Exercise training can attenuate the inflammatory milieu in women with systemic lupus erythematosus

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[1]*Rheumatology Division, School of Medicine, University of Sao Paulo, Sao Paulo, Brazil;* [2]*Laboratory of Transplantation Immunobiology, Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil; and* [3]*School of Physical Education and Sport, University of Sao Paulo, Sao Paulo, Brazil*

Perandini LA, Sales-De-Oliveira D, Mello SB, Camara NO, Benatti FB, Lima FR, Borba E, Bonfa E, Sá-Pinto AL, Roschel H, Gualano B. Exercise training can attenuate the inflammatory milieu in women with systemic lupus erythematosus. *J Appl Physiol* 117: 639–647, 2014. First published July 18, 2014; doi:10.1152/japplphysiol.00486.2014.— Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by chronic inflammation. This study sought to assess the effects of an exercise training program on cytokines and soluble TNF receptors (sTNFRs) in response to acute exercise in SLE women. Eight SLE women and 10 sex-, age-, and body mass index-comparable healthy controls (HC) participated in this study. Before and after a 12-wk aerobic exercise training program, cytokines and sTNFRs were assessed at rest and in response to single bouts of acute moderate/intense exercise. HC performed the acute exercise bouts only at baseline. After the exercise training program, there was a decrease in resting TNFR2 levels ($P = 0.025$) and a tend to reduction interleukin (IL)-10 levels ($P = 0.093$) in SLE. The resting levels of IL-6, IL-10, and TNF-α after the exercise training in SLE reached HC levels ($P > 0.05$). In response to a single bout of acute moderate exercise, the area under the curve (AUC) of IL-10 was significantly reduced after the exercise training program in SLE ($P = 0.043$), and the AUC of IL-10, IL-6, TNF-α, and sTNFR1 of SLE approached control values ($P > 0.05$). In response to a single bout of acute intense exercise, the AUC of IL-10 was significantly reduced in SLE ($P = 0.015$). Furthermore, the AUC of sTNFR2 tended to decrease after exercise training program in SLE ($P = 0.084$), but it did not reach control values ($P = 0.001$). An aerobic exercise training program attenuated the inflammatory milieu in SLE women, revealing a novel homeostatic immunomodulatory role of exercise in an autoimmunity condition.

chronic exercise training; cytokines; inflammation; immune system; autoimmune disease

SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) is an autoimmune disease characterized by chronic inflammation, as evidenced by exacerbated levels of interferon (IFN)-γ, interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-10, and soluble TNF receptors (sTNFRs) (29, 36, 39, 42), which have been implicated in a clustering of cardiovascular risk factors, such as dyslipidemia, dysautonomia, atherosclerosis, and insulin resistance (9, 21, 25, 38).

Recently, chronic exercise training has emerged as an adjuvant therapy in the treatment of SLE patients (4, 32). Importantly, it has been demonstrated that chronic exercise training can improve peak oxygen uptake ($\dot{V}o_{2peak}$), aerobic capacity,

cardiac autonomic control, fatigue, and quality of life in both adult and childhood SLE patients (10, 15, 28, 35, 41). Notwithstanding, there has been a concern that exercise could induce SLE flare by triggering a proinflammatory response. This notion is in contrast to the anti-inflammatory effects of exercise reported in some chronic diseases characterized by low-grade systemic inflammation, including type 2 diabetes (14, 26) and chronic heart failure (1, 13). Nonetheless, the effects of a chronic exercise training program on inflammation in autoimmune diseases remain inconclusive.

In this respect, Castellano et al. (11) reported an increase in resting TNF-α and IFN-γ levels, but no changes in IL-6 levels after 8 wk of aerobic exercise training in multiple sclerosis patients. In contrast, White et al. (45) demonstrated that 8 wk of resistance exercise training led to decreased levels of IFN-γ, IL-10, C-reactive protein, IL-4, and TNF-α in multiple sclerosis patients. In another three studies, however, exercise training failed to induce any changes in resting cytokine levels in patients with rheumatoid arthritis (6) or multiple sclerosis (5, 37). Collectively, these studies involving patients with autoimmune diseases did not reveal evidence of exercise-induced inflammation. In SLE women, our group recently showed that single bouts of acute moderate and intense exercise did not flare the disease (33); however, to our knowledge, there are no studies assessing the effects of chronic exercise training on inflammatory cytokines in these patients. From a clinical standpoint, it is essential to shed light on the apparent "paradoxical effect" of exercise in SLE, regarding which exercise training program would, on the one hand, improve cardiovascular health and, on the other hand, exacerbate inflammation.

This study aimed to investigate, for the first time, the effects of a 12-wk exercise training program on the cytokine (i.e., IFN-γ, IL-6, IL-10, TNF-α) and sTNFR (i.e., sTNFR1 and sTNFR2) response to single bouts of acute exercise (i.e., moderate and intense) in inactive SLE women. We hypothesized that the chronic exercise training program would not exacerbate the inflammatory response in these patients.

MATERIALS AND METHODS

Experimental design and patients. Eight consecutive women with inactive SLE and 10 sex-, age-, and body mass index (BMI)-comparable healthy controls (HC) were selected to participate in this study. The HC group participated in a previous study from our laboratory aimed to explore the effects of acute exercise bouts on inflammatory response (33).

All of the SLE patients fulfilled the American College of Rheumatology criteria for SLE diagnosis (22) and were regularly followed at the outpatient Lupus Clinic from the Rheumatology Division of the School of Medicine at University of Sao Paulo, Brazil. Disease

activity was determined by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (8). SLE manifestations were defined as follows: cutaneous disease (i.e., malar or discoid rash, oral ulcers, or photosensitivity), articular involvement (i.e., arthralgia or nonerosive arthritis involving 2 or more peripheral joints), neuropsychiatry disease (i.e., psychosis, depression, seizure, and peripheral neuropathy), renal disease (i.e., persistent proteinuria > 0.5 g/24 h; presence of cellular casts red or white blood cells; or mixed, persistent hematuria > 10 red blood cells per high-power field and 5/high power field leukocytes, excluding infection or stone), cardiopulmonary disease (i.e., serositis, myocarditis, restrictive lung disease, and pulmonary hypertension), and hematological complications (i.e., hemolytic anemia, leukopenia with a white blood cell count $< 4,000/mm^3$, lymphopenia $< 1,500/mm^3$ on 2 or more occasions, and thrombocytopenia with platelet count $< 100,000/mm^3$ in the absence of drugs).

The inclusion criteria for inactive SLE patients were the following: SLEDAI score < 4, not receiving glucocorticoid therapy for at least 6 mo before the beginning of the study, age between 20 and 40 yr, and physically inactive for at least 6 mo before selection. Exclusion criteria included the following: change in medication during study period, secondary rheumatic disease (e.g., Sjögren syndrome, antiphospholipid syndrome), BMI ≥ 30 kg/m^2, acute renal failure, cardiac and pulmonary involvement, fibromyalgia, and musculoskeletal and joint disorders, which could preclude exercise testing or training.

The SLE women underwent a 12-wk exercise training program. Before and after the chronic exercise training, cytokines and sTNFRs were assessed at rest and in response to single bouts of acute moderate and intense exercise. Additionally, aerobic condition (assessed by a graded exercise test), quality of life [assessed by Short-Form 36 questionnaire (SF-36)], fatigue [assessed by Fatigue Severity Scale (FSS)], and laboratory parameters were evaluated. HC was assessed at baseline for the same parameters, but did not undertake the chronic exercise training program. To assess the effects of the chronic exercise training program in SLE, pre- to postintervention comparisons were conducted. In addition, to test whether the chronic exercise training program was able to rescue possible abnormalities in SLE, between-group comparisons between SLE (pre- and postassessments) and HC (only baseline assessments) were performed. The experimental design is illustrated in Fig. 1.

This study was approved by the Local Ethical Committee (School of Medicine of University of Sao Paulo, no. 0185/11), and all of the subjects signed an informed consent. This trial was registered at www.clinicaltrials.gov as NCT01515163.

Fatigue and health-related quality of life assessments. Fatigue was assessed by FSS, and the health-related quality of life by the SF-36 questionnaire. FSS ranged from 9 to 81 points, where lower values indicate lower symptoms of fatigue. SF-36 scales (physical function, role-physical, bodily pain, general health, vitality, social function, role-emotional) ranged from 0 to 100, where a maximal score of 100 indicates the best health condition.

Cardiopulmonary exercise test. The maximal graded exercise tests were performed in a treadmill (Centurion 200, Micromed), with increments in velocity and grade at every minute until volitional exhaustion. Oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) 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). Heart rate (HR) was continuously recorded at rest, during exercise, and at recovery, using a 12-lead electrocardiogram (Ergo PC Elite, Micromed). Cardiopulmonary exercise test was considered maximal when one of the following criteria was met: $\dot{V}O_2$ plateau (i.e., <150 ml/min increase between two consecutive stages); respiratory exchange ratio value >1.10; and HR no less than 10 beats below age-predicted maximal HR (34). $\dot{V}O_{2peak}$ was considered as the average of the final 30 s of the test. Ventilatory thresholds were identified following previously described procedures (44). In brief, the ventilatory anaerobic threshold (VAT) was determined when ventilatory equivalent for $\dot{V}O_2$ ($\dot{V}E/\dot{V}O_2$) increased without concomitant increase in ventilatory equivalent for carbon dioxide ($\dot{V}E/\dot{V}CO_2$). The respiratory compensation point (RCP) was determined when $\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$ increased simultaneously.

Chronic exercise training program. The SLE women underwent a 12-wk, twice a week, supervised exercise training program in an intrahospital gymnasium (Laboratory of Assessment and Conditioning in Rheumatology, School of Medicine, University of Sao Paulo). The training sessions consisted of a 5-min warm-up, followed by 30–50 min of treadmill walking, and a 5-min cooling-down period. The walking duration was gradually increased at every 4 wk, from 30 to 50 min. The intensity of the exercise sessions was set at the HR correspondent to the interval between the VAT and 10% below the RCP.

Single bouts of acute moderate and intense exercise. Cytokine and sTNFRs in response to 30-min single bouts of acute moderate and intense exercise were assessed before and after the chronic exercise training program. The area under the curve (AUC) of each cytokine and sTNFR kinetics was calculated by the trapezoid model. The order of the single bouts of acute exercise (i.e., moderate or intense) were randomized and interspaced by at least 72 h. All of the acute exercise
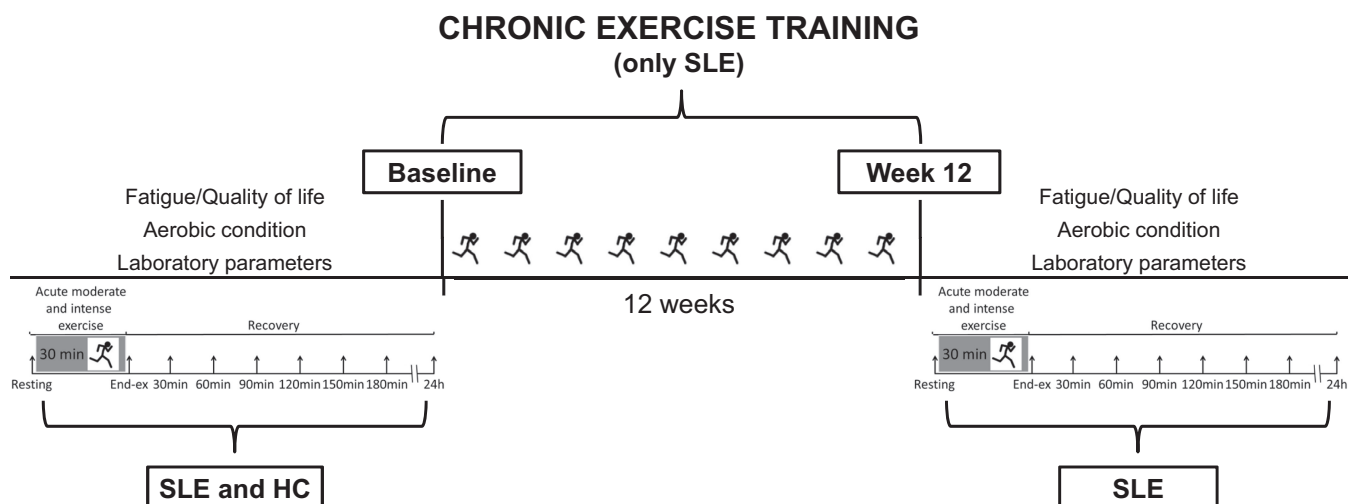

Fig. 1. Experimental design. SLE, systemic lupus erythematosus; HC, healthy controls; End-ex, end of the acute exercise bout.

bouts were performed at 7:00 AM. The single bout of acute moderate exercise was performed at an intensity correspondent to 10% below the VAT (SLE before the chronic exercise training program = 47.4 ± 6.4% of $\dot{V}O_{2peak}$; SLE after the chronic exercise training program = 50.8 ± 12.6% of $\dot{V}O_{2peak}$; HC = 47.5 ± 8.6% of $\dot{V}O_{2peak}$), whereas the single bout of acute intense exercise was set at an intensity correspondent to 50% of the delta difference between the VAT and the RCP (SLE before the chronic exercise training program = 68.1 ± 7.3% of $\dot{V}O_{2peak}$; SLE after the chronic exercise training program = 63.7 ± 13.0% of $\dot{V}O_{2peak}$; HC = 66.8 ± 6.7% of $\dot{V}O_{2peak}$). The room temperature was kept at 22°C during all of the experimental conditions. Before the single bouts of acute exercise was performed, the antecubital vein was cannulated for blood sampling. Blood (5 ml) was sampled and drawn into a dry tube at baseline, at the end of the acute exercise bout, every 30 min during a 3-h recovery period (Rec30, Rec60, Rec90, Rec120, Rec150 and Rec180), and 24 h after the end of exercise (Rec24h). Blood samples were centrifuged at 2,000 g for 15 min at 4°C, and the serum aliquot was stored at −80°C for subsequent analyses.

Resting cytokines and sTNFRs were calculated as the average values between the single bouts of acute moderate and intense exercise.

Cytokines and sTNFRs. IFN-γ, IL-10, IL-6, TNF-α, sTNFR1, and sTNFR2 were measured by a multiplex human panel using a Luminex 200 apparatus (Luminex, Austin, Texas). The immunoassays were performed according to the manufacturer procedures (Billerica, MA, EMD Millipore, Milliplex). The reliability of cytokines and sTNFR measurements was tested using the resting serum samples from the single bouts of acute moderate and intense exercise. The intraclass correlation coefficients, expressed as mean (95% of confidence interval) were as follows: IFN-γ, 0.93 (0.86–0.97); IL-10, 0.97 (0.95–0.98); IL-6, 0.98 (0.98–0.99); TNF-α, 0.84 (0.78–0.94); sTNFR1, 0.89 (0.78–0.94); and sTNFR2, 0.93 (0.85–0.96).

Laboratory parameters assessments. Serum levels of C-reactive protein and C3 and C4 complement fractions were measured using immunoturbidimetry method (Cobas 8000). Creatine phosphokinase and urea were assessed by automatic kinetic system (LKB 8600). A colorimetric kinetic assay was used to determine creatinine levels. Erythrocyte sedimentation rate was assessed by Westergren automated method. Erythrocytes, hematocrit, leukocytes, and platelets were obtained by fluorescence flow cytometry (Sysmex XT2000i). Reactivity of double-stranded DNA was assessed by indirect immunofluorescence on *Crithidia luciliae* (Euroimmun, Medizinische, Labordiagnostika, Lübeck, Germany). Serum samples with IgG reactivity at a dilution ≥ 1:10 were considered positive.

Statistical analysis. Data are presented as means ± SE. The Gaussian distribution of the data was tested by Kolmogorov-Smirnov's test (with Lilliefor's correction). Independent samples were compared with unpaired t-test when data were parametric and with Mann-Whitney U-test when data were nonparametric. Dependent samples were compared with paired t-test when data were parametric and with Wilcoxon test when data were nonparametric. Data analysis was performed using the Statistical Package for Social Sciences (SPSS), version 17.0 for Windows. The level of significance was set at $P \leq 0.05$, with a trend toward significance being accepted at $P \leq 0.1$.

RESULTS

The subjects' characteristics are presented in Table 1. Weight, height, and BMI were comparable in SLE and HC and were not affected by the chronic exercise training program in SLE ($P > 0.05$). SLEDAI remained unchanged in SLE after the chronic exercise training program ($P > 0.05$).

Aerobic condition, fatigue and quality of life, and laboratory parameters. Aerobic condition data are presented in Table 2. Before the chronic exercise training program, all aerobic pa-

Table 1. *Demographic, clinical, and therapy data of the SLE and HC groups*

	SLE PRE	SLE POST	HC
n	8	8	10
Age, yr	35.8 ± 6.5	35.8 ± 6.5	30.6 ± 5.2
Weight, kg	65.4 ± 5.9	64.3 ± 4.7	63.9 ± 8.9
Height, cm	161.2 ± 5.1	161.3 ± 5.5	162.6 ± 5.7
BMI, kg/m^2	25.2 ± 2.6	24.8 ± 2.1	24.1 ± 2.3
SLEDAI	1.3 ± 1.1	0.9 ± 1.0	
Disease duration, yr	11.6 ± 6.4	11.8 ± 2.2	
Drugs, n (%)			
Glucocorticoid	0	0	
Antimalarial	6 (75)	6 (75)	
Azathioprine	1 (12.5)	1 (12.5)	
Methotrexate	1 (12.5)	1 (12.5)	
Mycophenolate mofetil	2 (25)	2 (25)	

Values are means ± SD or percentage of total sample; n, no. of patients. SLE, systemic lupus erythematous; HC, healthy control; PRE, before the chronic exercise training program; POST, after the chronic exercise training program; BMI, body mass index; SLEDAI, SLE disease activity index.

rameters were lower in SLE compared with HC ($P < 0.05$). Following the chronic exercise training program, $\dot{V}O_{2peak}$ remained unchanged ($P > 0.05$), whereas time at VAT ($P = 0.005$), time at RCP ($P = 0.012$), time to exhaustion ($P = 0.030$), and peak HR ($P = 0.040$) increased in SLE. Importantly, after the chronic exercise training program in SLE, all variables but $\dot{V}O_{2peak}$ approached control levels ($P > 0.05$ for SLE vs. HC).

Fatigue and quality of life data are shown in Table 3. The FSS was comparable between SLE and HC at baseline ($P > 0.05$) and significantly decreased after the chronic exercise training program in SLE ($P = 0.050$).

The role-physical, general health, and vitality scales of the SF-36 were lower in SLE than in HC at baseline ($P < 0.05$), whereas the bodily pain, social function, role-emotional, and mental health scales were similar in both groups ($P > 0.05$). After the chronic exercise training program in SLE, all of the SF-36 scales approached control values ($P > 0.05$ for SLE vs. HC), except for the physical function scale ($P = 0.017$ for SLE vs. HC).

Table 4 shows the laboratory parameters. No between-group differences were observed at baseline ($P > 0.05$). None of the laboratory parameters were affected by the chronic exercise training program in SLE ($P > 0.05$). C4 levels were lower after

Table 2. *Cardiopulmonary exercise test data of the SLE group PRE and POST chronic exercise training program and of the HC group at baseline*

	SLE PRE	SLE POST	HC
n	8	8	10
Time at VAT, min	4.9 ± 0.8*†	7.0 ± 1.9	7.1 ± 1.1
Time at RCP, min	9.6 ± 1.6*†	10.6 ± 1.3	11.3 ± 1.5
Time to exhaustion, min	11.9 ± 1.2*†	12.8 ± 1.1	13.8 ± 1.6
$\dot{V}O_{2peak}$			
l/min	1.57 ± 0.17†	1.63 ± 0.22†	2.0 ± 0.2
ml·kg^{-1}·min^{-1}	24.1 ± 2.8†	25.4 ± 3.6†	31.0 ± 5.1
HR$_{peak}$, beats/min	181 ± 6*†	186 ± 6	191 ± 9

Values are means ± SD; n, no. of patients. VAT, ventilatory anaerobic threshold; RCP, respiratory compensation point; $\dot{V}O_{2peak}$, peak oxygen uptake; HR$_{peak}$, peak heart rate. *Significant differences between SLE PRE vs. SLE POST ($P < 0.05$). †Significant differences between SLE vs. HC ($P < 0.05$).

Table 3. *Fatigue and health-related quality of life data of the SLE group PRE and POST chronic exercise training program and of the HC group at baseline*

	SLE PRE	SLE POST	HC
n	8	8	10
FSS	33.4 ± 14.4*	26.4 ± 10.2	26.6 ± 13.3
Physical function	58.1 ± 15.3†	70.6 ± 11.2†	82.5 ± 4.2
Role-physical	78.1 ± 28.1†	93.8 ± 11.6	97.5 ± 7.9
Bodily pain	69.4 ± 18.1	69.3 ± 14.6	81.8 ± 17.6
General health	65.6 ± 19.2†	72.5 ± 19.8	85.5 ± 11.9
Vitality	56.3 ± 24.6†	66.3 ± 14.6	76.5 ± 13.8
Social function	78.1 ± 24.8	93.8 ± 13.4	97.5 ± 5.3
Role-emotional	79.1 ± 30.7	87.5 ± 24.9	83.4 ± 23.6
Mental health	67.0 ± 19.6	77.0 ± 10.8	78.8 ± 10.2

Values are means ± SD; *n*, no. of patients. FSS, fatigue severity scale. *Significant differences between SLE PRE vs. SLE POST ($P < 0.05$). †Significant differences between SLE vs. HC ($P < 0.05$).

the chronic exercise training program in SLE compared with HC ($P = 0.041$), but this parameter remained within normal range.

Resting cytokines before and after the chronic exercise training program. Before the chronic exercise training program, resting IL-6, IL-10, sTNFR2, and TNF-α levels were higher in SLE than in HC ($P < 0.05$), whereas resting IFN-γ and sTNFR1 levels were similar between the groups ($P > 0.05$) (Fig. 2). After the chronic exercise training program, there was a decrease in resting sTNFR2 levels ($P = 0.025$) and a tendency to a decrease in resting IL-10 levels ($P = 0.093$) in SLE. Interestingly, following the chronic exercise training program, resting levels of IL-6, IL-10, and TNF-α in SLE approached control values ($P > 0.05$ for SLE vs. HC). Resting IFN-γ and sTNFR1 levels remained comparable between SLE after the chronic exercise training program and HC ($P > 0.05$).

Effects of a single bout of acute moderate exercise on the kinetics of cytokines and sTNFRs before and after the chronic exercise training program. Cytokines and sTNFRs in response to a single bout of acute moderate exercise are presented in Fig. 3.

Before the chronic exercise training program, the AUC of IL-10, IL-6, TNF-α, sTNFR1, and sTNFR2 in response to a single bout of acute moderate exercise was higher in SLE compared with HC ($P < 0.05$). Importantly, the AUC of IFN-γ

in SLE was not affected by the chronic exercise training program ($P = 1.00$) and did not differ from HC ($P > 0.05$). In contrast, the AUC of IL-10 was significantly reduced after the chronic exercise training program in SLE ($P = 0.043$), reaching values comparable to those of HC ($P = 0.626$). Following the chronic exercise training program, the decline in the AUC of IL-6, TNF-α, and sTNFR1 in SLE did not reach statistical significance ($P > 0.05$), but it approached control levels ($P > 0.05$ for SLE vs. HC). Conversely, the AUC of sTNFR2 did not decrease after the chronic exercise training program ($P = 0.128$) in SLE and remained higher compared with HC ($P = 0.032$).

Effects of a single bout of acute intense exercise on the kinetics of cytokines and sTNFRs before and after the chronic exercise training program. Cytokines and sTNFRs in response to a single bout of acute intense exercise are presented in Fig. 4.

Before the chronic exercise training program, the AUC of IL-10, TNF-α, and sTNFR2 in response to a single bout of acute intense exercise was higher in SLE compared with HC ($P < 0.05$). After the chronic exercise training program, the AUC of IL-10 was significantly reduced in SLE ($P = 0.015$), reaching values similar to those of HC ($P = 0.965$). Furthermore, the AUC of sTNFR2 tended to decrease following the chronic exercise training program in SLE ($P = 0.084$), but it failed to reach control values ($P = 0.001$ for SLE vs. HC). The AUC of the remaining cytokines and sTNFRs was not altered by the chronic exercise training program in SLE ($P > 0.05$).

DISCUSSION

To our knowledge, this was the first study to investigate the effects of a 12-wk aerobic exercise training program on cytokine kinetics in inactive SLE women. Our main findings were that the chronic exercise training program did not exacerbate inflammation, and, perhaps more importantly, it mitigated the exacerbated inflammatory milieu observed in the SLE women.

In support of previous observations (29, 36, 39, 42), our SLE women showed an aberrant cytokine profile (e.g., increased TNF-α, IL-10, and IL-6 levels) compared with their healthy counterparts. Importantly, this pattern has been implicated in the increased prevalence of atherosclerosis, dyslipidemia, and insulin resistance in SLE, possibly increasing the risk for

Table 4. *Laboratory parameters of the SLE group PRE and POST chronic exercise training program and of the HC group at baseline*

	SLE PRE	SLE POST	HC
n	8	8	10
Anti-dsDNA positive, n (%)	1 (12.5)	1 (12.5)	
C3 (90–180 mg/dl)	102.3 ± 19.5	100.8 ± 10.8	114.6 ± 26.0
C4 (10–40 mg/dl)	13.1 ± 4.0	12.7 ± 3.3*	20.7 ± 8.3
CPK (26–192 U/l)	109.0 ± 33.8	103.4 ± 38.7	114.8 ± 58.1
Creatinine (0.50–0.90 mg/dl)	0.75 ± 0.07	0.74 ± 0.11	0.77 ± 0.11
Urea (10–50 mg/dl)	24.5 ± 5.4	24.9 ± 7.9	25.2 ± 8.0
Erythrocytes (4.0–5.4 million/mm^3)	4.22 ± 0.25	4.24 ± 0.45	4.38 ± 0.37
Hematocrit (35–47%)	38.4 ± 1.3	37.9 ± 2.1	37.9 ± 2.7
Leukocytes (4.0–11.0 million/mm^3)	4.44 ± 1.66	4.80 ± 1.5	6.19 ± 2.93
Platelets (140–450 million/mm^3)	156.3 ± 101.5	234.9 ± 58.4	253.9 ± 49.13
CRP (<5 mg/l)	2.3 ± 2.4	2.9 ± 2.6	2.8 ± 3.1
ESR (5.6–11.0 mm)	13.8 ± 11.4	18.1 ± 12.2	12.1 ± 16.9

Values are means ± SD or percentage of total sample; *n*, no. of patients. dsDNA, double-stranded DNA; CPK, creatine phosphokinase; CRP, C-reactive protein; ESR, erythrocytes sedimentation rate. *Significant differences between SLE vs. HC ($P < 0.05$).

Resting cytokine levels
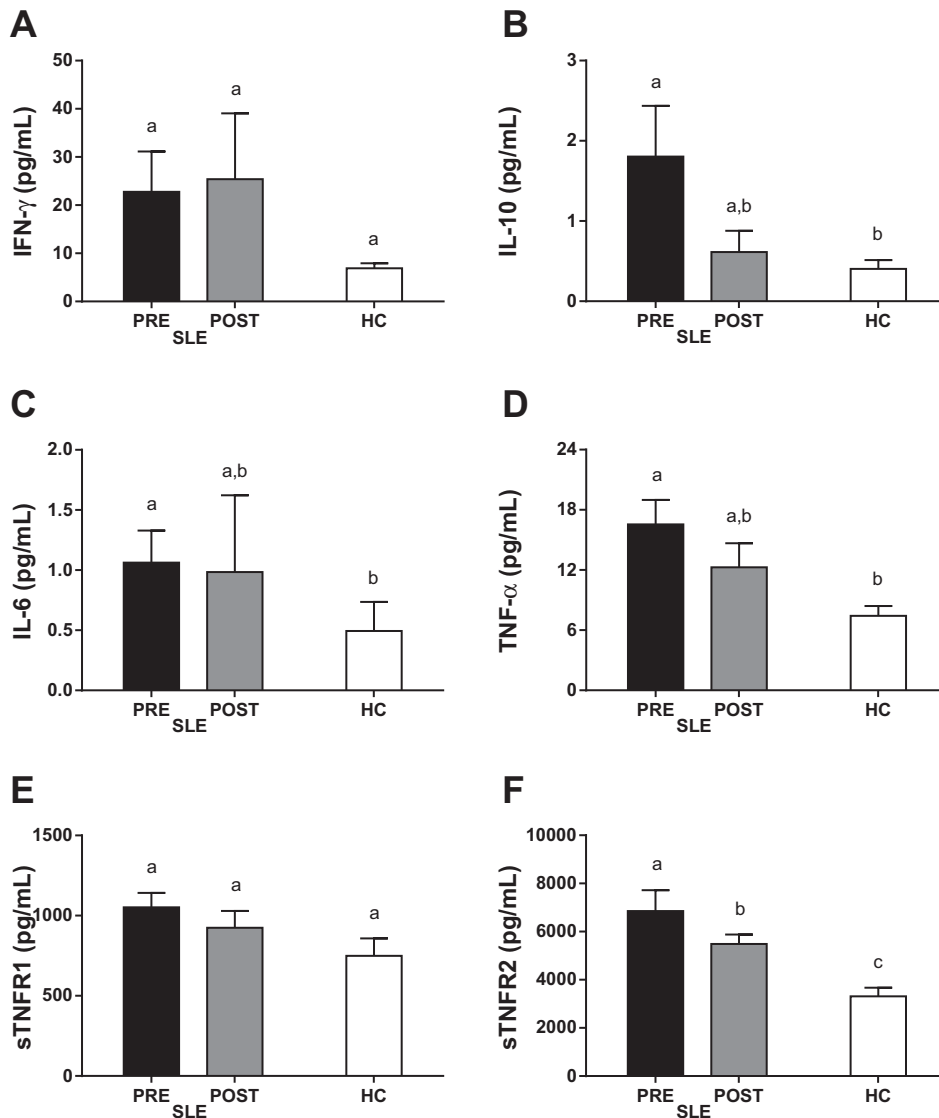
A

B

C

D

Fig. 2. Resting cytokine levels in the SLE group before (PRE) and after (POST) the chronic exercise training program and in the HC group at baseline. *A*: interferon (INF)-γ. *B*: interleukin (IL)-10. *C*: IL-6. *D*: tumor necrosis factor (TNF)-α. *E*: soluble TNF receptor (sTNFR) 1. *F*: sTNFR2. Values are means ± SE. [a,b,c] Means with different letters are significantly different from each other ($P < 0.05$).

E

F

cardiovascular events and mortality (9, 21, 25, 38). Although chronic exercise training has been considered as an efficient tool to reduce cardiovascular events in a variety of chronic diseases (7, 16), there has been a concern that chronic exercise training could flare inflammation in patients with autoimmune rheumatic diseases. To date, there is no empirical evidence to confirm this speculation, with few studies showing that well-designed supervised exercise training programs do not induce disease flare in some autoimmune rheumatic diseases, including idiopathic inflammatory myopathies (2, 3, 30), rheumatoid arthritis (23, 27), and SLE (10, 28, 41). However, none of these studies has provided a comprehensive analysis of the cytokine response to single bouts of acute exercise assessed before and after a chronic exercise training program, which is of upmost relevance considering the relationship between inflammatory markers (i.e., cytokines and sTNFRs) and the autoimmune disease activity (12, 36, 39). In the present study, we assessed the effects of a 12-wk exercise training program on the 24-h cytokine and sTNFR kinetics in response to single bouts of

acute exercise of different intensities (i.e., moderate and intense). Our findings revealed that the chronic aerobic training program does not exacerbate inflammation either at rest or in response to single bouts of acute moderate and intense exercise, as evidenced by no major changes in any of the measured cytokine at any time point. These results further support the safety of chronic exercise training upon inflammation, refuting the claims that exercise could be potentially harmful to SLE patients.

Interestingly, in contrast to the notion that exercise could negatively affect inflammatory milieu, the chronic exercise training program alleviated cytokine levels in the SLE women. This was evidenced by decreased resting levels of sTNFR2 and IL-10 after the training program. Furthermore, resting IL-6, IL-10, and TNF-α levels after the exercise training program in SLE were compared with those of HC at baseline. Likewise, the AUC of IL-10, IL-6, sTNFR1, and TNF-α was markedly reduced after the chronic exercise training program in SLE, approaching normal values. Chronic exercise training program
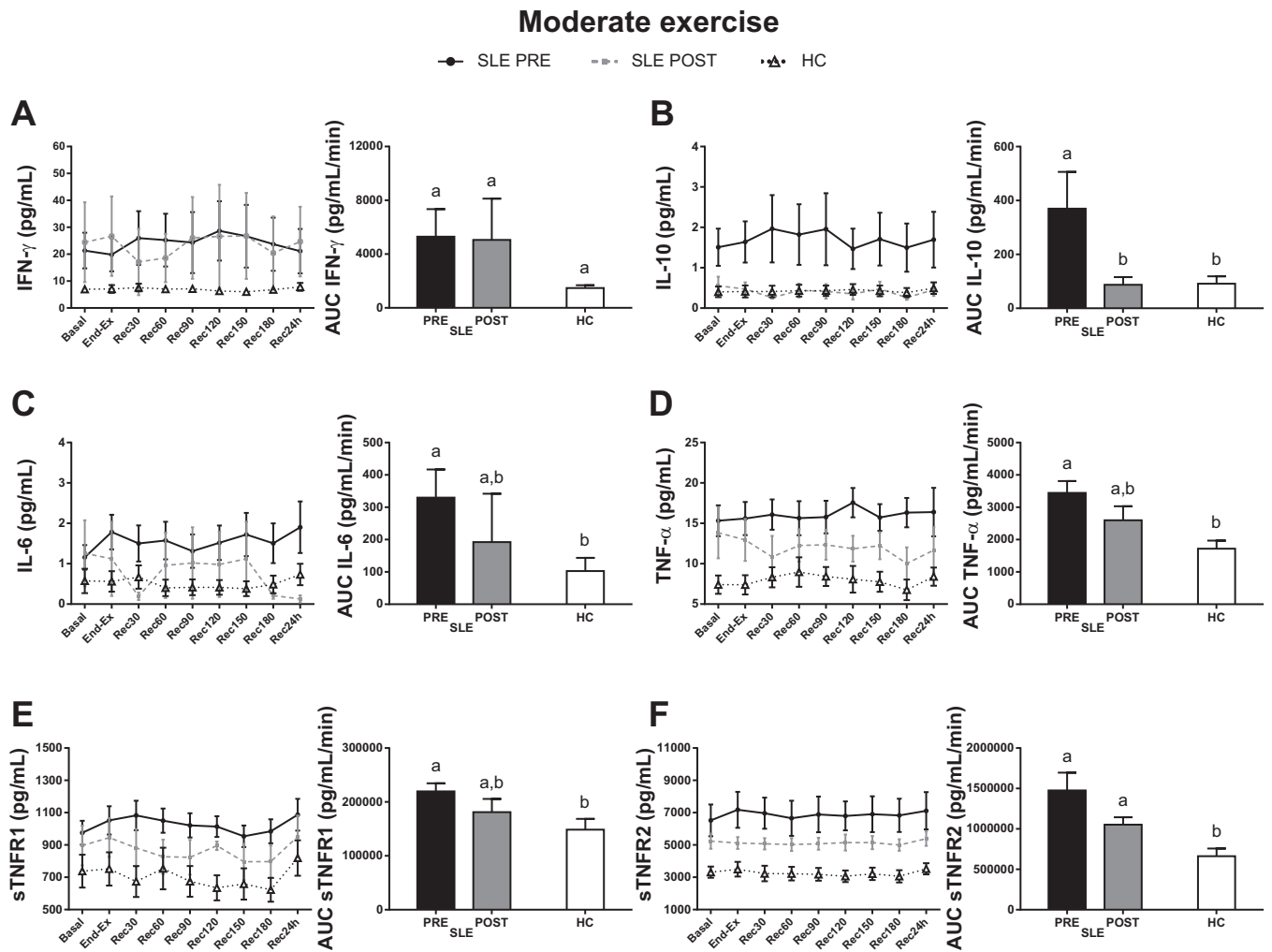
Moderate exercise

Fig. 3. Cytokine and sTNFR responses to a single bout of acute moderate aerobic exercise in the SLE group PRE and POST chronic exercise training program and in the HC group at baseline. Values are means ± SE. *A*: INF-γ. *B*: IL-10. *C*: IL-6. *D*: TNF-α. *E*: sTNFR1. *F*: sTNFR2. AUC, area under the curve. [a,b] Means with different letters are significantly different from each other ($P < 0.05$).

has been suggested to exert its potential anti-inflammatory effects via three main mechanisms (19, 43): *1*) a reduction of visceral fat (40); *2*) a reduction in the expression of toll-like receptors in monocytes and macrophages, with a subsequent reduction in the cytokine production (20); and *3*) an increase in the release of IL-6, followed by a release of anti-inflammatory cytokines from the skeletal muscle, the so-called myokines (31). In this regard, the effects of the chronic exercise training program in reducing the resting levels of the classically anti-inflammatory cytokines IL-10 and sTNFR2 could be interpreted as detrimental. However, chronic exercise training has been shown to attenuate the acute cytokine response to a single bout of exercise in healthy subjects (17), which concur with the present findings. Similarly, exercise-induced reductions in sT-NFR2 and IL-10 levels were also observed in patients with heart failure (13) and multiple sclerosis (45). Moreover, the SLE patients showed increased pro- and anti-inflammatory cytokines at baseline compared with their healthy peers. Importantly, an overproduction of both Th1 and Th2 cytokines has been shown to play a role in the immunopathology of SLE (18), and high levels of IL-6 and IL-10 have been reported as biomarkers for disease activity in SLE patients (12). In this

context, the attenuation in both pro- and anti-inflammatory cytokines may be proposed as a novel homeostatic immunomodulatory role of chronic exercise training in SLE. To date, it is difficult to speculate as to what extent a reduction in the cytokine milieu might be associated with exercise-induced cardiovascular improvements in SLE (e.g., improved aerobic conditioning, endothelial function, cardiac autonomic control). Given that antagonists of specific cytokines [e.g., anti-IL-10, (24)] have been considered as potential candidates for the treatment of SLE, further studies should determine whether long-term exercise training can be recommend as an adjuvant anti-inflammatory therapy to SLE patients.

Further to the investigation of the effects of the chronic exercise training on inflammation, this study provided evidence that chronic exercise training programs can effectively lead to improvements in a cluster of aerobic conditioning parameters, fatigue scores, and selected domains of quality of life, adding to an increasing body of knowledge showing positive clinical outcomes in exercise-trained SLE patients (10, 15, 28, 41). Therefore, these data pointed out that chronic exercise training can be useful as an adjuvant therapy in the management of the comorbidities associated with SLE.
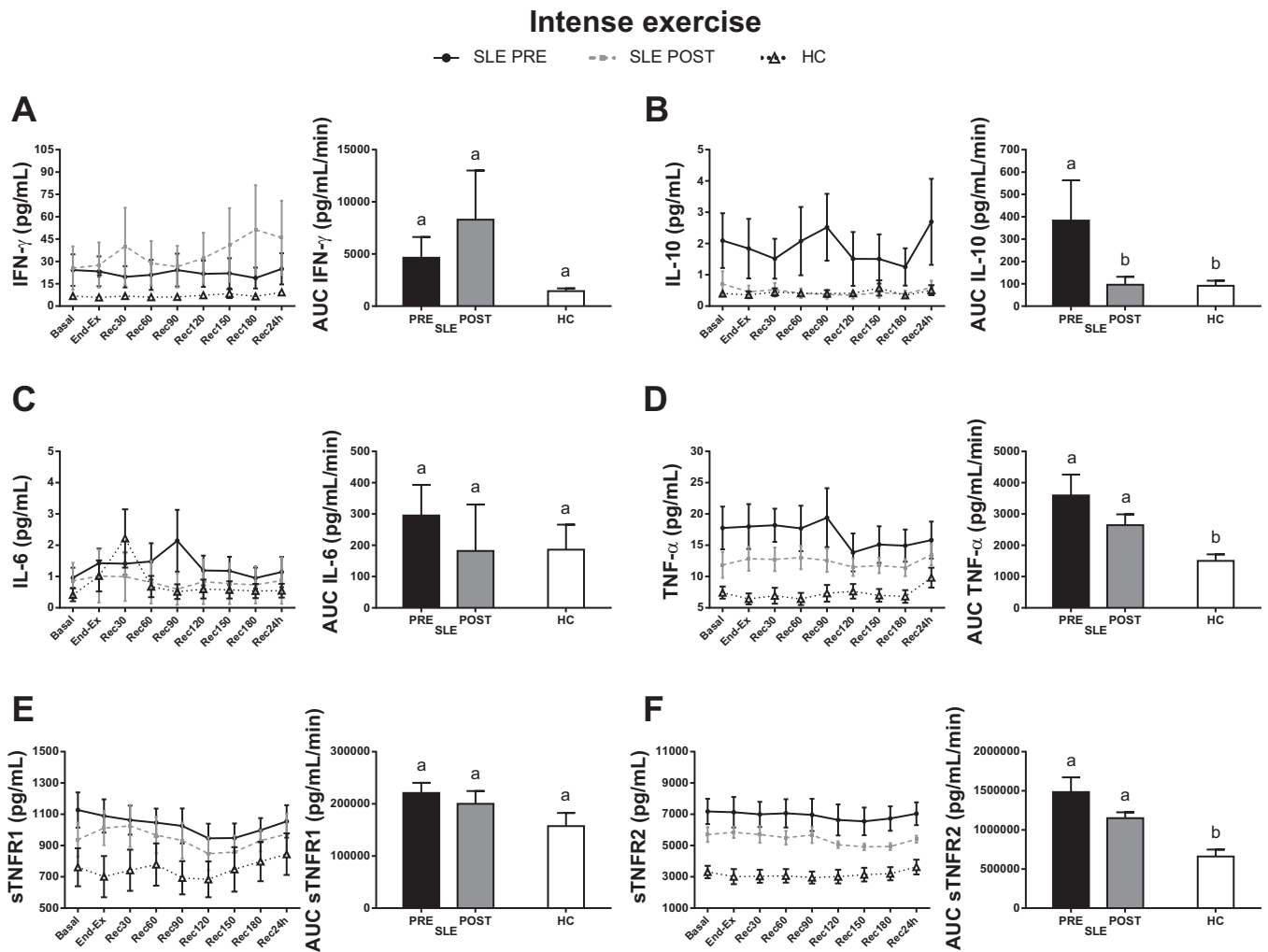
Fig. 4. Cytokine and sTNFR responses to a single bout of acute intense aerobic exercise in the SLE group PRE and POST chronic exercise training program and in the HC group at baseline. Values are means ± SE. *A*: INF-γ. *B*: IL-10. *C*: IL-6. *D*: TNF-α. *E*: sTNFR1. *F*: sTNFR2. [a,b] Means with different letters are significantly different from each other ($P < 0.05$).

This study is not without limitations. First, due to the complex experimental protocol adopted in this study, a relatively small sample was enrolled, precluding us to design a nontrained control group. However, as SLE is a very heterogeneous disease, the within-group analyses performed in this study may have reduced the interindividual variability, producing more reliable results. Additionally, the SLE women involved in this study were a homogenous group with regard to the lack of glucocorticoid use, disease activity, physical activity levels, age, BMI, sex, and physical capacity. Second, due to the sample's characteristics, these findings cannot be generalized to patients with active disease, under glucocorticoid treatment, or with secondary autoimmune diseases. Finally, the follow-up employed in this study was relatively short. Further long-term exercise training studies are needed to validate these findings.

In conclusion, a 12-wk aerobic exercise training program did not flare inflammation in inactive SLE women at rest or in response to single bouts of acute exercise, irrespective of their intensity. Moreover, this intervention attenuated the exacerbated inflammatory milieu, suggesting that regular exercise training may promote a homeostatic immunomodulatory effect in SLE.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: L.A.P., D.S.-dO., F.B.B., F.R.L., E. Bonfa, A.L.S.-P., H.R., and B.G. conception and design of research; L.A.P., D.S.-dO., S.B.V.M., N.O.C., F.R.L., E. Borba, and A.L.S.-P. performed experiments; L.A.P., S.B.V.M., N.O.C., F.B.B., F.R.L., E. Borba, E. Bonfa, A.L.S.-P., H.R., and B.G. analyzed data; L.A.P., D.S.-dO., S.B.V.M., N.O.C., F.B.B., E. Borba, E. Bonfa, A.L.S.-P., H.R., and B.G. interpreted results of experiments; L.A.P.,

D.S.-dO., S.B.V.M., F.R.L., E. Bonfa, H.R., and B.G. prepared figures; L.A.P., D.S.-dO., S.B.V.M., N.O.C., F.B.B., F.R.L., E. Borba, E. Bonfa, A.L.S.-P., H.R., and B.G. drafted manuscript; L.A.P., E. Bonfa, H.R., and B.G. edited and revised manuscript; L.A.P., D.S.-dO., S.B.V.M., N.O.C., F.B.B., F.R.L., E. Borba, E. Bonfa, A.L.S.-P., H.R., and B.G. approved final version of manuscript.

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