palo Alto, CA), following the manufacturer's recommendations.

PCR array analysis

The PCR array analyses of the mRNA were performed in two sets of six 96-well plates per group, following the manufacturer's recommendations (The Human Innate & Adaptive Immune Responses RT^2 Profiler™ PCR Array Kit; Qiagen, South Korea). The data analysis and validation were performed using the free online software provided by the manufacturer's website (Qiagen, South Korea). Gene expression levels were considered to be altered (up- or down-regulated) if fold changes of > 2.0 were observed.

Bioinformatics analysis

A protein-interaction network analysis was performed to evaluate the network representations of the signaling pathways and the physical relationships between the proteins encoded by the differentially expressed (DE) genes. The Cytoscape plugin GeneMania (31) was employed to scan for physical and pathway interactions between the seed nodes. Network descriptive parameters (*i.e.*, number of nodes, number of edges, average node degree, diameter, characteristic path length, clustering coefficient, and network density) were calculated using the Cytoscape Plugin Network Analyzer (5). After the networks were constructed, the twenty percent most connected nodes in each network (considered as hubs) were marked with yellow borders for a better visualization.

Statistical analysis

Data are presented as mean ± standard deviation. The Gaussian distribution of the data was tested by Kolmogorov-Smirnov's test (with Lilliefor's correction). Demographic data of the three groups ($SLE_{INACTIVE}$, SLE_{ACTIVE}, and HC) were compared using one way ANOVA followed by Bonferroni post hoc test. Drugs proportions of both SLE groups were compared with χ^2 test. All data analysis was performed using the Statistical Package for Social Sciences (SPSS), version 17.0 for Windows. The level of significance was set at $P \leq 0.05$.

RESULTS

Patients and healthy controls main characteristics' are presented in Table 1 and 2. Age, body mass, height, and BMI were comparable between the $SLE_{INACTIVE}$, SLE_{ACTIVE}, and HC groups ($P > 0.05$). The SLE_{ACTIVE} group showed higher SLEDAI score compared with the $SLE_{INACTIVE}$ group ($P < 0.05$) and lower C3 and C4 levels compared with the HC group ($P < 0.05$).

In the HC group, 46 DE genes (5 up-regulated and 41 down-regulated) were modulated at the end of the single bout of acute exercise, whereas 26 DE genes were up-regulated at Recovery compared with Baseline (Table 3). Additionally, the network analysis indicated that the main exercise-modulated genes were as follows: *IL2, IFNG, TNF, IL18, IL23A, IL1B,*

Table 3. Gene expression response to a single bout of exercise in healthy controls (HC).

Gene symbol	Gene name	End-ex *vs.* Baseline (FC)	Recovery *vs.* Baseline (FC)
	INNATE IMMUNITY		
C3	Complement component 3	-3.4826	NC
CASP1	Caspase 1	-2.0997	2.8927
CCL2	Chemokine (C-C motif) ligand 2	-4.1143	NC
CCL5	Chemokine (C-C motif) ligand 5	-3.1449	NC
CD14	CD14 molecule	NC	2.33
CD40LG	CD40 ligand	-2.467	NC
CD8A	CD8a molecule	-2.1748	NC
CRP	C-reactive protein	-4.1143	NC
CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	-3.1182	NC
CXCL10	Chemokine (C-X-C motif) ligand 10	-3.9112	NC
CXCR3	Chemokine (C-X-C motif) receptor 3	-3.0706	NC
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	-2.4561	2.3022
HLA-A	Major histocompatibility complex. class I, A	2.9903	NC
HLA-E	Major histocompatibility complex, class I, E	2.0173	2.0892
IFNA1	Interferon alpha 1	-4.1143	NC
IFNB1	Interferon beta 1	-3.9216	NC
IL18	Interleukin 18	-2.0383	NC
IL1A	Interleukin 1 alpha	-4.5095	NC
IL1B	Interleukin 1 beta	NC	4.6954
IL1R1	Interleukin 1 receptor, type I	NC	4.9493
IL2	Interleukin 2	-6.0684	NC
IRF3	Interferon regulatory factor 3	-2.1479	NC
ITGAM	Integrin, alpha	NC	3.2972
LY96	Lymphocyte antigen 96	-4.6578	NC
LYZ	Lysozyme	NC	2.0842
MBL2	Mannose-binding lectin (protein C) 2, soluble	-3.6605	NC
MX1	Interferon-inducible protein p78	-3.5097	NC
MYD88	Myeloid differentiation primary response gene (88)	-2.2664	NC
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	NC	3.1521
NOD1	Nucleotide-binding oligomerization domain containing 1	NC	3.6606
RAG1	Recombination activating gene 1	-3.5375	NC
STAT1	Signal transducer and activator of transcription 1	NC	2.4584
TICAM1	Toll-like receptor adaptor molecule 1	-2.9017	NC
TLR1	Toll-like receptor 1	NC	2.6445
TLR3	Toll-like receptor 3	-2.9082	NC
TLR7	Toll-like receptor 7	-2.0592	NC
TLR8	Toll-like receptor 8	NC	2.7166
TNF	Tumor necrosis factor	-3.1592	NC
	ADAPTIVE IMMUNITY		
C3	Complement component 3	-3.4826	NC
CCL2	Chemokine (C-C motif) ligand 2	-4.1143	NC
CCL5	Chemokine (C-C motif) ligand 5	-3.1449	NC
CCR4	Chemokine (C-C motif) receptor 4	2.2592	NC
CCR5	Chemokine (C-C motif) receptor 5	2.023	2.3474
CCR6	Chemokine (C-C motif) receptor 6	2.0789	2.4998
CCR8	Chemokine (C-C motif) receptor 8	-8.4509	NC
CD40LG	CD40 ligand	-2.467	NC
CD80	CD80 molecule	-5.0934	NC
CD8A	CD8a molecule	-2.1748	NC
CRP	C-reactive protein	-4.1143	NC
CSF2	Colony stimulating factor 2 (granulocyte-macrophage)	-3.1182	NC

CXCL10	Chemokine (C-X-C motif) ligand 10	-3.9112	NC
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	-2.4561	2.3022
FASLG	Fas ligand	-2.3892	NC
FOXP3	Forkhead box P3	-2.7225	NC
GATA3	GATA binding protein 3	-3.3916	NC
HLA-A	Major histocompatibility complex, class I, A	2.9903	NC
IFNA1	Interferon alpha 1	-4.1143	NC
IFNAR1	Interferon (alpha, beta and omega) receptor 1	NC	2.7179
IFNB1	Interferon beta 1	-3.9216	NC
IFNG	Interferon gamma	NC	2.1988
IL10	Interleukin 10	-4.0672	NC
IL13	Interleukin 13	-3.3514	3.2836
IL17A	Interleukin 17A	-4.1143	NC
IL18	Interleukin 18	-2.0383	NC
IL1A	Interleukin 1 alpha	-4.5095	NC
IL1B	Interleukin 1 beta	NC	4.6954
IL1R1	Interleukin 1 receptor, type I	NC	4.9493
IL2	Interleukin 2	-6.0684	NC
IL23A	Interleukin 23, alpha subunit p19	-3.1288	24.9473
IL4	Interleukin 4	-4.3607	NC
IL5	Interleukin 5	-4.1143	2.1612
IL6	Interleukin 6	-4.2226	NC
IRF3	Interferon regulatory factor 3	-2.1479	NC
ITGAM	Integrin, alpha	NC	3.2972
JAK2	Janus kinase 2	NC	2.8748
LYZ	Lysozyme	NC	2.0842
MAPK8	Mitogen-activated protein kinase 8	NC	2.524
MBL2	Mannose-binding lectin (protein C) 2, soluble	-3.6605	NC
MX1	Interferon-inducible protein p78	-3.5097	NC
MYD88	Myeloid differentiation primary response gene (88)	-2.2664	NC
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	NC	3.1521
NOD1	Nucleotide-binding oligomerization domain containing 1	NC	3.6606
RAG1	Recombination activating gene 1	-3.5375	NC
RORC	RAR-related orphan receptor C	-2.726	NC
SLC11A1	Solute carrier family 11, member 1	NC	3.2821
STAT1	Signal transducer and activator of transcription 1	NC	2.4584
STAT3	Signal transducer and activator of transcription 3	NC	2.5869
STAT4	Signal transducer and activator of transcription 4	NC	2.9454
STAT6	Signal transducer and activator of transcription 6	-2.2238	NC
TICAM1	Toll-like receptor adaptor molecule 1	-2.9017	NC
TLR1	Toll-like receptor 1	NC	2.6445
TLR3	Toll-like receptor 3	-2.9082	NC
TLR7	Toll-like receptor 7	-2.0592	NC
TLR8	Toll-like receptor 8	NC	2.7166
TNF	Tumor necrosis factor	-3.1592	NC
TYK2	Tyrosine kinase 2	NC	2.0777

Table 4. Gene expression response to a single bout of exercise in inactive systemic lupus erythematosus (SLE$_{INACTIVE}$).

Gene symbol	Gene name	End-ex *vs.* Baseline (FC)	Recovery *vs.* Baseline (FC)
	INNATE IMMUNITY		
C3	Complement component 3	-2.6061	NC
CASP1	Caspase 1	-2.3041	NC
CCL2	Chemokine (C-C motif) ligand 2	-2.2908	NC
CCL5	Chemokine (C-C motif) ligand 5	-2.3139	2.0725
CD14	CD14 molecule	2.2325	NC
CD40	CD40 molecule	NC	2.1064
CD40LG	CD40 ligand	2.1076	NC
CD8A	CD8a molecule	NC	2.0703
CRP	C-reactive protein, pentraxin-related	-3.8309	NC
CXCL10	Chemokine (C-X-C motif) ligand 10	-4.3281	2.9765
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	-3.2047	NC
HLA-A	Major histocompatibility complex, class I, A	3.9397	NC
IFNA1	Interferon alpha 1	-3.8309	NC
IFNB1	Interferon beta 1	-3.8309	NC
IL18	Interleukin 18	-3.8301	NC
IL1A	Interleukin 1 alpha	-3.021	2.8189
IL2	Interleukin 2	-3.5093	2.1392
IRF7	Interferon regulatory factor 7	2.2998	NC
LY96	Lymphocyte antigen 96	-4.9407	NC
MBL2	Mannose-binding lectin (protein C) 2, soluble	-3.8309	2.2082
NLRP3	NLR family, pyrin domain containing 3	2.1374	NC
NOD2	Nucleotide-binding oligomerization domain containing 2	2.4438	NC
TICAM1	Toll-like receptor adaptor molecule 1	-2.8619	2.0873
TLR3	Toll-like receptor 3	-3.0218	NC
TLR4	Toll-like receptor 4	NC	-2.3616
TLR6	Toll-like receptor 6	-2.1854	NC
TLR7	Toll-like receptor 7	NC	2.0258
TLR8	Toll-like receptor 8	NC	-2.2878
TLR9	Toll-like receptor 9	NC	2.4685
IFNB1	Interferon beta 1	-3.8309	NC
CCR4	Chemokine (C-C motif) receptor 4	2.4068	NC
CCR5	Chemokine (C-C motif) receptor 5	NC	2.1401
IL17A	Interleukin 17A	-3.8309	NC
IFNA1	Interferon alpha 1	-3.8309	NC
IL4	Interleukin 4	-2.9785	2.3649
IL6	Interleukin 6	-3.8309	2.1509
IL5	Interleukin 5	-5.684	NC
IL2	Interleukin 2	-3.5093	2.1392
IL18	Interleukin 18	-3.8301	NC
IL13	Interleukin 13	-3.7825	2.1634
IL10	Interleukin 10	-3.8309	2.0211
CXCL10	Chemokine (C-X-C motif) ligand 10	-4.3281	2.9765
IL1A	Interleukin 1 alpha	-3.021	2.8189
CCL2	Chemokine (C-C motif) ligand 2	-2.2908	NC
CCL5	Chemokine (C-C motif) ligand 5	-2.3139	2.0725
FASLG	Fas ligand	-3.07	2.0561
CXCR3	Chemokine (C-X-C motif) receptor 3	-3.9117	NC

	ADAPTIVE IMMUNITY		
C3	Complement component 3	-2.6061	NC
CCL2	Chemokine (C-C motif) ligand 2	-2.2908	NC
CCL5	Chemokine (C-C motif) ligand 5	-2.3139	2.0725
CCR4	Chemokine (C-C motif) receptor 4	2.4068	NC
CCR5	Chemokine (C-C motif) receptor 5	NC	2.1401
CCR8	Chemokine (C-C motif) receptor 8	-3.2653	NC
CD40	CD40 molecule	NC	2.1064
CD40LG	CD40 ligand	2.1076	NC
CD80	CD80 molecule	NC	3.0758
CRP	C-reactive protein, pentraxin-related	-3.8309	NC
CXCL10	Chemokine (C-X-C motif) ligand 10	-4.3281	2.9765
CXCR3	Chemokine (C-X-C motif) receptor 3	-3.9117	NC
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	-3.2047	NC
FASLG	Fas ligand	-3.07	2.0561
FOXP3	Forkhead box P3	-3.2481	NC
GATA3	GATA binding protein 3	-2.7182	NC
HLA-A	Major histocompatibility complex, class I, A	3.9397	NC
IFNA1	Interferon alpha 1	-3.8309	NC
IFNB1	Interferon beta 1	-3.8309	NC
IFNG	Interferon gamma	-2.8439	2.4089
IL10	Interleukin 10	-3.8309	2.0211
IL13	Interleukin 13	-3.7825	2.1634
IL17A	Interleukin 17A	-3.8309	NC
IL18	Interleukin 18	-3.8301	NC
IL1A	Interleukin 1 alpha	-3.021	2.8189
IL2	Interleukin 2	-3.5093	2.1392
IL4	Interleukin 4	-2.9785	2.3649
IL5	Interleukin 5	-5.684	NC
IL6	Interleukin 6	-3.8309	2.1509
IRF7	Interferon regulatory factor 7	2.2998	NC
MBL2	Mannose-binding lectin (protein C) 2, soluble	-3.8309	2.2082
NLRP3	NLR family, pyrin domain containing 3	2.1374	NC
NOD2	Nucleotide-binding oligomerization domain containing 2	2.4438	NC
RAG1	Recombination activating gene 1	-5.7506	NC
RORC	RAR-related orphan receptor C	-2.8572	NC
STAT4	Signal transducer and activator of transcription 4	NC	2.0954
TICAM1	Toll-like receptor adaptor molecule 1	-2.8619	2.0873
TLR3	Toll-like receptor 3	-3.0218	NC
TLR4	Toll-like receptor 4	NC	-2.3616
TLR6	Toll-like receptor 6	-2.1854	NC
TLR7	Toll-like receptor 7	NC	2.0258
TLR8	Toll-like receptor 8	NC	-2.2878
TLR9	Toll-like receptor 9	NC	2.4685

Table 5. Gene expression response to a single bout of exercise in active systemic lupus erythematosus (SLE_{ACTIVE}).

Gene symbol	Gene name	End-ex *vs.* Baseline (FC)	Recovery *vs.* Baseline (FC)
	INNATE IMMUNITY		
C3	Complement component 3	-3.4223	NC
CASP1	Caspase 1	-2.0748	NC
CCL2	Chemokine (C-C motif) ligand 2	-2.0748	NC
CCL5	Chemokine (C-C motif) ligand 5	-2.6933	NC
CRP	C-reactive protein, pentraxin-related	-3.7408	NC
CXCL10	Chemokine (C-X-C motif) ligand 10	-3.7408	2.1367
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	-2.3726	NC
HLA-A	Major histocompatibility complex, class I, A	2.6664	NC
IFNA1	Interferon alpha 1	-2.3368	NC
IFNB1	Interferon, beta 1	-3.0087	2.6837
IL18	Interleukin 18	-2.3353	NC
IL2	Interleukin 2	-3.5439	NC
IRF7	Interferon regulatory factor 7	2.4521	2.0215
LY96	Lymphocyte antigen 96	-4.7781	NC
MAPK8	Mitogen-activated protein kinase 8	NC	2.2523
MBL2	Mannose-binding lectin (protein C) 2, soluble	-3.7146	NC
TICAM1	Toll-like receptor adaptor molecule 1	-2.885	-2.0228
TLR3	Toll-like receptor 3	NC	3.0184
	ADAPTIVE IMMUNITY		
C3	Complement component 3	-3.4223	NC
CCL2	Chemokine (C-C motif) ligand 2	-2.0748	NC
CCL5	Chemokine (C-C motif) ligand 5	-2.6933	NC
CCR4	Chemokine (C-C motif) receptor 4	2.2639	3.5957
CCR5	Chemokine (C-C motif) receptor 5	NC	2.2158
CCR6	Chemokine (C-C motif) receptor 6	NC	2.1947
CCR8	Chemokine (C-C motif) receptor 8	-2.594	NC
CD80	CD80 molecule	-4.8174	3.6854
CRP	C-reactive protein, pentraxin-related	-3.7408	NC
CXCL10	Chemokine (C-X-C motif) ligand 10	-3.7408	2.1367
CXCR3	Chemokine (C-X-C motif) receptor 3	-2.1625	-2.3697
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	-2.3726	NC
FASLG	Fas ligand	-2.3726	-2.4915
FOXP3	Forkhead box P3	-3.6605	-4.0451
GATA3	GATA binding protein 3	-2.3368	NC
HLA-A	Major histocompatibility complex, class I, A	2.6664	NC
IFNA1	Interferon alpha 1	-2.3368	NC
IFNB1	Interferon, beta 1	-3.0087	2.6837
IFNG	Interferon gamma	-4.8211	NC
IL10	Interleukin 10	-2.9632	NC
IL13	Interleukin 13	-2.1952	NC
IL17A	Interleukin 17A	-2.9683	NC
IL18	Interleukin 18	-2.3353	NC
IL2	Interleukin 2	-3.5439	NC
IL4	Interleukin 4	-2.5493	2.1261
IL5	Interleukin 5	-2.8056	NC
IL6	Interleukin 6	-3.7408	2.1817
IRF7	Interferon regulatory factor 7	2.4521	2.0215
MAPK8	Mitogen-activated protein kinase 8	NC	2.2523
MBL2	Mannose-binding lectin (protein C) 2, soluble	-3.7146	NC

RAG1	Recombination activating gene 1	-4.8882	NC
STAT4	Signal transducer and activator of transcription 4	NC	2.1524
TICAM1	Toll-like receptor adaptor molecule 1	-2.885	-2.0228
TLR3	Toll-like receptor 3	NC	3.0184

Table 6. Network descriptive parameters in patients ($SLE_{INACTIVE}$ and SLE_{ACTIVE}) and healthy controls (HC).

Network parameters	SLE_{ACTIVE}	$SLE_{INACTIVE}$	HC
Number of nodes	29	40	58
Number of edges	29	60	195
Average node degree	2.0	3.0	6.72
Diameter	9	8	6
Characteristic path length	3.64	3.40	2.62
Clustering coefficient	0.15	0.33	0.39
Network density	0.07	0.08	0.12

IFNAR1, IL1R1, JAK2, STAT1, STAT3, STAT4, TYK2, IRF3, NFKB1 and *MYD88* (Figure 1; genes with yellow borders).

In the $SLE_{INACTIVE}$ group, there were 39 DE genes (7 up-regulated and 32 down-regulated) at End-ex compared with Baseline, whereas 22 genes (20 up-regulated and 2 down-regulated) were modulated at Recovery compared with Baseline (Table 4). Additionally, the network analysis revealed that the main exercise-modulated hubs were as follows: *IL2, IL13, IL18, GATA3, STAT4, CCL5, LY96, TLR4, TLR7, TLR8* and *TLR9* (Figure 2; genes with yellow borders).

In the SLE_{ACTIVE} group, 32 DE genes (3 up-regulated and 29 down-regulated) were modulated at End-ex when compared with Baseline, while 17 (13 up-regulated and 4 down-regulated) were modulated at Recovery when compared with Baseline (Table 5). Network analysis revealed that the main exercise-modulated hubs were *IL2, IFNG, IL18, IL13, GATA3, STAT4,* and *CCL5* (Figure 3; genes with yellow borders).

The analysis of the network descriptive parameters is shown in Table 6. Interestingly, the lower number of nodes and edges, average node degree, and network density in the SLE_{ACTIVE} and $SLE_{INACTIVE}$ groups indicated that a single bout of exercise modulated less dense gene networks in the SLE groups when compared with the HC group. In addition, SLE_{ACTIVE} had lower network connectivity when compared with $SLE_{INACTIVE}$.

DISCUSSION

To the best of our knowledge, this is the first study to assess the effects of acute exercise on the gene expression profiles of circulating leukocytes from SLE patients. The main findings of this study were two-fold: *i)* the SLE patients and healthy individuals displayed changes in the expression of their leukocyte inflammation-related genes following acute aerobic exercise; and *ii)* exercise regulated fewer immune-related genes and less connected networks in the leukocytes from the SLE patients compared with their healthy peers.

Leukocytes from the HC group showed a down-regulation of genes related to innate immunity (*i.e.*, cytokines and their receptors) at End-ex, in contrast with previous findings (30, 66). Although these previous studies did not detect any alterations in cytokine-related gene expression, cytokine serum levels did increase after exercise in accordance with a recent study from our group (46). In addition, the expression of Toll-like receptor pathway-related genes *(i.e., TLR3, TLR7, MYD88, IRF3,* and *IFNB1)*, which are associated with the inflammatory process (3), were down-regulated at End-ex in the HC group, suggesting that an anti-inflammatory response occurred immediately after exercise as previously reported (60). Conversely, at Recovery, the leukocytes of the HC group showed the up-regulation of the expression of genes related to the JAK/STAT pathway (*i.e., JAK2, TYK2, STAT1, STAT3, STAT4, IFNG,* and *IFNAR1*) and the pro-inflammatory pathway (*i.e., IL1B, IL1R1, NFKB1, TLR1, TLR8,* and *CD14)*, cor-

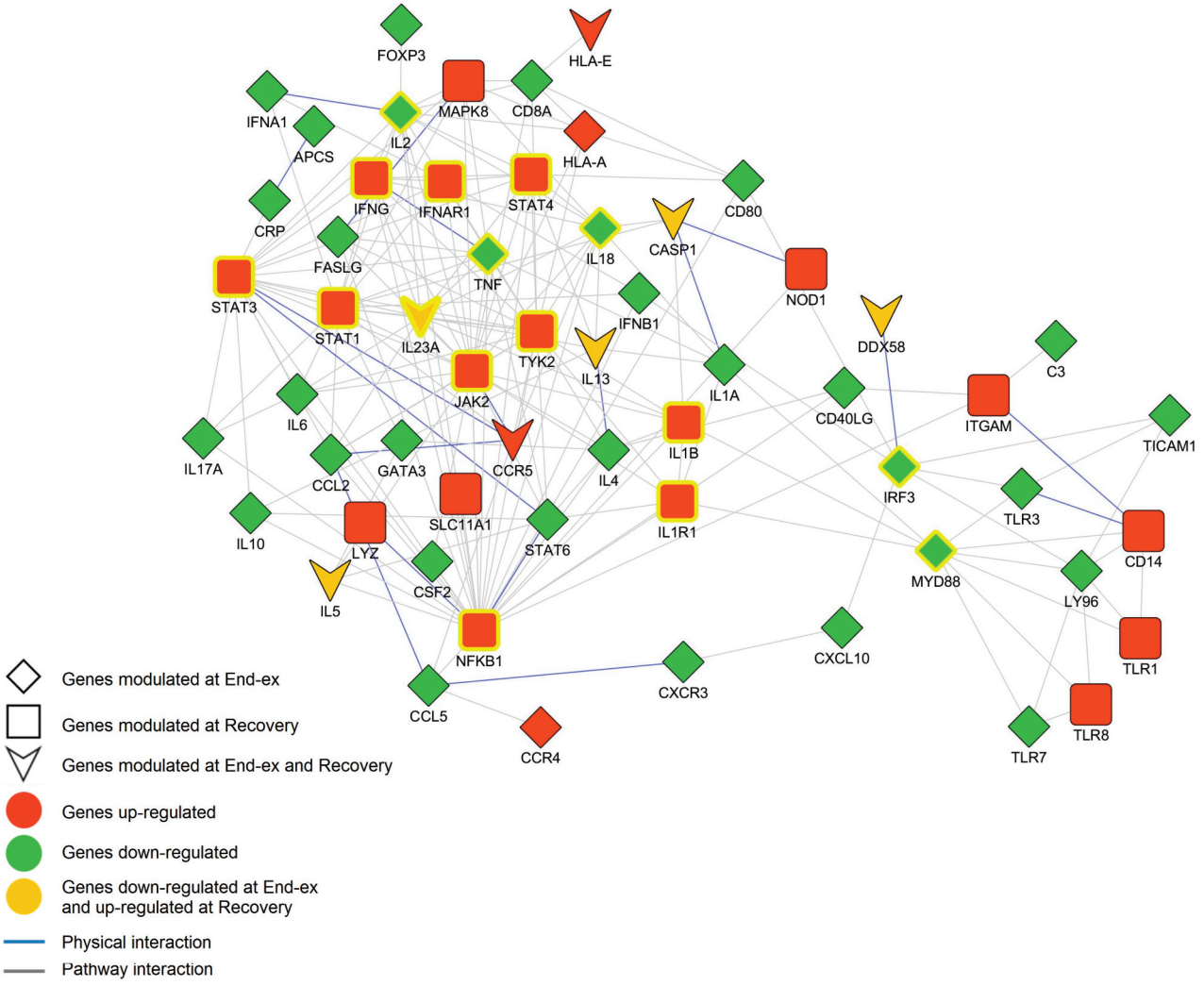

Figure 1. Network analysis of changes in gene expression obtained from comparisons between Baseline, End-ex and Recovery periods in healthy controls. Network analysis of the DE genes resulting from the comparisons of End-ex *vs.* Baseline and Recovery *vs.* Baseline in the HC. Networks were constructed using information obtained from the pathways (grey edges) and physical interactions (blue edges). Diamond and square nodes denote the DE genes regulated immediately after exercise (End-ex) and at Recovery, respectively. V-shaped nodes represent the genes regulated during both periods (End-ex and Recovery). The up- and down-regulated genes are depicted in red and green, respectively. The down-regulated genes at End-ex that were up-regulated at Recovery are depicted in orange.

roborating previous results (35, 54). The increased gene expression in relation to the JAK/STAT pathway has been associated with a variety of cellular functions (32); however, particularly in response to exercise, it has been linked to cell growth and the survival of neutrophils (54). Additionally, the increased expression of pro-inflammatory pathway-related genes at Recovery support previous findings (35). Neubauer et al. (35) suggested that this up-regulation might be explained by the appearance of muscle-derived damage-associated molecular mechanisms. Such molecules could be recognized by Toll-like receptors (TLR), triggering downstream pathways in a similar manner as the activation of sterile inflammation (14), but differing due to the transient nature of the exercise-induced inflammatory response (35).

The $SLE_{INACTIVE}$ group showed a similar response to the HC group, with the down-regulation of innate immunity genes such as cytokine-related *(i.e., IL13, IL2, IL18,* and *CCL5*) and TLR-related pathway (*i.e., TLR6, LY96*, and *TICAM1*) genes at End-ex. These genes were up-regulated at Baseline. Thus, it seems that exercise may negatively modulate the expression of genes over-expressed at baseline in SLE. However, while cytokine-related genes were altered in response to exercise, serum cytokine levels appeared to be not affected (46). The down-regulation observed at End-ex was not sustained during Recovery, where there was up-regulation of genes related to JAK/STAT pathway (*i.e., STAT4, IL13,* and *IL2*) and cytokines *(i.e., IL13, IL1A*, and *CCR5*). Although these genes have been implicated in SLE pathogenesis (4, 28), the same response has also been observed in healthy individuals (54) in a transitory manner (35). Moreover, adaptive immunity genes *(i.e., GATA3, RORC,* and *FOXP3*) were down-regulated at End-ex in $SLE_{INACTIVE}$, and *GATA3* was the highest connected gene in its corresponding network. *GATA3* is a transcription factor responsible for promoting the responses of T helper 2 cells and has been associated with the autoantibodies production in SLE patients (44), as well as autoimmune glomerulonephritis in mice (69). Therefore, it seems that a single bout of exercise could, at least transiently, down-regulate transcription factors associated with SLE disease pathogenesis.

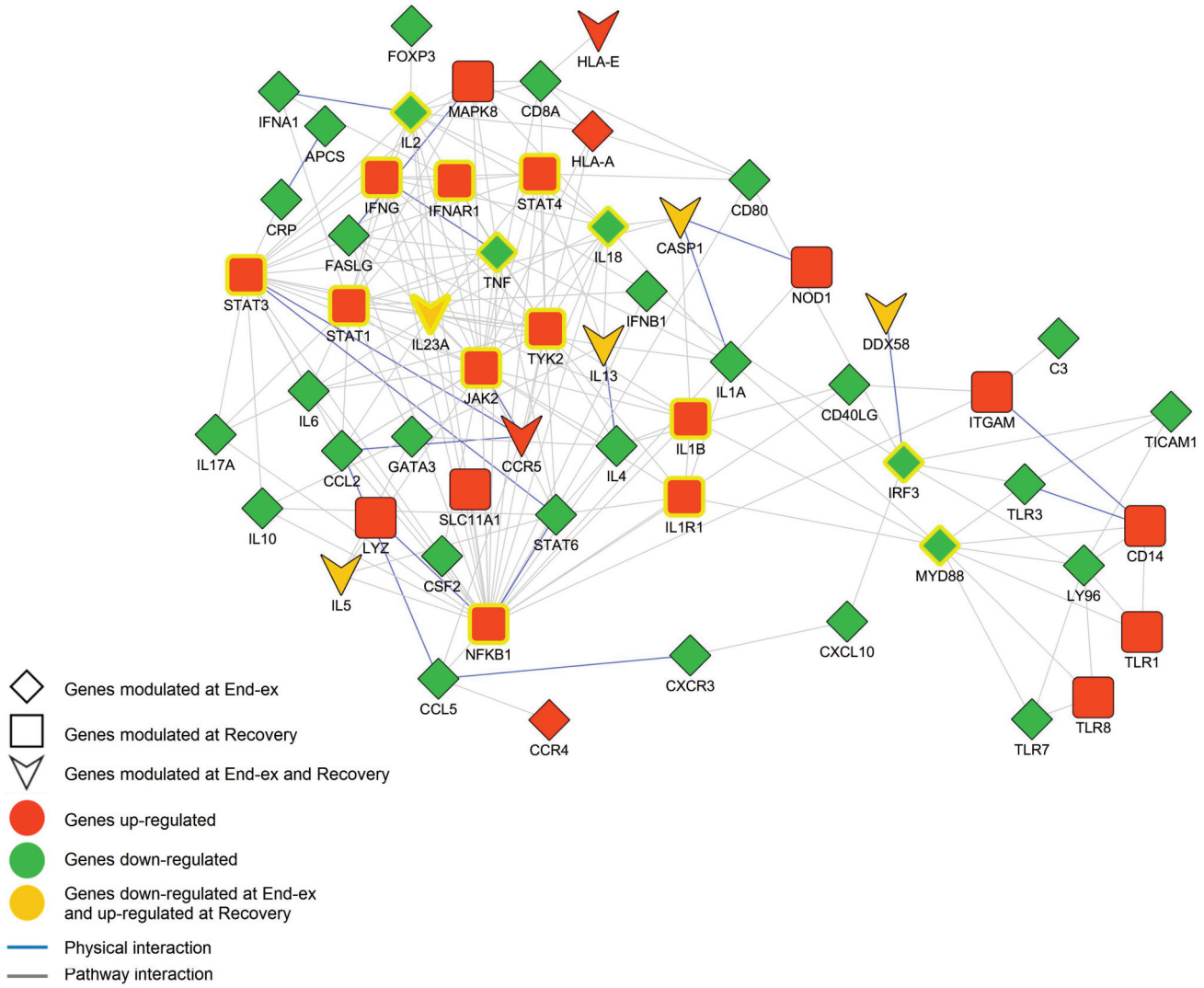

Figure 2. Network analysis of changes in gene expression obtained from comparisons between Baseline, End-ex and Recovery periods in $SLE_{INACTIVE}$ patients. Network analysis of DE genes resulting from the comparisons of End-ex *vs.* Baseline and Recovery *vs.* Baseline in the $SLE_{INACTIVE}$ patients. Networks were constructed using information obtained from the pathways (grey edges) and physical interactions (blue edges). Diamond and square nodes denote the DE genes regulated immediately after exercise (End-ex) and at Recovery, respectively. V-shaped nodes represent genes regulated during both periods (End-ex and Recovery). The up- and down-regulated genes are depicted in red and green, respectively. The down-regulated genes at End-ex phase that were up-regulated genes at Recovery are depicted in orange.

In the SLE_{ACTIVE} group, fewer genes were modulated by exercise, resulting in a less connected network. The main down-regulated network genes at End-ex were related to both innate and adaptive immunity, such as *IL2, IFNG, IL18, IL13, GATA3,* and *CCL5*. Interestingly, we observed a down-regulation of *IFNG*, which has been pointed out as a major effector molecule in SLE disease (64). Moreover, the reduction in *GATA3* gene expression was also observed in $SLE_{INACTIVE}$ at End-ex. At Recovery, fewer genes were up-regulated (*i.e., STAT4, TLR3, IRF7, CCR4,* and *CCR5*), and they possessed low degrees of connection, suggesting only the partial activation of the immune pathway.

Overall, we observed a similar response pattern of leukocyte gene expression in the $SLE_{INACTIVE}$, SLE_{ACTIVE} and HC groups following a single bout of acute exercise, since all groups showed a global down-regulation of blood leukocyte gene expression at End-ex compared with Baseline. These findings further support the notion that a single bout of aerobic exercise (up to ~70% VO_{2peak}) may not trigger a pro-inflammatory response in SLE patients (46, 47). Despite this, the analysis of network parameters (*i.e.,* number of nodes, number of edges, average node degree, and network density) suggested a lower network connectivity in the $SLE_{INACTIVE}$ and SLE_{ACTIVE} groups when compared with the HC group. Moreover, when compared the HC group, the SLE groups showed a higher network diameter with a lower clustering coefficient, which may suggest a less efficient and controlled flow of information (57). One hypothesis for the less connected networks and reduced number of modulated genes in SLE groups would be that an overexpression of inflammatory genes at Baseline might have precluded the additional exercise-induced transcriptional up-regulation in the leukocytes, as the expression of inflammatory genes might have approached maximum levels at Baseline. Alternatively, the drug therapies in SLE groups may have partially impeded the broader gene modulation observed in the HC group. In fact, treatment with immunosuppressive drugs (*e.g.*, glucocorticoid, chloroquine, and methotrexate) has been

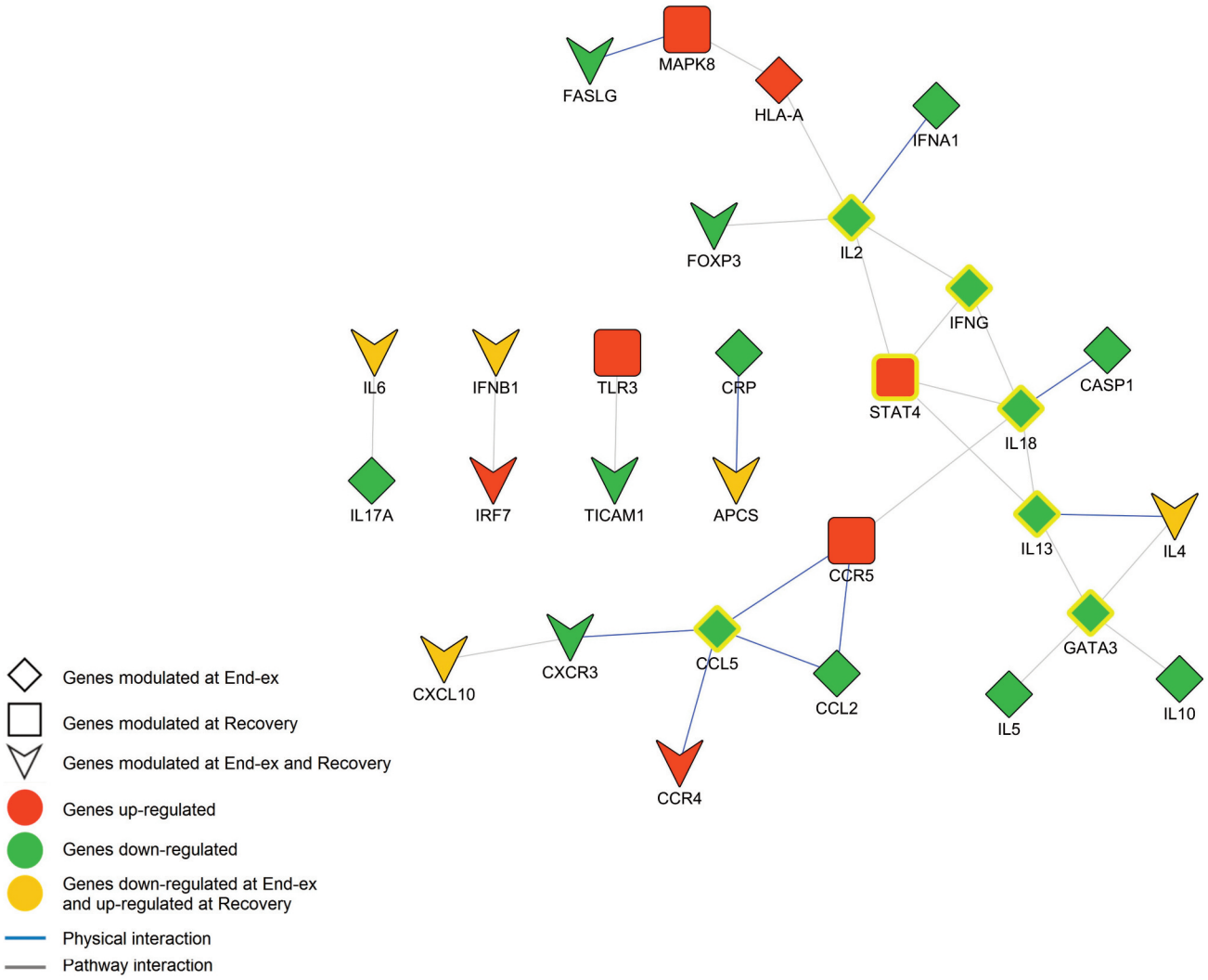

Figure 3. Network analysis of changes in gene expression obtained from comparisons between Baseline, End-ex and Recovery periods in SLE_{ACTIVE} patients. Network analysis of the DE genes resulting from the comparisons of End-ex *vs.* Baseline and Recovery *vs.* Baseline in the SLE_{ACTIVE} patients. Networks were constructed using information obtained from the pathways (grey edges) and physical interactions (blue edges). Diamond and square nodes denote the DE genes regulated immediately after exercise (End-ex) and at Recovery, respectively. V-shaped nodes represent genes regulated during both periods (End-ex and Recovery). The up- and down-regulated genes are depicted in red and green, respectively. Down-regulated genes at End-ex that were up-regulated at Recovery are depicted in orange.

shown to suppress the expression of inflammatory cytokine-related genes in human peripheral blood mononuclear cells (24, 67). Interestingly, lower network connectivity was observed in the SLE_{ACTIVE} group in comparison with the $SLE_{INACTIVE}$ group. Difference in drug therapy between the SLE groups may possibly account for this observation; although both SLE groups were receiving immunosuppressive therapy, only the SLE_{ACTIVE} group was treated with glucocorticoids, which may induce a very potent immunomodulatory response (24, 67).

Importantly, the gene expression changes in the leukocytes from the HC group formed more densely connected networks compared to those from the SLE groups, and some genes were exclusively regulated in the HC group, such as *IL23, JAK2, STAT1, STAT3,* and *TYK2*. The latter has been considered important in the maintenance of complex I-dependent mitochondrial respiration and ATP production. Notably, $Tyk2^{(-/-)}$ showed decreased exercise tolerance compared with wild-type mice, which was paralleled by deficiencies in Tyk2 kinase and other components of the JAK/STAT pathway, predisposing the animals to abnormal responses following inflammatory challenges (50).

One may argue that the main limitation of this study is the lack of gene expression assessment in specific sub-sets of leukocytes, such as neutrophils or peripheral blood mononuclear cells. However, our main purpose was to investigate the global effect of exercise on leucocyte gene response, rather than the specific response of each leukocyte subset. Furthermore, previous studies reported that a single bout of acute aerobic exercise elicited a similar gene expression in total leukocytes (10) and neutrophils (16), suggesting that leukocyte gene expression may be representative of its sub-populations. Notwithstanding, since we did not analyze possible changes in leukocyte number, which may have influenced gene expression, additional studies involving SLE patients are required to further clarify the role of acute aerobic exercise upon leukocyte sub-sets gene expression. Furthermore, the lack of biochemistry data (*e.g.*, cytokines and acute phase pro-

teins) at each time-point may be considered another limitation, thus a more integrative study comprising molecular, biochemical, and physiological responses remains necessary. Finally, caution should be exercised in generalizing the current findings since this is a relatively small-scale study (*i.e.*, n = 8 patients and 4 controls).

CONCLUSIONS

In conclusion, our results indicated that a single bout of acute aerobic exercise altered gene expression levels in circulating leukocytes from healthy individuals and SLE patients, irrespective of disease activity. Immediately after exercise, inflammatory genes were down-regulated in all groups compared with Baseline; at Recovery, an up-regulation was observed. Less connected networks were revealed in the SLE groups, suggesting that the leukocytes from these patients are deficient in triggering a normal exercise-induced immune transcriptional response. Further studies are needed to assess the association of exercise-induced molecular changes with the potential benefits and risks of exercise training in SLE management.

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COMPETING INTERESTS

The authors declare that they do not have conflict of interests.

AUTHOR CONTRIBUTIONS

- **Conception and design of the experiments:** L.A.P.; A.L.S..; E.B.; H.R.; B.G.
- **Collection, analysis and interpretation of data:** L.A.P.; D.S.; D.C.A.; H. A.; C.A.M.; M.A.C.; F.B.B.; F.R.L.; E.B.; E.B.; A.L.S.; R.H.; N.O.S.C.; B.G.
- **Drafting the article or revising it critically for important intellectual content:** L.A.P.; D.S.; D.C.A.; H. A.; C.A.M.; M.A.C.; F.B.B.; F.R.L.; E.B.; E.B.; A.L.S.; R.H.; N.O.S.C.; B.G.

All authors approved the final version of the manuscript

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