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

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2 Introduction and background

2.1 General objective

Systemic lupus erythematosus (SLE) is a rare chronic autoimmune disease with a varied phenotype and multisystemic involvement[1]. SLE shows a predilection for women of childbearing age in which the prevalence in Denmark is 20-40/100.000[2]. The disease involves a complex interplay of immunopathogenic pathways that is yet to be fully elucidated[3]. Notably, these patients often suffer from reduced exercise capacity and a related up to 50-fold increased risk of cardiovascular disease (CVD)[3,4]. The cause of impaired exercise capacity has been debated for decades[4,5], and remains to be fully elucidated, but likely reflects the unique link between immune function and exercise capacity.

SLE is characterised by an activation of the interferon (IFN) system with increased expression of IFN-regulated genes[6]. There are three types of IFNs that can be further divided into several subtypes and classes that affect distinct receptors[6]. Key subtypes of type 1 IFN are IFNα and IFNβ[7,8]. These subtypes display a high degree of homology and biological effects[9]. In healthy individuals, IFNα and IFNβ constitute an important part of the barriers against viral infections and modulation and activation of the innate and adaptive immune system[7]. The activation of IFNs are tightly regulated by several mechanisms[7], but in SLE patients, the balance between activation and regulation becomes skewed with a concomitant over-expression of IFNs[10]. Since IFNs in peripheral blood are measured in the order of picograms per millilitre, and they are incredibly short lived as they constantly interact with blood and endothelial cells, no method has produced effective measurements of this constant varying amount of IFN. Instead robust assessments of the underlying IFN gene signature (IFNGS) have been developed, by measuring the pool of transcribed mRNA related to prolonged IFN exposure. The individual patient's IFNGS has been linked to the subsequent clinical presentation and pathophysiology of disease[11,12].

These gene signatures can be differentiated between genes related to type 1 (like IFNα and IFNβ), type 2 (like IFNγ) and type 3 (like IFNλ) IFNs. Which creates type 1, type 2 and type 3 IFNGSs. These major groups have multiple subgroups, and these subgroups interfere and crosstalk with eachother, therefore single genes, or small gene groups coding to just one of these IFNs are hard to define.

Ex vivo studies have shown, that IFNα and IFNβ, which are both types of type 1 IFN interfere with the interleukin (IL)-6 induced transducer and activator of transcription 3 (STAT3), leading to an abrogation of IL-6 activated intracellular pathways[13,14]. IL-6 is a pleiotropic cytokine with both pro- and anti-inflammatory effects. During exercise, IL-6 is released from skeletal muscle resulting in increased circulating levels of IL-6[15]. Recently we found that the IL-6 receptor antibody tocilizumab (TCZ) completely abolish exercise-induced reductions in visceral and cardiac adipose tissue in healthy individuals as well as reducing the left ventricular mass growth of the TCZ treated group to match the non-exercising controls[16,17]. Thus, IL-6 is an important signalling molecule stimulating both cardiac and non-cardiac adaptations to exercise training.

Evidence suggests that the type 1 IFN largely regulates a chronic pathological process and is less involved in the acute disease manifestations[11,12].

Chaussabel et al. attempted to define a unifiable IFNGS for SLE[18] by dividing genes from pediatric SLE patients into multiple modular subset of gene signatures related to interferon profile. Defining 260 different modules of coclustered genesets that were upregulated in SLE.

Chiche et al. further defined three of these modules (see 3.9.9); M1.2, M3.4 and M5.12[19], relating M1.2 to IFNα, M3.4 to signalling by both IFNα and β but also some degree of IFNγ signalling and M5.12 to IFNα, β and γ.

M1.2 had no increase with increasing SLEDAI score, and likely represents a baseline characteristic of the SLE disease as noted by the type 1 IFNGS representing a chronic pathological process[11,12]. Increasing M3.4 and M5.12 scores correlated with increased SLEDAI scores[19]. Furthermore, Chiche et al. showed that in order to have M5.12 activity; SLE patients needed M3.4 activity creating a dose-response correlation of these modules. Measuring the levels of these genetic modules and correlating them to our primary outcomes will allow us to evaluate whether patients with more active type 1 IFN system have fewer benefits from exercise.

Based on our previous studies combining exercise training and IL-6 receptor blockade, we hypothesize that exercise-induced cardiac, metabolic and muscle adaptations are impaired in SLE patients with high- compared to low- IFNGS.

Exercise capacity in patients with SLE has previously been studied, in those studies focus was on fatigue severity and quality of life measures (QoLs), showing a non-significant improvement in fatigue scores, and a slightly significant increase in VO_2Max[20,21]. Only few studies have investigated the cardiac benefits, such as decreased sympathetic tonus, of exercise training in SLE patients[22], showing an overall, but reduced compared to healthy controls, positive effect of exercise in this patient group.

Studies have shown that endurance high intensity interval training (HIIT) is superior to resistance training with respect to improving QoLs in patients with SLE[23]. Exercise-induced IL-6 is intensity-dependent, thus, HIIT will lead to a higher release of IL-6 compared to exercise at lower intensity. It is tempting to speculate that SLE patients with high IFNGS will experience increased exercise-related fatigue due to the supressed IL-6 signalling[24].To the best of our knowledge, an exercise intervention study in patients with SLE regarding the differing effects for IFNGS has not been performed.

Thus, the aim of the present study is to compare the effects of regular supervised exercise training on cardiac adaptations, exercise tolerance and fatigue in SLE patients who will have measured their IFNGS before and after exercise intervention, so that post hoc analysis can show whether increases to any of the three IFNGS modules affect their outcomes. Changes in IL-6 signalling in the immune system will also be assessed.

2.2 Overall aim

The overall aim of this project is to understand whether patients with differing IFNGS differ in their cardiac, metabolic and muscle adaptations to exercise training, notably cardiac pump function and perfusion. Furthermore, to study the impact of exercise on patient-reported outcomes including measures of fatigue and SLE disease activity according to IFNGS.

We anticipate that the results will provide insight into the fundamental mechanisms of severe fatigue and exercise capacity in patients with SLE, this may improve and personalize physical rehabilitation and medical treatment strategies in this rare disease.

2.2.1 Primary aim

We aim to investigate whether maximal oxygen uptake adaptation to 12 weeks of regular supervised exercise training differs between SLE patients with increasing IFNGS.

In addition to this, we aim to investigate as a co-primary endpoint whether patients with increasing IFNGS will have less benefit of exercise on their fatigue severity scores.

2.2.2 Secondary aims

To investigate whether the cardiac and metabolic adaptations to 12 weeks of regular supervised exercise training differ between SLE patients with increasing IFN gene signature. Cardiac function will be assessed by left ventricular ejection fraction, ventricular muscle mass, and coronary perfusion reserve. Metabolic adaptations include changes in visceral adipose tissue mass, lean body mass, and various metabolic blood samples as well as lung function.

To investigate the effects of regular supervised exercise training on measures of SLE disease activity and patient-reported outcomes including but not limited to SLEDAI/SRI-50, FSS and SF-36.

2.3 Trial registration

We intend to register the trial at ClinicalTrials.Gov following acceptance by the regional ethics committee.

2.4 Hypotheses

We hypothesize that exercise-induced cardiac and metabolic adaptations are impaired and exercise intolerance and fatigue is aggravated in SLE patients with high compared to low IFNGS.

We hypothesize that improvements in peak oxygen uptake following 12 weeks of high intensity exercise is less pronounced in SLE patients with increasing IFNGS.

3 Methods

3.1 Study design and study settings

The study is designed as an investigator-blinded randomized controlled trial consisting of 12 weeks of endurance exercise (Figure 1). A total of 60 patients will be randomized to intervention in a 1:1 fashion, a total of 30 patients will follow an exercise intervention, while the remaining will be encouraged to maintain current exercise habits for the duration of the study (see Figure 1).

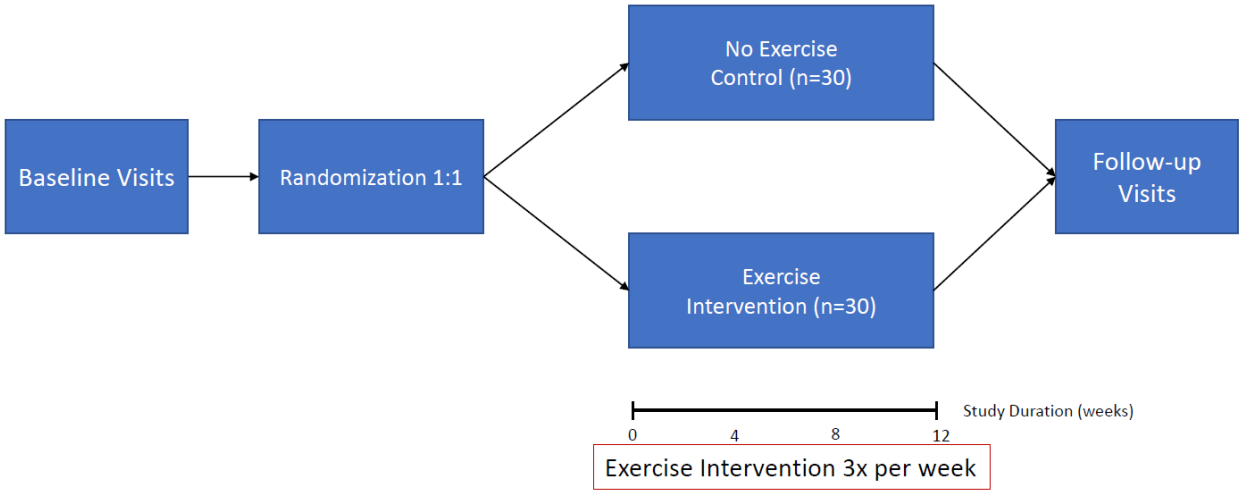

Figure 1 Study Design

The enrolment period is planned to start in early 2022 and run for approximately two years or when 30 patients are recruited to each group. Follow-up is estimated to be completed in the spring of 2023. All baseline and follow-up tests will be carried out at Centre for Physical Activity Research (CFAS), Rigshospitalet, Denmark. ^{82}Rb-PET scans will be performed at the Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet, Denmark. All data will be collected and analysed in Denmark.

3.2 Eligibility criteria

3.2.1 Inclusion criteria

- Age ≥ 18 years by inclusion.
- Able to provide informed consent.
- Diagnosed SLE and fulfilling the classification criteria for SLE based on the American College of Rheumatology/EULAR criteria[25] (SLICC)

3.2.2 Exclusion criteria

- Health conditions that prevent participating in the exercise intervention determined by the Research Coordinator these include but are not limited to
    - Major bone fracture at inclusion
    - Significant myalgias exacerbated by physical exercise
    - Active infectious disease such as Covid-19
    - Severe symptomatic pleuritis or pericarditis
- Corticosteroid use > 10mg/day at baseline
- Diagnosed with diabetes mellitus by physician
- Pregnancy
- SLEDAI-2k[26] (with the SELENA modifications to Proteinuria changes so as to not exclude patients with chronic proteinuria[27]) > 10
- Contraindications to ^{82}Rb-PET with adenosine stress (according to local guidelines at the Dept. of Clinical Physiology, Nuclear Medicine and PET, Rigshospitalet, which are in accordance with the recommendations of the European Association of Nuclear Medicine)[28]
    - Fever, myocarditis or endocarditis
    - Previous heart transplantation
    - Dysregulated atrial or ventricular tachyarrhythmias
    - Severe chronic obstructive pulmonary disease with a FEV_1 of less than 50% of predicted
    - Second or third degree sinoatrial or atrioventricular block
    - Active bronchospasm at the time of the scan
    - Systolic blood pressure <90 or >200 mmHg at the time of the scan

- Treatment with theophyllin within 7 days of the scan

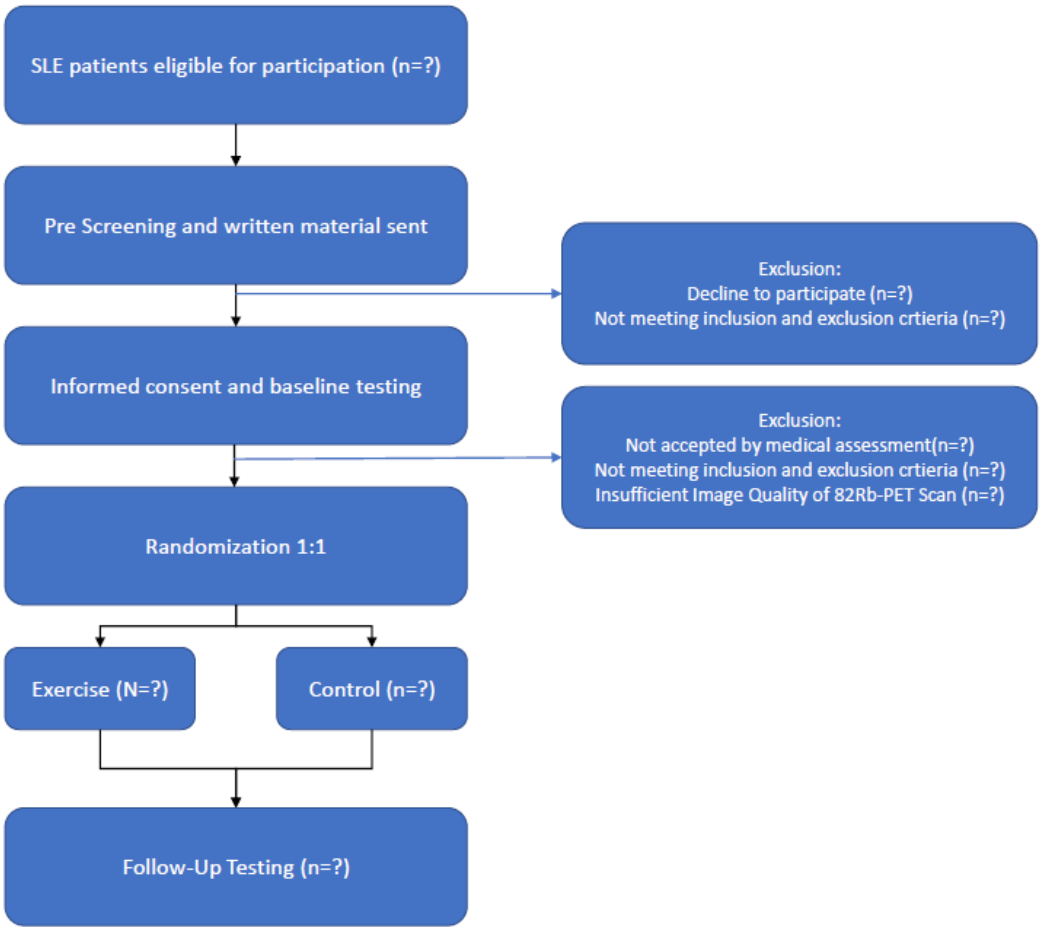

Figure 2 Flowchart of study. SLE; Systemic Lupus Erythematosus.

3.3 Intervention

The exercise training program includes three sessions per week over a 12-week period and will take place at CFAS or a local collaborator. The exercise intervention will start when baseline visits have been completed.

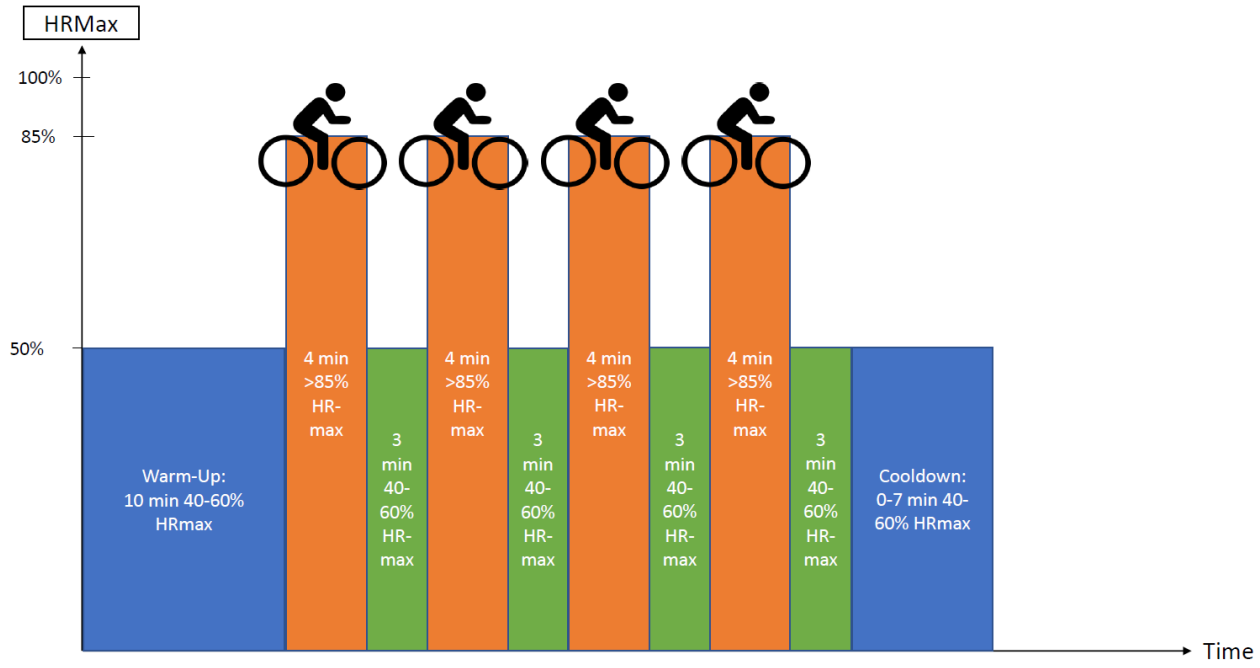

Figure 3 Exercise intervention, HRMax = Maximal Heart Rate.

The program consists of high intensity endurance training on ergometer bicycles. The intensity will progress throughout the 12 weeks of training. The training consists of 10 minutes of warm up at 40-60% maximum heart rate (HRmax), followed by 4 sets of 4 minutes of high intensity interval training (HR>85%HRmax) followed by 4 sets of 3 minutes of medium intensity (40-60% HRmax), for a total of 28minutes, and finally a 10 min cool-down of 50% HRmax fading to exercise cessation (see sFigure 3 Exercise intervention), for a total exercise time of 38-45 minutes. Previous studies in our centre have had significant results following this training program[17]. Strenuous exercise is considered beneficial for SLE patients, and not considered harmful[29].

All sessions will be supervised and administered by CFAS personnel or local collaborators with experience in handling endurance exercise. To ensure proper intensity, all patients will be wearing a heart rate monitor. The maximal heart rate (HR_{max}) will be measured as the peak heart rate during the final minute of the VO_{2max} test (see 3.9.2)[30]. To evaluate the precise amount of exercise completed for every patient, each training session will be documented (attendance, total time of HR_{max} > 85%, total time of exercise, average watt, average heart rate, and perceived exertion (BORG scale)).

The exercise session will be counted as successful if more than half of the high intensity training is above 85% of HR_{max} (so 8 minutes of the session), this is similar to previous studies. The time in each HR span of the patient for each training session will then be recorded to be measured for further compliance parameters.

Control groups will be encouraged to maintain current exercise habits for the duration of the study, and both groups will do a dietary and activity record at study baseline and followup.

3.4 Criteria for discontinuation

Prior to each exercise session, the patient will be assessed regarding chest pain (acute or recurring). The termination criteria during exercise include onset of angina, confusion, nausea, severe pain in joints or muscles that prohibit further exercise, physical signs of profound fatigue, and verbal

communication of profound fatigue. If chest pain is present prior to exercise or any of the above-mentioned criteria occurs during exercise, the instructors are obliged to call one of the study-affiliated medical doctors immediately. If a patient experiences increased joint or muscle pain during the exercise, the medical doctor will be called to decide (in dialog with the patient) whether the exercise can continue or not.

3.5 Concomitant care

The patients will follow standardized clinical care at their respective physician. The patients will be urged to continue their normal day-to-day activities and diet outside of this study. The patients will be advised to inform the study personnel about any new prescription or over-the-counter drugs used one month prior to, and during the study. All information regarding changes in medicine will be documented. We do not anticipate complications from participation in this study.

3.6 Outcomes

3.6.1 Primary Outcome:

Changes in maximal aerobic capacity (VO_2max)

3.6.2 Co-Primary Outcome:

Patient reported Fatigue, measured by Fatigue Severity Scale (FSS)[31]

3.6.3 Secondary Outcomes:

Physician evaluated measures of SLE, such as the year 2000 updated SLE disease activity (SLEDAI-2K)[26], with the SELENA modifcations[27], SLEDAI Responder Index-50 (SRI-50)[32] and Visual Analog Scale (VAS) of global disease by Physician.

Transcriptomic analysis of blood samples related to IFN-I signalling.

3.6.4 Exploratory Outcomes:

Patient reported outcome measures (PROMs) including VAS fatigue, VAS pain and Short Form (SF)-36 Health Survey[33].

Cardiac adaptations, including left ventricular mass, stroke volume, left ventricular and atrial end-diastolic volume, global longitudinal strain, left ventricular ejection fraction, and coronary perfusion reserve by echocardiography.

Metabolic adaptations, such as fat distribution, waist-to-height and waist-to-hip ratio, total fat mass, visceral fat mass, gynecoid fat mass, android fat mass and lean body mass, measured by DXA scan.

Blood samples analysed for markers related to exercise, metabolism, inflammation and cardiovascular function.

Transcriptomic analysis of blood samples related to IFN-II, IFN-III, IL-6, and TNF signalling.

Peripheral capillaries of the nailfold assessed by capillaroscopy.

Autonomic nervous system measurements by RR interval changes.

Change in physical activity measured by activity measurements and dietary changes will serve to help alleviate possible confounders

3.7 Recruitment plan

Patients will be recruited from the out-patient clinic at the Department of Rheumatology, Rigshospitalet. If determined eligible for the study by one of the nurses or the treating rheumatologist, patients will be informed about the possibility of participating in the current study. The patient will be informed that interest in this study will allow one of the Study MDs to screen their electronic and physical medical journal for inclusion criteria before contacting the patient.

If the patient shows interest, the nurse or rheumatologist will inform one of the CFAS investigators who will contact the patient by telephone and perform pre-screening.

In order to assess for inclusion and exclusion criteria, so as to not contact patients that would be excluded from the study, a study MD will pre-screen the patient based on the information accessed through the medical journal.

This information will include: Name, Social Security Number (Including age and sex), pharmaceutical products used (to assess for corticosteroid and other anti-rheumatic drugs for the treatment of SLE use), medical history (to rule out DM and check for other diagnoses that could pose an exercise risk), recent blood samples and the entries from the rheumatologic department at RH to asses for the classification criteria for SLE based on the American College of Rheumatology/EULAR criteria[25].

Alternatively, a clinician employed at the out-patient clinic at the Department of Rheumatology will provide a list of patients eligible for the study, who will then be contacted by the study personnel.

If the patient fulfils the inclusion criteria and none of the exclusion criteria at prescreening and the patient accepts; written information including ("Deltagerinformation om deltagelse I et videnskabeligt forsøg") a consent form ("Informeret samtykke til deltagelse I et sundhedsvidenskabeligt forskningsprojekt"), and the pamphlet "*Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt*" will be emailed, and a telephone call approximately 3 days later will be planned.

Through this the patient will be informed that further participation in the study will require access to the medical electronic and physical journal for investigator, the sponsor and her representatives (Bente Klarlund Pedersen and CFAS), and controlling instances that ascertain correct ethical conduct of this study. They will furthermore be informed that this consent can be recalled at any time with termination of study participation, and with no further effects on their future treatment of their disease.

If the patient still shows interest in participating by the second telephone call, the patient will be invited to CFAS for visit 1, if the prospective participant prefers so and cannot make time for a physical visit 1 it can be done by video call.

If, for any reason, the patient wishes additional time to consider participation, a telephone call will be planned within 3-5 days.

Informed consent will always be obtained prior to medical- and baseline assessments by a medical doctor or a delegated experienced study personnel working under supervision.

Following this oral and written information and written consent visit 1; visit 2 of the baseline visit will be planned (see 3.8.1 below), patients will be asked to arrive fasting at visit 2.

3.8 Patient visits

Following the visit to obtain consent all patients will undergo a total of six assessment visits. Four of the visits will take place at CFAS (visits 1, 3, 4 and 6) and two will take place at the Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet (visits 2 and 5).

The exercise intervention will take place at CFAS or one of our local collaborators. Baseline visits 1, 2, and 3 will take place prior to intervention. During follow-up visits 4, 5, and 6, patients will be met by staff blinded to the intervention. An overview of visits is presented in Table 1.

3.8.1 Visit 1 – Informed Consent

At CFAS or if the participant prefers, another setting such as video conference:

The patient will be informed about the possibility of bringing a trustee along. In a quiet setting without external interruptions, the patient will receive thorough information regarding purpose, procedure, potential side-effects and possible withdrawal of the study by one of the medical doctors or a delegated experienced study personnel.

The patient will be offered to use at least 24 hours or as long time as is needed for contemplation and explained that SLE treatment will not be jeopardized in any way by declination to join the study.

If the patient wishes to continue, a consent form will be signed. If the patient requests to consider their participation further; a new appointment about one week later will be scheduled.

Prior to this initial information, the patient has the opportunity to contact one of the study investigators, with contact information clearly listed on the written material they have received by email.

3.8.2 Visit 2 - Thorough medical examination - Baseline

At CFAS:

Patients will arrive fasted at CFAS. The patient will then undergo a medical assessment. If, for any reason, the examining medical doctor finds reason to exclude the patient, no further assessments will take place. The patient will be informed about the decision, and if deemed necessary, the patient will be informed to contact his/her general practitioner or rheumatologist.

Further OGTTs, DXA scan, VO_2-Max test, medical history and more as described in table 1 will then be done on visit 1.

Blood Samples including Pax Gene tubes for IFNGS are collected. These blood samples are the characterization and outcome packages of blood samples as specified in 3.9.7 as well as the IFNGS analysis as described in 3.9.9.

3.8.3 Visit 3 - ^{82}Rb PET Scan - Baseline

At Dept. of Clinical Physiology, Nuclear medicine & PET, Rigshospitalet:

Rest-adenosine stress ^{82}Rb-PET/CT scan. The patient will be informed to pause nitroglycerin for 1 hour, other long-lasting nitrite-based drugs, betablockers and persantin for 24 hours, prior to the ^{82}Rb-PET scan (if appropriate). Furthermore, the patient must refrain from consuming any methylxantin-containing drugs, foods or drinks for 18 hours prior to the scan. Methylxantins encompass theophyllin, caffeine, and theobromine, and are present in e.g. coffee, tea, chocolate, and cocoa.

3.8.4 Visit 4 - Acute Exercise bout - Baseline

At CFAS:

Patients will be asked to arrive fasting.

Acute exercise bout as specified in 3.9.3 and Figure 4 with the exercise related markers of inflammation as specified in 3.9.7.3.

1. Patient waits in a relaxed environment for at least 20 minutes.
2. During this time they will complete the autonomic nerve function test as described in 3.9.16.
3. Baseline ECG and Blood Pressure will be measured.
4. Blood tests as specified in the acute exercise bout package at 3.9.7 will be drawn twice, this is defined as t=-5 and t=0
5. Acute exercise bout of 45 minutes of acute exercise. As described below
6. Following warm up at 10 minutes, first bout at 14 minutes, final bout at 35 minutes, and total exercise at 45 minutes, (t=10, 14, 35, and 45 respectively) blood tests as specified in the small package at 3.9.7 will be drawn.
7. Exercise ceases and further blood tests as specified in the small package at 3.9.7 will be drawn at 15 (t=60), 45(t=90), and 60 (t=105) minutes after exercise
8. After these blood tests participants will be offered a light meal.
9. Participants will be instructed in dietary diaries and have applied activity axial accelerometer-based physical activity monitors (AX3; Axivity, Newcastle upon Tyne, UK) for a 3-to-5-day period.

Timeline	Baseline				Intervention (12 weeks)	Follow-up		
Visit	1	2	3	4		5	6	7
Study day	1	2	3	4		93	94	95
Written consent	X							
Medical history		X						
Questionnaires		X				X		
Clinical examination (Vital signs, BP, ECG)		X		X		X		X
Blood Sample[1]		X		X		X		X

PAX-Gene tube for IFNGS		X				X		
DXA scan and anthropometrics (weight, BMI)		X				X		
VO_2 max test	X	X				X		
^{82}Rb-PET scan			X				X	
Echocardiography		X				X		
OGTT		X				X		
Lung function		X				X		
Acute Exercise Bout				X				X
Muscle Biopsy		X				X		
Activity and Dietary record initiation				X				X
Nailfold Capillaroscopy		X		X		X		X
Autonomic Nerve Function tests				X				X

Table 1 Overview of the visits. BMI; Body mass index, BP; blood pressure, DXA; dual energy X-ray absorptiometry, ECG; Electrocardiogram, rubidium-82 positron emission tomography; 82Rb-PET scan, VO2 max; maximal oxygen consumption.

[1]Blood samples will be drawn as specified in 3.9.7 Blood samples

3.8.5 Visit 5 – Medical examination - Follow-up

At CFAS:

Follow-up assessments identical to Visit 2.

3.8.6 Visit 6 - ^{82}Rb PET Scan – Follow-up

Dept. of Clinical Physiology, Nuclear medicine & PET, Rigshospitalet:

Follow-up assessments Identical to visit 3.

3.8.7 Visit 7 - Acute Exercise bout – Follow-up

CFAS:

Follow-up assessments Identical to visit 4

3.9 Assessment of study outcome

All study outcomes will be collected after written signed consent.

3.9.1 Medical assessment

Prior to enrolment and at follow-up, each patient will undergo a medical examination and interview that includes auscultation of heart and lungs, a brief medical history, electrocardiogram, and blood pressure.

3.9.2 Maximal aerobic capacity

Patients will perform a graded exercise test on a bicycle ergometer (Monark LC4, Monark Exercise AB, Vansbro, Sweden) at CFAS, to determine the maximal oxygen consumption (VO_2 max).

Maximal workload (watt-max) will be estimated based on baseline physical activity, resting heart rate, weight, gender, age and waist circumference as per the method described by Nes et al[34].

Using this estimation participants will warm up for 5 minutes at 15% of their watt-max (rounded to nearest 10) and increase in steady increments of 1 minute each to reach their estimated 100% watt-max at 15 minutes (so an increase of 8,5% watt-max per minute). Following these 15 minutes, watt will be added in the same increments until exhaustion.

The patients will be encouraged to perform at their maximal effort by CFAS staff. The ventilation rate and expired CO_2 and O_2 will be measured by indirect calorimetric measurements (Quark b2, Cosmed, Rome, Italy.) The peak HR measured will serve as reference for relative intensity during the study.

This test will be done immediately following consent, and again at the first baseline day and at follow-up, to alleviate for habituation in the exercise group.

If for some reason the participant cannot do more than 7 minutes on this maximal aerobic capacity or they cannot increase their maximal heart rate above 120 minutes at the first test; another test will be done which is reduced to this new watt-max (reached at 15 minutes). This test will be done with a stress ECG monitoring to evaluate whether the participant suffers from a cardiac condition that limits their potential.

3.9.3 Acute Exercise Bout

At baseline and follow-up all patients, both control and intervention patients will perform an acute symptom-limited exercise bout on a bicycle ergometer (Monark LC4, Monark Exercise AB, Vansbro, Sweden) at CFAS. This exercise bout will be identical to the exercise interventions and consist of 10 minutes of warm up at 40-60% maximum heart rate (HRmax), followed by 4 sets of 4 minutes of high intensity interval training (HR>85%HRmax) these sets are followed by 4 sets of 3 minutes of medium intensity (40-60% HRmax), for a total of 28minutes, and finally a 0-7 min cool-down of 40-60% HRmax or below. This gives a total exercise of 38-45 minutes.

These acute exercise bouts are identical to the intervention and gives us an opportunity to study the changes in cytokine activity during exercise for patients with SLE following intervention.

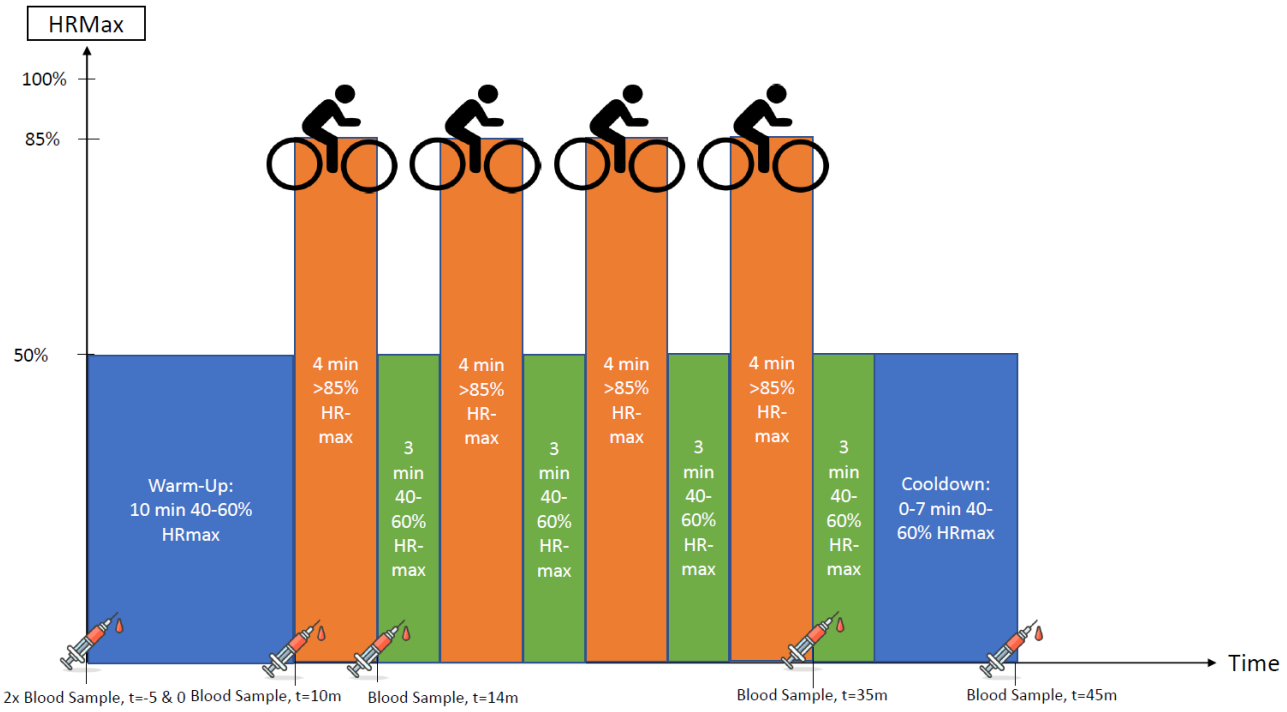

Figure 4 Acute Exercise Bout, HRMax = Maximal Heart Rate

During and following this bout, the small package of blood samples will be drawn to assess for markers of inflammation, as described in 3.9.7.3, for a total of 10 times, as described in 3.8.4 (see Figure 4).

3.9.4 Activity Measures and Dietary Diaries

At baseline and followup, patients will have applied axial accelerometers monitors (AX3; Axivity, Newcastle upon Tyne, UK) for a 3 to 5 day period, to study their Free-Living physical activity.

In addition to this dietary diaries will be handed out as questionnaires at baseline and follow-up for patients to fill in.

3.9.5 Transthoracic echocardiography

Transthoracic echocardiography (TTE) will be performed at CFAS to assess changes in cardiac function and structure. The images will be acquired and analyzed with GE Vivid E95 (GE Vingmed Ultrasound, Horten, Norway) and GE Echopac software. Patients will be examined in the left lateral decubitus position. All echocardiograms will be obtained and analyzed by one of the study clinicians and validated by a cardiologist who is blinded to the allocated study group. The acquisition of images will be done according to the American Society of Echocardiography and the European Association of Cardiovascular Imaging [35]. In parasternal long axis view, the left ventricular wall thicknesses will be determined at end-diastole at the level of the mitral valve leaflet tips. Ventricular mass will be calculated by the formula: $0.8 \cdot 1.04 \cdot [(\text{interventricular septum} + \text{LV internal diameter} + \text{posterior wall thickness})^3 - (\text{LV internal diameter})^3] + 0.6$ g. LV ejection fraction will be determined by Simpson's biplane method. Left atrial volume will be determined by the biplane area-length method. The early (E) to late (A) ventricular filling velocity ratio (E/A), will be determined by Pulsed-wave doppler in apical 4-chamber view. The early mitral annular diastolic

velocity (e') will be determined in both the septal and lateral region using the 4-chamber view. Global longitudinal strain measurements and ejection fraction will be performed by the semi-automatized algorithms in the GE software.

3.9.6 Body composition

To analyze fat and lean body mass, patients will undergo a dual x-ray absorptiometry (DXA) (Lunar Prodigy GE Heathcare, Madison, Wisconsin, USA. enCORE software version 14, 10, 022), under the supervision of experienced staff. Patients will be asked to empty their bladder prior to scanning.

3.9.7 Blood samples

Blood Samples will be taken at multiple times throughout the study, all samples, including the ones specified in 3.9.8 and 3.9.9, will be analyzed at Rigshospitalet unless otherwise noted.

Samples taken in this way will be accessible through the patients medical record at "Labka", and earlier data collected by other physicians will have been needed at the point of drawing these blood tests in order to assess for the SLICC criteria, as described under recruitment.

Blood samples will be referred in the protocol as the "Characterization Package", the "Outcome Package" and the "Exercise Package" in this protocol. A total of 200mL of blood will be drawn at baseline tests and at follow-up tests. For a total of 400mL of blood withdrawn within the 12 weeks of study.

3.9.7.1 Characterization package

Blood samples of approximately 25mL necessary to characterize type and severity of SLE, and also required for the SLICC since the characterization package will only be taken along with the outcome package at baseline (3.8.2), blood tests that are in the outcome package will not be included in the characterization package.

These include:

Antinuclear Antibodies by immunofluorescence, Sjögren Antibodies (SSA & SSB), Anti-Phospholipid antibodies, Anti-Smith Antibodies, Anti U1RNP, and Direct Antiglobulin test (DAT).

3.9.7.2 Outcome package

Fasting (minimum of 10 hours) blood samples of approximately 75mL will be drawn in the morning at CFAS at baseline and follow-up visits 1 and 4 respectively.

They will include

1. Markers of inflammation (Leukocyte differential counts, thrombocytes count and plasma measurements of highly sensitive C-Reactive Protein(hsCRP), ferritin, IL-1, IL-6, soluble IL-6 receptor, IL-10, TNF, IFNα, and IFNγ)
2. Specialized SLE markers of inflammation (Complement C3 and C4, Antibodies to double-stranded DNA, and β2-microglobulin).
3. Metabolic Markers (Glucose, Hba1c) and Lipid status (Total Cholesterol, Nonesterified Fatty Acids (NEFAs), low density lipoproteins (LDL), small dense LDL(sdLDL), triglycerides, high density lipoproteins (HDL))
4. Markers of hydration status (Hematocrit, Sodium, Potassium, Hemoglobin, Creatinine)
5. Liver and Muscle Biomarkers (ALAT, Creatine-Kinase, Myoglobin)

6. Cardiac markers (Troponin T, Troponin I, NT-ProBNP and ANP)
7. Markers of endothelial damage (VCAM, Tissue Plasminogen Activation Factor and von Willebrand Factor).
8. Peripheral whole blood cell mRNA expressions as specified under 3.9.9 (below)
9. Biobank blood test for the CFAS biobank.

3.9.7.3 Exercise Package

Blood sample of approximately 5-10ml to be drawn multiple times during the acute exercise bout: analyzed for markers of inflammation, such as HS-CRP, ferritin, Leukocyte Differential, Il-1, Il-6, soluble Il-6-receptor, Il-10, IFNα, IFNγ, hemoglobin, thrombocytes, sodium, potassium, and hematocrit.

3.9.7.4 OGTT Package

Two blood samples of approximately 5-10 ml to be drawn twice during OGTT, these samples will be analyzed for glucose, insulin, and c-peptide.

3.9.8 Biochemical analyses

Blood sampling will be conducted at baseline and at post-intervention by standard procedures. Blood sampling will include markers for inflammation as above, metabolites, haematology, electrolytes, liver- and renal status, endocrinology, and lipid status. White blood cells will be separated and flow cytometry by a 10-colored antibody panel consisting of seven prefabricated customized DuraClone tubes from Beckman Coulter (Beckman Coulter, Brea, CA, USA) with freeze dried antibodies will be used to analyse surface markers and cytokine expression levels in various white blood cell subpopulations, focusing primarily on IL-6 signalling in monocytes and B lymphocytes.

3.9.9 mRNA expression analyses

During withdrawal of whole blood from patients, 10mL will be transferred to a PAXgene Blood RNA tube (PreAnalytiX, Switzerland), which will then be incubated overnight at room temperature before processing, to complete lysis of nucleated blood cells.

RNA will then be isolated without external stimuli using the PAXgene Blood RNA Kit (Qiagen), following manufacturer instructions. The samples will then be spun in a Sigma 4k10 centrifuge to pellet the nucleic acids, which will then be resuspended, transferred to a PAXgene Shredder spin column and incubated in optimized buffers and Proteinase K to digest proteins. The shredder spin column will then be centrifuged again to homogenize the cell lysate and remove cell debris.

The supernatant of the flow-through fraction will then be transferred to a fresh microcentrifuge tube and ethanol will be added.

Then the lysate will be transferred to a PAXgene RNA spin column and spinned briefly. This will cause RNA to bind to the spin column membrane and with several following wash steps, contaminants will be removed. In between these wash steps the spin column membrane will be treated with DNAase I to remove trace amounts of DNA. Hereafter RNA will be eluted in a buffer (BR5) and heat denatured. Whereafter RNA will be stored at -80°C.

When ready for analysis, these tubes will be thawed on ice and total RNA will be quantified using RT-qPCR or Nanostring (Thermo Fisher, Waltham, MA, USA) technology.

We will then analyze these samples for mRNA gene expression analysis for IFNGS, TNF-related genes, IL-6-Related genes as well as housekeeping genes. In total we will analyze mRNA expression for around 100 different genes. The abbreviations and types of genes as well as their function have been looked up using Genecards.org[36].

The expression of each transcript will be normalized to an average expression of housekeeping genes. We plan to analyze the mRNA expression of three housekeeping genes.

Afterwards, like the process described by Kim et al. we will determine both a Z-score and a Geomean mRNA expression score for all subjects[37]:

3.9.9.1 Standardized mRNA expression scores

Samples will be compared to samples from 10 healthy in-house volunteers, to give a Z-Score:

$$Z - Score_{Gene} = \frac{[Gene\ Count - Mean\ (HC\ gene\ expression)]}{[Standard\ Deviation\ (HC\ Gene\ expression)]}$$

This Z-Score can then be averaged for each module to give a standardized mRNA expression score for all of the modalities below.

3.9.9.2 Geomean mRNA expression Score

Alternatively, the geometric mean of mRNA expressed genes can be calculated as:

$$Geomean - IFNScore = \sqrt[n]{g_1 \cdot g_2 \cdot g_3(\dots) \cdot g_n}$$

So for example:

$$GEOMEAN, M5.12 = \sqrt[8]{[\mathrm{ADAR}] \cdot [\mathrm{BST2}] \cdot [\mathrm{GBP2}] \cdot [\mathrm{IRF9}] \cdot [\mathrm{ISG20}] \cdot [\mathrm{SP100}] \cdot [\mathrm{TRIM21}] \cdot [\mathrm{TRIM25}]}$$

Which gives a score for M5.12 that can be compared across patients with SLE.

This geomean score can be done in absence of healthy volunteers and could be useful for further clinical practice.

3.9.9.3 Interferon Gene Signature Analysis

To analyse the interferon gene signature we will use both the methods by Chaussabel et al. and El-Sherbiny et al.

In summation we will analyse 58 genes for mRNA expression related to IFN.

3.9.9.3.1 Chiche and Chaussabel et al. method

The Chiche and Chaussabel et al. method will be done in WB of modules defined by them as related to IFNGS for SLE; M1.2, M3.4 and M5.12 (see Table 2).

These genes are the same as defined in the modular gene expression by Chaussabel et al. and have been used for SLE patients[18,19].

3.9.9.3.1.1 M1.2	**20 Genes**	
Abbreviation	**Gene**	**Function[36,38] (according to Genecards.org)**
CXCL10	chemokine (C-X-C motif) ligand 10	Protein Coding gene. Diseases associated with CXCL10 include Periapical Periodontitis and Viral Encephalitis. Among its related pathways are Innate Immune System and PEDF Induced Signaling. Gene Ontology (GO) annotations related to this gene include

		signaling receptor binding and chemokine activity. An important paralog of this gene is CXCL9.
DDX60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60	Protein Coding gene. Diseases associated with DDX60 include Lip Cancer and Autosomal Recessive Alport Syndrome. Gene Ontology (GO) annotations related to this gene include nucleic acid binding and hydrolase activity. An important paralog of this gene is DDX60L.
EPSTI1	epithelial stromal interaction 1	Protein Coding gene. Diseases associated with EPSTI1 include Lupus Erythematosus and Systemic Lupus Erythematosus.
HERC5	HECT and RLD domain containing E3 ubiquitin protein ligase 5	Protein Coding gene. Diseases associated with HERC5 include Influenza. Among its related pathways are RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways and Innate Immune System. Gene Ontology (GO) annotations related to this gene include ubiquitin-protein transferase activity. An important paralog of this gene is HERC6.
IFI44	interferon-induced protein 44	Protein Coding gene. Diseases associated with IFI44 include Hepatitis D and Potocki-Shaffer Syndrome. An important paralog of this gene is IFI44L.
IFI44L	interferon-induced protein 44-like	Protein Coding gene. Diseases associated with IFI44L include Lymph Node Tuberculosis and Immunodeficiency 38 With Basal Ganglia Calcification. An important paralog of this gene is IFI44.
IFIT1	interferon-induced protein with tetratricopeptide repeats 1	Protein Coding gene. Diseases associated with IFIT1 include Microphthalmia With Limb Anomalies and Vasculopathy, Retinal, With Cerebral Leukoencephalopathy And Systemic Manifestations. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include RNA binding. An important paralog of this gene is IFIT1B.
IFIT3	interferon-induced protein with tetratricopeptide repeats 3	Protein Coding gene. Diseases associated with IFIT3 include Lupus Erythematosus and Human Cytomegalovirus Infection. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include identical protein binding. An important paralog of this gene is IFIT2.
IFITM3	interferon induced transmembrane protein 3	Protein Coding gene. Diseases associated with IFITM3 include Influenza. Among its related pathways are Innate Immune System and Interferon gamma signaling. An important paralog of this gene is IFITM1.
ISG15	ISG15 ubiquitin-like modifier	Protein Coding gene. Diseases associated with ISG15 include Immunodeficiency 38 With Basal Ganglia Calcification and Influenza. Among its related pathways are RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways and Innate Immune System.

		Gene Ontology (GO) annotations related to this gene include protein tag. An important paralog of this gene is UBC.
LAMP3	lysosomal-associated membrane protein 3	Protein Coding gene. Diseases associated with LAMP3 include Syndromic Oculocutaneous Albinism and Rem Sleep Behavior Disorder.
LY6E	lymphocyte antigen 6 complex, locus E	Protein Coding gene. Diseases associated with LY6E include Acute Promyelocytic Leukemia and West Nile Virus. Among its related pathways are Ectoderm Differentiation and Metabolism of proteins. An important paralog of this gene is GPIHBP1.
MX1	myxovirus (influenza virus) resistance 1	Protein Coding gene. Diseases associated with MX1 include Influenza and Viral Encephalitis. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include GTP binding and GTPase activity. An important paralog of this gene is MX2.
OAS1	2'-5'-oligoadenylate synthetase 1, 40/46kDa	Protein Coding gene. Diseases associated with OAS1 include Infantile-Onset Pulmonary Alveolar Proteinosis-Hypogammaglobulinemia and Pulmonary Alveolar Proteinosis With Hypogammaglobulinemia. Among its related pathways are Interferon gamma signaling and Innate Immune System. Gene Ontology (GO) annotations related to this gene include RNA binding and transferase activity. An important paralog of this gene is OAS3.
OAS2	2'-5' oligoadenylate synthetase 2	Protein Coding gene. Diseases associated with OAS2 include Tick-Borne Encephalitis and Microphthalmia With Limb Anomalies. Among its related pathways are Interferon gamma signaling and Innate Immune System. Gene Ontology (GO) annotations related to this gene include RNA binding and transferase activity. An important paralog of this gene is OAS3.
OAS3	2'-5' oligoadenylate synthetase 3	Protein Coding gene. Diseases associated with OAS3 include Chikungunya and Tick-Borne Encephalitis. Among its related pathways are Interferon gamma signaling and Innate Immune System. Gene Ontology (GO) annotations related to this gene include RNA binding and transferase activity. An important paralog of this gene is OAS2.
OASL	2'-5'-oligoadenylate synthetase-like	Protein Coding gene. Diseases associated with OASL include West Nile Fever and West Nile Virus Infection. Among its related pathways are Interferon gamma signaling and Innate Immune System. Gene Ontology (GO) annotations related to this gene include double-stranded RNA binding. An important paralog of this gene is OAS1.
RSAD2	radical S-adenosyl	Protein Coding gene. Diseases associated with RSAD2 include Yellow Fever and Chikungunya. Among its related pathways are

	methionine domain containing 2	Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include protein self-association and iron-sulfur cluster binding.
RTP4	receptor transporter protein 4	Protein Coding gene. Among its related pathways are Signaling by GPCR. An important paralog of this gene is RTP3.
SERPING1	serine (or cysteine) peptidase inhibitor, clade G, member 1	Protein Coding gene. Diseases associated with SERPING1 include Type 1 Hereditary Angioedema and partial defiency of Complement Component 4. Among its related pathways are Immune response Lectin induced complement pathway and Formation of Fibrin Clot (Clotting Cascade). Gene Ontology (GO) annotations related to this gene include serine-type endopeptidase inhibitor activity. An important paralog of this gene is SERPINF2.
3.9.9.3.1.2 M3.4	**17 Genes**	
Abbreviation	**Gene**	**Function[36,38] (according to Genecards.org)**
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	Protein Coding gene. Diseases associated with DDX58 include Singleton-Merten Syndrome 2 and Singleton-Merten Syndrome. Among its related pathways are RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways and Cytosolic sensors of pathogen-associated DNA. Gene Ontology (GO) annotations related to this gene include nucleic acid binding and hydrolase activity. An important paralog of this gene is ENSG00000288684.
GBP1	guanylate binding protein 1, interferon-inducible	Protein Coding gene. Diseases associated with GBP1 include Chronic Active Epstein-Barr Virus Infection and Aneurysmal Bone Cysts. Among its related pathways are Interferon gamma signaling and NF-kB (NFkB) Pathway. Gene Ontology (GO) annotations related to this gene include identical protein binding and enzyme binding. An important paralog of this gene is GBP3.
GBP3	guanylate binding protein 3	Protein Coding gene. Diseases associated with GBP3 include Retinitis Pigmentosa 7. Among its related pathways are Interferon gamma signaling and Calcineurin-regulated NFAT-dependent transcription in lymphocytes. Gene Ontology (GO) annotations related to this gene include GTP binding and GTPase activity. An important paralog of this gene is GBP1.
GBP5	guanylate binding protein 5	Protein Coding gene. Diseases associated with GBP5 include Chronic Active Epstein-Barr Virus Infection. Among its related pathways are Interferon gamma signaling and NF-kB (NFkB) Pathway. Gene Ontology (GO) annotations related to this gene include identical protein binding and GTPase activity. An important paralog of this gene is GBP3.
HERC6	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase Family Member 6	Protein Coding gene. Diseases associated with HERC6 include Marburg Hemorrhagic Fever. Among its related pathways are Innate Immune System and Class I MHC mediated antigen processing and presentation. Gene Ontology (GO) annotations related to this gene include ligase activity and ubiquitin-protein

		transferase activity. An important paralog of this gene is ENSG00000287542.
IFI35	interferon-induced protein 35	Protein Coding gene. Diseases associated with IFI35 include Stomatitis and Mouth Disease. Among its related pathways are Innate Immune System and Interferon gamma signaling. An important paralog of this gene is NMI.
IFIH1	interferon induced with helicase C domain 1	Protein Coding gene. Diseases associated with IFIH1 include Singleton-Merten Syndrome 1 and Aicardi-Goutieres Syndrome 7. Among its related pathways are RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways and Cytosolic sensors of pathogen-associated DNA. Gene Ontology (GO) annotations related to this gene include nucleic acid binding and hydrolase activity. An important paralog of this gene is DDX58.
IFIT2	interferon-induced protein with tetratricopeptide repeats 2	Protein Coding gene. Diseases associated with IFIT2 include Japanese Encephalitis and Microphthalmia With Limb Anomalies. Among its related pathways are Innate Immune System and Interferon gamma signaling. An important paralog of this gene is IFIT3.
IFIT5	interferon-induced protein with tetratricopeptide repeats 5	Protein Coding gene. Gene Ontology (GO) annotations related to this gene include tRNA binding. An important paralog of this gene is IFIT1.
IFITM1	interferon induced transmembrane protein 1	Protein Coding gene. Diseases associated with IFITM1 include West Nile Virus and Dengue Virus. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include obsolete signal transducer activity, downstream of receptor. An important paralog of this gene is IFITM3.
LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein	Protein Coding gene. Diseases associated with LGALS3BP include Henoch-Schoenlein Purpura and Varicose Veins. Among its related pathways are Response to elevated platelet cytosolic Ca2+. Gene Ontology (GO) annotations related to this gene include scavenger receptor activity. An important paralog of this gene is DMBT1.
PARP9	poly (ADP-ribose) polymerase family, member 9	Protein Coding gene. Diseases associated with PARP9 include B-Cell Lymphoma and Lymphoma. Among its related pathways are Metabolism of water-soluble vitamins and cofactors and Nicotinate metabolism. Gene Ontology (GO) annotations related to this gene include NAD+ ADP-ribosyltransferase activity. An important paralog of this gene is PARP14.
PLSCR1	phospholipid scramblase 1	Protein Coding gene. Diseases associated with PLSCR1 include Scott Syndrome. Among its related pathways are EGF/EGFR Signaling Pathway. Gene Ontology (GO) annotations related to this gene include calcium ion binding and proximal promoter DNA-binding transcription activator activity, RNA polymerase II-specific. An important paralog of this gene is PLSCR2.

SOCS1	suppressor of cytokine signaling 1	Protein Coding gene. Diseases associated with SOCS1 include Autoinflammatory Syndrome, Familial, With Or Without Immunodeficiency and Lipoprotein Quantitative Trait Locus. Among its related pathways are Interferon gamma signaling and TGF-Beta Pathway. Gene Ontology (GO) annotations related to this gene include protein kinase binding and insulin-like growth factor receptor binding. An important paralog of this gene is SOCS7.
STAT1	signal transducer and activator of transcription 1	Protein Coding gene. Diseases associated with STAT1 include Immunodeficiency 31B and Immunodeficiency 31C. Among its related pathways are CNTF Signaling and p70S6K Signaling. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and protein homodimerization activity. An important paralog of this gene is STAT4.
STAT2	signal transducer and activator of transcription 2	Protein Coding gene. Diseases associated with STAT2 include Pseudo-Torch Syndrome 3 and Immunodeficiency 44. Among its related pathways are CNTF Signaling and ERK Signaling. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and identical protein binding.
ZBP1	Z-DNA binding protein 1	Protein Coding gene. Diseases associated with ZBP1 include Herpes Simplex and Influenza. Among its related pathways are Cytosolic sensors of pathogen-associated DNA and Innate Immune System. Gene Ontology (GO) annotations related to this gene include RNA binding and left-handed Z-DNA binding.
3.9.9.3.1.3 M5.12	**8 Genes**	
Abbreviation	**Gene**	**Function[36,38] (according to Genecards.org)**
ADAR	adenosine deaminase, RNA-specific	Protein Coding gene. Diseases associated with ADAR include Dyschromatosis Symmetrica Hereditaria and Aicardi-Goutieres Syndrome 6. Among its related pathways are Innate Immune System and Formation of editosomes by ADAR proteins. Gene Ontology (GO) annotations related to this gene include adenosine deaminase activity. An important paralog of this gene is ADARB1.
BST2	bone marrow stromal cell antigen 2	Protein Coding gene. Diseases associated with BST2 include Stomatitis and Human Immunodeficiency Virus Type 1. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include obsolete signal transducer activity.
GBP2	guanylate binding protein 2	Protein Coding gene. Among its related pathways are Interferon gamma signaling and NF-kB (NFkB) Pathway. Gene Ontology (GO) annotations related to this gene include GTP binding and GTPase activity. An important paralog of this gene is GBP3.
IRF9	interferon regulatory factor 9	Protein Coding gene. Diseases associated with IRF9 include Immunodeficiency 65. Among its related pathways are Interferon gamma signaling and Jak/STAT Signaling Pathway. Gene Ontology (GO) annotations related to this gene include DNA-binding

		transcription factor activity. An important paralog of this gene is IRF4.
ISG20	interferon stimulated exonuclease gene 20kDa	Protein Coding gene. Diseases associated with ISG20 include Hepatitis C and Yellow Fever. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include nucleic acid binding and 3'-5'-exoribonuclease activity. An important paralog of this gene is AEN.
SP100	nuclear antigen Sp100	Protein Coding gene. Diseases associated with SP100 include Autoimmune Cholangitis and Primary Biliary Cholangitis. Among its related pathways are Interferon gamma signaling and SUMOylation. Gene Ontology (GO) annotations related to this gene include protein homodimerization activity and chromatin binding. An important paralog of this gene is SP140.
TRIM21	tripartite motif-containing 21	Protein Coding gene. Diseases associated with TRIM21 include Heart Block, Congenital and Sjogren Syndrome. Among its related pathways are Interferon gamma signaling and Cytosolic sensors of pathogen-associated DNA. Gene Ontology (GO) annotations related to this gene include identical protein binding and ligase activity. An important paralog of this gene is TRIM27.
TRIM25	tripartite motif-containing 25	Protein Coding gene. Diseases associated with TRIM25 include Swine Influenza and Middle East Respiratory Syndrome. Among its related pathways are RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include acid-amino acid ligase activity. An important paralog of this gene is TRIM7.

Table 2 Genes needed to measure IFNGS in the M1.2, M3.4 and M5.12 modules; The interferome Database, Genecards.org and Chiche et al.s supplementals have been used to create this table[19,38–40].

3.9.9.3.2 El-Sherbiny et al. method

In addition to these we will evaluate mRNA for IFNGS as per El-Sherbiny et al.[41] Who has segregated two IFNGS based on the above measurements into a characterizing type 1 IFN related gene signature as well as an activity-dependent type 2 IFN related gene signature.

3.9.9.3.2.1 Type 1 IFN relation			**13 genes (6 unique)**
Abbreviation	**Gene**	**Included in**	**Function**[36,38] **(according to Genecards.org)**
ISG15	ISG15 ubiquitin-like modifier	M1.2	Protein Coding gene. Diseases associated with ISG15 include Immunodeficiency 38 With Basal Ganglia Calcification and Influenza. Among its related pathways are RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways and Innate Immune System. Gene Ontology (GO) annotations related to this gene include protein tag. An important paralog of this gene is UBC.

IFI44	interferon-induced protein 44	M1.2	Protein Coding gene. Diseases associated with IFI44 include Hepatitis D and Potocki-Shaffer Syndrome. An important paralog of this gene is IFI44L.
IFI27	Interferon Alpha Inducible Protein 27	-	Protein Coding gene. Diseases associated with IFI27 include Hepatitis C and Oral Leukoplakia. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include RNA polymerase II activating transcription factor binding and lamin binding. An important paralog of this gene is IFI27L2.
CXCL10	chemokine (C-X-C motif) ligand 10	M1.2	Protein Coding gene. Diseases associated with CXCL10 include Periapical Periodontitis and Viral Encephalitis. Among its related pathways are Innate Immune System and PEDF Induced Signaling. Gene Ontology (GO) annotations related to this gene include signaling receptor binding and chemokine activity. An important paralog of this gene is CXCL9.
RSAD2	radical S-adenosyl methionine domain containing 2	M1.2	Protein Coding gene. Diseases associated with RSAD2 include Yellow Fever and Chikungunya. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include protein self-association and iron-sulfur cluster binding.
IFIT1	interferon-induced protein with tetratricopeptide repeats 1	M1.2	Protein Coding gene. Diseases associated with IFIT1 include Microphthalmia With Limb Anomalies and Vasculopathy, Retinal, With Cerebral Leukoencephalopathy And Systemic Manifestations. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene Ontology (GO) annotations related to this gene include RNA binding. An important paralog of this gene is IFIT1B.
IFI44L	interferon-induced protein 44-like	M1.2	Protein Coding gene. Diseases associated with IFI44L include Lymph Node Tuberculosis and Immunodeficiency 38 With Basal Ganglia Calcification. An important paralog of this gene is IFI44.
CCL8	C-C Motif Chemokine Ligand 8	-	Protein Coding gene. Diseases associated with CCL8 include Tenosynovitis and Tendinitis. Among its related pathways are p70S6K Signaling and TGF-Beta Pathway.

			Gene Ontology (GO) annotations related to this gene include protein kinase activity and chemokine activity. An important paralog of this gene is CCL11.
XAF1	XIAP-Associated Factor 1	-	Protein Coding gene. Among its related pathways are Innate Immune System and Apoptosis and Autophagy. An important paralog of this gene is TRAFD1.
IFI6	Interferon Alpha Inducible Protein 6	-	Protein Coding gene. Diseases associated with IFI6 include Dengue Virus. Among its related pathways are Innate Lymphoid Cells Differentiation and Innate Immune System. An important paralog of this gene is IFI27L2.
GBP1	guanylate binding protein 1, interferon-inducible	M3.4	Protein Coding gene. Diseases associated with GBP1 include Chronic Active Epstein-Barr Virus Infection and Aneurysmal Bone Cysts. Among its related pathways are Interferon gamma signaling and NF-kB (NFkB) Pathway. Gene Ontology (GO) annotations related to this gene include identical protein binding and enzyme binding. An important paralog of this gene is GBP3.
IRF7	interferon regulatory factor 7	-	Protein Coding gene. Diseases associated with IRF7 include Immunodeficiency 39 and Predisposition To Severe Viral Infection Due To Irf7 Deficiency. Among its related pathways are Interferon gamma signaling and Regulation of nuclear SMAD2/3 signaling. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity. An important paralog of this gene is IRF6.
CEACAM1	Carcinoembryonic-Antigen-Related Cell Adhesion Molecule 1	-	Protein Coding gene. Diseases associated with CEACAM1 include Acute Gonococcal Endometritis and Adenomatoid Tumor. Among its related pathways are Adhesion and Innate Immune System. Gene Ontology (GO) annotations related to this gene include protein homodimerization activity. An important paralog of this gene is CEACAM6.

3.9.9.3.2.2 Type 2 IFN relation		**14 genes (7 unique)**	
Abbreviation	**Gene**	**Included in**	**Function[36,38] (according to Genecards.org)**
LAMP3	Lysosomal Associated Membrane Protein 3	M1.2	Protein Coding gene. Diseases associated with LAMP3 include Syndromic Oculocutaneous Albinism and Rem Sleep Behavior Disorder.
IFIH1	interferon induced with helicase C domain 1	M3.4	Protein Coding gene. Diseases associated with IFIH1 include Singleton-Merten Syndrome 1 and Aicardi-Goutieres Syndrome 7. Among its related pathways are RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways and Cytosolic sensors of pathogen-associated DNA. Gene Ontology (GO) annotations related to this gene include nucleic acid binding and hydrolase activity. An important paralog of this gene is DDX58.
PHF11	PHD Finger Protein 11	-	Protein Coding gene. Diseases associated with PHF11 include Asthma and Dermatitis. An important paralog of this gene is PHF6.
SERPING1	serine (or cysteine) peptidase inhibitor, clade G, member 1	M1.2	Protein Coding gene. Diseases associated with SERPING1 include Angioedema, Hereditary, 1 and Partial Deficiency Of Complement Component 4. Among its related pathways are Immune response Lectin induced complement pathway and Formation of Fibrin Clot (Clotting Cascade). Gene Ontology (GO) annotations related to this gene include serine-type endopeptidase inhibitor activity. An important paralog of this gene is SERPINF2.
IFI16	Interferon Gamma-Inducible Protein 16	-	Protein Coding gene. Diseases associated with IFI16 include Herpes Simplex and Genital Herpes. Among its related pathways are Senescence and Autophagy in Cancer and Cytosolic sensors of pathogen-associated DNA. Gene Ontology (GO) annotations related to this gene include transcription factor binding. An important paralog of this gene is PYHIN1.
BST2	bone marrow stromal cell antigen 2	M5.12	Protein Coding gene. Diseases associated with BST2 include Stomatitis and Human Immunodeficiency Virus Type 1. Among its related pathways are Innate Immune System and Interferon gamma signaling. Gene

			Ontology (GO) annotations related to this gene include obsolete signal transducer activity.
SP100	nuclear antigen Sp100	M5.12	Protein Coding gene. Diseases associated with SP100 include Autoimmune Cholangitis and Primary Biliary Cholangitis. Among its related pathways are Interferon gamma signaling and SUMOylation. Gene Ontology (GO) annotations related to this gene include protein homodimerization activity and chromatin binding. An important paralog of this gene is SP140.
NT5C3B	5'-Nucleotidase, Cytosolic III-Like	-	Protein Coding gene. Among its related pathways are Gene Expression and Deadenylation-dependent mRNA decay. Gene Ontology (GO) annotations related to this gene include nucleotide binding and 5'-nucleotidase activity. An important paralog of this gene is NT5C3A.
SOCS1	suppressor of cytokine signaling 1	M3.4	Protein Coding gene. Diseases associated with SOCS1 include Autoinflammatory Syndrome, Familial, With Or Without Immunodeficiency and Lipoprotein Quantitative Trait Locus. Among its related pathways are Interferon gamma signaling and TGF-Beta Pathway. Gene Ontology (GO) annotations related to this gene include protein kinase binding and insulin-like growth factor receptor binding. An important paralog of this gene is SOCS7.
TRIM38	Tripartite Motif Containing 38	-	rotein Coding gene. Diseases associated with TRIM38 include Fanconi Renotubular Syndrome 2. Among its related pathways are Interferon gamma signaling and Innate Immune System. Gene Ontology (GO) annotations related to this gene include ligase activity and obsolete signal transducer activity. An important paralog of this gene is TRIM21.
UNC93B1	Unc-93 Homolog B1, TLR Signaling Regulator	-	Protein Coding gene. Diseases associated with UNC93B1 include Encephalopathy, Acute, Infection-Induced 1 and Herpes Simplex Encephalitis. Among its related pathways are Innate Immune System and Activated TLR4 signalling. Gene Ontology (GO) annotations related to this gene include Toll-like receptor binding. An important paralog of this gene is UNC93A.

UBE2L6	Ubiquitin/ISG15-Conjugating Enzyme E2 L6	-	Protein Coding gene. Among its related pathways are RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways and Innate Immune System. Gene Ontology (GO) annotations related to this gene include ligase activity and acid-amino acid ligase activity. An important paralog of this gene is UBE2L5.
STAT1	signal transducer and activator of transcription 1	M3.4	Protein Coding gene. Diseases associated with STAT1 include Immunodeficiency 31B and Immunodeficiency 31C. Among its related pathways are CNTF Signaling and p70S6K Signaling. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and protein homodimerization activity. An important paralog of this gene is STAT4.
TAP1	Transporter 1, ATP Binding Cassette Subfamily B Member	-	Protein Coding gene. Diseases associated with TAP1 include Immunodeficiency By Defective Expression Of Mhc Class I and Bare Lymphocyte Syndrome, Type I. Among its related pathways are Thrombopoetin signaling via JAK-STAT pathway and Innate Immune System. Gene Ontology (GO) annotations related to this gene include protein homodimerization activity and ATPase activity, coupled to transmembrane movement of substances. An important paralog of this gene is TAP2.

Table 3 Genes related to type 1 and 2 IFN according to El-Sherbiny et al.[41]

3.9.9.3.3 Siddiqi et al. method

These genes will be analyzed due to the findings by Siddiqi et al.[42]

3.9.9.3.3.1 Type 2 IFN relation		3 genes (3 unique)	
Abbreviation	Gene	Included in	Function[36,38] (according to Genecards.org)
S100A9	S100 Calcium Binding Protein A9	Siddiqi et al	Protein Coding gene. Diseases associated with S100A9 include Cystic Fibrosis and Juvenile Rheumatoid Arthritis. Among its related pathways are MyD88 dependent cascade initiated on endosome and Diseases of Immune System. Gene Ontology (GO) annotations related to this gene include calcium ion binding

			and microtubule binding. An important paralog of this gene is S100A12.
S100A8	S100 Calcium Binding Protein A8	Siddiqi et al.	Protein Coding gene. Diseases associated with S100A8 include Peptic Ulcer Disease and Duodenal Ulcer. Among its related pathways are MyD88 dependent cascade initiated on endosome and Diseases of Immune System. Gene Ontology (GO) annotations related to this gene include calcium ion binding and RAGE receptor binding. An important paralog of this gene is S100A12.
FCGR1A	Fc Gamma Receptor Ia	Siddiqi et al.	Protein Coding gene. Diseases associated with FCGR1A include Peritonitis and Pharyngitis. Among its related pathways are ADORA2B mediated anti-inflammatory cytokines production and Regulation of actin dynamics for phagocytic cup formation. Gene Ontology (GO) annotations related to this gene include obsolete signal transducer activity, downstream of receptor and IgG binding. An important paralog of this gene is FCRLB.

3.9.9.3.4 Assumed presentation of IFNGS

Assuming our patient population is similar to the one described by Chiche et al. we should see three groups of increasing IFNGS in the M1.2 then the M3.4 and then the M5.12 modules as well as increasing SLEDAI-2K scores (see Figure 5)[19], since our population is selected for a lower SLEDAI -2Kscore in order to tolerate the intervention better, we will likely see a skew towards fewer patients with a high M3.4 and M5.12 scores. But as our hypotheses is related to the activity of IFNα, all three groups with positive scores in any module should have reduced effect of exercise as described in the outcomes (see section 3.6).

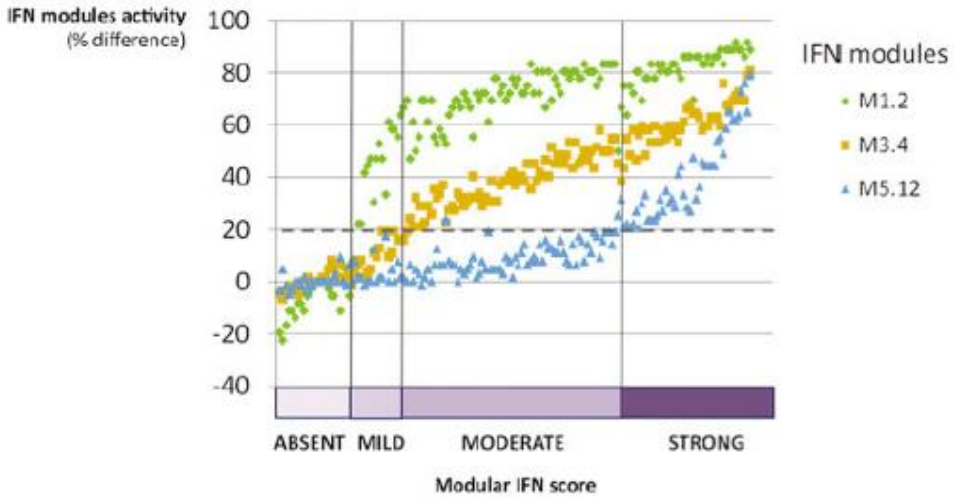

Figure 5 IFN score from Chiche et al.[19]

3.9.9.4 Analysis of Il-6 and TNFα related mRNA in peripheral blood

In addition to the analysis for IFNGS we will analyse mRNA in peripheral blood for transcripts of mRNA induced by IL-6 and TNFα. In summation we will analyse 38 genes for mRNA expression related to IL-6 and TNFα.

Whole blood will be procured and stored in a Pax-Gene tube as per 3.9.9 above, but will be analysed for mRNA transcripts for IL-6 and TNFα related genes as outlined below.

3.9.9.4.1 IL-6 Related mRNA expression

For Il-6 related gene expression, as done by other studies we will analyse Monocyte Chemotactic Protein 1 (MCP-1, which is also induced by IFN-γ)[43], SOCS3[44] and the IL-6 responsive genes of circulating macrophages which are important drivers of the acute phase response denoted by Jura et al.[45].

In addition to this, Robson-Ansley et al. has done methylation evaluation on healthy volunteers and their DNA demethylation in peripheral blood (which increases the transcription of the gene) as a result of the myokine activity of Il-6[46], we will analyse these genes as well, knowing that we are analysing mRNA expression in contrast to the original study.

For the list of genes will analyse and relate to IL-6 activity see Table 4.

3.9.9.4.1.1 IL-6	**27 Genes (27 Unique)**		
Abbreviation	**Gene**	**From Study**	**Function[36,38] (according to Genecards.org)**
α2M	Alpha-2-Macroglobulin	Fey 1991[47]	Protein Coding gene. Diseases associated with A2M include Alzheimer Disease and Alpha-2-Macroglobulin Deficiency. Among its related pathways are A-beta Uptake and Degradation and IL6-mediated signaling events. Gene Ontology (GO) annotations related to this gene include signaling receptor binding and serine-type endopeptidase inhibitor activity. An important paralog of this gene is PZP.
ADCY7	Adenylate Cyclase 7	Robson-Ansley 2014[46]	Protein Coding gene. Diseases associated with ADCY7 include Inhalation Anthrax. Among its related pathways are Aquaporin-mediated transport and Activation of cAMP-Dependent PKA. Gene Ontology (GO) annotations related to this gene include phosphorus-oxygen lyase activity and adenylate cyclase activity. An important paralog of this gene is ADCY2.
AER61/EOGT	EGF Domain-Specific O-Linked N-Acetylglucosamine (GlcNAc) Transferase	Robson-Ansley 2014[46]	This gene encodes an enzyme that acts in the lumen of the endoplasmic reticulum to catalyze the transfer of N-acetylglucosamine to serine or threonine residues of extracellular-targeted proteins. This enzyme modifies proteins containing eukaryotic growth factor (EGF)-like domains, including the Notch receptor, thereby regulating

			developmental signalling. Mutations in this gene have been observed in individuals with Adams-Oliver syndrome 4. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Aug 2015]
Ar [45]	Androgen Receptor		Protein Coding gene. Diseases associated with AR include Androgen Insensitivity, Partial and Spinal And Bulbar Muscular Atrophy, X-Linked 1. Among its related pathways are Coregulation of Androgen receptor activity and Integrated Breast Cancer Pathway. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and chromatin binding. An important paralog of this gene is NR3C2.
BRCA2	Breast And Ovarian Cancer Susceptibility Gene, Early Onset	Robson-Ansley 2014[46]	Protein Coding gene. Diseases associated with BRCA2 include Fanconi Anemia, Complementation Group D1 and Breast Cancer. Among its related pathways are Cell Cycle, Mitotic and Integrated Breast Cancer Pathway. Gene Ontology (GO) annotations related to this gene include protease binding and histone acetyltransferase activity.
C10orf89/PSTK	Phosphoseryl-TRNA Kinase	Robson-Ansley 2014[46]	Protein Coding gene. Diseases associated with PSTK include Ivic Syndrome and Ischiocoxopodopatellar Syndrome With Or Without Pulmonary Arterial Hypertension. Among its related pathways are Peptide chain elongation and Metabolism. Gene Ontology (GO) annotations related to this gene include kinase activity and tRNA binding.
CD11a/ITGAL[48,49]	Cluster of Differentiation 11a / Integrin Subunit Alpha L		Protein Coding gene. Diseases associated with ITGAL include Immune-Complex Glomerulonephritis and Chronic Venous Insufficiency. Among its related pathways are Integrin cell surface interactions and ERK Signaling. Gene Ontology (GO) annotations related to this gene include protein heterodimerization activity and cell adhesion molecule binding. An important paralog of this gene is ITGAX.
CD70/ TNFSF7[48,49]	Cluster of Differentiation 70 / Tumor Necrosis Factor Ligand Superfamily Member 7		Protein Coding gene. Diseases associated with CD70 include Lymphoproliferative Syndrome 3 and Acute Myocarditis. Among its related pathways are TRAF Pathway and Innate Immune System. Gene Ontology (GO) annotations related to this gene include

			signaling receptor binding and protease binding.
CDK9	Cyclin dependant kinase 9	Robson-Ansley 2014[46]	Protein Coding gene. Diseases associated with CDK9 include Herpes Simplex and Human Cytomegalovirus Infection. Among its related pathways are HIV Life Cycle and Formation of HIV-1 elongation complex containing HIV-1 Tat. Gene Ontology (GO) annotations related to this gene include transferase activity, transferring phosphorus-containing groups and protein tyrosine kinase activity. An important paralog of this gene is CDK13.
CREB1[45]	Cyclic AMP-Responsive Element-Binding Protein 1		Protein Coding gene. Diseases associated with CREB1 include Histiocytoma, Angiomatoid Fibrous and Melanoma Of Soft Tissue. Among its related pathways are PI3K/AKT activation and Activated TLR4 signalling. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and enzyme binding. An important paralog of this gene is CREM.
FCRL2	Fc Receptor-Like 2	Robson-Ansley 2014[46]	Protein Coding gene. Diseases associated with FCRL2 include Spastic Paraplegia 62, Autosomal Recessive. Gene Ontology (GO) annotations related to this gene include SH3/SH2 adaptor activity. An important paralog of this gene is FCRL3.
Foxq1[45]	Hepatocyte Nuclear Factor 3 Forkhead Homolog 1		Protein Coding gene. Diseases associated with FOXQ1 include Anterior Segment Dysgenesis 3 and Ectodermal Dysplasia 5, Hair/Nail Type. Among its related pathways are Preimplantation Embryo. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and DNA-binding transcription factor activity, RNA polymerase II-specific. An important paralog of this gene is FOXD1.
GADD45β[45]	Growth Arrest and DNA Damage Inducible Beta		Protein Coding gene. Among its related pathways are Development TGF-beta receptor signaling and miRNA Regulation of DNA Damage Response. An important paralog of this gene is GADD45A.
GRP1/ CYTH3	General receptor for phosphoinositides 1-associated scaffold protein / Cytohesin-3	Robson-Ansley 2014[46]	Protein Coding gene. Among its related pathways are Arf6 signaling events and Vesicle-mediated transport. Gene Ontology (GO) annotations related to this gene include phosphatidylinositol-3,4,5-trisphosphate

			binding and ARF guanyl-nucleotide exchange factor activity. An important paralog of this gene is CYTH1.
HSP90AB1	Heat Shock Protein 90kDa Alpha (Cytosolic), Class B Member 1	A. STEPHANOU et al. 1997[50]	Protein Coding gene. Among its related pathways are Cell Cycle, Mitotic and PI3K-Akt signaling pathway. Gene Ontology (GO) annotations related to this gene include RNA binding and protein kinase binding. An important paralog of this gene is HSP90AA1.
IER3[45]	Immediate Early Response 3		Protein Coding gene. Diseases associated with IER3 include Cortical Dysplasia, Complex, With Other Brain Malformations 6 and Gilles De La Tourette Syndrome. Among its related pathways are PI3K/AKT activation and RET signaling.
IRAK3	Interleukin-1 receptor-associated kinase	Robson-Ansley 2014[46]	Protein Coding gene. Diseases associated with IRAK3 include Asthma-Related Traits 5 and Asthma. Among its related pathways are IL-1 Family Signaling Pathways and ERK Signaling. Gene Ontology (GO) annotations related to this gene include protein homodimerization activity and protein kinase activity. An important paralog of this gene is IRAK1.
JDP2	Jun Dimerization protein 2	Robson-Ansley 2014[46]	Protein Coding gene. Diseases associated with JDP2 include Granulomatous Amebic Encephalitis and Primary Amebic Meningoencephalitis. Among its related pathways are IL-1 Family Signaling Pathways and Tacrolimus/Cyclosporine Pathway, Pharmacodynamics. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and protein heterodimerization activity. An important paralog of this gene is ATF3.
LRG-1[51]	Leucine Rich Alpha-2-Glycoprotein 1		Protein Coding gene. Diseases associated with LRG1 include Appendicitis and Normal Pressure Hydrocephalus. Among its related pathways are Innate Immune System. Gene Ontology (GO) annotations related to this gene include transforming growth factor beta receptor binding. An important paralog of this gene is CHADL.

LDB2	LIM Domain Binding 2	Robson-Ansley 2014[46]	
MCP-1/CCL2[43]	Monocyte Chemotactic Protein 1 / C-C Motif Chemokine Ligand 2		Protein Coding gene. Diseases associated with CCL2 include Neural Tube Defects and Human Immunodeficiency Virus Type 1. Among its related pathways are p70S6K Signaling and A-beta Uptake and Degradation. Gene Ontology (GO) annotations related to this gene include protein kinase activity and heparin binding. An important paralog of this gene is CCL7.
MGP	Matrix GLA Protein	Robson-Ansley 2014[46]	Protein Coding gene. Diseases associated with MGP include Keutel Syndrome and Peripheral Pulmonary Stenosis. Among its related pathways are NOTCH1 regulation of human endothelial cell calcification and Validated transcriptional targets of AP1 family members Fra1 and Fra2. Gene Ontology (GO) annotations related to this gene include calcium ion binding and structural constituent of bone.
PLAUR[45]	Plasminogen Activator, Urokinase Receptor		Protein Coding gene. Diseases associated with PLAUR include Cancer of the Ureter and Paroxysmal Nocturnal Hemoglobinuria. Among its related pathways are Post-translational modification- synthesis of GPI-anchored proteins and Transcription Receptor-mediated HIF regulation. Gene Ontology (GO) annotations related to this gene include signaling receptor binding. An important paralog of this gene is LYPD3.
RAG 1[52,53]	recombination activating gene 1		Protein Coding gene. Diseases associated with RAG1 include Combined Cellular And Humoral Immune Defects With Granulomas and Alpha/Beta T-Cell Lymphopenia With Gamma/Delta T-Cell Expansion, Severe Cytomegalovirus Infection, And Autoimmunity. Among its related pathways are Interleukin-7 signaling and RET signaling. Gene Ontology (GO) annotations related to this gene include protein homodimerization activity and ubiquitin-protein transferase activity.
RAG 2[46,52,53]	recombination activating gene 2	Robson-Ansley 2014[46] (downregulated)	Protein Coding gene. Diseases associated with RAG2 include Omenn Syndrome and Combined Cellular And Humoral Immune Defects With Granulomas. Among its related pathways are Interleukin-7 signaling and RET signaling. Gene Ontology (GO)

			annotations related to this gene include chromatin binding and methylated histone binding.
RELA[45]	RELA Proto-Oncogene, NF-KB Subunit / Nuclear Factor of Kappa Light Polypeptide Gene Enhancer In B-Cells 3		Protein Coding gene. Diseases associated with RELA include Mucocutaneous Ulceration, Chronic and Rela Fusion-Positive Ependymoma. Among its related pathways are Activated TLR4 signalling and TNFR1 Pathway. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and identical protein binding. An important paralog of this gene is REL.
SOCS3[44,54]	Suppressor of cytokine signaling 3		Protein Coding gene. Diseases associated with SOCS3 include Dermatitis, Atopic, 4 and Overnutrition. Among its related pathways are Interferon gamma signaling and TGF-Beta Pathway. Gene Ontology (GO) annotations related to this gene include protein kinase inhibitor activity. An important paralog of this gene is CISH.
VEGFA[55]	Vascular Endothelial Growth Factor A		Protein Coding gene. Diseases associated with VEGFA include Microvascular Complications Of Diabetes 1 and Poems Syndrome. Among its related pathways are ERK Signaling and VEGF ligand-receptor interactions. Gene Ontology (GO) annotations related to this gene include protein homodimerization activity and protein heterodimerization activity. An important paralog of this gene is PGF.
STAT-3	Signal Transducer and Activator of Transcription 3	Suggested by Kanwal Siddiqi[42]	Protein Coding gene. Diseases associated with STAT3 include Autoimmune Disease, Multisystem, Infantile-Onset, 1 and Hyper-Ige Recurrent Infection Syndrome 1, Autosomal Dominant. Among its related pathways are IL-9 Signaling Pathways and Prolactin Signaling. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and sequence-specific DNA binding. An important paralog of this gene is STAT1.

Table 4 Genes related to IL-6 to be tested for mRNA expression

3.9.9.4.2 TNFα Related mRNA expression

TNFα related genetic expression of mRNA were found from the study by Smiljanovic et al.[56] who did in vitro stimulation of whole blood with TNFα to study the mRNA expression of circulating macrophages. As well as other authors, noted in Table 5.

Only the genes upregulated by Il-6 and TNFα respectively will be included in the geomean and standardised scores for these genes (see above)

3.9.9.4.2.1 TNFα	**15 Genes (13 Unique)**	
Abbreviation	**Gene**	**Function[36,38] (according to Genecards.org)**
ADAM17[56]	A Disintegrin And Metalloproteinase domain 17	Protein Coding gene. Diseases associated with ADAM17 include Inflammatory Skin And Bowel Disease, Neonatal, 1 and Neonatal Inflammatory Skin And Bowel Disease. Among its related pathways are Signaling by NOTCH1 and NOTCH2 Activation and Transmission of Signal to the Nucleus. Gene Ontology (GO) annotations related to this gene include SH3 domain binding and integrin binding. An important paralog of this gene is ADAM10.
ARNTL2[56]	Aryl Hydrocarbon Receptor Nuclear Translocator Like 2	Protein Coding gene. Diseases associated with ARNTL2 include Hepatorenal Syndrome and Acute Kidney Failure. Among its related pathways are Circadian rythm related genes and BMAL1-CLOCK,NPAS2 activates circadian gene expression. Gene Ontology (GO) annotations related to this gene include DNA-binding transcription factor activity and protein dimerization activity. An important paralog of this gene is ARNTL.
CASP3[56]	Caspase 3	Protein Coding gene. Diseases associated with CASP3 include Oropharynx Cancer and Ischemia. Among its related pathways are TNFR1 Pathway and ERK Signaling. Gene Ontology (GO) annotations related to this gene include peptidase activity and cysteine-type peptidase activity. An important paralog of this gene is CASP7.
CASP5[56]	Caspase 5	Protein Coding gene. Diseases associated with CASP5 include Cowpox and Familial Mediterranean Fever. Among its related pathways are TRAF Pathway and Apoptosis and Autophagy. Gene Ontology (GO) annotations related to this gene include cysteine-type endopeptidase activity and scaffold protein binding. An important paralog of this gene is CASP4.
CCR1[56]	C-C Motif Chemokine Receptor 1	Protein Coding gene. Diseases associated with CCR1 include Behcet Syndrome and Immune-Complex Glomerulonephritis. Among its related pathways are Innate Immune System and Chemokine Superfamily: Human/Mouse Ligand-Receptor Interactions. Gene Ontology (GO) annotations related to this gene include G protein-coupled receptor activity and chemokine receptor activity. An important paralog of this gene is CCR3.
CD22/ SIGLEC2 [56]	Sialic Acid-Binding Ig-Like Lectin 2	Protein Coding gene. Diseases associated with CD22 include Refractory Hematologic Cancer and Hairy Cell Leukemia. Among its related pathways are Antigen activates B Cell Receptor (BCR) leading to generation of second messengers and Innate Immune System. Gene Ontology (GO) annotations related to this gene include carbohydrate binding. An important paralog of this gene is SIGLEC1.

GJA3[56]	Gap Junction Protein Alpha 3	Protein Coding gene. Diseases associated with GJA3 include Cataract 14, Multiple Types and Early-Onset Posterior Polar Cataract. Among its related pathways are Myometrial Relaxation and Contraction Pathways and Gap junction trafficking. Gene Ontology (GO) annotations related to this gene include gap junction channel activity. An important paralog of this gene is GJA8.
GPR35[56]	G Protein-Coupled Receptor 35	Protein Coding gene. Diseases associated with GPR35 include Cholangitis, Primary Sclerosing and Short-Rib Thoracic Dysplasia 10 With Or Without Polydactyly. Among its related pathways are Peptide ligand-binding receptors and Signaling by GPCR. Gene Ontology (GO) annotations related to this gene include G protein-coupled receptor activity. An important paralog of this gene is GPR55.
MAP3K7IP3/ TAB3[56]	Mitogen-Activated Protein Kinase Kinase Kinase 7 Interacting Protein /TGF-Beta Activated Kinase 1 (MAP3K7)	Protein Coding gene. Among its related pathways are Activated TLR4 signalling and MAP Kinase Signaling. An important paralog of this gene is TAB2.
MCP-1/CCL2[43]	Monocyte Chemotactic Protein 1 / C-C Motif Chemokine Ligand 2	Protein Coding gene. Diseases associated with CCL2 include Neural Tube Defects and Human Immunodeficiency Virus Type 1. Among its related pathways are p70S6K Signaling and A-beta Uptake and Degradation. Gene Ontology (GO) annotations related to this gene include protein kinase activity and heparin binding. An important paralog of this gene is CCL7.
PLAUR (downregulated) [56]	Plasminogen Activator, Urokinase Receptor	Protein Coding gene. Diseases associated with PLAUR include Cancer of the Ureter and Paroxysmal Nocturnal Hemoglobinuria. Among its related pathways are Post-translational modification-synthesis of GPI-anchored proteins and Transcription Receptor-mediated HIF regulation. Gene Ontology (GO) annotations related to this gene include signaling receptor binding. An important paralog of this gene is LYPD3.
PPAP2A / PLPP1 [56]	Phospholipid Phosphatase 1	Protein Coding gene. Diseases associated with PLPP1 include Myxosarcoma. Among its related pathways are Androgen receptor signaling pathway and triacylglycerol biosynthesis. An important paralog of this gene is PLPP2.
PPP1CA[56]	Protein Phosphatase 1 Catalytic Subunit Alpha	Protein Coding gene. Diseases associated with PPP1CA include Venezuelan Equine Encephalitis and Encephalitis. Among its related pathways are Circadian rythm related genes and Development Ligand-independent activation of ESR1 and ESR2. Gene Ontology (GO) annotations related to this gene include hydrolase activity and phosphoprotein phosphatase activity. An important paralog of this gene is PPP1CC.
SPP1[56]	Secreted Phosphoprotein 1	Protein Coding gene. Diseases associated with SPP1 include Pediatric Systemic Lupus Erythematosus and Urolithiasis. Among its related pathways are Integrin cell surface interactions and Development_Hedgehog and PTH signaling pathways in

		bone and cartilage development. Gene Ontology (GO) annotations related to this gene include cytokine activity and extracellular matrix binding.
SYNGR3[56]	Synaptogyrin 3	Protein Coding gene. Diseases associated with SYNGR3 include Deafness, Autosomal Recessive 74 and Deafness, Autosomal Recessive 86. Gene Ontology (GO) annotations related to this gene include protein N-terminus binding and SH2 domain binding. An important paralog of this gene is SYNGR1.

Table 5 Genes related to TNFa to be tested for mRNA expression

3.9.10 Urine Samples

Urine samples will be collected, and analyzed by dipstick at CFAS screening for hematuria, proteinuria, glucosuria, leukocyturia and nitrituria.

3.9.11 SLE disease activity measures

At baseline and follow-up, PROMs will be collected from questionnaires including Fatigue Severity Scale and VAS fatigue, VAS pain and Short Form (SF)-36 Health Survey[33].

Patients will be evaluated by a blinded study MD, using the SLEDAI-2K[26] system (With the SELENA modifications), the SR-50, Q-SLAQ score[57], as well as VAS Physician of overall disease at both inclusion and follow-up.

3.9.12 ^{82}Rb-PET Scan

A subgroup of patients, chosen from their Lupus Anticoagulant and Sjögren antibody status, up to 40, will undergo a serial rest–stress imaging on a Biograph mCT 128-slice scanner (Siemens Healthcare) using list-mode 3D acquisition after ^{82}Rb infusion (Bracco Diagnostics). Low-dose CT (0.4 mSv; 120 kVp; effective tube current, 26 mA [11-mAs quality reference]; 3.3 seconds) will be performed for attenuation correction during normal breathing. Stress will be induced with adenosine infused at 140 cg/kg/min for 6 minutes, with acquisition starting 2.5 minutes later. Coronary artery calcium score (CACS) images and the quantitation of pericardial fat will be acquired from a non-contrast breath-hold CT at the first scan. Myocardial blood flow will be quantitated from dynamic rest and stress images with quantitative-gated nuclear imaging based on a single compartment 82Rb tracer kinetics model using syngo.MBF (Siemens Medical Solutions, Version VB15, Illinois, USA), and Corridor4DM v. 2015.0.0.44 (INVIA, LLC, Ann Abor, USA). The myocardial flow reserve, which may be used to unveil microvascular disease in these patients will be calculated as myocardial blood flow at adenosine-induced maximal hyperaemia divided by resting myocardial blood flow. Based on left ventricular contours, ventricular volumes and ejection fraction will be determined.

3.9.13 Oral glucose tolerance test

A standard 2-hour 75 g OGTT will be performed with a serial of blood drawn at baseline, after 60 min and after 120 min. These samples will be analyzed for glucose, insulin, and c-peptide as per 3.9.7.4.

3.9.14 Lung function

Standardised lung function testing will be performed in accordance with consensus guidelines at using equipment at CFAS. This encompasses dynamic spirometry[58], body plethysmography[59], and diffusing capacity[60]. Based on summary equations[61,62], the expected values according to height, age and sex will be calculated.

3.9.15 Nailfold Capillaroscopy

Peripheral capillaries will be assessed at baseline and follow-up by nailfold capillaroscopy, a technique routinely used in the clinic for patients with systemic scleroderma, SLE or dermatomyositis. This is a non-invasive examination where a clinician uses a capillaroscope, in essence a powerful digital microscope, to examine the peripheral capillaries of the subjects nailfolds[63].

3.9.16 Autonomic Nerve Function tests

Using a handheld external device that measured the first lead of an ECG, participants will be instructed to stand up from supine position, do a controlled breathing exercise and then a Valsalva manoeuvre while measuring the RR interval.

3.9.17 Muscle biopsies

Muscle biopsies will not be required for participation in the study and will be entirely optional.

After anesthetising the skin with up to 10ml Lidocaine 20mg/Noradrenalin5microgram/ml, approximately 100-200 mg of skeletal muscle tissue will be extracted from the quadriceps muscle under sterile conditions. The samples are then immediately frozen in liquid nitrogen and stored at −80°C until analysis.

Upon completion of the study, the samples will be analysed en bloc for fiber type, inflammatory markers, expression of multiple genes, including, but not limited to: NF-κB p65 DNA binding activity (ELISA), phosphorylated and total JNK, phosphorylated AMPK (p-AMPK) total AMPK (Western blotting), mRNA expression of genes as described in 3.9.9, as well as NF-κB binding activity (Western blotting).

3.9.18 Data analysis

At least 10 participants in each intervention group and 10 participants in each control group, will be included to obtain a significance level of 0.05 with a power of 80% to observe an increase in VO_2max of 1.14ml/kg/min with a SD of 2.15 in the control group and an increase in VO_2max of 3.80ml/kg/min with a SD of 3.34 in the intervention group based on a previous publication[5]. If we assume a drop-out rate of less than 30% with 15 participants in each group, we will have at least a power of 80% even with 30% drop-out. With 14 participants in each group, we will achieve a power of 90%.

Sample Size	
Group 1	10
Group 2	10
Total	20
Study Parameters	
Mean, group 1	1.14
Mean, group 2	3.8
Alpha	0.05
Beta	0.2
Power	0.8

Similar results were gained when examining the study by Boström et al.[64] were the control group had an increase in VO_2max of 1.7ml/kg/min with an SD of 1.3 and the intervention group had an increase of 3.2ml/kg/min with an SD of 1.2. A significance of 0.05 and a power of 80% requires 12 subjects in each group.

Since we hope to be able to part our groups into patients with higher IFNGS being the patients with M3.4 and M5.12 and we assume one third of our patients will be in this group, including 30 patients for intervention will give us 10 “high IFNGS intervention” patients in total for this power calculation.

Results will be analysed according to a pre-defined statistical analysis plan and initial modelling of the primary outcome will be based on Analysis of Covariance (ANCOVA) performed with change (post-measure minus pre-measure) as the dependent outcome, with a fixed factor for group (two levels) and applying the value at baseline as a covariate.

$$k = \frac{n_2}{n_1} = 1$$

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2/K)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

$$n_1 = \frac{(2.15^2 + 2.15^2/1)(1.96 + 0.84)^2}{2.66^2}$$

$$n_1 = 10$$

$$n_2 = K * n_1 = 10$$

$\Delta = |\mu_2 - \mu_1|$ = absolute difference between two means
σ_1, σ_2 = variance of mean #1 and #2
n_1 = sample size for group #1
n_2 = sample size for group #2
α = probability of type I error (usually 0.05)
β = probability of type II error (usually 0.2)
z = critical Z value for a given α or β
k = ratio of sample size for group #2 to group #1

4 Ethical considerations

4.1 General information

This study will be conducted in accordance with Regional Ethics Committee (REC) and the Declaration of Helsinki. All participants must sign an informed consent form before enrolment. The protocol will be submitted to the Capital Regional Ethics Committee and to the Danish Data Protection Agency. Information regarding participants of the project will be protected in accordance with the applicable laws of Denmark including laws concerning the processing of personal data (“databeskyttelsesforordningen” and “databeskyttelsesloven”). Amendments and modifications to

the protocol will be sent to the Regional Ethics Comittee and the Danish Data Protection Agency for approval and no changes to the protocol will be established before approval.

4.2 Initiative for the project

The idea and development of the project was done by Center for Physical Activity Research by BKP as well as HEL, RHC, RMB collaborated with PHØ and SJA to formulate the foundation of the study. Since then MLA, SJØ, RHC, PHØ, HEL, RMB and SJA have adapted the study protocol. Further revisions have been done by MLA and SJA in collaboration. There are no economic interests in the study, funding has been sought by RMB, RHC, MLA and SJA.

4.3 Access to patient information

The medical doctors affiliated with this study, will seek access to the patients' medical chart ("Sundhedsplatformen"), to obtain information regarding previous diagnoses that could jeopardize the participation in the study. The patients' blood samples that are drawn as part of this study will be analyzed by the Department of Clinical Biochemistry at Rigshospitalet. Information regarding these blood samples will also be obtained through access to the patients' medical chart. Any health information regarding the patient will only be obtained following informed oral and written consent from the patient.

4.4 Novelty and importance

We expect this study to provide novel insight into the physiological response to exercise in SLE patients that differ in their IFN gene signature. As well as give clinicians an idea of the differing needs of patients in this heterogenous group of patients. Allowing physicians to further tailor medical practice to the individual. Furthermore, this research will help elucidate the complex relationship between auto-immune diseases and exercise.

4.5 Risks and disadvantages of interventions

Evidence shows that exercise for stable patients with SLE is safe[5,20], and it has proven to be clinically beneficial on patient reported outcome measures of fatigue, but not necessarily on their SLE activity. Therefore, we are not asking our control group to seize their current physical activities, but rather we are adding on exercise in the intervention group.

We believe that these 12 weeks of not increasing exercise hold little risk for the control groups, who after the study will be instructed in the exercise methods that the intervention group received.

4.6 Risks and disadvantages of baseline and follow-up tests

During the study, blood samples of up to 400 ml will be drawn from the patient. Half of this over multiple days on the baseline assessments and half of this 12 weeks after at follow-up assessments.

The risks of dizziness, infections or anemia are minimal. Patients will be informed to contact one of the medical doctors affiliated with this study, if the patient suspects infection surrounding the penetrated skin (redness, swelling, fever, heat).

A VO_2max test is considered safe but may cause patients to feel dizzy immediately following the test. An oral glucose tolerance test is considered safe. A lung function test is considered safe but may cause patients to feel dizzy for a limited time. An echocardiography following standard clinical procedures is considered safe and does not in itself pose any risks. ^{82}Rb-PET/CT will be performed by specialized medical technologists at the Dept. of Clinical Physiology, Nuclear Medicine and PET at Rigshospitalet, who routinely do this. The scan itself, including the injection of tracer, is not associated with any discomfort, but an intravenous line must be placed which is associated with the same risks as normal blood sampling as described above. Adenosine infusion may cause some

temporary discomfort, notably palpitations, breathlessness, headache, dizziness, and fatigue; adenosine has a half-life in the blood stream of less than 30 seconds, and these symptoms will cease within minutes after the 6-minute adenosine infusion is discontinued. The stress scan is performed under the immediate supervision of a nuclear medicine physician.

Prior to the muscle biopsy, local anesthesia will be administered, which is associated with short-lasting discomfort. After a few minutes, the area is numbed, and a small skin incision is made. The Bergstrom cannula is inserted in M. vastus lateralis and the biopsy is taken. This part of the procedure might be associated with slight pain. The procedure takes a few minutes and after the biopsy is taken, the wound will be closed with Steri-Strips, which must be kept on for five days. There will be an additional patch, which can be removed after two days. Subjects might experience some degree of muscle pain after the biopsy. Paracetamol,1000 mg, max. four times a day will be recommended as pain killers. The procedure can leave small bruises, but generally heals nicely. Temporary decreased sensation at the incision area or where the local anesthetic has been injected can be seen. Usually, nerve lesions and altered sensation at the incision area heals within months. In theory, infections can appear when piercing the skin. This occurs in 1 out of 25,000 times and can in some cases require treatment with antibiotics. In order to avoid infection all participants will be informed to abstain from swimming in seawater or swimming pools within the first five days. Furthermore, they will be informed to contact a project doctor in case of any signs of infection (heat, redness, swelling or fever). We believe that the potential outcome of this study to future SLE patients are significant, compared to any potential risk or disadvantages that patients must endure by participating.

4.7 Radiation exposure

A DXA scan will provide an effective radiation dose in the range of 3-30 µSv - equivalent of 3 days of background radiation in Denmark and is considered safe (Category I in "Appendiks 2: Retningslinjer om anvendelse af ioniserende stråling i sundhedsvidenskabelige forsøg"; NVK 2011). The radiation exposure due to each rest-stress ^{82}Rb-PET/CT scan is 4 mSv. The maximal total effective radiation exposure associated with participation in this study in thus less than 9 mSv and is thus risk category IIb according to the International Commission on Radiation Protection and the European Commission. Due to this radiation exposure, the lifetime risk of cancer is increased from 25% to 25.045%. As the current study is directly targeted at unveiling means of preventing and curing cardiovascular disease in patients with SLE, we consider this reasonable. If pregnancy is deemed possible, a pregnancy test will be performed prior to the scans.

4.8 Safety and precautions

Patients will undergo a medical assessment to ensure eligibility and safety. At all patient interventions (including exercise) at CFAS, a medical doctor is present and reachable. During exercise at one of the local collaboration-centers a medical doctor will not be present but be reachable through telephone. The Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet has great experience in conducting PET scans and will perform the necessary precautions both prior to and after the scan.

4.8.1 Safety evaluation

All adverse events (AEs) and serious adverse events (SAEs) observed by the investigators will be reported in the case report form (CRF) and to REC as requested, from the start of the trial (after written consent) to the final day of protocol procedure. An AE is defined as any untoward medical

occurrence in a participant, which does not necessarily have a causal relationship with the trial intervention. AEs are graded as the following:

Grade 1: The AE is noticeable to the subject but does not interfere with subject's every day's life activities; it may or may not require additional concomitant therapy.

Grade 2: The AE interferes with the subject's daily activities; it usually requires additional therapy.

Grade 3: The AE is intolerable and requires additional therapy.

Grade 4: The individual is at immediate risk of death at the time of the AE; it does not refer to an event which hypothetically might have caused death if it was more severe. Life-threatening events result in a SAE.

An SAE is any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.

4.9 Procedure for recording and handling AEs and SAEs

Full description of these procedures is attached in Appendix A. All AEs/SAEs related to the study assessments (including the exercise intervention) will be recorded immediately and notified in the CRF. All AEs/SAEs (both those that are anticipated and unanticipated) will be recorded on adverse event forms and published with study results. We will also collect patients' self-report of AEs/SAEs, and report these in the CRF. These forms will include a description and classification of the event, date of onset, date resolved, whether the event was serious or not (ICH criteria), relationship of the event to the study (1=none, 2=unlikely, 3=possible, 4=probable, 5=definitely), action taken, and whether the study was suspended or not.

All SAEs, regardless of causation, will be reported to REC within one week (in any SAE resulted in death, an immediate report within 24 hours will be sent.) Once a year the sponsor will summarize all SAEs and report these to REC. If at any point it is considered medically unsafe by the principal investigator for a patient to continue in the study, the patient will be excluded from the study and offered appropriate support.

4.10 Dissemination of study results

The results of the study, whether positive, negative or inconclusive, will be disseminated through a systematic dissemination and publication strategy. After registration on ClinicalTrials.org, the study protocol will be published in an international peer reviewed journal, and once the study has been completed and the data analysed, a preprint of the manuscript will be submitted to medRxiv. After this, the manuscript will then be submitted to peer reviewed scientific journals. After publication, the original data will be made available in a public repository. The results will also be presented at national and international conferences. Patients will be informed of the study and the results by public outreach in the form of layman articles, short study reports at websites and in newsletters and presentations. Authorships will be assigned according to the Vancouver rules as determined by the PI RHC. All participants are guaranteed access to more information about the project. In need of information, participants will be invited to contact the Research Coordinator Malte Lund Adamsen.

4.11 Withdrawal from study

A patient may choose to end their participation in the study at any time. The patient will not be obliged to provide a reason, and the termination of participation will not influence future medical treatment nor have any other consequences for that patient, and this will be made clear to the patient when obtaining oral and written informed consent. The reason for discontinuation will be

documented (including "unknown reason"). In exceptional circumstances, the study investigator may choose to withdraw a patient from the study due to safety reasons determined by the study investigator.

The patient must have completed no less than 80% of the prescribed exercise (no less than 29 session or less than 32 minutes per session on average). This will be assessed at two weeks intervals, and subjects will be given two weeks to catch up on the missed sessions.

4.12 Insurance

All patients will be covered by The Danish Patient Insurance Association for any injury that may occur as a direct consequence of study-related procedures.

4.13 Practical setups

The study related procedures will be performed at the following locations:

1. Eligibility and recruitment: Department of rheumatology, Rigshospitalet and CFAS
2. Enrolment and baseline testing including VO_2 max, echocardiography, muscle biopsies**,** and DXA: CFAS
3. PET scan: Department of Clinical Physiology, Nuclear Medicine & PET, Rigshospitalet
4. Exercise intervention: CFAS or a local collaborator with experience in exercise therapy for patients.

4.14 Economy

The economy for this project will be maintained through a research fund created and maintained by center for activity research at Rigshospitalet by our economist Inge Holm.
MLA is employed by CFAS, Rigshospitalet with a one-year guarantee of salary, through use of some of the Novo Nordisk Foundation grant (see below). The Danish Rheumatism Association (Gigtforeningen) has pledged 1,000,000DKK for payment of 2 more years of his salary, dependent on re-application after the first year (grant ID: R202-A7503).
MD RHC has received a grant from "Region Hovedstadens Forskningsfond" for DKK540,000 for her salary.
MD Simon Jønck has received a grant from Gangstedforeningen for DKK 500,000 for his salary for this project as well as another project.
The project has received a grant from The Novo Nordisk Foundation (1,753,832 DKK, Grant ID: NNF20OC0065929). To cover all expenses new funding will be sought throughout the study and in case of further grants, the Regional Ethics Committee and participant information will be updated.

Participants will not receive economic compensation. However, transport allowance may be granted at attendance (a maximum of DKK 4000). As well as payment for temporal disability and pain with muscle biopsies of 500 DKK per biopsy.
None of the investigators involved in the research group have any financial interests in the project.

4.15 Biobank & Data Storage

Data and associated biological material will be stored in an individual database and research biobank during the study. At the end of the study remaining biological material will be transferred to the general research biobank of CFAS. The biological material will be stored in this research biobank for a maximum of 10 years. After that, it will be destroyed. If any later studies want to make use of the biological material, this will only take place following approval by the ethical committee. Confidentiality of the subjects will be maintained by assigning subjects a study number,

keeping identifiers separate from the data and storing data in a locked file and secure computer database. Scientific reports generated from the study will not contain information that would identify the participants.

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