Statistical analysis plan (SAP)

Type 1 interferon induced changes to exercise adaptations in systemic lupus erythematosus patients

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1 BACKGROUND AND RATIONAL

Systemic lupus erythematosus (SLE) is a rare chronic autoimmune disease with a varied phenotype and a predisposition for women of childbearing age[1]. Systemic involvement of skin, joints and internal organs are based on a complex interaction between distinct immunopathogenic pathways including overexpression of interferons (IFN) [2,3]. Disease manifestations comprise flares interspaced by periods of inactive disease, cumulative accrual of organ damage and constitutive symptoms such as pain and fatigue[3–7].

Patients with SLE also suffer from reduced exercise capacity with lower improvement in maximal oxygen uptake ($\dot{V}O_2max$) and fatigue severity scores (FSS) following moderate continuous exercise training (MCT) compared to healthy controls[7–9].

High-intensity interval-training (HIIT) is a broad category of aerobic exercise programs. High intensity typically refers to exercising above a certain heart rate, ranging from 75% to 95% of the maximal heart rate (HR_{max})[10,11]. Interval training refers to interspacing these high intensity intervals with durations ranging from 15 seconds to 5 minutes with lower intensity buffers. Multiple sets of these intervals will then compromise a total exercise duration of one session ranging from 20 to 180 minutes[11,12]. These individual HIIT sessions are then repeated 2-5 times weekly[10–12].

Compared to MCT, HIIT has proven particularly effective at increasing $\dot{V}O_2max$ and alleviating fatigue in several other populations than SLE-patients, including healthy[13,14], middle-aged[15], and elderly women[16,17], heart failure patients[10,12], as well as pre- and perioperative cancer patients[11,18]. As of now, no randomised controlled studies have investigated the impact of HIIT on fatigue or $\dot{V}O_2max$ in SLE.

The fatigue and exercise impairment reported by patients with SLE remains unexplained but may reflect aberrations in immune function and physical adaptations to exercise[19]. Pathway analyses indicate a central role of IFN driven immune activation in SLE[20]. In this respect, overexpression of type I IFNs (IFN-I) is of particular interest; often reported as a composite score reflecting whole blood mRNA expression of IFN regulated genes, denoted as the IFN gene signature (IFNGS)[21].

Among the various effects of IFN-I is suppression of interleukin 6 (IL-6) signalling; in ex vivo studies, IFN-I has been found to attenuate IL-6 -induced phosphorylation of transducer and activator of transcription 3 (STAT3) leading to an abrogation of IL-6 activated intracellular pathways. Furthermore, IFN-I induces transcription of the suppressor of cytokine signalling 3 (SOCS3) gene further inhibiting IL-6 signaling[22,23].
IL-6 is a cytokine with both pro- and anti-inflammatory effects, it has multiple sources including contracting skeletal muscle[24]. IL-6 in humans can cause lipolysis [25], increase glucose uptake and glycogen storage in skeletal muscle[26], and it may supress production of proinflammatory cytokines such as TNF and IL-1β [27,28]. Furthermore, it has been shown that IL-6 is required for exercise induced loss of visceral adipose tissue in overweight adults[29].

Based on these observations, this study hypothesises that in patients with SLE followed in routine care, a high IFNGS, predicts poorer improvement in aerobic capacity and fatigue following a 12- week HIIT intervention.

2 OBJECTIVES

2.1 Primary aim:

The primary aim is to investigate whether 12-week supervised HIIT has an effect on aerobic exercise capacity measured by $\dot{V}O_2max$ and fatigue measured by FSS in patients with SLE and if IFN-I activity determined by IFNGS negatively influences these effects.

2.2 Secondary aims:

The secondary aim is to investigate whether 12-week supervised HIIT has an effect on health related quality of life measured by SF-36[30] and disease activity measured by SLEDAI (with SELENA modifications)[31,32] scores in patients with SLE and if IFN-I activity determined by IFNGS negatively influences these effects.

2.3 Other objectives:

Examine the effect of 12 weeks of HIIT on the pulmonary function of SLE patients. Considering effect modulation by IFNGS.

Examine the effects of exercise on the cardiac adaptations of SLE patients. Considering effect modulation by IFNGS.

Examine the effects of exercise on metabolic markers of SLE patients. Considering effect modulation by IFNGS.

Examine the effects of exercise on body composition in SLE patients. Considering effect modulation by IFNGS.

Examine the effect of 12 weeks of HIIT on the transcriptome related to IFN-I, IFN-II, TNF, and IL-6 in SLE patients.

3 HYPOTHESES

3.1 Primary Hypotheses

We hypothesize that 12 weeks of HIIT exercise will increase aerobic capacity and decrease fatigues score compared to no intervention, and further that the effects are modulated by IFNGS, more specifically:

1.
    a. HIIT is superior to no intervention control at improving aerobic capacity.
    b. The effect of HIIT on aerobic capacity is modulated by IFNGS, such that a higher IFNGS results in lower benefits of HIIT.

2.
    a. HIIT is superior to no intervention control at alleviating fatigue.
    b. The effect of HIIT on fatigue is modulated by IFNGS, such that a higher IFNGS results in less decrease of initial fatigue from HIIT.

Superiority is claimed if the difference is statistically significant and favors the HIIT intervention. In order to control the family-wise error rate, hypotheses 1 and 2 will be tested in parallel with Bonferroni-adjustment and hypotheses a and b will be tested hierarchically, so that hypothesis b is only tested if a is significant.

3.2 Secondary Hypotheses

Secondary hypotheses are not hierarchically tested, instead exploratory hypotheses will be controlled for false discovery rate by the Benjamini-Hochberg method[33].Secondary hypotheses are (in random order):

- HIIT is superior to no intervention control at maintaining current SLE symptoms as evaluated using SELENA-SLEDAI[34] by a physician.
    - This effect is modulated by IFNGS.
- HIIT is superior to no intervention control at improving health related quality of life as estimated by the SF-36 domains.
    - This effect is modulated by IFNGS.

3.3 Exploratory Hypotheses

Further exploratory hypotheses will be tested from the exploratory outcomes. There is no hierarchy of the exploratory hypotheses, instead exploratory hypotheses will be controlled for false discovery rate[33].

4 TRIAL DESIGN, DATA COLLECTION AND OUTCOMES ASSESSMENT

The study protocol, detailing the hypotheses, methods, recruitment and conduct of the study has been published in a non-peer reviewed openly accessible preprint database[35]. In brief, the study is a randomized control trial, consisting of 2 arms, labeled exercise and control. As outlined in the publicly available protocol[35]; initial sample size calculations were based on a t-test for changes in VO2max. Assuming a between group difference in aerobic capacity change scores similar to the 1989 Robb-Nicholson study[36] (mean: 2.66, 95%CI [0.36, 4.96]); resulted in 10 completers needed in each group, if we assumed a median split of IFN-score we would require 40 completers for four groups (low IFN control, Low IFN exercise, High IFN control and high IFN exercise). We assumed as much as a 30% dropout; which required a sample size of 60 participants. After reaching 40 completers, recruitment ceased, and only further participants already enrolled for baseline examination were included, this resulted in 55 participants included in the study. These were then stratified for sex and randomly allocated 1:1 to exercise or control. At www.clinicaltrials.gov the study is identified by NCT05478018. Prior to commencement the scientific ethics committee of the capital region of Denmark approved to trial with identifier H-21039032. The trial primarily took place at Centre for Physical Activity on Rigshospitalet, Ole Maaløes Vej 24, in Copenhagen, Denmark. Recruitment took place primarily at the center for vasculitis and spine diseases on Rigshospitalet in Blegdamsvej 9, Copenhagen, Denmark. 82-Rb-Pet-CT scans were conducted at the department of clinical physiology at Rigshospitalet, Blegdamsvej 9, Copenhagen, Denmark.

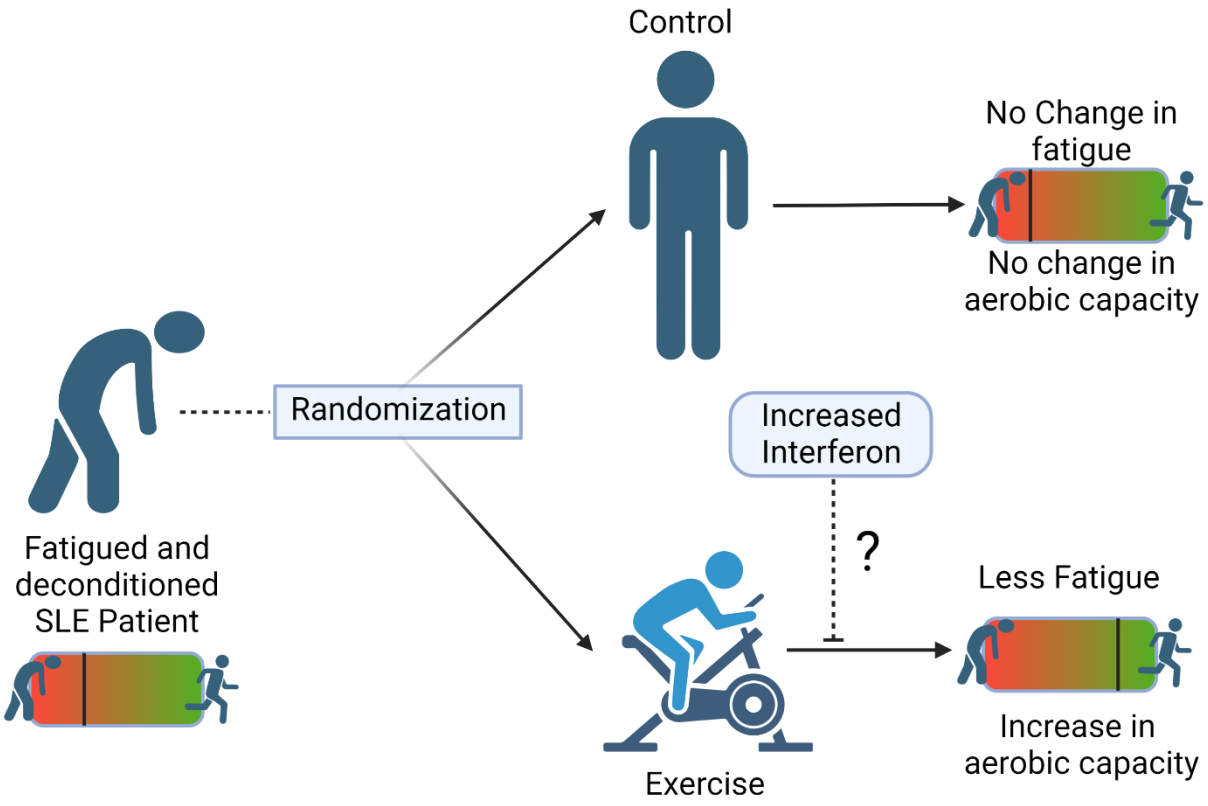

Figure 1 - Graphical hypothesis

The exercise group underwent 12 weeks of thrice weekly 45 minute HIIT exercise sessions, with 4 intervals of 4 minutes of high intensity exercise, defined as a heart rate above 85% of the maximal heart rate interspaced with 3 minute active breaks of pedaling against light resistance (less than 20% wattmax).

As noted above the hypotheses were that these 12 weeks would improve aerobic capacity and fatigue in SLE patients, and that it would improve more in patients with lower IFNGS.

Participants flow through the project with a screening visit, where aerobic capacity is assessed, a baseline visit with most of the outcomes, and a baseline-acute bout session consisting of one exercise bout and the measurements of the autonomic nerve system. A subgroup of patients will then undergo a 82-Rb-rest-stress-PET-CT evaluating cardiac adaptation. Patients are then randomized to exercise or control. Whereafter they will undergo a followup visit, another acute bout (termed the follow-up acute bout) and at last if they did one at baseline, they will do a repeat 82-Rb-rest-stress-PET-CT.

5 OUTCOMES

5.1 Primary Outcomes

5.1.1 Domain: Aerobic Capacity

Timeframe: -2, 0 to 12 weeks

Measurement: Maximal volume of inspired oxygen per minute per kilogram of body weight of the participant (VO_2Max).

- Measured during a maximal exercise bout. Maximal defined as two out of three of the following:
  - Plateau in volume of inspired oxygen per minute. So that increase in workload results in no increase in inspired oxygen.
  - Ratio between volume of inspired oxygen and expired carbondioxide more than 1.1
  - Exertion by BORG scale 18, 19 or 20.

  If the bout is considered a maximal bout, the VO2max will be defined as the highest 30 second average oxygen uptake per minute per kilogram of bodyweight. (in ml/min/kg)
- Volumes of gases measured by indirect calorimetry.
- Heart rate during the bout is measured by PolarFlow™ and added to the CosMed system.
- In order to test whether changes in this measurement is just due to changes in weight, absolute VO2max (not corrected for patient weight) will also be reported.

5.1.2 Domain: Fatigue – Patient reported

Timeframe: 0 to 12 weeks.

Measurement:

- Patient reported outcome measure (PROM).
- Krupp's Fatigue severity scale[37], as the average of all 9 domains (0-7).
- This scale has been verified for use in Danish SLE patients[38].

5.2 Secondary Outcomes

5.2.1 Domain: Physician evaluated changes in measures of SLE

Timeframe: 0 to 12 weeks

Measurement: Y2K updated SLE disease activity (SLEDAI-2K) with the SELENA modifications[31].

5.2.2 Domain: Patient reported outcome quality of life.

Timeframe: 0 to 12 weeks, and again as an exploratory analysis, at 1, 2 and 3 months following the intervention.

Measurement:

- Short Form (SF)-36 Health Survey (0-100)[39] - Possible scores range from 0 to 100, with higher scores representing better health status.
- Summed into the 8 domains.
- Summed into the 2 aggregate scores.

5.2.3 Domain: Type 1 Interferon gene signature

Timeframe: 0 to 12 weeks

Measurement: Pax-gene tubes withdrawn from fasting participants at baseline.

- Analyzed by Nanostring for genes related to IFN-signaling.[40]
- Normalized to housekeeping genes
- Calculating a standardized z-score compared to the expression from 9 healthy controls

5.3 Exploratory Outcomes

5.3.1 Domain: Physician evaluated changes in disease activity

Timeframe: 0 to 12 weeks

Measurement:

- Itemized Physician evaluated changes in measures of SLE on a scale from 0-22 that account for partial improvements in condition. Evaluated by the Systemic Lupus Erythematosus Disease Activity Index 2000 Responder Index-50 (SRI-50)[41].
- Disease activity as evaluated by a physician familiar with SLE diagnosis based on physical examination and patient history on a scale of 0 to 100, higher score indicating more active disease.

5.3.2 Domain: Patient reported outcome measures

Timeframe: 0 to 12 weeks, and again as an exploratory analysis, at 1, 2 and 3 months following the intervention.

Measurements:

- Total fatigue as assessed by the participant on a visual analog scale from 0-100, higher scores equal less fatigue.
- Total pain on a visual analog scale of 0-100, higher scores equal more pain (worse).
- Quick systemic lupus activity questionnaire (Q-SLAQ) as reported by the Q-SLAQ questionnaire (translated to Danish by author MLA)[42]. Possible scores range from 0 to 33, with higher scores representing more active SLE (worse)

- SLE activity on a visual analog scale of 0-10 higher scores equal more active disease (worse).

5.3.3 Domain: Kidney disease

Timeframe: 0 to 12 weeks

Measurements:

- Measured by proteinuria.
- Measured by plasma-creatinine.

5.3.4 Domain: Body Composition

Timeframe: 0 to 12 weeks

Measurements:

By DEXA scan:

- Total adipose tissue by weight/percentage.
- Android adipose tissue by weight/percentage.
- Gyneoid adipose tissue by weight/percentage.
- Total lean mass by weight.
- Bone Mass density by weight per area.

By tape measure

- Waist to height ratio

5.3.5 Domain: Lung Function

Timeframe: 0 to 12 weeks

Measurements:

By dynamic spirometry

- Forced Expiratory Volume at 1 second (FEV1) as volume / percentage of expected.
- Forced vital capacity as volume / percentage of expected.
- Forced expiratory volume by forced vital capacity – ratio between volumes / percentages.
- Total lung capacity – volume / percentage of expected.
- Residual Lung volume – volume / percentage of expected.
- Alveolar volume – volume / percentage of expected.
- Diffusing capacity for carbon mono-oxide – volume / percentage of expexted.
- Carbon mono-oxide transfer coefficient – diffusing capacity per liter of lung volume – ratio / percentage of expected

5.3.6 Domain: Metabolic adaptations

Timeframe: 0 to 12 weeks

Measurements: Measured during an OGTT at 0, 15, 30, 60, 90, and 120 minutes following consumption of 83g of glucose in 250mL of water.

- Plasma concentration of glucose.
- Plasma concentration of insulin.
- Plasma concentration of pro-insulin c-peptide.
- Overall changes in the curves will be compared.
- Matsuda Index will be calculated
- Disposition Index will be calculated
- AUC will be calculated

Measurements: Measured following an overnight fast, at the 0 minute mark of the OGTT

- Plasma concentration of total cholesterol
- Plasma concentration of triglycerides
- Plasma concentration of LDL-cholesterol
- Plasma concentration of VLDL-cholesterol
- Plasma concentration of HDL-cholesterol

5.3.7 Domain: Peripheral Capillary adaptations

Timeframe: 0 to 12 weeks

Measurements: Measured by nailfold capillaroscopy by a trained physician, twice before and twice after intervention. Analyzed for:

- Capillary density - score of 1-4, higher scores equal fewer capillaries
- Average capillary width – in µm
- Average capillary length – in µm
- Count of avascular areas - score of 1-4, higher scores indicate more avascular areas
- Capillary disorganization - score of 1-4, higher scores indicate more disorganization
- Microhemmorhages – count per finger
- Bushy capillaries – average number per millimeter
- Mega capillaries – average number per millimeter
- Meandering capillaries – average number per millimeter
- Tortuous capillaries – average number per millimeter
- Other findings – physicians comment

5.3.8 Domain: Cardiac adaptations

Timeframe: 0 to 12 weeks

Measurements:

Measured bedside:

- Systolic blood pressure at rest
- Diastolic blood pressure at rest
- Heart rate at rest

Measured by echocardiography by a trained physician

- Left ventricular end-diastolic volume
- Left atrial end-diastolic volume
- Global longitudinal strain
- Stroke volume
- Left ventricular ejection fraction
- Left ventricular mass
- Coronary perfusion reserve

Measured by ^{82}Rb-rest-stress-Pet-CT on a subset of patients (30-40 depending on patient opt-in), stress will be conducting with low-dose adenosine:

- Coronary perfusion reserve
- Myocardial flow reserve
- Ventricular volumes at rest and stress
- Atrial volumes at rest and stress
- Ventricular and atrial ejection fractions at rest and stress
- Splenic response ratio
- Splenic stress-to-rest intensity ratio
- Heart rate at rest and stress
- Rate pressure product
- Systolic blood pressure at rest and stress
- Cardiovascular resistance at rest and stress

5.3.9 Domain: Free-living physical activity

Timeframe: 0 to 12 weeks

Measurements:

- Free-living physical activity measured using axial accelerometer-based physical activity monitors (AX3; Axivity, Newcastle upon Tyne, UK) for a 5 day period

5.3.10 Domain: Acute exercise bout

Timeframe: 0 to 12 weeks

Description: Subjects arrive fasted and then undergo a 45minute exercise sessions consisting of 4 intervals of 4 minutes and interspaced with active rest, starting with a 10 minute warmup and ending with a 10 minute cooldown, peripheral blood will be sampled 9 times at the following timepoints (in minutes): -5 (before going on the bike), 0 (on the bike before first pedaling), 10 (after warmup), 14 (after first interval), 35 (after last interval), 45 (after cooldown), 60 (resting for 15 minutes after getting off the bike), 90 (resting for 45 minutes after getting off the bike), and 105 (resting for 60 minutes after getting off the bike)

Measurements: concentration in peripheral blood:

- High Sensitivity C-Reactive Protein
- Interleukin-6
- Soluble Interleukin-6 receptor
- Interleukin-1
- Interleukin-10
- Interferon α
- Interferon γ
- Hemoglobin
- Thrombocytes
- Sodium
- Potassium
- Chloride
- Hematocrit
- Ferritin
- Leukocyte Differential count

Measurements: Subject adaptations to exercise

- Heart rate (continuous throughout session)
- Resistance in high intensity intervals
- Subject exhaustion by Borg Scale (6-20 higher scores equal more exhaustion) during, warmup, each interval and following cooldown.

5.3.11 Domain: Pro- and anti-inflammatory related mRNA expression

Timeframe: 0 to 12 weeks

Type 1 interferon induced changes to exercise adaptations in systemic lupus erythematosus patients.

Description: as with the secondary outcome, mRNA segments of genes related to the following signaling pathways will be measured using Nanostring™

Measurements:

- Expression related to IFN-β
- Expression related to IFN Gamma
- Expression related to TNF
- Expression related to IL-6

5.3.12 Domain: Dietary diaries

Timeframe: 0-2 to 12-14 weeks

Description: Subjects will be tasked to fill in dietary diaries for three consecutive representative days at baseline and followup. Using standard calculations energy intake and macronutrients will be calculated from this diary.

Measurements:

- Energy intake (kJ/day)
- Carbohydrate intake (g/day)
- Lipid intake (g/day)
- Protein intake (g/day)
- Other intake (categorical)

5.3.13 Domain: Muscular adaptation

Timeframe: 0 to 12 weeks

Description: A subset of volunteering subjects who will undergo muscle biopsy can deliver baseline and followup muscular biopsies

Measurements:

- Muscle Biopsy transcriptomic analysis of genes related to TNF, IL-6, IFN alpha, beta and Gamma signalling.
- NF-κB p65 DNA binding activity (ELISA), phosphorylated and total JNK, phosphorylated AMPK (p-AMPK) total AMPK (Western blotting).
- NF-κB p65 DNA binding activity (ELISA) & NF-κB binding activity (Western blotting).
- Phosphorylated and total c-Jun N-terminal kinase
- AMP-activated protein kinase

5.3.14 Domain: Autonomic Nerve Function testing

Timeframe: 0-2 to 12-14 weeks

Description: By Vagus™, a device measuring heart rate and heart rate variability will measure:

Measurements:

- Resting heart rate
- Ratio between minimal and maximal heart rate when the subject is:
    - Rising from supine.
    - Controlled breathing exercises.
    - Doing the Valsalva maneuver.

6 STUDY POPULATION, ANALYSIS SET AND STATISTICAL PRINCIPLES

Eligibility criteria have been previously published [35]. In brief, the participants suffered from systemic lupus erythematosus, and fulfilled 10 or more points on the 2017 ACR/EULAR SLICC[43], were not pregnant, did not suffer from diabetes mellitus and were 18 years or older, both males and females were included.

As stated earlier, sample size calculation required 40 completers, the study included 55 participants.

Randomization was done in a 1:1 ratio, stratified on sex.

The statistical analyses will be carried out primarily in the modified intention to treat (mITT) population, excluding the participants who were randomized but were found ineligible as per the ineligibility criteria within the first week of randomization, while retaining the data from all other study participants as randomized regardless of compliance and study completion. Supplementary per protocol (PP) analyses will exclude data from participants in the exercise group who failed to show up for 80% or more of the exercise sessions (29 or more sessions needed attendance to be considered in the PP group).

6.1 Statistical Methods

Evaluation of the treatment effect in the primary analyses will be performed using a constrained linear mixed effect model including sex, follow-up time (categorical), treatment, and the constrained treatment*time interaction as fixed effects, and with an unstructured covariance pattern to account for repeated measurements on each study participant; as a sensitivity analysis, the interaction between sex*time will also be considered, but not included in the main model, as there seem to be no interaction for this in exercisers without SLE at 12 weeks[44]. Effect modification by IFNGS will be evaluated by further including IFNGS (continuous scale), the IFNGS- -time interaction, and the constrained treatment*time*IFNGS interaction in the linear mixed model. Missing data will be handled implicitly by maximum likelihood estimation in the linear mixed model.

For the exploratory outcomes in the oral glucose tolerance test and acute exercise bout domains blood was sampled 6 or 9 times (respectively) throughout the measurements, a linear mixed effect model will be used on summary scores (such as DI or Matsuda Index).

6.2 Multiple testing and significance

Multiple testing of the co-primary endpoints will be handled by Bonferroni-adjustment (i.e. by multiplying p-values by two and presenting estimated treatment effects with 97.5% confidence intervals). Tests of treatment effect and effect modification by IFNGS will be carried out sequentially, so that effect modification will only be tested if a significant treatment effect is found. This is in accordance with the alpha set at p=0.05 in the original protocol without Bonferroni correction. The p-values from the secondary and exploratory analyses will be adjusted for multiple testing using the method of Benjamini and Hochberg[33], which control the false discovery rate (FDR). Adjustment will be carried out for secondary and exploratory end points separately and for tests of treatment effect and effect modification separately. In all analyses an adjusted p-value < 0.05 will be considered statistically significant.

6.3 Tools for statistical analysis

Analysis will be done in an updated version of Rstudio with an updated version of R[45]. The LMMstar package[46] will be used for linear mixed model analyses. The code will be available on Github (https://github.com/Malte-Lund/Lupex-Statistics).

7 DEVIATIONS FROM THE ORIGINAL PROTOCOL

During the study, participants who were interested in the further scientific process were invited to online meetings to discuss the study, the outcomes, and the potential effects of the study for SLE patients. During these meetings a suggestion to email questionnaires to participants every month for the three months following the intervention was suggested by the participant panel. These additions were implemented, such that the last 25 participants received these questionnaires at 1, 2 and 3 months following the intervention.

One participant (ID = LUP021) had a plasma-HCG within the unsure level for pregnancy at baseline. By mistake she was randomized before the pregnancy could be verified. The participant was pregnant and was therefore excluded by ineligibility following randomization.

During the study, the following changes were done to the original protocol:

Version	Change Date	Comments
1.0	18/1-2022	First protocol accepted by the ethical committee
1.1	2/2-2022	Added supplementary outcomes, correctly outlined that activity measuring by AX3 will be done at baseline and at followup.
1.2	15/2 2022	Added tissue-plasminogen activator, VCAM and vWF to blood sample outcomes.
1.3	11/3 2022	Added measurements on the autonomiv nervous system by Vagus™ to outcomes. Updated recruitment procedure to let clinicians provide a list of eligible patients to the researcher. Updated fatigue severity score in the description of outcomes. Due to concern for repeated COVID-19 quarantine rules, added option to conduct some visits by video-call (unused in the study, but possible in the protocol)
1.4	05/04-2022	Minor updates to the acute exercise bout.
1.5	25/5-2022	Cleared up the individualized VO2max test (15% watt-max warm up with 8,5% wattmax increments). As well as the possibility for stress ECG.

1.6	11/10-2022	Added Louise Diederichsen (LD) as co-author. She has experience in exercise studies for connective tissue diseases and is familiar with muscle biopsies on these patients.
1.7	2/11-2022	Added HSP-90 as an IL-6 induced gene expression outcome, based on a paper by A. Stephanou [47]
1.8	22/5-2023	Added Kanwal Zahid Siddiqi (KS) as a co-author due to her experience with IFN-signatures
1.9	14/6-2023	Added alterations to the protocol and prospected genes to be measures, 3 additional IFN-related genes and one additional IL-6 related gene as suggested by KS.
2.0	27/9-2023	Changed Principal Investigator to Malte Lund Adamsen. Removed Peter Godsk as co-author upon his request. Offered Laura Langkjær Johnsen co-authorship if she could recruit a significant quantity of patients from Gentofte hospital (she attempted to but was unsuccessful). Updated participant information to reflect this possibility of recruitment. Changed description of how the muscle biopsy is done as suggested by LD Kept Transcriptomics and Physician Evaluated outcomes as secondary outcomes, cleared up that other outcomes must be seen as exploratory.

8 IMPLEMENTATION OF THE STASTITICAL ANALYSIS PLAN

Following approval of the statistical analysis plan by the writing committee it will be published at the Github account(https://github.com/Malte-Lund/Lupex-Statistics/tree/main/Documents), the clinicaltrials.gov website, and the Centre for Physical Activity website (www.aktisundhed.dk) prior to commencing statistical analysis.

9 EXPECTED WRITING COMMITTEE

Malte Lund Adamsen, Simon Jønck, Iben Rasmussen, Marie Louise L Petersen, Clara Sofie Egeberg, Mark Lyngbæk, Julie Lyng Forman, Anna A. Lützen, Kanwal Zahid Siddiqi, Helga Ellingsgaard, Phillip Hasbak, Louise Diederichsen, Regitse H Christensen, Ronan M. G. Berg, Bente K. Pedersen, Pil Højgaard, Søren Jacobsen

Acknowledgements

We would like to thank the patient panel (in alphabetical order, last names omitted to preserve anonymity): Anne-Maren, Ea, Julie, Mette, Rasmus

10 EXPECTED OUTLINE OF REPORTS

The study report will be aimed at a publication in clinical journals, thus each report will contain 3500-4000 words and 4 to 6 main figures and tables depending on the journal.
The 2010 CONSORT guidelines[48] will be followed when reporting the data.

The primary endpoints (fatigue and aerobic capacity) and the secondary endpoints, along with relevant related exploratory endpoints such as diet, physical activity measured by accelerometers, body composition and adverse events will be published in one paper.
The cardiometabolic outcomes, such as results from the 82-Rb-Pet-CTs, the pulmonary function tests and the echocardiographic outcomes as a second paper. The direct intra-bout related changes from exercise, such as the cytokine measurements will make up a third paper.

11 OVERVIEW OF CONTENT IN REPORTS

Paper 1: Aerobic Capacity, Fatigue, IFN-signature, SF-36, Diet, OGTT, physical activity AX3, Body composition, Adverse events – Overuse pain & discomfort vs overuse injury.

Paper 2: 82-Rb-Rest/Stress-PET-CT, Echocardiography, Capillaroscopy, & Pulmonary Function testing.

Paper 3: Cytokine measurements doing acute exercise bout, Vagus™ measurements.

12 Paper 1 – Primary & Key Secondary outcomes

Working title: Adaptations in **aerobic capacity and fatigue** induced by 4x4 HIIT in systemic lupus erythematosus patients and the modulation by type 1 Interferon gene signature. – An outcome assessor blinded randomized controlled single-blinded clinical trial.

Outcomes reported in this paper:

Aerobic Capacity, Fatigue, IFN-signature, SF-36, Diet, physical activity AX3, Body composition, Adverse events.

12.1 Tables in paper

Table 1. Baseline characteristics separated by randomization group. Aerobic capacity, weight & waist circumference separated by screening/baseline.
Table 2. Changes from baseline and estimated treatment differences for the Primary and secondary outcomes, and related exploratory outcomes as suggested by the CONSORT statement.
Table 3. As table 2 but with randomization groups further divided according to percentiles of the IFN-score at baseline and the estimated treatment difference replaced by the estimate for de IFN-score*randomization group interaction.

12.2 Figures in paper

Figure 1. CONSORT flow diagram.

Figure 2. Trends in the primary outcome over time divided by percentiles of the IFN-score.
Figure 3. Trends in secondary and related exploratory outcomes over time.

12.3 Tables in supplement

eTable 1. Adherence to prescribed exercise.
eTable 2. Descriptive statistics of per protocol vs. protocol violators.
eTable 3. Self-reported diet, macronutrients and energy intake divided by intervention group.
eTable 4. Daily steps and METs in free-living conditions as reported by accelerometers.
eTable 5. Adverse reactions & events following randomization.
eTable 6. Other relevant characteristics measured at screening, baseline and followup, separated by intervention group at followup.
eTable 7. Baseline data grouped into the same percentiles of the IFN-score as table 3.

12.4 Figures in supplement

eFigure 1. Flow of participants.
eFigure 2. Volcano plots depicting change in gene counts from baseline to followup in both intervention groups.
- eFigure 2a Intervention group changes in genes
- eFigure 2b Control group changes in genes
- eFigure 2c Between group differences in gene count changes.

eFigure 3. Overview of the intervention.
eFigure 4. Overview of the OGTT, levels of Insulin, Glucose and C-peptide at different timepoints separated by intervention and IFN-percentiles.
eFigure 5. False discovery rate of exploratory endpoints – Histogram of p-values.

13 Paper 2 – Cardiopulmonary outcomes

Working title: Adaptations in **cardiopulmonary functions** induced by 12 weeks of 4x4 HIIT in systemic lupus erythematosus patients and the modulation by type 1 Interferon gene signature on exercise. – A secondary analysis of an outcome assessor blinded randomized controlled clinical trial.

Outcomes reported in this paper: 82-Rb-Rest/Stress-PET-CT, Echocardiography, Capillaroscopy, & Pulmonary Function testing.

13.1 Tables in paper

Table 1. Baseline characteristics, including the
Table 2. Changes in 82-Rb-Rest/Stress-PET-CT, Echocardiography and Lung Function findings, separated by intervention group.
Table 3. As table 2 but further separated into low/mid/high IFN groups.

13.2 Figures in paper

Figure 1. CONSORT flow diagram.
Figure 2. Trends in outcomes over time divided by percentiles of the IFN-score.

13.3 Tables in supplement

eTable 1. Adverse reactions & events following randomization.

13.4 Figures in supplement

eFigure 1. False discovery rate – Histogram of p-values in this paper.

14 Paper 3 – Intra-bout cytokine response and resting vagal tone outcomes

Working title: Adaptations in **resting vagal tone and intra-bout cytokine response to singular exercise bout** induced by 12 weeks of 4x4 HIIT in systemic lupus erythematosus patients and the modulation by type 1 Interferon gene signature. – A tertiary analysis of an outcome assessor blinded randomized controlled clinical trial.

Outcomes in this paper: Acute Bout – Cytokine levels during exercise, sensitivity adjusted by hematocrit. Vagus™ measurements

14.1 Tables in paper

Table 1. Baseline characteristics, separated by intervention group.
Table 2. Caloric and macronutrient intake, physical activity measured in steps/calories burned and METs in the randomization groups at follow-up.
Table 3. Estimated marginal means of blood sample concentration with 95%CI from the 9 timepoints in the acute exercise bout.

14.2 Figures in paper

Figure 1. CONSORT flow diagram.
Figure 2. Trends in outcomes over time divided by percentiles of the IFN-score.

14.3 Tables in supplement

eTable 1. Self-reported Diet
eTable 2. Adverse reactions & events following randomization.

eTable 3. Estimated marginal means of all the blood samples measured at all timepoints during the acute exercise bout.

14.4 Figures in supplement

eFigure 1. Figure detailing the acute exercise bout.
eFigure 2. Outcomes from the Vagus™ measurements at the specific timepoints
- Resting heart rate
- Rise from supine heart rate variability (HRV)
- Expiratory/inspiratory HRV

eFigure 3. False discovery rate – Histogram of p-values in this paper.

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16 UNFORMATTED TABLES WITH INTENDED CONTENT

Table 1 Baseline characteristics			
	Control	Exercise	Total
Age (years)			
Sex (N (%) female)			
SLE duration (years)			
SLE activity markers			
SLEDAI			
SLICC			
B2MG			
LYMPHOCYTES			
THROMBOCYTES			
Antibodies			
Anti-Sm			
ANTI-DsDNA			
NEITHER ANTI-DsDNA or ANTI-sm			

Table 2 – Change scores without IFN-difference			
Outcome	Change from 0 to 12 weeks Mean ± SD		Estimated treatment difference (95% CI, p-Value)
	Control (n=xx)	Exercise (n=yy)	
VO2max			
FSS			
SF-36 Aggregate Physical Component			
SF-36 Aggregate Mental Component			
Etc.			

Table 3 – Change scores with IFN quantiles							
Outcome	Change from 0 to 12 weeks Mean ± SD						Estimated IFN:Treatment interaction (95% CI, p-Value)
	Control (n=xx)			Exercise (n=yy)			
	IFN-low	IFN-Mid	IFN-High	IFN-Low	IFN-Mid	IFN-High	
VO2max							
FSS							
SF-36 Aggregate Physical Component							
SF-36 Aggregate Mental Component							
Etc.							

IFN-Low = Lower 33% percentile of the IFN-scores, IFN-Mid 33%-66% percentile of the IFN-scores, IFN-High 67%-100% of IFN-scores.