Effects of 12-week Aerobic Exercise on Arterial Stiffness, Inflammation,and Cardiorespiratory Fitness in Women with Systemic LUPUS Erythematosus: Non-Randomized Controlled Trial

NCT: NCT03107442

Document Date: 26/03/2024

Study Protocol and Statistical Analysis Plan

1. Objective

The primary aim of this study was to assess the effect of a 12-week aerobic exercise intervention following the ACSM guidelines on arterial stiffness in women with SLE in comparison with usual care. Secondary aims were to assess the effects of the exercise intervention on inflammation, oxidative stress, and cardiorespiratory fitness.

2. Design and Protocol Registration

This non-randomized controlled trial was registered at clinicaltrials.gov [NCT03107442] on 11 April 2017, before the enrolment of participants started (i.e., on 12 April), and no deviations occurred regarding the primary outcome and the secondary outcomes analyzed here.

3. Setting and Eligibility Criteria

Participants were recruited from the Systemic Autoimmune Diseases Unit of the "Virgen de las Nieves" and the "San Cecilio" University Hospitals. Women with a diagnosis of SLE according to the ACR criteria [26], a follow-up of 12 months, clinical and treatment stability during the previous six months, and not performing regular exercise (defined as 60 min/week of structured exercise) were included. Exclusion criteria were to have been under biological treatment in the previous six months or to need a prednisone dose of >10 mg/day; a background of CVD in the previous year; to present contraindications to perform exercise; other associated rheumatic conditions; pregnancy; active acute or chronic infection; neoplasms; acute renal failure; cardiac or pulmonary involvement; body mass index (BMI) >35; or not being able to read, understand, and sign written informed consent.

All participants received detailed information about the study procedures, and signed written informed consent. The Research Ethics Committee of Granada approved the protocol on 11 November 2016 (reference No.: 10/2016).

4. Procedures

A telephone screening was conducted. Potentially eligible participants were invited to a personal screening and, if included, day 1 of the baseline examination was performed. The baseline examination comprised two assessment days. On day 1, pulse wave velocity (PWV) was assessed. Thereafter, cardiorespiratory fitness testing was performed, and socio-demographic and clinical information was collected. On day 2 (i.e., between two and four days after day 1), 8-h fasting blood samples were collected between 8:00 a.m. and 10:00 a.m. This article follows the TREND statement for improving the reporting of non-randomized experiments of behavioral and public health interventions (downloadable at EQUATOR Network: https://goo.gl/ZSyLrj; Supplementary Table S1) [27]. The funding source had no role in the study.

5. Methods

5.1.Interventions

5.1. 1. Exercise Group

To maximize transparency and replicability, the exercise program described in this manuscript follows the Consensus on Exercise Reporting Template (CERT; Supplementary Table S2) [28]. The patients assigned to exercise performed two 75-min sessions per week during a total of 12 weeks (i.e., 24 sessions) of moderate to vigorous intensity aerobic exercise on a treadmill (BH, Serie i.RC12 Dual, Vitoria-Gasteiz, Spain) from 24 April to 14 July 2017. The sessions took place in a quiet room of the "Virgen de las Nieves" Hospital, Granada (Spain).

All sessions were performed in groups of a maximum of five persons (depending on the patients' schedule preferences) and were supervised by both exercise professionals with a degree in Sports Sciences and residents from the Internal Medicine Department.

Attendance at the sessions was registered daily and patients were contacted upon any missing session to ask for the reason and motivate them to replace it on an alternative day of the same week. Adherence to exercise is reported as the median attendance frequency and the proportion of patients attending >75% (i.e., 18 sessions; the minimum pre-defined attendance to assess efficacy) and >90% of the sessions. All the sessions began with a warm-up comprising 3–4 min of activation on the treadmill at about 35–40% of the heart rate reserve (HRR) and 3–4 min of active stretching of major muscle groups, and ended with a cool down phase of static stretching of major muscle groups and relaxation. Exercise was individually prescribed to represent moderate-to-vigorous intensity, with training intensity ranging from 40% to 75% of each patient's HRR. The maximum heart rate (HRmax) was estimated with the formula by Tanaka et al. (HRmax = 208 x (0.7 x age)) [29]. The training (or target) heart rate (tHR) was calculated with the formula tHR = HRrest + (%HRR). Heart rate was continuously monitored during all sessions (Polar V800, Kempele, Finland). We used the session rating of perceived exertion (RPE) as a measure of subjective training load [30], and the feeling scale to assess positive affective responses experienced before and after each session [31].

The starting level was specific for each individual according to her previous exercise experience and physical fitness. During the first half of the program, only continuous exercise was performed so that the patients got used to the treadmill and felt confident at increasing intensities. Continuous sessions comprised several bouts of exertion at constant intensity, followed by a couple of minutes of recovery (i.e., rest) to drink water. During month 2, there were alternated continuous and interval sessions, and at month 3, the patients undertook interval training sessions, where there were periods of lower and periods of higher intensity efforts followed by some minutes of rest for hydration (Table 1). The progression in volume and/or intensity was patient-limited and was undertaken by increasing the treadmill speed (first) or inclination according to the symptoms and perceived exertion. There were no home-based or non-exercise components within this intervention. However, if a participant was eventually not able to attend a particular session, we provided

her with a heart rate monitor and allowed recovery of that session out of the Hospital (a total of seven sessions were recovered in this fashion). Finally, the exercise intensity progressions had to be slightly modified from the initial plan. For instance, several patients perceived a 5% HRR intensity increase (i.e., from one week to another) as very heavy and difficult-to-follow. Consequently, there were weeks in which exercise intensity increased by 2.5% instead of 5% (Table 1).

5.1.2. Control Group

After the baseline evaluation, the SLE patients assigned to the (usual care) control group received verbal information about a healthy lifestyle, including physical activity guidelines and basic nutritional information.

5.2. Outcome Measures

Primary Outcome Measure: Arterial Stiffness

Arterial stiffness was assessed in a sitting position by PWV [9], using the Mobil-O-Graph® 24 h pulse wave analysis monitor (IEM GmbH, Stolberg, Germany), whose operation is based on oscillometry recorded by a blood pressure cuff placed on the brachial artery. The coefficient of variation (CV) of Mobil-O-Graph for consecutive PWV analyses is 3.4% and its intraclass correlation coefficient is 0.98 [0.96–0.99] [32]. This device has been largely shown to be valid and reliable for measuring PWV and central blood pressure in different populations [33,34], meets the accuracy requirements of the

British Hypertension Society (BHS) standard [35], and can be recommended for clinical use [36].

Secondary Outcome Measures

Blood Samples and Biochemical Analyses

Fasting blood specimens for biochemical and immunological tests were collected and routinely processed by the central laboratory of our hospital. Among other measurements, they included lipids, insulin (BioRad, Marne-la-Coquette, France), and a routine biochemical profile. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated (HOMA-IR = glucose (mmol/L) insulin (U/L)/22.5).

Inflammatory Markers Serum high-sensitivity CRP was assessed by an immunoturbidimetric method using the ARCHITECT cSystems (MULTIGENT CRP Vario assay); the limit of quantitation was 0.2 mg/L and the upper limit for normal serum was 5 mg/L (coefficient of variation <6%). Interleukin 6 and TNF- , as well as myeloperoxidase (MPO; as marker of oxidative stress), were measured in plasma. Serum was initially separated by centrifugation and stored at □70 *C*.

Bioserum concentrations of IL-6/TNF- (pg/mL) and MPO (ng/mL) were measured by an immunoradiometric assay using commercial kits (MILLIPLEX MAP Kit Human High Sensitivity T Cell Magnetic Bead Panel (HSTMAG-28SK) and Human Cardiovascular Disease Magnetic Bead Panel 2 (HCVD2MAG-67K)), Millipore) following the manufacturer's instructions. Quantitative data were obtained by using the Luminex-200 system (Luminex Corporation, Austin, TX, USA), and data analysis was performed on XPonent 3.1 software (Austin, TX, USA). The detections limits were 0.73 pg/mL for IL-6, 0.43 pg/mL for TNF-a, and 0.024 ng/mL for MPO.

Cardiorespiratory Fitness

Cardiorespiratory fitness was assessed with the Bruce submaximal treadmill protocol [37]. The test comprised five increasing workload stages of 3 min each (stage 1: 2.7 km/h and 10% inclination; stage 2: 4 km/h and 12% inclination; stage 3: 5.5 km/h and 14% inclination; stage 4: 6.8 km/h and 16% inclination; stage 5: 8 km/h and 18% inclination). The test concluded when the participant achieved 85% of the individual's HRmax, as

estimated with the formula by Tanaka et al. [29]. As validated SLE-specific formulas to estimate VO2max are not available, we used the total time to reach 85% HRmax as the outcome of interest.

Other Measurements

All participants filled out a socio-demographic and clinical data questionnaire. Height (cm) was measured using a height gauge, weight (kg) with a bioimpedance device (InBody R20, Biospace, Seoul, Korea), and body mass index (BMI) was calculated (kg/m2). Blood pressure was measured with Mobil-O-Graph® (IEM GmbH, Stolberg, Germany) [36]. Disease activity was assessed through the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI, range 0–105 where a higher score indicates higher degree of disease activity). Physical activity was self-reported at baseline and at week 12 with the International Physical Activity Questionnaire [38].

Sample Size

The sample size was calculated for the primary outcome (i.e., PWV). Ashor et al. found an average effect of aerobic exercise on PWV of □0.63 m/s in adults aged 18 years [21]. A total of 52 patients (26 per group) were needed to detect an effect of □0.63 (SD 0.75) m/s, with a power of 85% and an error of 0.05. Anticipating a maximum loss to follow-up of 15%, we aimed at recruiting a total of 60 patients.

Treatment Allocation and Blinding

Randomization was not feasible because more than half of the patients who regularly attend the Autoimmune Disease Units lived far from the Hospital and were not able to attend twice per week in case of being randomized to exercise. Therefore, participants from the city of Granada were included in the exercise group and participants living outside Granada were included in the control group.

To minimize potential selection bias, we aimed to match the groups by age (2 years), BMI (1 kg/m2), and SLEDAI (1 unit). The data analyzer was blinded to the patient allocation.

Statistical Analysis

The distribution of the main study variables was assessed through histogram and Q-Q plots. As the main outcomes were non-normally distributed, their descriptive characteristics were presented using the median and interquartile range instead of the mean and standard deviation and we used non-parametric tests for the main analyses. Between-group baseline characteristics were compared with the Student t-test (when normally distributed) or Kruskal-Wallis test (when non-normally distributed) for continuous variables and the Chi-square test for categorical variables. The between-group differences in the studied outcomes were assessed through quantile regression with baseline values, resting heart rate (bpm) [39], and changes in physical activity (min/week; since the change in self-reported physical activity at week 12 was >60 min higher in the control compared to the exercise group and this change was associated with changes in PWV; rPearson = 0.27, p = 0.048) as potential confounders, after checking baseline group comparisons. As we aimed at assessing efficacy, the primary analyses were definedas per-protocol, where patients from the exercise group were included if attendance at the exercise sessions was 75%. To assess the robustness of the results, subsequent sensitivity analyses (i.e., baseline observation carried forward (BOCF) imputation; per-protocol with minimum attendance of 90%, and complete-case analyses) were conducted. A blinded investigator (AS-M) undertook the data handling and all hypothesis testing. All the

analyses were conducted with Stata v.13.1 (StataCorp LP., College Station, TX, USA). Statistical significance was set at $p < 0.05$.

Results

There were no between-group differences in the changes in arterial stiffness (median PWV difference □0.034, 95% CI □0.42 to 0.36 m/s; p = 0.860) or hsCRP, TNF-, IL-6, and MPO (all $p > 0.05$) at week 12. In comparison to the control group, the exercise group significantly increased cardiorespiratory fitness (median difference 2.26 minutes, 95% CI 0.98 to 3.55; p = 0.001). These results suggest that 12 weeks of progressive treadmill aerobic exercise increases cardiorespiratory fitness without exacerbating arterial stiffness, inflammation, or oxidative stress in women with SLE.