# Statistical analysis plan (SAP)

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

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Table of Contents

[Statistical analysis plan (SAP) 1](#_Toc165628288)

[Type 1 interferon induced changes to exercise adaptations in systemic lupus erythematosus patients 1](#_Toc165628289)

[1 BACKGROUND AND RATIONAL 4](#_Toc165628290)

[2 OBJECTIVES 5](#_Toc165628291)

[2.1 Primary aim: 5](#_Toc165628292)

[2.2 Secondary aims: 5](#_Toc165628293)

[2.3 Primary objective: 5](#_Toc165628294)

[2.4 Key secondary objective: 5](#_Toc165628295)

[2.5 Other objectives: 5](#_Toc165628296)

[3 HYPOTHESES 5](#_Toc165628297)

[3.1 Primary Hypotheses 5](#_Toc165628298)

[3.2 Secondary Hypotheses 6](#_Toc165628299)

[3.3 Exploratory Hypotheses 6](#_Toc165628300)

[4 TRIAL DESIGN, DATA COLLECTION AND OUTCOMES ASSESSMENT 10](#_Toc165628301)

[5 OUTCOMES 11](#_Toc165628302)

[5.1 Primary Outcomes 11](#_Toc165628303)

[5.2 Key Secondary Outcome 12](#_Toc165628304)

[5.3 Other Secondary Outcomes 12](#_Toc165628305)

[5.4 Exploratory Outcomes 12](#_Toc165628306)

[6 STUDY POPULATION, ANALYSIS SET AND STATISTICAL PRINCIPLES 19](#_Toc165628307)

[6.1 Statistical Methods 19](#_Toc165628308)

[6.2 Significance levels of the claims 19](#_Toc165628309)

[6.3 Tools for statistical analysis 19](#_Toc165628310)

[7 DEVIATIONS FROM THE ORIGINAL PROTOCOL 20](#_Toc165628311)

[8 IMPLEMENTATION OF THE STASTITICAL ANALYSIS PLAN 22](#_Toc165628312)

[9 EXPECTED WRITING COMMITTEE 22](#_Toc165628313)

[10 EXPECTED OUTLINE OF REPORTS 23](#_Toc165628314)

[11 OVERVIEW OF CONTENT IN REPORTS 23](#_Toc165628315)

[12 Paper 1 – Primary & Key Secondary outcomes 23](#_Toc165628316)

[12.1 Tables in paper 23](#_Toc165628317)

[12.2 Figures in paper 23](#_Toc165628318)

[12.3 Tables in supplement 24](#_Toc165628319)

[12.4 Figures in supplement 24](#_Toc165628320)

[13 Paper 2 – Cardiopulmonary outcomes 25](#_Toc165628321)

[13.1 Tables in paper 25](#_Toc165628322)

[13.2 Figures in paper 25](#_Toc165628323)

[13.3 Tables in supplement 26](#_Toc165628324)

[13.4 Figures in supplement 26](#_Toc165628325)

[14 Paper 3 – Dietary and physical activity outcomes 26](#_Toc165628326)

[14.1 Tables in paper 26](#_Toc165628327)

[14.2 Figures in paper 26](#_Toc165628328)

[14.3 Tables in supplement 26](#_Toc165628329)

[14.4 Figures in supplement 26](#_Toc165628330)

[15 REFERENCES 28](#_Toc165628331)

[16 UNFORMATTED TABLES WITH INTENDED CONTENT 31](#_Toc165628332)

## BACKGROUND AND RATIONAL

Systemic lupus erythematosus (SLE) is a rare chronic autoimmune disease with a varied phenotype and a predisposition for women of childbearing age1. 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 fatigue3–7.

Patients with SLE also suffer from reduced exercise capacity with lower improvement in maximal oxygen uptake (V̇O2max) and fatigue severity scores (FSS) following moderate continuous exercise training (MCT) compared to healthy controls7–9.

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 (HRmax)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 minutes11,12. These individual HIIT sessions are then repeated 2-5 times weekly10–12.

Compared to MCT, HIIT has proven particularly effective at increasing V̇O2max and alleviating fatigue in several other populations than SLE-patients, including healthy13,14, middle-aged15, and elderly women16,17, heart failure patients10,12, as well as pre- and perioperative cancer patients11,18. As of now, no randomised controlled studies have investigated the impact of HIIT on fatigue or V̇O2max 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 exercise19. Pathway analyses indicate a central role of IFN driven immune activation in SLE20. 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 signaling22,23.   
IL-6 is a cytokine with both pro- and anti-inflammatory effects, it has multiple sources including contracting skeletal muscle24. IL-6 in humans can cause lipolysis 25, increase glucose uptake and glycogen storage in skeletal muscle26, 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 adults29.

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.

## OBJECTIVES

### Primary aim:

The primary aim is to investigate if increasing IFN-I activity determined by IFNGS negatively influences any effect of a 12-week supervised HIIT vs. no intervention on aerobic exercise capacity by V̇O2max or fatigue by FSS in patients with SLE.

### Secondary aims:

The secondary aim is to investigate if IFNGS negatively influences any effects of 12-week HIIT on measures of SLE disease activity and health related quality of life.

### Primary objective:

Investigate the effect of HIIT relative to the no exercise control comparator on changes in aerobic capacity and fatigue following a 12-week intervention.

Furthermore, to investigate the effect modulation of IFNGS on this effect.

### Key secondary objective:

Examining the effects of physical activity on SLE-disease activity and health related quality of life, and investigating any effect modulation of IFNGS on this effect.

### 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 paitents. 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.

## HYPOTHESES

### Primary Hypotheses

12 weeks of HIIT exercise will increase aerobic capacity as compared to no intervention control.

This effect can be modulated by IFNGS.

12 weeks of HIIT exercise will decrease fatigue scores, as compared to no intervention control.

This effect can be modulated by IFNGS.

The hierarchy of the hypotheses and subsequent claims for the primary outcome are as follows; both primary hypotheses are regarded as equal and significance will be corrected as suggested by Bonferroni30.

1. HIIT is superior to no intervention control at improving aerobic capacity. Superiority is claimed if the difference is statistically significant and favors the HIIT intervention.
   1. The effect of HIIT on aerobic capacity is modulated by IFNGS, such that a higher IFNGS results in lower benefits of HIIT.
2. HIIT is superior to no intervention control at alleviating fatigue. Superiority is claimed if the difference is statistically significant and favors the HIIT intervention.
   1. The effect of HIIT on fatigue is modulated by IFNGS, such that a higher IFNGS results in less decrease of initial fatigue from HIIT.

### Secondary Hypotheses

The hierarchy of the secondary hypotheses and subsequent claims are as follows;

1. HIIT is not inferior to no intervention control at maintaining current SLE symptoms as evaluated using SELENA-SLEDAI31 by a physician.
   1. This effect is modulated by IFNGS.
2. HIIT is superior to no intervention control at improving health related quality of life as estimated by the SF-36 domains.
   1. This effect is modulated by IFNGS.

### Exploratory Hypotheses

The hierarchy of the exploratory hypotheses and subsequent claims are as follows;

1. HIIT is superior to no intervention control at reducing severity of other (than SLEDAI) physician evaluated changes in SLE.
   1. This effect is modulated by IFNGS.
2. HIIT is superior to no intervention control at reducing severity of other (than FSS) patient reported outcomes in SLE.
   1. This effect is modulated by IFNGS.
3. HIIT is not inferior to no intervention control at inducing renal disease in SLE patients.
   1. This effect is modulated by IFNGS.
4. HIIT is superior to no intervention control at changing the body composition in SLE patients, the hypothesis should be tested in the following order;
   1. increasing the lean mass of SLE patients.
      1. This effect is modulated by IFNGS.
   2. Decreasing the total adipose tissue in SLE patients
      1. This effect is modulated by IFNGS.
   3. Decreasing the android adipose tissue in SLE patients
      1. This effect is modulated by IFNGS.
   4. Decreasing the gyneoid adipose tissue in SLE patients
      1. This effect is modulated by IFNGS.
   5. Decreasing the Waist-circumference to height ratio in SLE patients.
      1. This effect is modulated by IFNGS.
5. HIIT is superior to no intervention control at improving lung function tested in the following order:
   * 1. Improving forced expiratory volume at 1 second (FEV1) as percentage of expected.
        1. Improving FEV1 as total volume.
        2. These effects are modulated by IFNGS.
     2. Improving forced vital capacity (FVC) as percentage of expected.
        1. Improving FVC as total volume.
        2. These effects are modulated by IFNGS.
     3. Improving forced expiratory volume by forced vital capacity – ratio (FEV/FVC) between volumes
        1. FEV/FVC ratio between percentages of expected.
        2. These effects are modulated by IFNGS.
     4. Increasing total lung capacity (TLC) – by percentage of expected
        1. Increasing TLC by total volume.
        2. These effects are modulated by IFNGS.
     5. Decreasing residual lung volume (RLV) by percentage of expected
        1. Decreasing RLV by volume
        2. Effects are modulated by IFNGS
     6. Increasing alveolar volume (AV) by percentage of expected.
        1. Increasing AV by volume
        2. Effects are modulated by IFNGS
     7. Increasing diffusing capacity for carbon monoxide (DLCO) by percentage of expected.
        1. Increasing DLCO by total capacity.
        2. Effects are both modulated by IFNGS.
     8. Improving the carbon monoxide transfer coefficient (KCO) as percentage of expected.
        1. Improving KCO as total ratio
        2. Effects are modulated by IFNGS.
6. HIIT is superior to no intervention control at inducing beneficial changes to the metabolism.
   1. Measured by Disposition Index
   2. Measured by Matsuda Index
   3. Measured during an OGTT, changes of insulin, c-peptide and glucose, as the area under the curves
   4. Measured during an OGTT; insulin, c-peptide and glucose as the individual timepoints in the curve.
   5. Measured by lower total cholesterol, LDL, VLDL, HDL and TAGs
   6. These effects are modulated by IFNGS
7. HIIT compared to no-intervention control changes the peripheral capillaries when viewed through a capillaroscope.
   1. Measured by an increase in total density.
   2. Measured by fewer microhemorrhages.
   3. Measured by fewer meandering capillaries.
   4. Measured by fewer tortuous capillaries.
   5. Measured by fewer avascular areas.
   6. Measured by less capillary disorganization.
   7. Measured by fewer bushy capillaries.
   8. Measured by fewer megacapillaries.
   9. Measured by a decrease in average capillary width.
   10. Measured by a decrease in average capillary length.
   11. All the above are modulated by IFNGS.
   12. Measured by more benign other findings as reported by physician.
8. HIIT is superior to no intervention control at inducing cardiac remodeling.
9. HIIT does not change regular daily activity in SLE patients when compared to no-exercise controls.
10. HIIT increases total calorie intake in SLE patients.
    1. This effect is modulated by IFNGS.
11. A single HIIT physical activity bout produces pro-inflammatory cytokines in SLE patients.
    1. 12 weeks of HIIT reduce the inflammatory cytokine production to exercise.
    2. These effects are modulated by IFNGS.
12. HIIT reduces the transcriptomic profile of peripheral blood in SLE patients
    1. HIIT reduces the IFN-1 related mRNA signalling in peripheral blood.
    2. HIIT reduces the IFN-2 related mRNA signalling in peripheral blood.
    3. HIIT reduces the TNF related mRNA signalling in peripheral blood.
    4. HIIT moves the IL-6 related mRNA signalling in peripheral blood from a more pro-inflammatory to an anti-inflammatory state.
13. HIIT induces improved signaling in the vagal nerve in SLE patients.
    1. These changes modulate fatigue by FSS.
    2. These changes are modulated by IFNGS.
14. HIIT induces muscular changes in biopsies.

## 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 database32. In brief, the study is a randomized control trial, consisting of 2 arms, labeled exercise and control. Participants are stratified for sex and randomly allocated 1:1 to exercise or control. At [www.clinicaltrials.gov](http://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.

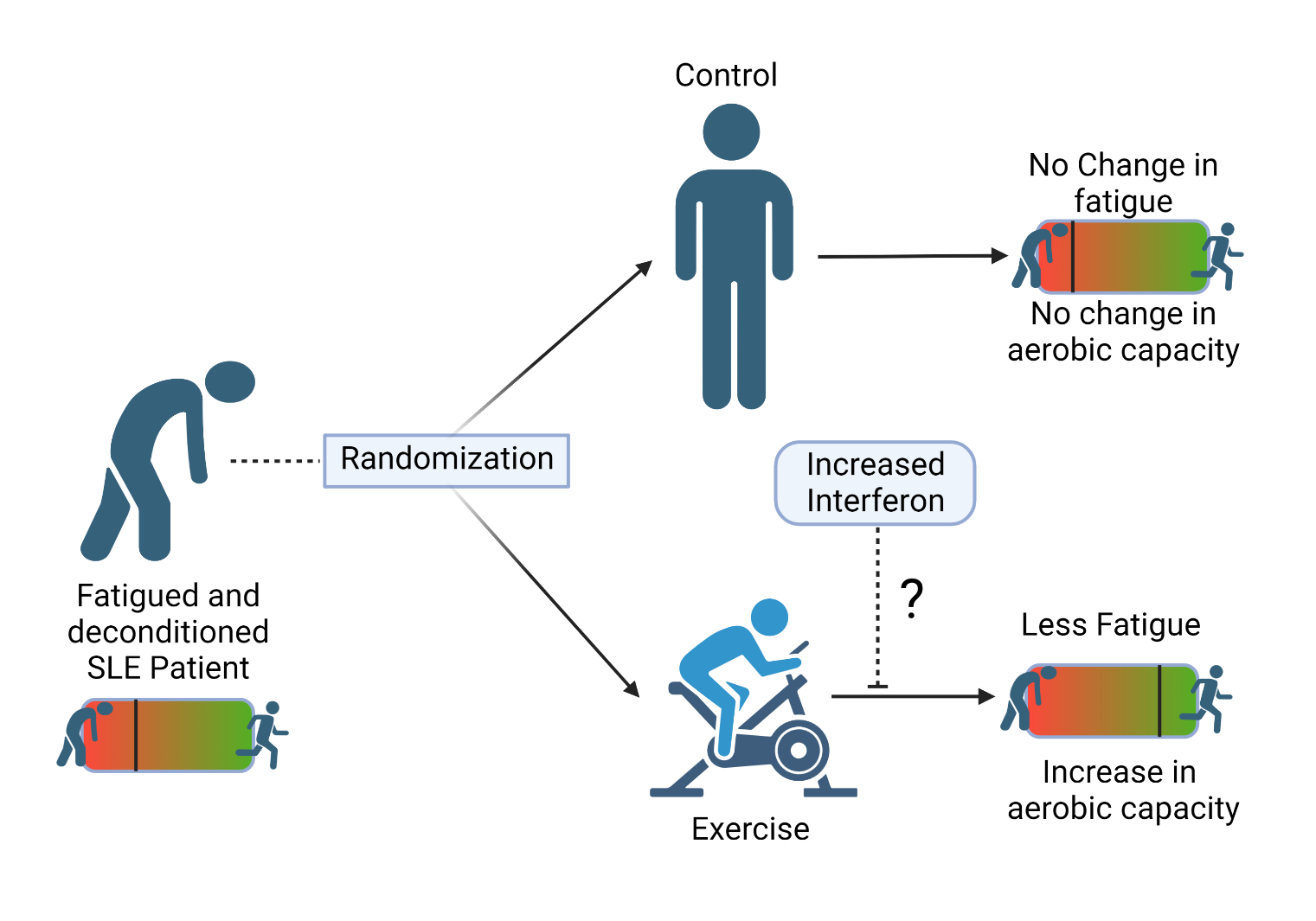


Figure 1 - Graphical hypothesis

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 hypothesis was that these 12 weeks would improve aerobic capacity and fatigue in SLE patients. But 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.

## OUTCOMES

### Primary Outcomes

#### 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 (VO2Max).

* 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.

#### Domain: Fatigue – Patient reported

Timeframe: 0 to 12 weeks

Measurement:

* Patient reported outcome measure (PROM).
* Krupp’s Fatigue severity scale33, as the average of all 9 domains (0-7).
* This scale has been verified for use in Danish SLE patients34.

### Key Secondary Outcome

#### Domain: Physician evaluated changes in measures of SLE

Timeframe: 0 to 12 weeks

Measurement: Y2K updated SLE disease activity (SLEDAI-2K) with the SELENA modifications35.

### Other Secondary Outcomes

#### Domain: Patient reported outcome quality of life.

Timeframe: 0 to 12 weeks

Measurement:

* Short Form (SF)-36 Health Survey (0-100)36 - Possible scores range from 0 to 100, with higher scores representing better health status.

#### 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.37
* Normalized to housekeeping genes
* Calculating a standardized z-score compared to the expression from 9 healthy controls

### Exploratory Outcomes

#### 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)38.
* 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.

#### Domain: Patient reported outcome measures

Timeframe: 0 to 12 weeks

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)39. 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).

#### Domain: Kidney disease

Timeframe: 0 to 12 weeks

Measurements:

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

#### 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

#### 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

#### 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

#### 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

#### 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 82Rb-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

#### 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

#### 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.

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

Timeframe: 0 to 12 weeks

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

#### 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)

#### 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

#### 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.

## STUDY POPULATION, ANALYSIS SET AND STATISTICAL PRINCIPLES

Eligibility criteria have been previously published 32. In brief, the participants suffered from systemic lupus erythematosus, and fulfilled 10 or more points on the 2017 ACR/EULAR SLICC40

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

The analysis will be done primarily as intention to treat (ITT), and secondarily as per protocol (PP). Participants in the exercise group who fail to show up to 80% or more of exercise sessions will be excluded from the PP analysis. Participants who fail to show up to follow-up will be excluded from the PP analysis but included in the ITT as restricted estimated maximum likelihood.

### Statistical Methods

The primary analysis will be performed using a linear mixed effect model with an unstructured covariance matrix. The primarily analysis will be done in an intention-to-treat manner. The linear mixed effect model implicitly handles missing data with a Restricted Maximum Likelihood analysis. The primary model will be outcome by time and an interaction between treatment and time + stratification for sex. The model will use a repetition for visit.

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 both on summary scores (such as DI or Matsuda Index) and for each individual value with a repetition for visit and timepoint.

### Significance levels of the claims

Since this study includes both a primary and a co-primary endpoint the cut-off for significance will be Bonferroni corrected to p=0.025 and the associated 95% confidence interval adjusted for this. This is in accordance with the alpha set at p=0.05 in the original protocol without Bonferroni correction. Secondary outcomes will be adjusted for false discovery rate (FDR) in the Benjamini-Hochberg (BH) method41 as a group, and the exploratory outcomes will be adjusted for FDR in the BH method as a separate group.

### Tools for statistical analysis

Analysis will be done in an updated version of rstudio with an updated version of R42. The LMMstar package43 will be used for the primary analysis, the LME4 package44 will be used as a comparator, but assuming the results are similar only the LMMstar results will be published. The code will be available on Github (<https://github.com/Malte-Lund/Lupex-Statistics> ).

## DEVIATIONS FROM THE ORIGINAL PROTOCOL

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 45 |
| 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. |

## 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> ), and the Centre for Physical Activity website ([www.aktisundhed.dk](http://www.aktisundhed.dk) ) prior to commiencing statistical analysis.

## 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

## 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 primary endpoints and the secondary endpoints, along with relevant related exploratory endpoints will be published in one paper. Planned publication scopes the cardiometabolic outcomes, such as results from the 82-Rb-Pet-CTs and the echocardiographic outcomes as a second paper. The behavioral changes from exercise, such as the diet diaries, the activity measurements, the adaptations to the singular exercise bout and capillaroscopic changes will likely make up a third paper. Relevant end points to include in each paper will be further discussed by the writing committee and therefore this outline of contents is not complete but may change between papers.

## OVERVIEW OF CONTENT IN REPORTS

(Unformatted tables with specific variables are placed at the end of the text)

## Paper 1 – Primary & Key Secondary outcomes

Working title: Adaptations in aerobic capacity and fatigue induced by 4x4 HIIT in systemic lupus erythematosus patients is [not] modulated by type 1 Interferon gene signature on exercise. – An outcome assessor blinded randomized controlled single-blinded clinical trial.

Words in [ ] brackets included based on the outcome on the primary analysis. If they differ the title will be edited accordingly (e.g. adaptations in aerobic capacity but not fatigue (…)).

### Tables in paper

Baseline characteristics not separated by randomization group as we assume a constrained baseline for the primary analysis. Aerobic capacity & weight separated by screening/baseline.

Primary and key secondary outcomes grouped by randomization group at followup.

As table 2 but with baseline and randomization groups subgrouped into lowest 25% quartile of IFN-score, mid 50% quartile of IFN-score and highest 25% quartile of IFN-score.

Other outcomes grouped by randomization group.

### Figures in paper

Table of 3x2 graphs: 2 Spaghetti-plots with lines for 10 individuals sampled at random from each intervention group depicting the primary outcomes, colored by intervention group.

2 model plots with the constrained baseline linear mixed model estimations and Bonferroni-adjusted 95% CI for the primary outcomes. Possibly with the individual data points.

2 plots with change in Primary Outcome against IFN-signature as individual data points with a regression line from the interaction in the appropriate linear mixed effect model.

Y = VO2Max, X = Timepoint(Screening visit, Baseline Visit, Followup Visit). Colored by intervention (exercise or control) group. As a point plot with lines connecting ID.

Y = Estimated VO2max with 95% CI, averaged over sex, X = Timepoint, Colored by intervention group. Three different percentiles of IFN-score depicted as well.

Y = FSS-Score, X = Baseline Visit, Followup Visit. Colored by Exercise or control group. As a point plot with lines connecting ID.

Y = Estimated FSS with 95% CI, averaged over sex, X = Timepoint, Colored by intervention group. Three different percentiles of IFN-score depicted as well.

Y = Change in VO2Max, X = IFN-Signature, colored by intervention group. With a linear regression line (and confidence intervals for this) from the linear mixed model IFN-signature coefficient.

Y = Change in FSS, X = IFN-Signature, colored by intervention group. With a linear regression model from the linear mixed model IFN-signature coefficient.

Key secondary outcomes depicted as constrained baseline models, with three different percentiles of IFN-score depicted as well.

### Tables in supplement

1. Adherence to prescribed exercise.
2. Adverse reactions & events following randomization.
3. Other relevant characteristics measured at screening, baseline and followup, separated by intervention group at followup.
4. Baseline data grouped into quartiles of the IFN-score.

### Figures in supplement

Flow of participants.

Outcomes from the Vagus™ measurements at the specific timepoints

Resting heart rate

Rise from supine heart rate variability (HRV)

Expiratory/inspiratory HRV

Overview of the intervention.

False discovery rate of secondary endpoints – Histogram of p-values.

False discovery rate of exploratory endpoints – Histogram of p-values.

## Paper 2 – Cardiopulmonary outcomes

Working title: Adaptations in **cardiopulmonary functions** induced by 12 weeks of 4x4 HIIT in systemic lupus erythematosus patients is [not] modulated by type 1 Interferon gene signature on exercise. – A secondary analysis of an outcome assessor blinded randomized controlled clinical trial.

Words in [ ] brackets included based on the outcome on the primary analysis

### Tables in paper

Baseline characteristics

82-Rb-Rest/Stress-PET-CT, Echocardiography and Lung Function findings at baseline and followup. Separated by intervention group at followup.

### Figures in paper

Table of 3x1 graphs: a spaghetti-plots with lines for 10 individuals sampled at random depicting the PET-CT outcomes.

A model plot with the constrained baseline linear mixed model estimations and unadjusted 95% CI

A plot with change in MFR against IFN-signature as individual data points with a regression line from the interaction in the appropriate linear mixed effect model.

Y = Myocardial Flow Reserve (MFR), X = Timepoint (Baseline Visit, Followup Visit). Colored by intervention (exercise or control) group. As a point plot with lines connecting ID.

Y = Estimated MFR with 95% CI, averaged over sex, X = Timepoint, Colored by intervention group. Three different percentiles of IFN-score depicted as well.

Y = Change in MFR; X = IFN-Signature, colored by intervention group. With a linear regression model from the linear mixed model IFN-signature coefficient.

Graphs depicting change in diffusing capacity similarly to figure 1.

Graphs depicting changes to stroke volume similarly to figure 1.

### Tables in supplement

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

### Figures in supplement

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

## Paper 3 – Dietary and physical activity outcomes

Working title: Adaptations in **daily diet, physical activity and cytokine response to singular exercise bout** induced by 12 weeks of 4x4 HIIT in systemic lupus erythematosus patients is [not] modulated by type 1 Interferon gene signature on exercise. – A tertiary analysis of an outcome assessor blinded randomized controlled clinical trial.

Words in [ ] brackets included based on the outcome.

### Tables in paper

Baseline characteristics – constrained between randomization groups.

Caloric and macronutrient intake, physical activity measured in steps/calories burned and METs in the randomization groups at followup.

Estimated marginal means of blood sample concentration with 95%CI from the 9 timepoints in the acute exercise bout.

### Figures in paper

Acute-Bout domain: tableau of multiple graphs, Y = Cytokine (or leukocyte) concentration in plasma with 95% CIs X = Timepoint (from 0 to 105), Colored by baseline (pooled), followup exercise or followup control.

OGTT domain: 3 graphs Y = insulin, glucose and pro-insulin. X = Timepoint. Colored by baseline (pooled), followup exercise or followup control.

### Tables in supplement

1. Self-reported Diet
2. Adverse reactions & events following randomization.
3. Estimated marginal means of all the blood samples measured at all timepoints during the acute exercise bout.

### Figures in supplement

Figure detailing the acute exercise bout.

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

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## 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 |  |  |  |