The Effects of Exercise on Vascular Physiology in Systemic Sclerosis Patients

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Abstract

The original research in this thesis aimed to investigate the efficacy and feasibility of exercise in people with systemic sclerosis (SSc). The heralding symptom in the pathophysiology of SSc is vascular dysfunction in the digital area which is the primary cause of Raynaud's phenomenon (RP). Digital disfiguration, ulcers and RP affect the quality of life (QoL) in people with SSc. Medical treatment does not have dramatic improvements and is also accompanied by short- and long-term side effects leading to further health complications. Exercise could be considered as a safe and cost-effective adjunct therapy that could potentially reduce the use of medication.

The primary outcomes in study 1 were the physiological differences between the arm crank (ACE) and cycler ergometer (CE) protocols in sedentary adults. Study 2 investigated the microvascular function, quality of life, cardiorespiratory fitness, functional capacity and body composition in people with SSc. Study 3 explored the feasibility of exercise in people with SSc with primary outcomes being the recruitment and attrition rates as well as the adherence rates to exercise.

The novel findings of this research were: Study's 1 novelty was 1) the predictive equation for the cycle ergometer peak oxygen uptake ($CE\dot{V}O_{2peak}$) trough the physiological responses of ACE and body composition features (Study 1). The equation estimated with this model is: $CE\dot{V}O_{2peak}$ = 11.776 + 1.418 X arm crank ergometer peak oxygen uptake ($ACE\dot{V}O_{2peak}$) (ml·kg-1·min-) – 1.454 x total lean body mass (TLBM) + 3.967 X lower limb lean body mass (LLLBM). This predictive equation was later used in study 2 to compare ACE to CE $\dot{V}O_{2peak}$ as a correlation between the improvement of microcirculation (laser-Doppler fluximetry) and $\dot{V}O_{2peak}$ has been demonstrated in rheumatoid arthritis patients before (Metsios et al., 2014). Study's 2 novelties were 2) ACE seems more potent to improve the microcirculation in the digital area in people with SSc compared to CE, 3) the exercise programme that consisted of a high intensity interval training (HIIT) protocol that was performed for 12 weeks twice per week seems capable to prevent the formation of digital ulcers in people with SSc and the concomitant hospitalisations and/or in some occasions digital amputations, 4) QoL in people with SSc significantly improved after the exercise intervention. Study's 3 novelties were that 5) the exercise programme (12 weeks, twice/week) was feasible in people with SSc with very high recruitment and adherence rates, 6) our combined exercise protocol (HIIT and resistance training) was enjoyable and fairly easy to be performed by our participants, 7) individuals experiences confirmed the feasibility of our intervention and exercise protocol and highlighting the importance of applying supervised exercise programmes. Study 2 acted as a guiding study as to which mode of exercise could induce better results in the microcirculation in the digital area. Afterwards, study 3 utilised the upper limb exercise with weight training to assess its feasibility in people with systemic sclerosis.

These findings contribute to the growing evidence base for the effects of exercise in people with SSc. Our study is the first to investigate the effects of HIIT on digital microcirculation in people with SSc and the first to explore the feasibility of a combined exercise protocol in this clinical population. Future research should explore the effects of exercise in people with SSc in larger clinical trials.

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The production of this thesis has been an amazing experience and challenge that eventually came to a successful completion. There were times of paramount stress and times of enjoying those small steps of progress towards the completion of the research studies. Throughout the whole PhD process there were people who provided their greatest help and I would like to thank each one of them separately.

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Finally, I would like to dedicate this to my family. I would like to express my sincere thanks to my parents, my grandparents and my brother for their unreserved support throughout those three years that kept me alive despite the distance (Greece) that separated us. I am grateful and indebted for their support.

Research outputs

Publications

- Mitropoulos A., Gumber A., Crank H., & Klonizakis M. Validation of an arm crank ergometer test for use in sedentary adults. Journal of Sports Science and Medicine, 2017; 16 (4): 558-564.
- Mitropoulos A., Gumber A., Crank H., Akil M., & Klonizakis M. The effects of upper and lower limb exercise on the microvascular reactivity in systemic sclerosis patients. Arthritis Research & Therapy, 2018; 20 (1): 112.

- Mitropoulos A., Gumber A., Crank H., Akil M., & Klonizakis M. Investigating the effectiveness and feasibility of exercise on microvascular reactivity and quality of life in people with systemic sclerosis: Study protocol for a feasibility study. BMC Trials, 2018.
- Mitropoulos A., Gumber A., Akil M., & Klonizakis M. Exploring the microcirculatory effects of an exercise programme including aerobic and resistance training in people with limited cutaneous systemic sclerosis. Microvascular Research, 2019; 125: 103887.

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- Mitropoulos A., Gumber A., Crank H., Akil M., & Klonizakis M. Exploring the feasibility of an exercise programme including aerobic and resistance traning in people with limited cutaneous systemic sclerosis.

Conference and Research Presentations

- Annual European Congress of Rheumatology-EULAR 13-16 June 2018, Amsterdam, Netherlands. **Poster presentation:** Mitropoulos A., Gumber A., Crank H., Akil M., & Klonizakis M. The effects of upper and lower limb exercise on the microvascular reactivity in systemic sclerosis patients.
- 'Future Physiology', The Physiological Society, two days conference, 13-14 December 2017, Leeds, UK. **Poster presentation:** Mitropoulos A., Gumber A., Crank H., Akil M., & Klonizakis M. The effects of upper and lower limb exercise on the microvascular reactivity in systemic sclerosis patients.

Statement of originality

I hereby certify that I am solely responsible for the work contained in this thesis, unless otherwise acknowledged. Other work has been duly cited and the locations of source material can be found in the epilogue. I confirm that neither this thesis, nor the data included within it, has been submitted to Sheffield Hallam University or any other institution in partial or complete fulfilment for an undergraduate or postgraduate degree.

The initial research project idea was conceived by Dr. Markos Klonizakis and established by me specifically with respect to the exercise part (e.g., programme, protocol). The overwhelming majority of study activities such as ethical approval, participant recruitment, data collection, statistical analysis, delivery and supervision of exercise intervention and writing up study's results were conducted by myself.

Abbreviations

6MWT	Six-minute walking test
ACE	Arm crank ergometer
ACE$\dot{V}O_{2peak}$	Arm crank ergometer peak oxygen uptake
ACR	American college of rheumatology
ACSM	American college of sport medicine
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CE$\dot{V}O_{2peak}$	Cycle ergometer peak oxygen uptake
CG	Control group
cGMP	Cyclic guanosine monophosphate
CMV	Cytomegalovirus
CPET	Cardiopulmonary exercise test
CVC	Cutaneous vascular conductance
dcSSc	Diffuse cutaneous systemic sclerosis
DUs	Digital ulcers
EC	Endothelial cells
ECG	Electrocardiogram
ECM	Extracellular matrix
EG	Exercise group

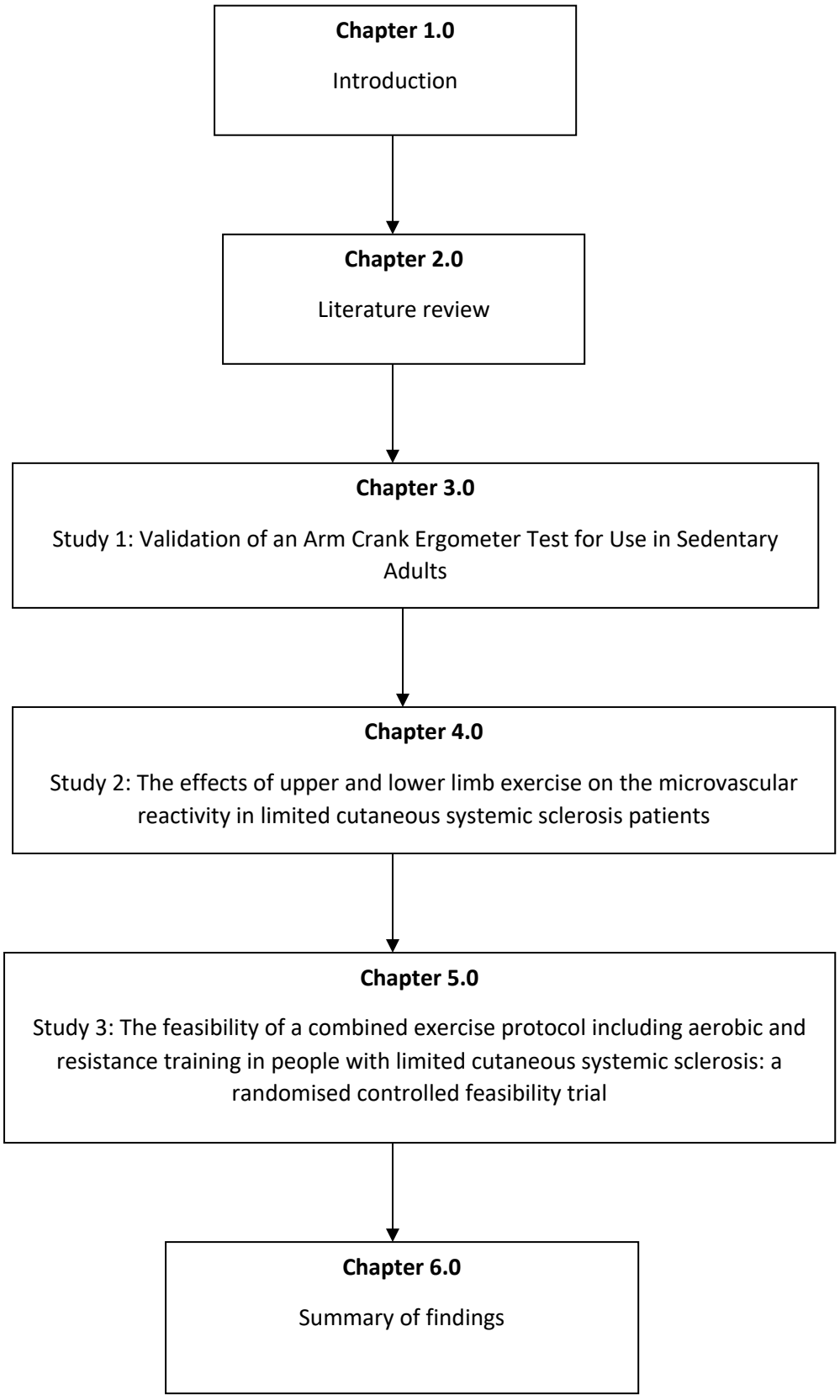
eNOS	Endothelial nitric oxide synthase
EPCs	Endothelial progenitor cells
ET-1	Endothelin-1
EULAR	European league against rheumatism
EUSTAR	European scleroderma trials and research
FMD	Flow mediated dilatation
FMV	Flow mediated vasodilatation
GAVE	Gastric antral vascular ectasia
GTN	Glyceryl trinitrate
HIF-1a	Hypoxia-inducible factor 1-alpha
HIIT	High intensity interval training
HR_{max}	Maximum heart rate
HR-QoL	Health related quality of life
IL-6	Interleukin-6
IL-8	Interleukin-8
ILD	Interstitial lung disease
KCI	Potassium chloride
LBMLL	Lean body mass lower limbs
LBMUL	Lean body mass upper limbs
lcSSc	Limited cutaneous systemic sclerosis

LDF	Laser-Doppler fluximetry
L-NAME	N^G-nitro-L-arginine-methyl-ester
MLC	Myosin light chains
MICT	Moderate intensity continuous training
NO	Nitric oxide
NYHA	New York heart association
PAH	Pulmonary arterial hypertension
PDE5	Phosphodiesterase 5
PDE-I's	Phosphodiesterase inhibitors
P_{mean}	Mean load
P_{peak}	Peak workload intensity
PPO	Peak power output
P_{rec}	Recovery load
QoL	Quality of life
QUALY	Quality-adjusted life years
RER	Respiratory exchange ratio
ROS	Reactive oxygen species
RM	Repetition maximum
RP	Raynaud's phenomenon
RPE	Rating of perceived exertion

RT	Resistance training
SRC	Scleroderma renal crisis
SSc	Systemic sclerosis
sICAM	Soluble intercellular adhesion molecule-1
sVCAM	Soluble vascular cell adhesion molecule-1
$TcpO_2$	Transcutaneous oxygen tension
TGF-β	Transforming growth factor beta
TLBM	Total lean body mass
T_{max}	Peak vascular response in relation to time
t_{peak}	Peak workload duration
t_{rec}	Recovery duration
$\dot{V}CO_2$	Volume of exhaled carbon dioxide
$\dot{V}E$	Minute ventilation
VEGF	Vascular endothelial growth factor
$\dot{V}O_{2peak}$	Peak oxygen uptake
VT	Tidal volume
vWF	von Willebrand factor

Structure of the thesis

Flow diagram of the thesis



Chapter summary

Chapter one

This chapter provides an overview of this thesis. It includes in brief the pathophysiology of systemic sclerosis, the medical treatment that is currently being used to treat Raynaud's phenomenon and the effects of exercise on vascular function.

Chapter two and three

Chapter two is a detailed literature review of the pathophysiology of systemic sclerosis with emphasis on the vascular abnormalities which are the main cause of Raynaud's phenomenon. Furthermore, the chapter also covers other aspects of the condition such as epidemiology and the available therapeutic approaches. Moreover, it elaborates in detail the effects of exercise on vascular function with focus on high intensity interval training in other clinical conditions with vascular involvement in their pathophysiology. Quality of life in people with systemic sclerosis is also extensively reviewed. Chapter two is closing with the aims and objectives of the general research project. Moreover, chapter three reviews the theory and literature behind the methods used to and selected for all the studies included in this PhD research project.

Chapter four

This chapter discusses the rationale, methods, results and discussion for the research study that examined the physiological differences between arm cranking and cycling in sedentary middle-aged adults. The primary aim of this chapter is to validate an arm crank ergometer protocol for use in sedentary populations and to create an equation that will accurately predict the peak oxygen uptake through the physiological responses of an arm crank ergometer test.

Chapter five

This chapter includes the rationale, methods, results and discussion for the pilot study that investigated the effects of exercise (arm cranking vs cycling) on the vascular function and quality of life in people with systemic sclerosis. The primary aim of this chapter is to compare the effects of two different modes of exercise on vascular function as measured via laser Doppler fluximetry and to assess the clinical

outcome (e.g., prevention of digital ulcers) for this clinical population as a result of the exercise intervention.

Chapter six

This chapter presents the rationale, methods, results and discussion for the feasibility study that investigated the feasibility of a combined exercise protocol (aerobic and resistance training) in people with systemic sclerosis. This chapter aims to explore the recruitment, attrition and adherence rates to the research study and the exercise programme. Moreover, it explores through questionnaires and interviews the feasibility of exercise in terms of enjoyment levels, intentions to engage to exercise, task-self efficacy, individual's experiences and potential barriers to exercise. It also explores the exercise-induced microcirculatory and quality of life changes.

Chapter seven

Chapter seven summarises and discusses the main findings of this research, describes what implications these findings may have on practice and proposes future research.

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Chapter 1 Introduction

1.1 Systemic Sclerosis - Scleroderma

The word scleroderma originates from the Greek words “skleros-σκληρός” and “derma-δέρμα”, which mean “hard” or “indurate” and “skin” respectively. Scleroderma used to be the official term to describe systemic sclerosis (SSc) until scientists discovered that tissue fibrosis is not limited to the hands rather it progresses in several internal organs. Therefore, the word systemic describes the whole-body involvement and reflects the extension of the clinical manifestations.

SSc is an autoimmune disease; its pathogenesis is mainly described by vasculopathy (Wollheim, 2005). Vascular dysfunction in the digital area is a common manifestation of SSc and can precede organ involvement by several years (Koenig et al., 2008). Approximately 50% of patients with SSc develop severe digital ischaemia and/or ulceration (Walker et al., 2007), which can be painful, difficult to heal, susceptible to infections, influencing also heavily the quality of life (QoL) and consequently increasing SSc-related disability (Nihtyanova et al., 2008; Steen et al., 2009). It is noteworthy that SSc has the highest case-specific mortality and morbidity amongst all rheumatic diseases (Altman et al., 1991).

Pharmacological agents (e.g., nifedipine) are commonly used as a first-line medical treatment approach. Although it can be effective and provide pain-relief to patients, the short-term (e.g., oedema, headaches, heart palpitations, dizziness and constipation) and long-term (e.g., heart dysfunction, increased cardiovascular risk) side effects of the medical treatment should also be considered for effects on QoL of SSc patients as well as the financial cost of treatment. Therefore, adjunct therapies with less side effects and cost implications are warranted (Pope, 2007; Prescribing & Medicines Team Health, 2015), with a view to reducing dependency on medication.

1.2 Exercise

A complementary approach to medical treatment would be to implement an exercise modality that would be suitable for this patient group; the exercise therapy has been successful in improving endothelial-

dependent vasodilation in a number of studies on patients with various diseases (Klonizakis et al., 2009; Meyer et al., 2012; Aksoy et al., 2015; Ramos et al., 2015), implicating an enhanced nitric oxide (NO) bioavailability via shear stress-mediated increases in endothelial NO synthase activity (Dyakova et al., 2015), a significant decrease in the serum markers of adhesion molecules (Aksoy et al., 2015), and enhanced anti-oxidants defences (Ji and Zhang, 2014) or increased substrate (L-arginine) availability (De Meirelles et al., 2009). As vascular function is the focal point for SSc, improving those mechanisms would be very beneficial for people with SSc, having the unquestionable benefits (i.e., increase in physical fitness and functional ability) of exercise as additional positive outcomes.

To our knowledge, the feasibility and efficacy of an exercise programme in the microcirculatory parameters in patients with SSc has not been previously examined. High intensity interval training (HIIT) has recently come to prominence for its effectiveness in inducing greater improvements in vascular function than moderate-intensity continuous training in clinical populations with cardiovascular disease (i.e., Ramos et al., 2015). Due to variation in HIIT protocols, limited evidence exists to support which exercise protocol could be more beneficial for SSc patients. A HIIT protocol with short intervals (30s exercise/30s passive recovery) may elicit more favourable patient reported satisfaction/enjoyment levels compared to other longer duration exercise protocols (Meyer et al., 2012). In chronic heart failure patients, a short HIIT protocol (30s exercise/30s passive recovery) was reported as being well tolerated, a preferred protocol with a low perception of effort, patient comfort and patients spent a longer time at higher percentage of peak oxygen uptake ($\dot{V}O_{2peak}$) than a longer HIIT protocol with active recovery phases (Meyer et al., 2012). Furthermore, when enjoyment levels in an overweight/obese cohort were examined after a short HIIT protocol it was found that performing a HIIT protocol on a cycle ergometer (CE) resulted in enjoyment rating of an average of 4.5 (moderately to quite a bit) out of 7 (extremely) rating (Smith-Ryan, 2017).

Although HIIT may improve the micro-and macro-vascular function in several clinical populations such as heart failure (Guiraud et al., 2012) and cardiometabolic conditions (Kessler et al., 2012) using treadmill

and cycle ergometer as modes of exercise; no evidence exists about the mode of exercise that would be effective on digital microcirculation where RP attacks are present in SSc patients. Assumptions could be made that utilising an upper-body aerobic exercise would potentially be more beneficial for the digital microcirculation rather than lower-body exercise, where the working muscles promote the blood flow in the lower limbs. Hence, the differential effects that may occur by the upper- and lower-limb exercise on the digital microcirculation in SSc patients should be examined.

Resistance training (RT) alone has produced significant improvements in the function of the vasculature in people with obesity (Dias et al., 2015); moreover, a combination of aerobic and RT have demonstrated improvements both in the macro- and micro-vascular function (Metsios et al., 2014; Maiorana et al., 2000). However, to our knowledge the effects of a combined exercise protocol (RT and aerobic exercise), utilising HIIT and circuit training for the aerobic and RT part, respectively, on microcirculation are yet to be examined in people with SSc.

1.3 Purpose of thesis

Several studies have examined the effects of different exercise protocols upon the QoL in SSc patients (Oliveira et al., 2017). Nevertheless, there is lack of evidence regarding the effects of exercise in SSc pathophysiology and more specifically, in the microvasculature. Through a thorough literature review, we have concluded that a combined exercise protocol (HIIT and RT) may be able to induce improvements in vascular function. However, it remained unclear which mode of exercise (e.g., lower or upper limb) would induce greater improvements (Study 2). At this point there was a need to compare $\dot{V}O_{2peak}$ between upper and lower limb exercise as it has been demonstrated in the past that $\dot{V}O_{2peak}$ was strongly linked to improvements in microcirculation in rheumatoid arthritis patients (Metsios et al., 2014). Therefore, we validated an ACE exercise test for use in inactive adults (Study 1). Moreover, there was a need to test the feasibility of a combined exercise protocol (HIIT and circuit weight training) in people with SSc (Study 3). Current literature also lacks safer adjunctive therapies alongside medical treatment that would potentially be able to minimise the use of medication or even replace them at some occasions.

1.4 Study 1

Title: Validation of an arm crank ergometer (ACE) test for use in inactive adults.

1.4.1 Research question

How $\dot{V}O_{2peak}$ could be compared between ACE and CE?

1.4.2 Aims

1. To validate an ACE exercise test for use in inactive adults

2. To produce a predictive equation for CE $\dot{V}O_{2peak}$ through the physiological responses of ACE.

1.4.3 Objective

1. To perform a cardiopulmonary exercise test on an ACE and CE and compare the physiological responses

1.5 Study 2

Title: The effects of upper and lower limb aerobic exercise on the microvascular reactivity in systemic sclerosis patients.

1.5.1 Research question

Which mode of aerobic exercise might optimally improve microvascular function in SSc?

1.5.2 Aims

1. To compare the effects of two different modes (arm cranking and cycling) of exercise in improving the health status of patients with SSc - a pilot study.

2. To qualitatively assess the patient's acceptability of each assessed exercise protocol.

1.5.3 Objectives

1. To define the optimal mode of aerobic exercise (arm cranking or cycling) for producing the best effect on microvascular function (laser Doppler fluximetry - LDF) and quality of life (based on EQ-5D-5L questionnaire).

2. To investigate the efficacy of a HIIT protocol with short intervals (30s 100% peak power output/ 30s passive recovery) to improve microvascular function assessing its effects on microcirculatory parameters and quality of life pre- and post-exercise intervention.
3. To assess adherence rates to the exercise-intervention (arm cranking and cycling) in the pilot study.
4. To explore enjoyment and motivational levels during exercise (arm cranking and cycling) sessions via questionnaires in the pilot study.

1.6 Study 3 - Feasibility study

Title: The feasibility of a combined exercise protocol including aerobic and resistance training in people with SSc: a randomised controlled feasibility trial

Study 2 investigates whether a combined exercise (HIIT and RT) is a feasible to be implemented in people with SSc. Furthermore, the microcirculatory parameters and quality of life in people with SSc after a combined exercise protocol were reported.

1.6.1 Research question

Is supervised combined exercise (HIIT and RT) training feasible to be implemented in people with SSc?

1.6.2 Aims

1. To investigate the feasibility of a combined exercise protocol (HIIT and RT) to be performed in people with SSc.

2. To qualitatively assess patients experience of enjoyment, adherence and motivation during the training intervention period in people with SSc.

1.6.3 Objectives

1. To define the feasibility and characteristics of the potential outcome measures and also assess the time taken, burden and completeness of the various outcome measures in order to identify an appropriate subset of measures to use in a randomised controlled trial.

2. To estimate the likely effect size (intervention minus control) or the variability for each potential outcome variable (point estimate and its uncertainty) as well as the standard deviation of the outcome measure. This will reflect the effectiveness of the intervention and the precision of the measure and will inform the choice of primary outcome but also the estimated sample size for a randomised controlled trial.
3. To assess the numbers of eligible patients according to the inclusion criteria but also the percentage of the recruited patients.
4. To assess the follow-up/retention rate at 6 months to estimate likely dropouts and study post-intervention effects.
5. To assess rates of adherence, compliance and attrition, exploration of enjoyment levels, assessment of exercise tolerance, number of adverse events and exploration of individual experiences. Also, to report on rates of screening (total number of people with SSc that were screened through the medical records regardless of eligibility), eligibility, and recruitment.

Chapter 2: Literature review

2.1 Chapter overview

This chapter reviews the current literature on systemic sclerosis and the beneficial effects of exercise. Firstly, it describes the disease epidemiology analysing factors such as prevalence and the current classification criteria of the disease. Secondly, it analyses the pathophysiology and more specifically vascular disease underlying systemic sclerosis and the currently available medical and non-medical treatment. Thirdly, it describes the damaging effects of systemic sclerosis on quality of life. Fourthly, it reviews the beneficial effects of exercise (intensity, mode, protocol) on vascular function and quality of life in several clinical conditions as well as in systemic sclerosis patients. Lastly, it concludes with the rationale of the research project identifying the knowledge gap and sets the aims and objectives in order to answer to the main research question (e.g., Is exercise effective and feasible to be implemented in people with systemic sclerosis?).

2.2 Systemic sclerosis epidemiology

2.2.1 Prevalence of systemic sclerosis

The diversity and the large spectrum in SSc clinical manifestations and severity, with various diagnosis and classification criteria over time among individuals renders this condition a challenge for epidemiologists. This affects SSc epidemiology, which is characterised by considerable variability in prevalence, incidence, risk factors and mortality rates estimations, mainly due to the diversity in the design and methods used to estimate these parameters (Ranque and Mouthon, 2010). Nevertheless, critical determinants of risk and causal factors can be identified.

The first European epidemiologic study of SSc was conducted in West Midlands, UK (Silman et al., 1988). Silman et al. (1988) utilised seven different sources and was population-based, estimating a prevalence of 13/million in men and 48/million in women. Further European studies were also conducted utilising a variety of ascertainment sources, most using a capture-recapture method (Le Guern et al.,

2004; Mayes et al., 2003): Allcock et al., (2004) estimated a prevalence of 88/million in Northeast England, which appears to be higher than the 3.08/100 000 found in the West Midlands by Silman et al., (1988) and lower to the 14.6/100 000 prevalence observed in south and west London (Silman et al., 1990). In Northeast England women with SSc outnumber men by a ratio of 5.2:1 (Allcock et al., 2004) which comes in agreement with previous findings (Englert et al., 1999; Mayes, 1996; Medsger, 1994). Moreover, the ratio of lcSSc to dcSSc found to be 4.7:1 and lcSSc was correlated with the presence of anticentromere antibodies, whereas dcSSc was associated with anti-Scl 70 antibodies which are compatible with the diagnosis of SSc (Allcock et al., 2004). In France (Le Guern et al., 2004) and Greece (Alamanos et al., 2005), epidemiologists concluded in a rather similar estimation of SSc prevalence of 158 and 154/million, respectively. These two studies highlight the presence of a North-South ratio of SSc in Europe, with lower prevalence in northern countries (Chifflot et al., 2008).

Moreover, Chifflot et al., (2008), proposed a North-South gradient in Europe with lower rates in Northern European countries [UK, Finland, and Iceland (Allcock et al., 2004; Kaipiainen-Seppanen & Aho, 1996; Geirsson et al., 1994; Silman et al., 1988)] compared to Southern European ones [France and Greece (Alamanos et al., 2005; Le Guern et al., 2004)]. Studies published since this have continued to report high incidences in Southern Europe (Spain, Croatia and Italy) (Lo Monaco et al., 2011; Radic et al., 2010; Arias-Nunez et al., 2008) but contradictory rates in Northern Europe with low annual incidence of 6–11 per million in Norway (Hoffmann-Vold et al., 2012) and a higher rate 19/million person-years in Southern Sweden (Andreasson et al., 2014). Incidence and prevalence in the USA, Canada, and Australia are reported at the higher end of this range (Bernatsky et al., 2009; Roberts-Thompson et al., 2006; Mayes et al., 2003).

Over the past years, there have been several studies regarding incidence and prevalence of SSc. The reported studies continue to show variation by geographic region. Incidence rates and prevalence estimates are fairly similar for Europe, the United States, Australia, and Argentina suggesting a

prevalence of 150-300 cases per million with a lower prevalence noted in Scandinavia, Japan, the UK, Taiwan, and India (Barnes & Mayes, 2012).

2.2.2 Risk factors

Ethnicity

People of a black ethnic background have a higher age-specific incidence rate and more severe clinical manifestations than other ethnicities according to several studies conducted in USA (Nietert et al., 2006; Mayes et al., 2003; Laing et al., 1997; Steen et al,. 1997). The dcSSc cases summed up to 60% versus 27% of the incidents in black versus white dcSSc women (Mayes et al., 2003). Interestingly, no difference reported in incidence according to race between men. It was also observed that people of a black ethnic background had an earlier age of onset of SSc than Caucasian people in USA (Nietert et al., 2006; Mayes et al., 2003; Laing et al., 1997; Steen et al., 1997). In Australia, the incidence rate was higher among continental European-born men, presumably because of greater silicates exposure in this population (Roberts-Thompson et al., 2006). In France, the incidence rate of SSc was 140/million (95% CI 122-170) in European versus 210 (128-293) in non-European (Northern and Sub-Saharian Africans, Asians and Carribeans). The dcSSc was more likely in non-European (34 versus 17, p=0.04; Le Guern et al., 2004). Generally, SSc is less frequent to European origin when compared to people of other ethnicities.

Gender

SSc is more common in women compared to men, with a mean gender ratio around 3:1 (Chifflot et al., 2008). This observation might be attributed to different environmental exposures, as well as hormonal background. Moreover, men are more likely to have dcSSc (Al-Dhaher et al., 2008; Ferri et al., 2002) than women. The gender ratio was apparently greater during the child bearing years, compared to older age groups; 3.4:1 versus 2.4:1 (Steen et al., 1997).

Gender preferences, clinical dissimilarities and intrinsic psychological perspectives also influence considerably the HR-QoL (Chularojanamontri et al., 2011). In general men gender is deemed a factor of poor prognosis in SSc (Nguyen et al., 2011; Wynn et al., 1985; Peters-Golden et al., 1984). Women have

more concern about daily and leisure activities whereas men's concern is work or study (Chularojanamontri et al., 2011). It has been reported that men may have renal failure, elevated blood pressure, inflammatory myopathy, arrhythmia, myositis, positive nuclear antibodies, dcSSc, echocardiography pulmonary artery pressure > 35mm Hg, ILD (Nguyen et al., 2011; Wynn et al., 1985) and greater risk to develop lung cancer (Colaci et al., 2013), and less frequently anti-centromere antibodies and sicca syndrome (Nguyen et al., 2011) more often than women. On the other hand, women present calcinosis, arthralgias and lcSSc more often than men (Nguyen et al., 2011). Moreover, a study reported that perceived health status is not associated with gender and that women have more self-reported symptoms of anxiety compared to men who were free of self-reported symptoms of both depression and anxiety (Nguyen et al., 2011). The same study concluded that perceived disability and impaired HR-QoL in SSc is not determined by gender, but functional and social issues should be considered as severe in both.

Age

Although SSc is rare during childhood or among very elderly adults, it is possible to occur at any age. Nevertheless, peak occurrence is usually in the fifth decade (Nietert et al., 2006; Mayes et al., 2003; Laing et al., 1997; Steen et al,. 1997), again with ethnicity being considered a contributing factor (Laing et al., 1997; Steen et al., 1997; Mayes et al., 2003; Le Guern et al., 2004).

Environmental risk factors

Silica

Manufacturing and rural activities have been associated with SSc incidence. This is mainly due to the exposure to silica, dust and hydrocarbons (Ranque et al., 2010). Initially, the association between silica and SSc was excluded by limited numbers of patients (Nietert et al., 2000), however, retrospective comparative studies followed to confirm that exposure to silica constitute a high risk to develop SSc (Janowsky et al., 2000). Odds ratio of 3.93 (1.84-8.54) (Englert et al., 2000) and 5.57 (1.69-18.37) (Diot

et al., 2002) were calculated in studies conducted in Australia and France, respectively, and thus a number of countries such as France, Germany, Canada and South Africa consider SSc as an occupational disease.

Solvents

Previous exposure to solvents was also associated with SSc (Nietert et al., 2000) in case control studies with discrepancies among studies with respect to the types of solvents involved and/or patient gender. Amongst solvents that have been considered as high risk are paint thinner or removers, mineral spirits, trichloroethylene, trichloroethane, perchloroethane, gasoline, aliphatic hydrocarbons, halogenated hydrocarbons, benzene, toluene and xylene-solvents. For example, men with SSc have been observed to be more frequently exposed to organic solvents (namely trichloroethylene) than controls (Odds ratio 2.9 [1.1-7.6]) (Nietert et al., 2000), which was not the case for women (Nietert et al., 1998). In a recent meta-analysis incidence of SSc was correlated with increased odds ratio for silica, chlorinated solvents, trichloroethylene and welding fumes for men with SSc, aromatic solvents and ketones for women with SSc and white spirit for both genders (Marie et al., 2014).

Occupational risk factors

Various toxic products such as epoxy resins and pesticides, paints, adhesive, hair dye, contact lenses and fabric eyes have been explored in SSc case controls studies but were not demonstrated significantly are risk for SSc. Moreover, despite the fact that silicone breast implants were suspected as potential risk factors for SSc induction (Varga et al., 1989), two meta-analyses (Marie et al., 2014; Janowsky et al., 2000) inferred to the absence of correlation between SSc and breast implants. In addition, outbreaks of SSc-like disorders have apparently been associated to chemical exposure, including polyvinyl chloride intoxication (Garabrant et al., 2003) or toxic oil syndrome (Nietert et al., 2000).

Other risk factors that may trigger SSc include viruses or certain chemicals. For example, Lunardi et al., (2000) reported that 93% of people with SSc present the "SSc peptide" that is assessed by serum immunoglobulin. Notably, this peptide demonstrates an essential homology with UL94, a

cytomegalovirus (CMV) late protein than can be identified by purified lgG from patients (Lunardi et al., 2000). In vitro, patient antibodies targeting UL94 also appear to induce apoptosis of endothelial cells (EC) comparably to SSc specific antibodies, recommending a possible role of CMV in the activation and the maintenance of SSc. Consequently, it is inferred that a cascade of extrinsic or intrinsic events seems necessary for the initiation of the disease (Halenious and Hengel, 2014).

Genetic factors

A large number of studies have been conducted in an attempt to identify genetic risk factors for SSc. A USA-based study involving 703 families, demonstrated that first degree relatives of people with SSc were found to be more susceptible to develop SSc themselves, with a relative risk close to 13 (10-16 across cohorts), with a recurrence rate of 1.6% versus 0.026% in the general population (Arnett et al., 2001). The relative risk in siblings (λs) varies between 3 and 15 (10-27) across cohorts. The only study that has been performed in twins demonstrated a poor agreement with the clinical expression of the disease (4.7%) after the analysis of 42 twin pairs (24 monozygotic; Feghali-Bostwick et al., 2003). Nevertheless, there is a higher consistency with the presence of anti-nuclear antibodies: 40% for dizygotic and 90% for monozygotic. The inference from these results indicates that genetic predisposition alone is not adequate to develop SSc but might affect the autoantibody profile.

Vascular Related Genes

Endothelin-1 is one of three isoforms and is synthesized by vascular endothelial (VE) cells, fibroblasts, bone marrow mast cells, neutrophils, macrophages, and cardiac myocytes (Shiwen et al., 2009). Various triggers induce synthesis of ET-1 including TGF-β and other growth factors, cold exposure, low shear stress, hypoxia, and angiotensin II (Shiwen et al., 2009); but its synthesis is reduced by nitric oxide (NO), natriuretic peptides, increased blood flow, and prostacyclin (Ortega & de Artinano, 1997). ET-1 is also degraded by MMP-1, which is reduced in SSc (Shiwen et al., 2009). Two types of receptors for ET-1 (ETα and ETβ) are variably expressed on endothelial cells, vascular smooth muscle cells, adventitial fibroblasts, tissue fibroblasts, neutrophils, mast cells; and monocytes and ET receptor engagement on

these cells triggers a variety of pro-inflammatory or fibrotic response, includ-ing vasoconstriction of vasculature (Shiwen et al., 2009). ET-1 increases surface expression of ICAM-1 on fibroblasts, stimulates CI synthesis, promotes formation of myofibroblasts, and facilitates binding of T cells to fibroblasts (Shiwen et al., 2009; Sakkas et al., 2006). ET-1 acts as a downstream mediator of TGF-β, and its induction by TGF-β in fibroblasts is via small mother against decapentaplegic (Smad)-independent signaling that involves c-Jun N-terminal kinase (JNK) and activin receptor-like kinase (ALK)5 pathways (Shi-Wen et al., 2006). Polymorphisms of ET-1 receptors are associated with SSc. For example, there is an association of *EDNRB* polymorphisms and dcSSc and *EDNR-A* polymorphism with anti-RNA polymerase autoantibodies in SSc (Shiwen et al., 2009). Polymorphisms were also described in the promoter of the NOS2 gene that confers susceptibility to pulmonary arterial hypertension (PAH) in SSc (Kawaguchi et al., 2006). Potassium voltage-gated channel shaker-related subfamily 5 (*KCNA5*) has a role in the regulation of vascular tone. It is inhibited by hypoxic conditions leading to vasoconstriction. *KCNA5* may have a protective role against PAH-associated SSc, this protective role was identified with variant rs10744676 (Bossini-Castillo et al., 2012).

2.2.3 Hospitalisations

A recent study analysed 4981 hospitalisations with hospital discharge records of SSc, in 736 patients, between 2001 and 2012 (Piga et al., 2016); women accounted for 84.8% of admission. Stratification for admission type revealed that 67.8% were in-patients and 32.2% were day-hospital (a programmed one-day hospitalisation). SSc was the primary diagnosis in 3631 (72.9%) hospitalisations of which 65.4% were inpatient admissions; this percentage increased to 74.4% for SSc as non-primary diagnosis. The overall mean length of stay for in-patient admissions was 8.0 days (±5.57; median 7 days) and 8.8 days (±9.6; median 6 days) for SSc as primary and non-primary discharge diagnosis, respectively. During study period, a remarkable increase in SSc hospitalisations, both inpatient and day-hospital, was detected as effect of a significant rise in number of hospitalisations with primary discharge diagnosis of SSc. In contrast, the number of hospitalisations with SSc as non-primary discharge diagnosis showed an initial

decline and then remained steady. The ratio between hospitalisations resulting in SSc as primary diagnosis and total number of admissions in regional area was 0.36 per 1000 in 2001, but it underwent a statistically significant ($p < 0.0001$), gradual and progressive annual increase raising to 1.33 per 1000 in 2012. Increased number of hospitalisations for SSc was mostly secondary to the raised number of day-hospital; the inpatient/day-hospital ratio was 4.0:1 in 2001, while it reached 1.9:1 in 2012. Major reasons for day-hospital admissions were therapeutic procedures, mainly intravenous treatment with prostacyclin receptor agonists.

Patient characteristics

For the previously mentioned hospitalisation studies, and among the 736 patients recorded, 84.4% were women (Women/Men ratio 5.4:1) (Piga et al., 2016). Stratification by age and sex revealed additional, interesting information: Only six patients (4 women and 2 men) were <16 years old. The largest proportion of women hospitalized was aged 45–64 years (43.16%) followed by those older than 64 years (34.94%). An even more significant difference emerged for hospitalised males; those aged 45–64 years were 47.8 % and those older than 64 were 27.8 %.

2.2.4 Mortality

Mortality rates in SSc have improved significantly compared with earlier published reports. A longitudinal study reported a 10-year cumulative mortality rate showing an improvement in survival from 54% in the 1970s to 66% in the 1990s (Steen and Medsger, 2007). Improvement in survival was ascribed to improvements in diagnostic processes and to the development of more effective treatments for scleroderma renal crisis. It was demonstrated that pulmonary fibrosis and PAH replaced scleroderma renal crisis as the leading causes of death in SSc-related mortality. Scleroderma renal crisis is a major complication in patients with SSc. It is characterized by malignant hypertension and oligo/anuric acute renal failure. Scleroderma renal crisis occurs in 5% of patients with SSc, particularly in the first years of disease evolution and in the diffuse form.

The European League against Rheumatism (EULAR) Scleroderma Trials and Research (EUSTAR) database has reported the causes and risk factors for death in SSc (Tyndall et al., 2010). The database comprised 5860 patients with SSc who fulfilled the American college of rheumatology (ACR) 1980 classification criteria (Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee, 1980). Data for death and comorbidity causes were available for 234 out of 284 fatalities. To calculate predictors of mortality they used a multivariate Cox proportional hazards model reporting a direct association to SSc for 55% of deaths and 41% to non-SSc causes with the remainder 4% of cases considered non-classifiable. Among the 284 patients, 54.6% had dcSSc and 40.5% had lcSSc. The medial disease duration was 7.1 years for dcSSc and 15 years for lcSSc. The 19% percent of deaths were accounted for pulmonary fibrosis and 14% for pulmonary arterial hypertension (PAH). Arrhythmias were the most common cause for the 14% of SSc-related myocardial disease deaths. Renal cases of death only justified 4%, all of which were associated to scleroderma renal crisis. Gastrointestinal-related causes accounted for 3% of patient deaths.

As regards to the non-SSc related deaths, the following causes identified: infections (13% of all deaths), neoplasia (13%), and cardiovascular disease (12%). An analysis performed to identify the SSc-related comorbidities from patients with non-SSc-related deaths. The results demonstrated that a large number of patients who died from pneumonia also presented gastroesophageal reflux with or without documented aspiration. The 64.2% of those patients died from lung cancer had concomitant pulmonary fibrosis. In the EUSTAR report, independent predictors of reduced survival comprised of presence of proteinuria, PAH, pulmonary restriction with a forced vital capacity of less than 80% predicted, presence of dyspnoea greater than New York Heart Association (NYHA) Class II, higher age at onset of RP, decreased diffusion capacity for carbon monoxide, and a modified Rodman skin score higher than 10. This report is supportive to previous findings (Tyndall et al., 2010) which showed that pulmonary fibrosis and PAH are the most significant causes of SSc-related deaths and likely are partly responsible for non-SSc-related deaths.

Moreover, Hissaria et al., (2011) examined South Australian based population and reported the survival rates in 786 scleroderma patients using standardised mortality ratios compared with the general population. As anticipated, people with SSc had lower survival compared with a standardised mortality ratio of 1.46 [95% confidence interval (CI) 1.28-1.69]. The factors that identified to be responsible for the increase in mortality were older age of onset, gender (men), scleroderma renal crisis, pulmonary fibrosis, PAH, cancer, and antitopoisomerase and anti-U1 antibodies.

2.3 Classification criteria

The EULAR 2013 classification criteria (van den Hoogen et al., 2013) are considered as the gold standard criteria for the condition. Previous criteria lacked sufficient sensitivity, specifically in patients with early SSc or with limited cutaneous SSc (Hachulla and Launay, 2011; Hudson et al., 2007; Lonzetti et al., 2001). The EULAR 2013 criteria include clinical manifestations of the three hallmarks of SSc: vasculopathy, fibrosis of the skin and/or internal organs and production of certain autoantibodies. The four items including in the 1980 ACR classification criteria [scleroderma proximal to the metacarpophalangeal joints, sclerodactyly, digital pitting scars (not pulp loss), and bilateral basilar pulmonary fibrosis] are also incorporated, as are the items in the criteria proposed by LeRoy and Medsger (2001): RP, autoantibodies, nailfold capillaroscopy abnormalities, skin fibrosis.

The EULAR 2013 classification criteria are presented in Table 1. The table demonstrates the one criterion that, if present, is adequate to classify an individual with SSc, the two exclusionary criteria, and the seven items with a combined threshold above which cases are classified as SSc. Classification criteria are set for patients that may have SSc and are being examined for inclusion in an SSc study. The criteria are not applicable to patients with SSc-like disorder that accounts for their manifestations; and patients are not classified as having SSc when presenting 'skin thickening sparing the fingers'.

Skin thickening of the fingers of both hands that extends proximal to the metacarpophalangeal joints, is a manifestation that the classification system assigns nine points. This is adequate to classify the patient as having SSc, and no further implementation of the point system is required. Otherwise, the point system

is implemented by adding the scores for manifestations that are present. The items are skin thickening of the fingers, fingertip lesions, telangiectasia, abnormal nailfold capillaries, PAH and/or interstitial lung disease (ILD), RP, and SSc-related autoantibodies. Two different possible manifestations are included within two items, skin thickening of the fingers and fingertip lesions. In the case where a patient presents both manifestations, the score for the category is the higher score of the two manifestations. For example, in the item fingertip lesions, if a patient has both manifestations, that is, digital tip ulcers (weighted 2) and fingertip pitting scars (weighted 3), the total score for the item would be 3. The highest possible score is 19, and patients with a score of ≥ 9 are classified as having SSc. The definitions of the items used in the criteria are provided in table 2.

2.3 Table 1 ACR/EULAR criteria for the classification of systemic sclerosis

Item	Sub-item(s)	Weight/score**
Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints (sufficient criterion)	-	9
Skin thickening of the fingers (only count the higher score)	Puffy fingers Sclerodactyly of the fingers (distal to the metacarpophalangeal joints but proximal to the proximal interphalangeal joints)	2 4
Fingertip lesions (only count the higher score)	Digital tip ulcers Fingertip pitting scars	2 3
Telangiectasia	-	2
Abnormal nailfold capillaries	-	2
PAH and/or ILD (maximum score is 2)	PAH ILD	2 2
Raynaud's phenomenon	-	3
SSc-related autoantibodies (anticentromere, anti–topoisomerase I [anti–Scl-70], anti–RNA polymerase III) (maximum score is 3)	Anticentromere Anti-topoisomerase I Anti-RNA polymerase III	3

*These criteria are applicable to any patient considered for inclusion in a systemic sclerosis study. The criteria are not applicable to patients with skin thickening sparing the fingers or to patients who have a

scleroderma-like disorder that better explains their manifestations (e.g., nephrogenic sclerosing fibrosis, generalised morphoea, eosinophilic fasciitis, scleroderma diabetic rum, scleromyxedema, erythromyalgia, porphyria, lichen sclerosis, graft-versus-host disease, diabetic cheiroarthropathy).

**The total score is determined by adding the maximum weight (score) in each category. Patients with a total score of ≥9 are classified as having definite systemic sclerosis.

2.3 Table 2 Definitions of items/sub-items in the ACR/EULAR criteria for the classification of systemic sclerosis

Item	**Definition**
Skin thickening	Skin thickening or hardening not due to scarring after injury, trauma, etc.
Puffy fingers	Swollen digits–a diffuse, usually nonpitting increase in soft tissue mass of the digits extending beyond the normal confines of the joint capsule. Normal digits are tapered distally with the tissues following the contours of the digital bone and joint structures. Swelling of the digits obliterates these contours. Not due to other causes such as inflammatory dactylitis.
Fingertip ulcers or pitting scars	Ulcers or scars distal to or at the proximal interphalangeal joint not thought to be due to trauma. Digital pitting scars are depressed areas at digital tips as a result of ischaemia, rather than trauma or exogenous causes.
Telangiectasia	Telangiectasia are visible macular dilated superficial blood vessels, which collapse upon pressure and fill slowly when pressure is released. Telangiectasia in a scleroderma-like pattern are round and well demarcated and found on hands, lips, inside of the mouth, and/or are large mat-like telangiectasia. Distinguishable from rapidly filling spider angiomas with central arteriole and from dilated superficial vessels.
Abnormal nailfold capillary pattern consistent with systemic sclerosis	Enlarged capillaries and/or capillary loss with or without pericapillary haemorrhages at the nailfold. May also be seen on the cuticle.
Pulmonary arterial hypertension	Pulmonary arterial hypertension diagnosed by right-sided heart catheterisation according to standard definitions.
Interstitial lung disease	Pulmonary fibrosis seen on high-resolution CT or chest radiography, most pronounced in the basilar portions of the lungs, or occurrence of ‘Velcro’ crackles on auscultation, not due to another cause such as congestive heart failure.

Raynaud's phenomenon	Self-reported or reported by a physician, with at least a 2-phase colour change in finger(s) and often toe(s) consisting of pallor, cyanosis, and/or reactive hyperaemia in response to cold exposure or emotion; usually one phase is pallor.
SSc-related auto antibodies	Anticentromere antibody or centromere pattern seen on antinuclear antibody testing, anti–topoisomerase I antibody (also known as anti–Scl-70 antibody), or anti– RNA polymerase III antibody. Positive according to local laboratory standards.

2.4 Vascular anatomy and physiology

The peripheral vascular system includes all the blood vessels that exist outside the heart. The peripheral vascular system is classified as follows:

1. The aorta and its branches.
2. The arterioles.
3. The capillaries.
4. The venules and veins are returning blood to the heart.

The function and structure of each segment of the peripheral vascular system vary depending on the organ it supplies. Aside from capillaries, blood vessels are all made of three layers:

1. The adventitia or outer layer which provides structural support and shape to the vessel.
2. The tunica media or a middle layer composed of elastic and muscular tissue which regulates the internal diameter of the vessel.
3. The tunic intima or an inner layer consisting of an endothelial lining which provides a frictionless pathway for movement of blood.

Within each layer, the amount of muscle and collagen fibrils varies, depending on the size and location of the vessel.

2.4.1 Arteries

Arteries play a major role in nourishing organs with blood and nutrients. Arteries are always under high pressure. To accommodate this stress, they have an abundance of elastic tissue and less smooth muscle. The presence of elastin in the large blood vessels enables these vessels to increase in size and alter their diameter. When an artery reaches a particular organ, it undergoes further division into smaller vessels which have more smooth muscle and less elastic tissue. As the diameter of the blood vessels decreases, the velocity of blood flow also diminishes. It is estimated that about 10% to 15% of the total blood volume is contained in the arterial system. This feature of high systemic pressure and low volume is typical of the arterial system.

There are two main types of arteries found in the body: the elastic arteries (Andall et al., 2016), and the muscular arteries (Matsushima et al., 2015). Muscular arteries include the anatomically named arteries like the brachial artery, the radial artery, and the femoral artery. Muscular arteries contain more smooth muscle cells in the tunica media layer than the elastic arteries. Elastic arteries are those nearest the heart (aorta and pulmonary arteries) that contain more elastic tissue in the tunica media than muscular arteries. This feature of the elastic arteries allows them to maintain a relatively constant pressure gradient despite the constant pumping action of the heart.

2.4.2 Arterioles

Arterioles provide blood to the organs and are primarily composed of smooth muscle. The autonomic nervous system influences the diameter and shape of arterioles. Arterioles respond to the tissue's need for more nutrients/oxygen and simultaneously they play a significant role in the systemic vascular resistance because of the lack of significant elastic tissue in the walls.

The arterioles vary from 8 to 60 micrometres. The arterioles are further subdivided into meta-arterioles.

2.4.3 Capillaries

Capillaries are thin-walled vessels composed of a single endothelial layer. Because of the thin walls of the capillary, exchange of nutrients and metabolites occurs primarily via diffusion. The arteriolar lumen regulates the flow of blood through the capillaries.

2.4.4 Venules

Venules are the smallest veins and receive blood from capillaries. They also play a role in the exchange of oxygen and nutrients for water molecules. There are post-capillary sphincters (vascular shunt) located between the capillaries and venules. The venule is very thin-walled and easily prone to rupture with excessive volume.

2.4.5 Veins

Blood flows from venules into larger veins. Similarly, to the arterial system, three layers make up the vein walls. In contrast to the arteries, the venous pressure is low. Veins are thin walled and are less elastic. This feature permits the veins to hold a very high percentage of the blood in circulation. The venous system can accommodate a large volume of blood at relatively low pressures, a feature termed high capacitance. During the systemic circulation, nearly three-fourths of the circulating blood volume is contained in the venous system. Veins are also equipped by one-way valves which allow for blood flow, toward the heart, in a forward direction. The blood flow in the lower limb veins is promoted through muscle contractions. The forward blood flow from the lower limbs to the heart is also affected by respiratory alterations that influence pressure gradients in the abdomen and chest cavity. This pressure differential is highest during deep inspiration, but a small pressure differential can be observed during the entire respiratory cycle.

2.4.6 Structure and function

One of the blood vessels function is to transport nutrients to organs/tissues and remove wastes away from organs/tissues in the blood. A primary purpose and significant role of the vasculature is its participation in oxygenating the body (Andall et al., 2016). Deoxygenated blood from the peripheral veins is transported back to the heart from capillaries, to venules, to veins, to the right side of the heart, and then to the lungs. Oxygenated blood from the lungs is transported to the left side of the heart, into the aorta, then to arteries, arterioles, and finally capillaries where the exchange of nutrients occurs. Loading and unloading of oxygen and nutrients occur mostly in the capillaries.

2.4.7 Nerves

Blood vessels are primarily innervated by the sympathetic nervous system. The smooth muscles of vasculature contain alpha-1, alpha-2, and beta-2 receptors. A fine balance between the influence of the sympathetic and parasympathetic nervous systems is responsible for the underlying physiological vascular tone. Specialised receptors located in the aortic arch and the carotid arteries acquire information regarding blood pressure (baroreceptors) and oxygen content (chemoreceptors) through blood flow. This information is then delivered to the nucleus of the solitary tract via the vagus nerve (Reyes et al., 2008). Blood vessel constriction or relaxation then follows accordingly regulated by the sympathetic response.

2.4.8 Muscles

Blood vessels are consisted primarily by smooth muscle cells. These muscle cells are located within the tunica media along with elastic fibres and connective tissue. It is noteworthy to mention that the contraction of skeletal muscle plays a critical role in the promotion of peripheral blood flow towards the heart in the venous system.

2.5 Vascular Physiology

2.5.1 Endothelial cell function

Endothelial cells occupy a pivotal location between blood and tissue, which facilitates their involvement in numerous physiological processes. Blood vessels consist of a layer of smooth muscle surrounding an inner layer of endothelium. In addition to providing a selectively permeable barrier to blood, endothelial cells are vital to maintaining a physiological equilibrium relating to the processes of inflammation, platelet aggregation, thrombosis and vascular smooth muscle proliferation (Henderson, 1991). Endothelial cells also modulate vascular tone and blood flow and, in doing so, have profound effects on the overall function of the cardiovascular system (Henderson, 1991).

The endothelium responds to various humoral, neural and mechanical stimuli by releasing both contractile and relaxing signals that affect the underlying smooth muscle and thus, vascular tone. Endothelial cells are influenced by shear force, by platelets and the coagulation system, and by hormones and neurotransmitters (Quyyumi, 1998; Henderson, 1991). The endothelium, in turn, influences vascular tone through a variety of signals, including the vasoconstrictors angiotensin II, endothelin (ET)-1 and thromboxane, as well as vasodilators prostacyclin and NO (Quyyumi, 1998; Henderson, 1991).

NO-dependent vasodilation

Endothelial cells contribute to the regulation of blood flow, in part through NO-dependent vasodilation (Bivalacqua et al., 2003; Quyyumi, 1998; Henderson, 1991). NO-dependent vasodilation is initiated when agonists (such as acetylcholine and shear stress) activate the endothelial cell's phosphoinositol pathway and increase cytosolic calcium levels. In the endothelial cell calcium binds to calmodulin, which then activates endothelial nitric oxide synthase (eNOS) to form NO from its precursor substrate, L-arginine. NO diffuses to the adjacent smooth muscle cells where it activates soluble guanylate cyclase to increase cyclic guanosine monophosphate (cGMP) levels. cGMP relaxes vascular smooth muscle by

decreasing calcium levels through inhibition of the phosphoinositol pathway. In summary, agonists stimulate eNOS to produce NO, which increases smooth muscle cGMP and reduces smooth muscle calcium levels and tone. NO continuously fluctuates at low concentrations and therefore constantly influences the vascular tone (Henderson, 1991).

2.5.2 Shear stress and endothelial function

Shear stress is defined as the force exerted by the blood flow on blood vessel walls. This stress generates a response in the vascular wall, characterised by release of endothelial mediators, which in turn stimulate structural remodelling through activation of gene expression and protein synthesis (Hudlicka and Brown, 2009). Hemodynamic forces exerted by the heart during the cardiac cycle, PP (difference between systolic and diastolic pressure) and tangential stress, change the structure of vascular wall. PP induces distention of the vascular wall which increases the radial tension on the blood vessels. Tangential stress or shear stress depends on the inner diameter of the vessel, blood flow rate, viscosity of the blood, and pulsatility of blood flow. It is estimated using Poiseuille's law, through the product of shear on the wall and blood viscosity: $\tau = 4 * \eta * q / \pi * r^3$ where η is fluid viscosity, q is flow, π is defined as the ratio of the circumference of a circle to its diameter and r is radius. It is worth noting that this formula should be considered only for a blood vessel with circular cross section and in laminar flow regime. On the other hand, in clinical studies, shear stress is calculated through blood viscosity and shear rate (γ), which is estimated from the values of blood flow velocity (V) and internal arterial diameter (d) according to the following equation (Reneman et al., 2006): $\gamma = 8 * V / d$. Shear stress values calculated in this way might be held for *in vitro* assays, provided that the conditions meet Poiseuille's law. The latter statement cannot be applied to blood vessels *in vivo*, considering the presence of non-Newtonian fluid, distensible vessels, pulsatile flow, and branching of the arterial tree. Moreover, blood flow velocity, and wall shear stress, is high in systole and relatively low in diastole. Thus, diastole comprises approximately two thirds of the cardiac cycle, and the level of wall shear stress during this phase of the cardiac cycle contributes substantially to the mean wall shear stress (Reneman et al., 2006).

Other important factors that regulate vascular response to shear stress are blood flow characteristics (magnitude and shape) and vascular tree anatomy (Friedman et al., 1987). For instance, it is well known that turbulence in zones of arterial branching, where oscillatory shear stress is generated, constitute areas of vascular remodelling associated with starting events leading to atherosclerosis (Giddens et al., 1993). It has been demonstrated that the flow patterns in ascending aorta contribute to pro-atherosclerotic environment, mainly that low and oscillator shear stress, specifically near of the aortic sinus. There is a correlation between low shear stress and increased incidence of vascular damage, especially near to the coronary arteries (Suo et al., 2008).

2.5.3 Endothelial progenitor cells and endothelial function

Somatic stem and progenitor cells provide a source of tissue-specific cellular elements that permit appropriate tissue and organ functions through replacement of injured, diseased, and senescent cells in many organ systems throughout the lifespan (Weissman et al., 2001). Hematopoietic (Ivanov et al., 2017; Eaves, 2015), intestinal (Beumer & Clevers, 2016; Grun et al., 2016), skin (Ge Yejing et al., 2017; Fuchs, 2016) and skeletal muscle stem cells (Chal & Pourquie, 2017; Joanisse & Parise, 2016) have been identified and rigorously studied. Nevertheless, little is known about the cellular and molecular mechanisms that are related to homeostatic repair and replacement of vascular endothelial cells. Asahara et al. (1997) reported on the identification of circulating progenitor cells for the endothelial lineage. These putative endothelial progenitor cells (EPC) were bone marrow-derived cells that displayed an upregulation of “endothelial” cell surface markers and downregulation of “hematopoietic” markers during in vitro culture, suggesting that some hematopoietic stem cells displayed the ability to transdifferentiate into EPC to regenerate endothelial cells in vitro (Asahara et al., 1999). These putative EPCs also displayed colony-forming activity and were capable of migrating to sites of ischemic injury in vivo in animal models of human disease. Since these putative EPCs were isolated from human blood but participated in neovascularization in the tissues of injured animals, the authors proposed that the cells were engaged in postnatal vasculogenesis as a means to repair the damaged blood vessels. Numerous

publications followed where various cell surface markers were identified as enriching for the putative hematopoietic stem cells that could transdifferentiate into EPC subsets to enhance vascular repair (Majka et al., 2003; Jackson et al., 2001) (reviewed Kovacic et al., 2008) or could serve as a biomarker for the presence or severity of cardiovascular disease in humans (Fadini et al., 2012; Yoder, 2009).

2.6 Pathophysiology of systemic sclerosis

Vascular involvement is a common clinical manifestation in patients with connective tissue disorders and it is an important cause of death in established disease. Vasculopathy can be directly involved in the pathogenesis of the clinical condition, constituting an acute manifestation of rheumatoid arthritis (e.g., vasculitis), lupus (e.g., antiphospholipid syndrome), or SSc (e.g., PAH, digital ulcers).

Vasculitis is frequent to several connective tissue disorders, being triggered by a vascular inflammatory cascade of the vessel walls that may take numerous clinical forms due to its ability to affect vessels of different sizes (arteries, veins, and/or capillaries) and areas (involving either skin or internal organs), with a prognosis that may range from mild to life-threatening (Toubi et al., 2004).

Auto-immune connective tissue diseases can also be related to a considerable spectrum of cardiovascular manifestations that affect myocardium, pericardium, cardiac valves and the conduction system (Prasad et al., 2015). Although clinically-silent, these cardiovascular manifestations can have various impacts on the patient's condition increasing considerably the co-morbidity and mortality.

In the pathophysiology of SSc vascular disease is fundamental all along its development from early onset to late complications. One of the three key features that characterize the SSc connective tissue disorder is vasculopathy, along with fibrosis and auto-immunity. Both forms of SSc (lcSSc and dcSSc) display symptoms of vasculopathy (Gabrielli et al., 2009; Koch and Distler, 2007; LeRoy et al., 1988). On one hand, lcSSc presents a skin involvement that is limited to the face, neck, and areas distal to elbow and knees. On the other hand, dcSSc the skin involvement extends proximal to upper arms, thighs and/or trunk. The vasculature is a direct target in SSc, as demonstrated by the range of clinical manifestations

that occur from the initiation to the development of the disease and have a significant effect on the quality of life of those patients.

2.6.1 Microvascular complications in SSc

Vascular disease can affect several organs in SSc including kidneys, lungs, heart and digital arteries, leading to various clinical manifestations in people with SSc.

RP constitutes the hallmark of the clinical manifestations observed in SSc. The main sites affected by this microvasculature disorder are fingers and toes, however, it can also affect other extremities. Over 95% of people with SSc present evidence of RP that can initiate many years before any other clinical manifestation of SSc. RP is a result of hypoxia in the extremities in response to cold and is described by a triphasic colour pattern: pallor (constricted blood-flow), cyanosis (tissue hypoxia) and rubor (reperfusion) (Block and Sequeira, 2001). Evidently, RP is triggered by endothelial injuries in association with dysregulations in the production of nitric oxide (NO) and vasoactive factors (Kahaleh, 2004).

RP can result in the formation of digital ulcers that is also one of the earliest complications of SSc. Healing of digital ulcers is often complex and difficult, and the most threatening complication is the amputation that is secondary to infections.

Telangiectasias are cause by a dilatation of postcapillary venules (Walker et al., 2005) and are also common in SSc, reflecting the systemic microvascular dysfunction of the disease. They are confined on the hands, face, lips and oral cavity.

Pulmonary vascular involvement in the form of PAH, diagnosed by right-heart catheterization, is presented in roughly 12% of patients with SS, and is observed in both lcSSc and dcSSc (Proudman et al., 2007; Mukerjee et al., 2003). The progressive remodelling of the small- to medium-sized pulmonary vasculature results in pulmonary artery vasoconstriction and cellular proliferation and concomitantly to SSc-PAH. The vascular remodelling, fibrosis, and intraluminal micro-thrombosis are triggered and maintained by hypoxemia and ischemia-reperfusion injury in the pulmonary vasculature (Farber et al.,

2004). These events leading to a progressive increase in pulmonary vascular resistance, pulmonary arterial pressure, and right ventricular pressure overload. Compensatory mechanisms in the right ventricle eventually result in cardiac failure, rendering PAH life-threatening.

Approximately 10% of patients with dcSSc and 2% of patients with lcSSc are affected by scleroderma renal crisis as a result of the vascular disease that can also affect renal vessels. This vascular complication is commonly related to the presence of anti-RNA polymerase III antibodies (Steen et al., 1984). Scleroderma renal crisis typically displays an acute onset of severe hypertension and renal failure, caused by a proliferative obliterative vasculopathy of arterioles result in a glomerular ischemia, as demonstrated by histopathological studies of renal biopsies.

Similarly, vascular remodelling of the gastrointestinal mucosa closely resembling telangiectasias (Gastric Antral Vascular Ectasia-GAVE) is able to induce gastrointestinal manifestations in people with SSc (Hung et al., 2013). GAVE is typically characterised, through microscopy, by dilatation of mucosal capillaries, focal fibrin thrombosis, fibromuscular hyperplasia, and fibrohyalinosis. This typical gastrointestinal characteristic can be found in autoimmune connective tissue disorders including SSc and is also correlated to liver diseases. Several reports estimate its prevalence in people with SSc between 1 and 20% (Ghrenassia et al., 2014; Hung et al., 2013; Marie et al., 2008).

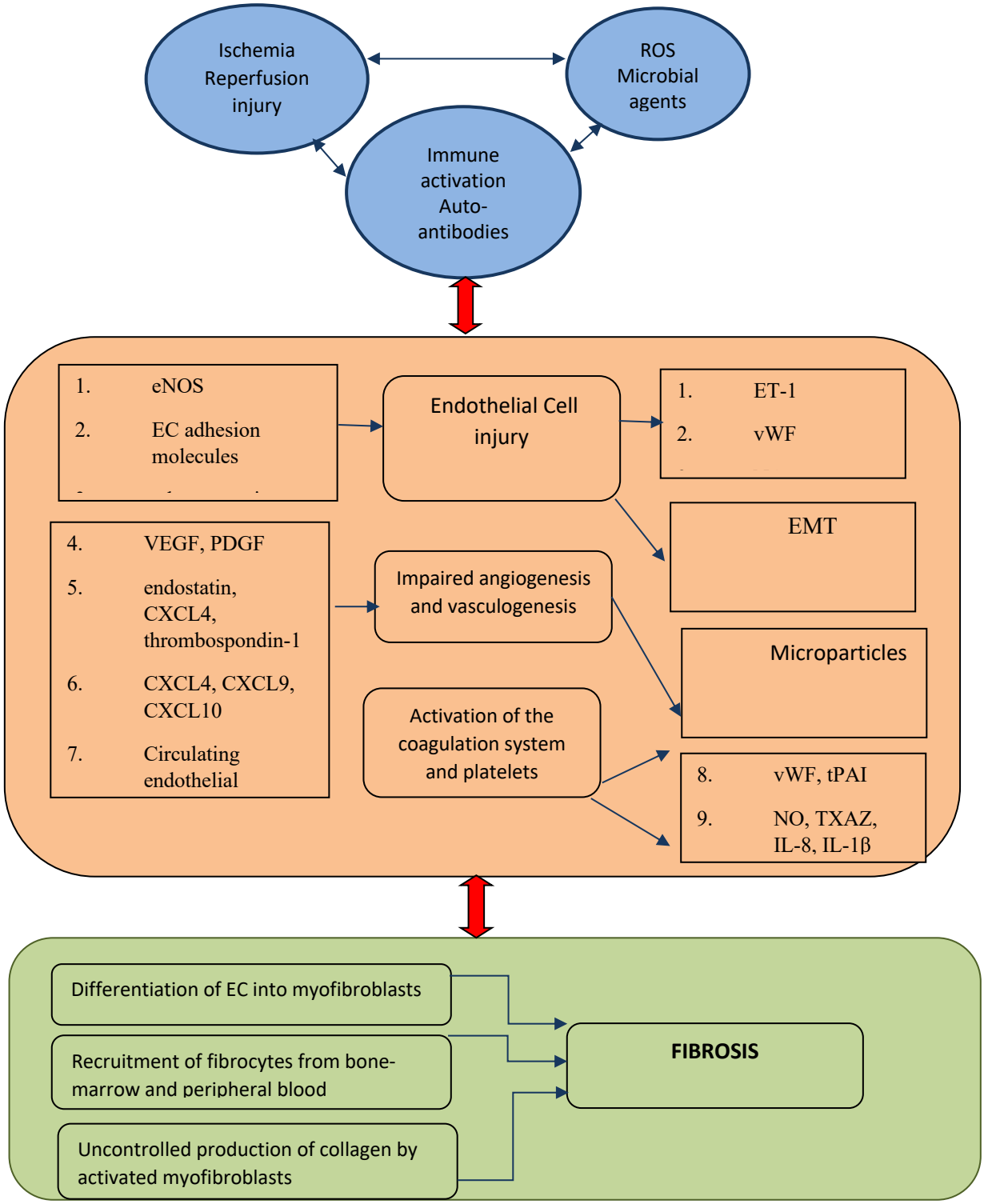
2.6.2 Vasculopathy in systemic sclerosis

SSc vasculopathy affects mainly small and medium-size arteries that present intimal hyperplasia and media thickening. Vasculopathy manifestations in the skin and in the lungs of patients with PAH can be associated with perivascular inflammation. In addition to the arteries, capillaries can also be affected by the vascular disease observed in SSc (Trojanowska, 2010). The involvement of capillaries is highlighted in nailfold capillaroscopy that demonstrates dilatation of the capillaries in early stages of SSc, and loss of the capillaries in the later stages (Cutolo et al., 2000). Impaired vascular permeability and tone are the earliest manifestations of vasculopathy in SSc. An imbalance between the vasoconstrictor molecules including endothelin-1 (ET-1) and the vasodilator NO contributes to the vascular dysfunction.

The aetiology of vasculopathy in SSc remains unknown, however, various events have been proposed to result in vascular injury in SSc, including free radicals, infectious agents, auto-antibodies and cytotoxic T cells directed against endothelial cells (Figure 1).

2.6.3 Microvasculature changes

Dysregulation of the vascular tone in arterioles and capillaries are an early and major sign of SSc. RP is the first clinical manifestation due to these abnormalities and affects primarily the fingers and toes. The structural modifications of the vasculature in SSc have been examined and observed using capillary microscopy in the nailfold of the fingers (Campbell and LeRoy, 1975). As expected, nailfold capillaroscopy allows the distinction of early and late microvascular disease (Cutolo et al., 2000). An early stage of the disease is characterised by a few giant capillaries and no capillary loss (Kavian and Batteux, 2015). At the next stage, the active disease is typically characterised by an increased number of giant capillaries as well as capillary microhaemorrhages without significant capillary loss. The last pattern characteristic of a late microvascular disease correlates the lack of giant capillaries with a significant loss of capillaries with avascular areas and marked dis-organisation of the normal capillary array. As previously noted, vascular changes are not limited to skin but also extend to the lungs, kidneys and other organs. For example, the lungs of people with SSc with PAH display vascular lesions in small- and medium-sized vessels that are distinguished by marked luminal obstruction, concentric intimal proliferation and the presence of infiltrating immune cells (Farber and Loscalzo, 2004). On the other hand, renal vessels of people with SSc can also show signs of media hyperplasia, intimal proliferation and obliteration of the lumen (Rabquer and Koch, 2012).



2.6 Figure 1 Pathophysiology of Vascular Damage

Sequence of events that could take part in the pathogenic process leading to vasculopathy in SSc. Various causative agents [ischemia-reperfusion injury, reactive oxygen species (ROS), microbial agents] could induce immune activation in predisposed subjects leading to chronic inflammation. Activated immune cells and auto-antibodies along with altered NO release cause endothelial cell (EC) activation. Altered production of several chemokines, cytokines and growth factors also contribute to an impaired angiogenesis and vasculogenesis, particularly VEGF, PDGF, chemokine ligand (CXCL)-4, CXCL-9, and CXCL-10. The EC injury along with endo-mesenchymal transition (EMT) process contribute to the activation of myofibroblasts and the production of exaggerated amounts of extra-cellular matrix (ECM) resulting in tissue fibrosis.
Adapted from Kavian and Batteux, (2015).

2.6.4 Endothelial cell injury

Increased levels of von Willebrand factor (vWF) and ET-1 are observed in the serum of patients with SSc, reflecting the endothelial cells dysfunctions and the active vascular disease (Kahaleh et al., 1981). The endothelial dysfunction is attested by the large gaps between endothelial cells, related to the vacuolization of the cytoplasm and the cytoskeletal rearrangement of these cells (Trojanowska, 2010; Freemont et al., 1992). Evidence suggests that endothelial cell apoptosis is increased in SSc. These apoptotic cells could initiate the activation of the innate immunity and lead to tissue injury, as well as coagulation activation. The endothelial injury could originate from cytoxic T cells, infections, auto-antibodies against endothelial cells or an ischemia-reperfusion phenomenon including reactive oxygen species (ROS) production. Indeed, endothelial cell apoptosis originates from the interplay of endothelial cells with cytotoxic T cells, either by Fas or granzyme/perforin pathway mechanisms. A cytotoxic endothelial cell apoptosis could be triggered by a viral infection of the endothelium, either directly or through recognition of infected cells by cytotoxic T cells. Cytomegalovirus (CMV) has been suspected to be involved in this process, since people with SSc present elevated levels of anti-CMV antibodies (Kahaleh and LeRoy, 1999). CMV-infected endothelial cells can separate and travel to distant capillary beds, thus spreading the virus and leading to systemic endothelial cell apoptosis and potentially to auto-immunity, via the production of anti-endothelial cell antibodies. This hypothesis has not been established and further studies are needed to define the exact role of CMV infection in endothelial injury and SSc pathophysiology.

ET-1 is a vasoconstrictor and its expression is elevated in the blood vessels, lungs, kidneys and skin of people with SSc (Liakouli et al., 2011). ET-1 is mainly a product of endothelial cells and its function is to mediate the vascular wall proliferation along with inflammation and fibrosis. This molecule seems to play a significant role in the maintenance of endothelial injury. Increased levels of ET-1 are related to digital ulcers in SSc (Sulli et al., 2009), and ET-1 is involved in the progression of microvascular damage in SSc as suggested by several clinical manifestations (Avouac et al., 2013). Two types of ET-1 receptors

have been identified: ET-1 type-A receptors are expressed by vascular smooth muscle cells and can regulate vasoconstriction, smooth muscle cell proliferation, fibrosis and inflammation. ET-1 type-B receptors are mainly expressed on endothelial cells and regulate vasodilation through the release of NO. The type-B receptors are down-regulated on endothelial cells in people with SSc. This event might contribute to the reduction of their vasodilatory properties (Abraham et al., 1997). Molecules that hinder ET-1 type- A and B receptors (e.g., bosentan) are often used for the prevention of new digital ulcers and the treatment of PAH related to SSc. Targeting the endothelial injury and ET-1 appears as an important therapeutic approach in the management of people with SSc.

The expression of endothelial NO synthase (eNOS) is reduced together with the NO release from vascular endothelium in SSc (Sinici et al., 2010). Transforming growth factor beta (TGF-β) is involved in fibrotic process of SSc - it appears to play a critical role in the metabolism of NO via the mediation of both inducible- and eNOS (Lafyatis, 2014; Higley et al., 1994). In turn, the alteration of NO production results in the alteration of vascular tone and platelet aggregation. NO also has an adverse regulatory effect on cytokine-induced endothelial cell activation and confines the endothelial release of pro-inflammatory cytokines such as interleukin 6 and 8 (IL-6 and -8). Therefore, the impaired NO production has an important role in SSc vascular disease. Moreover, several studies have reported that oxidative stress is increased in SSc and that it plays a part in endothelial injury by the peroxidation of cell membrane lipids and by activating the inflammatory process (Rosato et al., 2009; Servettaz et al., 2009; 2007; Allanore et al., 2004; Tikly et al., 2004).

A therapeutic approach that could enhance the sensitivity to endogenously NO production could have beneficial effects in SSc (Ghofrani et al., 2013; Grimminger et al., 2009). Indeed, riociguat, a molecule that targets and stimulates the soluble guanylate cyclase, has been demonstrated in a phase 2 trial to be beneficial in the treatment of PAH by enhancing exercise capacity (6-minute walking test) as well as pulmonary vascular resistance (Ghofrani et al., 2013).

Furthermore, phosphodiesterase 5 (PDE5) inhibitors have demonstrated beneficial effects in SSc vasculopathy. In the last decade, clinical trials have announced a successful treatment of RP with two PDE5 inhibitors (sildenafil or tadalafil), that have been initially developed for erectile dysfunction (De LaVega and Derk, 2009). As shown from a meta-analysis of the available randomised controlled trials, PDE5 inhibitors are capable to significantly improve Raynaud's condition score and frequency and duration of RP attacks compared with placebo in secondary RP (Roustit et al., 2013).

Endothelial cell adhesion molecules play a pivotal role in angiogenesis along with angiogenic factors and are involved in both cell-cell and cell-extracellular matrix (ECM) interactions as well as in the early steps of SSc vasculopathy (Prasad et al., 2015; Toubi et al., 2004). Indeed, the activation of endothelial cells in the early onset of SSc is correlated to elevated levels of soluble molecule such as soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1) and E-selectin (Distler et al., 2005). Increased levels of sICAM-1 can be found in the plasma of people with SSc with digital ulcers, compared to those that do not display any digital manifestations (Koch and Distler, 2007; LeRoy et al., 1988). In addition, E-selectin seems to be a promising biomarker of disease activity, as its circulating levels are related to the presence of avascular areas in nailfold capillaroscopy (Akimoto et al., 1996).

2.6.5 Digital ischemia in systemic sclerosis

Insufficient blood flow to living tissue is an excruciatingly painful experience and threatens the life of the tissue involved. Digital ischemic events are comparable to a myocardial infarction or a pulmonary embolism in that viability of the affected tissue is frequently lost and can critically affect the patient's quality of life. Death of digital tissue not only results in both functional disability and disfigurement, but it also is the clinical manifestation of an underlying systemic disease process (Macmahan and Wigley, 2010).

Digital ischemia is considered a serious complication in people with SSc. Morbidity from digital ischemia estimated to 30% of patients with persistent digital ulcers which results in irreversible tissue

loss (Ingraham and Steen, 2006) and it often requires hospitalisation. As a result, rapid effective actions must be taken to prevent permanent damage when ischemia threatens the livelihood of a digit. It has been reported that 20.4% of people with SSc proceed for amputation in one or more digits due to ischemia, 9.2% of which have multiple digit loss (Wigley et al., 1992).

Digital tissue vitality can be threatened by several pathological complications that compromise arterial blood supply such as vasculopathy, thrombosis, embolic, vasculitis, and traumatic; all complicated by secondary vasospasm. The aetiologies of digital ischemia are different; thus, it is important to comprehend the pathophysiology underlying each ischemic event in order to target therapy accordingly. More than 95% of people with SSc experience digital ischemia; research has made significant progress in defining the pathophysiological processes leading to that manifestation. This knowledge has led to many new treatment options.

2.6.6 Neural regulation and vascular dysfunction

Cold-induced signalling in cutaneous vasculature

Our body reduces heat loss by cold-induced vasoconstriction. This is a local effect of the cutaneous circulation, which responds to cold by an increase in sympathetic activity and the release of norepinephrine. Cold-induced vasoconstriction is particularly evident in RP and SSc patients (Block & Sequeira, 2001). However, it is paradoxical that cold-induced vasoconstriction occurs at all, as cooling would normally be expected to reduce the release of norepinephrine and slow down or inhibit vital biochemical pathways (intracellular calcium release, glycolytic activity) involved in vascular smooth muscle cell activation. The breakthrough in the understanding of digital thermoregulation was provided by a series of studies (Bailey et al., 2005, Bailey et al., 2004) that revealed firstly that α2 adrenoreceptors/adrenergic receptors (a2C-ARs) translocate from the *trans* Golgi to the cell surface of the vascular smooth muscle cell where they can respond to stimulation and, secondly, that signalling of these responses involves the RhoA/Rho (ROCK) kinase signalling pathway.

Cold stimulation in cutaneous arteries results in the immediate generation of reactive oxygen species in vascular smooth muscle cell mitochondria (Bailey et al., 2005). Reactive oxygen species are involved in REDOX signalling through the RhoA/Rho pathway (Kajimoto et al., 2007). RhoA is a guanosine triphosphate-binding protein, whose role is the regulation of actin–myosin dependent processes such as migration and cell contraction in vascular smooth muscle cell (Somlyo & Somlyo, 2004; Fukata & Kaibuchi, 2001). The myosin light chains (MLC) are phosphorylated /dephosphorylated by MLC kinase and MLC phosphatase respectively and this mechanism regulates the formation of functional actin/ myosin complexes. In the absence of an increase in intracellular calcium, activation of the RhoA/ROCK pathway leads to increased vascular smooth muscle cell contraction by inhibiting the activity of MLC phosphatase, thereby resulting in an increase of the levels of phosphorylated MLC by the MLC kinase. In addition, activation of the RhoA/ROCK pathway mediates the translocation of a2C-ARs from the *trans* Golgi network to the cell surface where they can respond to norepinephrine, thus promoting vasoconstriction. In the absence of a2C-AR stimulation and under normal conditions, cutaneous exposure to a cold stimulus results in vasodilation (Thompson-Togerson et al., 2007; Bailey et al., 2004; Flavahan et al., 1985). Mitochondrial ROS can increase the frequency of intracellular localised Ca^{2+} species known as calcium sparks which in turn can activate calcium activated potassium channels resulting in membrane hyperpolarisation which may ultimately result in vasodilation. Moreover, cold-induced ROS as well as Rho /ROCK may also result in downregulation of NO signalling (Liao et al., 2007; Rikitake & Liao, 2005). Thus, it has been speculated that vascular smooth muscle cell mitochondrial generation of ROS may initiate both cold-induced cutaneous vasoconstriction and vasodilation (Flavahan, 2008). Indeed, in RP and SSc patients, cold-sensitivity can be prevented by a2-AR antagonists (Freedman et al., 1995). In addition, it has been proposed that abnormal REDOX pathways (Herrick & Matucci-Cerinic, 2001) and enhanced a2C-AR activity in the cutaneous vasculature of these patients as a result of oxidative stress promotes the conditions that ultimately manifest as vasospastic episodes, that are so typical of the pathophysiology of this clinical condition (Flavahan, 2008; Bailey et al., 2005).

2.6.7 Biomarkers in systemic sclerosis

Autoantibodies as SSc diagnostic biomarkers

At present there are no specific diagnostic tests for SSc, and the disorder is diagnosed primarily based on the collective appearance of a cluster of clinical symptoms, such as RP, telangiectasias, oesophageal dysfunction with gastro oesophageal reflux, characteristic pigmentary changes, or presence of digital ulcers or calcinotic lesions accompanying clinically detectable skin induration. Indeed, the diagnostic criteria commonly employed for the classification of SSc, are based entirely on clinical manifestations and do not include any measurable serologic or laboratory parameters. However, it is well recognized that the presence of specific autoantibodies is one of the most common manifestations of SSc and more than 90% of people with SSc harbour antinuclear antibodies in their serum (Koenig et al., 2008; Steen, 2005; Ho & Reveille, 2003; Pollard et al., 1989). Some autoantibodies that are highly specific for SSc, such as anti-Scl-70 and anticentromere antibodies, have been used as diagnostic biomarkers to support or confirm the clinical diagnosis of SSc. Anti-Scl-70 antibodies are directed against DNA topoisomerase I and are almost exclusively present in the sera of patients with the diffuse form of SSc (Czompoly et al., 2009; Basu & Reveille, 2005). Anti-Scl-70 antibodies also correlate with the development of severe interstitial lung disease. Anticentromere antibodies recognize several protein components of the trilaminar kinetochore (Kallenberg, 1990). These antibodies are usually present in patients with the limited form of SSc and are found in 45–50% of these patients. In contrast to anti-Scl-70 antibodies, anticentromere antibodies are only found in approximately 10% of patients with diffuse SSc. These two autoantibodies are mutually exclusive, rarely coexisting in the same patient. There are numerous other autoantibodies less commonly present in SSc patients, including anti-RNA polymerase I and III antibodies in patients with rapidly progressive diffuse disease and a high frequency of SSc renal crisis (Grassegger et al., 2008; Meyer, 2006; Derk & Jimenez).

Biomarkers of endothelial cell dysfunction

Vascular dysfunction is considered to be one of the earliest clinical manifestations of SSc: it has been suggested to be a crucial initiating event in SSc pathogenesis (Wigley, 2009; Fleming et al., 2008; Kahaleh, 2008; Mulligan-Kehoe, 2008; Koch & Distler, 2007; LeRoy, 1996; Kahaleh et al., 1981). Endothelial injury leads to vascular fibro-proliferative lesions in multiple organs. However, the effects of vascular dysfunction are most dramatic when they involve the pulmonary and renal arterioles, causing renal crisis and PAH, respectively the two most prevalent causes of morbidity and mortality in patients with SSc.

Since the pioneering studies by Kahaleh and LeRoy, focusing attention on the important role of endothelial cells in SSc pathogenesis and originally demonstrating that specific endothelial cell proteins such as the von Willebrand factor (vWf) are abnormally elevated in the sera of patients with SSc (Kahaleh et al., 1986), there has been intense investigation and numerous studies have described potentially important biomarkers that may provide information about the functional status of endothelial cells and their dysfunction in SSc (Davies et al., 2006; Kuryliszyn-Moskal et al., 2005; Cerinic et al., 2003; Herrick et al., 1996). In the original study of Kahaleh, vWf was found elevated in the plasma of patients with SSc and patients with RP in comparison with normal controls (Kahaleh et al., 1986). These studies have subsequently been confirmed, and it has been suggested that this biomarker correlates with SSc severity (Herrick et al., 1996), the presence of pulmonary involvement (Scheja et al., 2001) and the extent of radiologically demonstrated interstitial lung disease (Kumanovics et al., 2008). Of interest was the observation that ADAMTS-13, an enzyme involved in the cleavage and processing of vWf, was found to be reduced in patients with SSc, suggesting that measurements of the activity of this enzyme may represent a biomarker of vascular involvement or endothelial cell dysfunction in patients with the disorder (Mannucci et al., 2003).

Numerous other molecules involved in different aspects of the pathogenesis of endothelial dysfunction in SSc have also been suggested as potential biomarkers for endothelial perturbations in the disorder. Among these are circulating levels of adhesion molecules, thrombospondin, thrombomodulin, endothelin (ET)-1, the N-terminal pro-peptide of the brain natriuretic peptide, vascular endothelial growth factor (VEGF), endostatin, plasminogen activator and metabolites of the arachidonic acid cascade, such as prostacyclin and thromboxane or nitrous oxide circulating metabolites.

Endothelin-1 is a 21-amino acid polypeptide produced by endothelial cells that is capable of potent vasoconstriction and is able to stimulate proliferation of smooth muscle cells. Numerous studies have conclusively demonstrated that ET-1 and its specific cellular receptors play a crucial role in the proliferative vasculopathy of SSc, in particular, in the vascular alterations of SSc-associated PAH (Abraham & Distler, 2007; Sticherling, 2006; Braun-Moscovici et al., 2004; Yamane et al., 1991). Thus, there has been intense interest in ET-1 measurement as a biomarker of SSc vasculopathy. Serum ET-1 levels have been found to be elevated in the plasma of SSc patients and to increase following cold exposure and triggering of RP. Elevated ET-1 levels correlated with other indicators of endothelial cell activation, such as increased vWf, as well as with the levels of other endothelial cell proteins, such as thrombomodulin and adhesion molecules, including soluble intercellular adhesion molecule 1 (ICAM-1) and soluble vascular cell adhesion protein 1 (VCAM-1). Furthermore, immunohistochemistry studies demonstrated the presence of an elevated expression of ET-1 and ET receptors in pulmonary parenchyma at early stages of development of interstitial lung disease and fibrosing alveolitis of SSc (Abraham et al., 1997). These observations suggested that ET-1 measurements may not only reflect crucial alterations in endothelial cell function involved in the pathogenesis of PAH but may also be indicators of the profibrotic activity responsible for the exaggerated production of connective tissue macromolecules characteristic of the disease.

Adhesion molecules involved in cell–cell and cell–extracellular matrix interactions are also important in the pathogenesis of the earlier stages of vascular alterations in SSc and have been suggested as potential

biomarkers for SSc vasculopathy. Increased expression of endothelial-leukocyte adhesion molecule 1 (ELAM-1), ICAM-1, VCAM-1, E-selectin and P-selectin have been found in affected skin from SSc patients, with higher levels present in samples from the diffuse form of the disease, indicating that these proteins may participate in the early stages of tissue fibrosis as well. Elevated serum levels of these adhesion molecules have been found in SSc patients compared with nor- mal individuals (Ihn et al., 1998; Blann et al., 1995) and other studies demonstrated that these levels correlated with increased severity and extent of visceral organ involvement in the disease (Denton et al., 1995).

Numerous recent studies have also demonstrated that, in addition to SSc functional abnormalities in endothelial cells, there might be abnormalities in angiogenesis and endothelial repair. The rarefaction of small capillaries with a reduction in capillary density in affected SSc tissues is consistent with abnormal and disordered angiogenesis. Therefore, markers that may reflect the angiogenesis process have been suggested to be important in the evaluation of vascular alterations in SSc (Distler et al., 2002). One of the key mediators of angiogenesis, VEGF, has been studied extensively as a potential biomarker for the vascular abnormalities in SSc (Chitale et asl., 2008; Davies et al., 2006; Kuryliszyn-Moskal et al., 2005; Choi et al., 2003; Distler et al., 2002). Indeed, high VEGF levels have been found in patients with early SSc, and these levels correlated with the presence of pulmonary fibrosis and abnormalities in pulmonary function, including reductions in vital capacity and the diffusing capacity of the lung for carbon monoxide. High levels of VEGF were also found to correlate with a shorter disease duration as well as with aggressive and rapidly progressive diffuse cutaneous SSc, although other studies failed to show such a correlation (Viac et al., 2000).

2.6.8 Oxidative stress

Oxidative stress plays a critical role in the pathogenesis of SSc, and data have accumulated over the past 15 years to document it. More specifically, ischemic phenomena leading to superoxide anions production take place in SSc patients (Herrick et al., 2001). Remarkably, silica, an environmental agent related to

the occurrence of SSc, is responsible for the induction of oxidative stress and NF-kB pathways in lungs of a luciferase reporter mouse model of respiratory insufficiency (Fubini & Hubbard, 2003). Indirect markers of ROS involvement have also been reported in sera from patients with SSc, such as oxidative proteins and lipid peroxidation (Allanore et al., 2004; Solans et al., 2000). Finally, monocytes and fibroblasts isolated from patients with SSc show an increased synthesis of superoxide anions (Sambo et al., 1999) that could directly upsurge fibroblasts proliferation and ECM production (Sasmbo et al., 2001.

2.6.9 Auto-immunity

The immune system plays an essential role in the pathophysiology of SSc. Autoantibodies imbalance in B and T lymphocytes subpopulations and perturbations of dendritic cells have been reported in SSc. The disease cannot be qualified as autoimmune, since autoantibodies have not been demonstrated to induce the disease, however, the identification of specific autoantibodies is of critical help in the diagnosis and the evaluation of prognosis of SSc (Tyndal et al., 2013). The distribution of autoantibodies in SSc patients is associated with phenotypes. Thus, anti-topoisomerase 1, anti-RNA polymerase III and antifibrillarin (anti-U3 RNP) correlate with dcSSc, while anticentromere, anti-Pm/Scl, anti-Th/To and anti-U1 RNP are associated with lcSSc. Various other antibodies have also been reported in SSc patients, directed against several targets such as fibrillin-1, metalloproteinases or platelet derived growth factor (PDGF) receptor. These autoantibodies argue for a pathogenic role of B cells (Arnett, 2006). Thus, circulating B cells from people with SSc differ in their phenotype as compared to healthy controls, with increased proportions of naïve B cells and decreased numbers of memory B cells and plasma cells (Sato et al., 2004). Cluster of differentiation (CD)19 and CD21, two activation coreceptors of the B-cell receptor (BCR), are overexpressed in these naïve and memory B cells. The activation receptors CD80, CD86 and CD95 are upregulated on memory B cells, suggesting their participation to the pathogenetic process.

High levels of B cell activating factor (BAFF) have been measured in the serum of patients with SSc, together with an overexpression of BAFF-R at the surface of peripheral B cells from SSc patients (Matsushita & Sato, 2005). BAFF activates the NF-kB pathway, promotes B cells survival and

participates to the differentiation of autoreactive B cells. In addition, it has been documented that B cells infiltrate the dermis of SSc patients (Kraaij & van Laar, 2008; Bosello et al., 2007), that circulating levels of IL-6 are increased in SSc patients as compared to healthy controls and correlated with the extent of skin fibrosis (Sato et al., 2001). In tight skin mouse (Tsk-1), the depletion of B cells leads to a decrease of total IL-6 messenger ribonucleic acid (mRNA) and improvement of fibrotic lesions (Hasegawa et al., 2006). Finally, IL-6 is known to stimulate collagen secretion by fibroblasts and represent a potential ‘‘B cell link’’ to fibroblast activation.

T cells are also involved in the pathogenesis of SSc. Thus, a type 2 T helper (Th-2) bias has been documented in people with SSc (Sunderkötter & Riemekasten, 2006). Increased levels of IL-4 and IL-13 have been measured in the serum of people with SSc. These two cytokines have been classically described as synergic Th-2 polarization cytokines via signal transducer and activator of transcription 6 (STAT6). Although the trigger contributing to the initiation of secretion of these cytokines is not identified, it has been documented that mature and activated Th-2 secrete IL-4, IL-6 and IL-13, enhancing a positive loop of Th-2 polarization and B cells stimulation (Chizzolini, 2008). Interestingly enough, the treatment of Tsk-1 mice with an anti- IL-4 monoclonal antibody prevented the induction of skin lesions (Ong et al., 1998), underlining the major role this cytokine in SSc physiopathology.

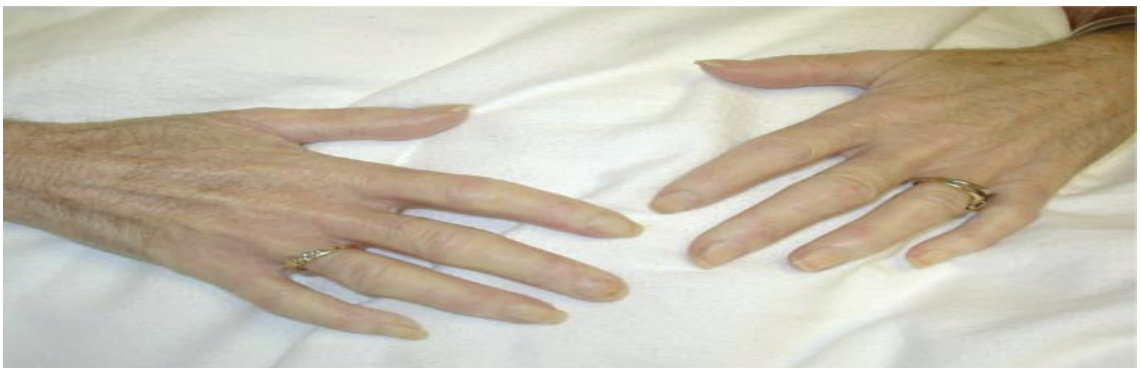
Targeting B cells (anti-CD20), IL-6, IL-4 and also T cells and mature lymphocytes (anti-CD52) may lead to substantial clinical improvement in mouse models and in open series of patient with SSc (Hasegawa et al., 2006; Stratton et al., 2001; Ong et al., 1998; Isaacs et al., 1996). These findings argue for the important role of B and T cells in the pathogenesis of SSc. In addition to B and T lymphocytes, dendritic cells appear of utmost importance in SSc. Thus, a recent proteome-wide analysis of the culture supernatant of plasmacytoid dendritic cells (pDC) isolated from patients with SSc allowed identification of increased levels of CXCL4, with higher levels in diffuse than in limited forms, and increased levels in early diffuse vs. late diffuse people with SSc (van Bon et al., 2014). Interestingly, CXCL4 was also detected in the skin of people with SSc and not in healthy control skin. Serum levels of CXCL4 correlated

with clinical status of people with SSc. Notably, in patients with lung fibrosis and/or PAH, high levels of CXCL4 correlated with a poor prognosis. Finally, infusion of CXCL4 leads to increased leukocytes infiltration, skin thickening and C-C chemokine ligand 2 mRNA expression in the bleomycin-induced model of SSc. Added together, these observations argue for a possible role of CXCL4 in the pathogenesis of SSc. Further studies need to confirm the interest of targeting CXCL4 in SSc patients.

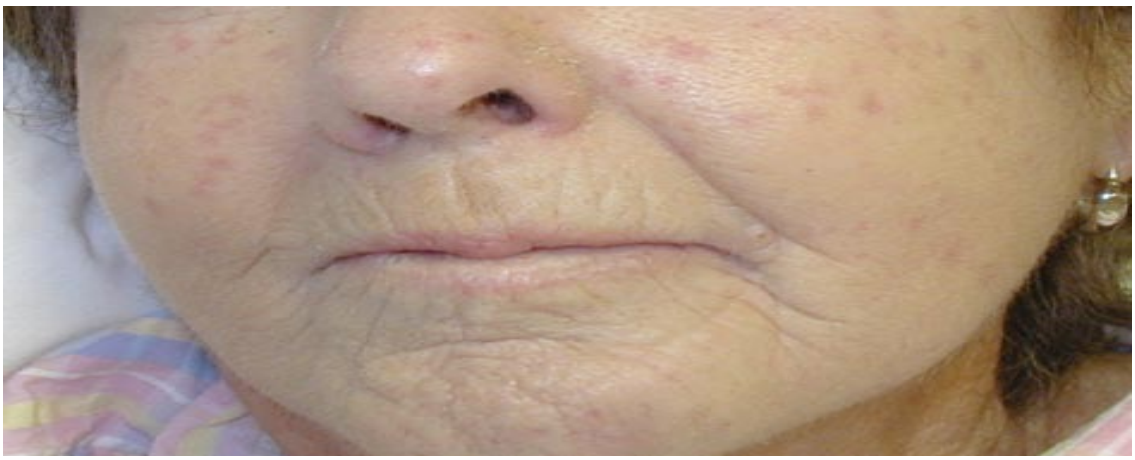
2.6.10 Clinical manifestations

Skin

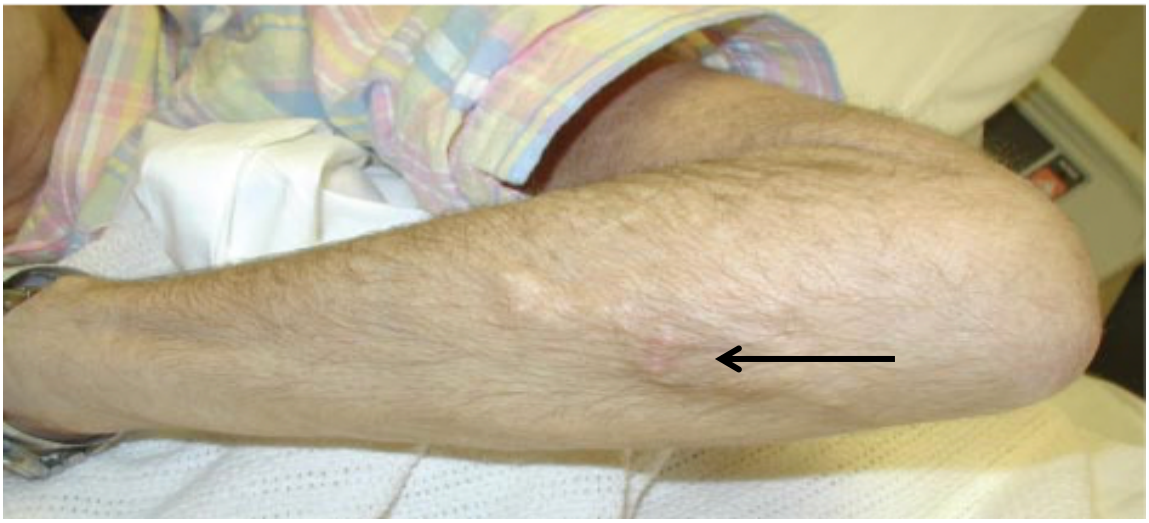
Skin involvement is very common in systemic sclerosis. Early symptoms may be puffiness, swelling, and decreased flexibility of the joints and tendons. Therefore, the affected skin appears shiny, taut, and thickened, tightly adhering to the underlying cutis (Figure 2). Skin thickening is usually accompanied by hyperpigmentation, providing a salt-and-pepper appearance. As systemic sclerosis advances to the fibrotic stage, the skin becomes more thickened until atrophy occurs, especially over the bony prominences and extensor surfaces of the proximal interphalangeal joints. During the atrophic stage, the dermis may soften and revert to normal or below average thickness. Other skin findings include nail-fold capillary alterations (dilated loops at the nail bed and distended venules), telangiectasias (Figure 3), painful ulcerations from ischemia (with or without necrosis), subcutaneous calcinosis, and RP. When ulceration occurs, healing is slow with frequent secondary infection. Subcutaneous calcifications (Figure 4) composed of amorphous calcium hydroxyapatite occur mainly in periarticular tissues. Although radiographs are not necessary for a diagnosis of subcutaneous calcifications, they can show the radiopaque deposits (Figure 5).



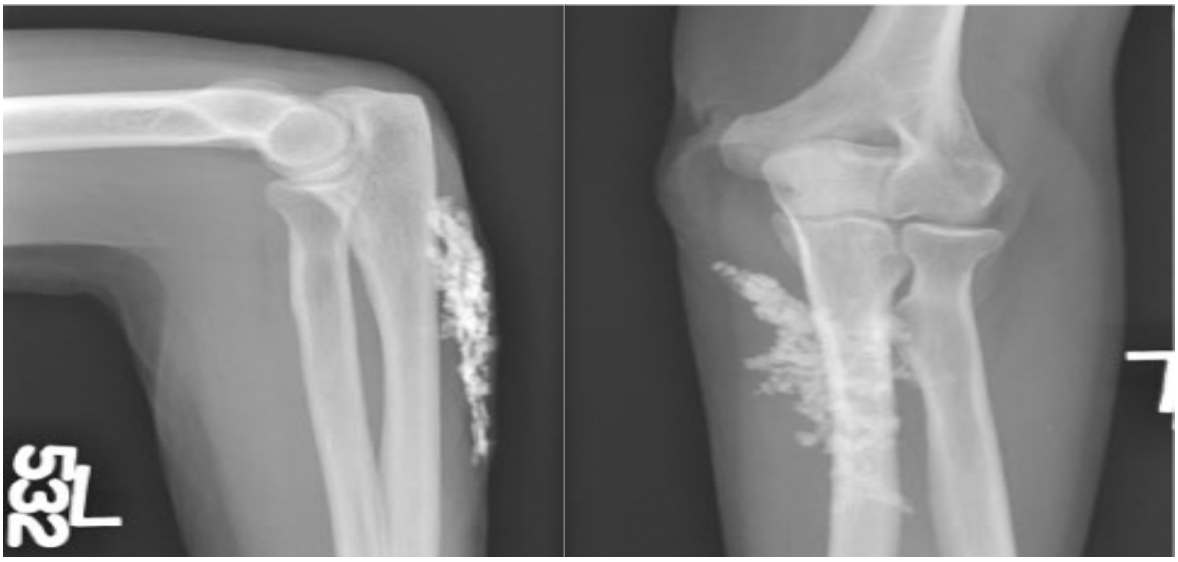
2.6 Figure 2 The affected skin in scleroderma appears shiny, taut, and thickened, tightly adhering to the underlying cutis. This is most notable in the hands.



2.6 Figure 3 Telangiectasias scattered on the face.



2.6 Figure 4 Palpable subcutaneous calcifications (arrow).



2.6 Figure 5 Radiographs showing subcutaneous calcifications as radiopaque deposits (arrows).

Raynaud's phenomenon

RP occurs almost universally in systemic sclerosis and is manifested by episodic pallor followed by cyanosis and/or rubor of the distal portions of the digits after exposure to cold. RP often predates other manifestations in the limited subtype and is often found concurrently in dcSSc (Wigley, 2002). Capillary nailfold microscopic abnormalities seen in association with RP predict later development of rheumatic disease. Vascular occlusion can occur and has been associated with the anticardiolipin antibody, but this is very rare in SSc (Shapiro, 1990). Arterial occlusive disease and digital ulcers can occur in extremities and may require amputation.

Pulmonary

Pulmonary manifestations of SSc include ILD, PAH, pleuritis and pleural effusion, and aspiration pneumonia (Deepa et al., 2016). Dyspnoea and non-productive cough in people with SSc should raise the possibility of lung disease, and a work-up for ILD should be performed. However, chronic cough may be the only sign of pulmonary disease in SSc. Interstitial fibrosis is more likely to occur among persons with dcSSc than in those with lcSSc; it can occur without prior warning symptoms and can occur early in the disease course. On physical examination, end-inspiratory rales (fine or Velcro crackles) are often heard. Pulmonary function abnormalities can reveal a restrictive ventilatory defect, suggested by a

reduction in forced vital capacity and decreased lung compliance and diffusing capacity. Chest radiograph shows reticular interstitial thickening in a linear or nodular pattern most evident in the lower lung bases. A high-resolution computed tomography scan is more sensitive and can detect early disease when chest radiographs are normal (Wells, 2008). A "ground glass" appearance is a feature of pneumonitis rather than fibrosis. This lung manifestation is seen more frequently with diffuse disease, in African Americans, and in those with antibodies to topoisomerase-I (Wells, 2008).

Pulmonary hypertension is more frequently seen with limited systemic sclerosis than with diffuse disease and often occurs late in the disease course. A common presenting symptom of pulmonary hypertension is dyspnoea on exertion. Physical examination may reveal accentuation of the S2 and signs of right-sided heart failure (elevated jugular venous pressure, pitting oedema, right ventricular heave). An echocardiogram or right-sided cardiac catheterization can confirm the diagnosis. Pleuritis and plural effusion can occur without symptoms. In late-stage systemic sclerosis, lung cancer can occur independent of tobacco use but is rare (Zeineddine et al., 2016).

Cardiovascular

Cardiac manifestations may affect people with either lcSSc or dcSSc and, when clinically evident, are often associated with mortality (Ferri et al., 2002; Scussel-Lonzetti, 2002; Steen & Medsger, 2000, Hegedus & Czirjak, 1995). Vlachoyiannopoulos et al. (2000) retrospectively analysed the clinical files of 254 people with SSc over a 4-year period. They estimated the mortality rate to be 2% per year, and the incidence of cardiac disease to be between 7% in lcSSc and 21% in dcSSc patients (Vlachoyiannopoulos et al. 2000). Similarly, a review of 1095 SSc patients between 1959 and 1988 estimated the overall mortality of SSc to be 33%, with deaths of 42 patients (4.5%) resulting from cardiac manifestations (Follansbee et al., 1993). Another review of 405 SSc patients followed for 5 years between 1990 and 2000, determined that 21 out of a total of 145 (14%) patient deaths were due to cardiac manifestations, at a rate of 1% per year.

Cardiac manifestations of SSc can affect all heart elements and may result in pericardial effusion, arrhythmias, conduction system defects, valvular impairment (in rare cases), myocardial ischaemia, myocardial hypertrophy and heart failure (Kahan & Allanore, 2006). These primary myocardial manifestations those without systemic or PAH and without significant pulmonary or renal disease likely result from the underlying vascular pathology of SSc, i.e. the characteristic vascular lesions and fibrosis that impair microcirculation and myocardial function, respectively (Kahan & Allanore, 2006). The early myocardial manifestations of SSc are often non-specific, making evaluation of susceptible patients problematic. Patients with cardiac manifestations may therefore remain undiagnosed, potentially enabling the disease to progress silently. Early diagnosis is therefore very important. For patients with SSc undergoing autologous haematopoietic stem cell transplantation, a full cardiological assessment before and during the transplant is recommended, as patients with cardiac abnormalities are known to be at increased risk of mortality (Saccardi et al., 2004).

Renal

Scleroderma renal crisis (SRC) occurs in 10–15% of the patients with dcSSc and only vary rarely (1–2%) in lcSSc (Teixeira et al., 2008; Penn et al., 2007). Most cases occur within the first 12 months of the disease and in up to a quarter of patients with SRC, the diagnosis of SSc is made at the time of the renal presentation. Typically, patients present with accelerated hypertension and progressive renal impairment. End-organ damage can result in encephalopathy with generalized seizures or flash pulmonary oedema. Microangiopathic anaemia is common and disseminated intravascular coagulation may develop. Approximately two-thirds of the cases of SRC require renal replacement therapy (Penn et al., 2007). Of these, half eventually recover sufficiently to discontinue dialysis. This can occur for up to 24 months, and so decisions about renal transplantation should be postponed until that time. The possibility for delayed renal recovery distinguishes SRC from other causes of end-stage renal failure. Historically, SRC was the commonest form of scleroderma-associated death (Steen & Medsger, 2007). Dramatically improved outcomes in the short-term are achieved with the use of angiotensin converting enzyme

inhibitors as routine therapy for established SRC. It remains unclear whether these or related drugs, such as angiotensin receptor blockers, are effective in preventing or abrogating SRC. Corticosteroids, along with cyclosporin (Denton et al., 1994), have been implicated as precipitants of SRC (DeMarco et al., 2002; Steen et al., 1998).

Gastrointestinal

Gastrointestinal manifestations are common in SSc, and the most common is oesophageal dysfunction. Abnormal propulsive peristalsis and hypomobility resulting from selective atrophy of the circular smooth muscle layer cause dysphagia, reflux esophagitis, and the abnormal sensation of food "sticking," which necessitates drinking of fluids for relief. People with SSc may experience retrosternal burning pain and acid regurgitation, especially when in a supine position. If reflux esophagitis remains untreated, a distal oesophageal stricture may develop, requiring periodic dilatation. Chronic esophagitis also may lead to Barrett's oesophagus, but this complication seems to be diminishing with widespread use of proton pump inhibitors. Rarely, telangiectasias may cause bleeding in the stomach and result in a "watermelon stomach" visible as stripes on endoscopy (Elkayam et al., 2000). Gastroparesis can aggravate reflux and contribute to bloating, abdominal cramps, and distention. These symptoms may lead to a functional ileus, which can be managed medically with nasogastric suction and bowel rest. Hypomobility of the intestines can lead to overgrowth of intestinal microorganisms, malabsorption, and cachexia (Lundberg et al., 1992). Also, volvulus of the small intestine has been observed (McFarlane et al., 2018). Patients who have colon involvement may present with constipation, which may be relieved by judicious use of increased dietary bulk, stool softeners, and increased fluid intake (McFarlane et al., 2018).

Musculoskeletal

Muscle involvement

The most frequent clinical symptoms for people with SSc are muscle pain and weakness. The frequency of muscle pain may reach up to 86% (Ranque et al., 2009; Medsger et al., 1968) among SSc patients. Scleroderma patients with myopathy have usually symmetric proximal limb weakness that is

indistinguishable from that seen in patients with idiopathic inflammatory myositis. Distal weakness may be also present (Hausmanowa-Petrusewicz et al., 1982; Medsger et al., 1968), although it can be difficult on certain occasions to distinguish myopathic weakness from the limitation of movement due to skin sclerosis, articular changes in proximity to the assessed muscles or fibrosis of underlying tissues. Muscle weakness reported by the treating physician was 18.9% in the lcSSc and 33.5% in the dcSSc subset in patients fulfilling the ACR classification criteria, and 36.5% in the ‘‘other’’ subgroup, consisting of patients with skin sclerosis distal to metacarpophalangeal joints in the EUSTAR database comprising data of 9165 SSc patients (Meier et al., 2012). This latter group included most probably patients with early SSc as well as cases with overlap syndromes. In other studies, the prevalence of abnormal muscle strength tested manually varied widely, from 10% up to 96% (Clements et al., 1999a; 1999b; Brick et al., 1989; Medsger et al., 1968). The lower prevalence of self-reported muscle weakness in the majority of the studies may suggest that muscle involvement in SSc patients is frequently rather mild and/or that the level of physical activity of SSc patients is reduced due to other reasons, such as malaise, synovitis, and heart or lung disease. However, in a study by Clements et al. (1999a) the prevalence of self-reported muscle weakness was higher (26-40%) if compared to decreased muscle strength by manual muscle testing (10%), indicating that sometimes muscle weakness may not be due to a primary myopathy but due to other scleroderma-associated disease symptoms, such as joint involvement, skin contractures or fatigue

Skeletal involvement

Synovitis can be present in patients with SSc in all disease stages, but it is most frequent in the early stage of the disease. The frequency of synovitis is higher in patients with the diffuse cutaneous subset compared to the limited cutaneous subtype, but only in early disease (Avouac et al., 2010; Su et al., 2009; Avouac et al., 2006). Arthritis-related pain is closely associated with SSc patients’ health related quality of life (Hyphantis et al., 2007). According to Baron et al. (1982) arthritis can be detected most often in the metacarpophalangeal joints, wrists, knees, distal interphalangeal joints, and proximal interphalangeal

joints, in decreasing order. Arthralgia and hand stiffness were among the four highest rated symptoms in terms of frequency and impact on daily activities in the Canadian National Survey (Bassel et al., 2011). Arthralgia was found to be significantly more common in patients with dcSSc, than with lcSSc (Ostojic & Damjanov, 2006). Moreover, Skare et al. (2011) reported that pain and stiffness were the symptoms that most affected functionality. Contractures are one of the main sources of disability in SSc. They are frequent in both subtypes; however, the prevalence of joint contracture is higher in dcSSc, than in lcSSc. Moreover, diffuse cutaneous subset is an independent predictor of the progression of flexion contractures. Though the development of contractures is relatively slow and gradual, it can be present in the early stages of the disease, too (Au et al., 2010; Erre et al., 2008; Ostojic & Damjanov, 2008; 2006; Avouac et al., 2006).

Malignancy

Malignancy and scleroderma have been studied for their relation by several studies in the past, with conflicting findings (Siau et al., 2011). A Danish study conducted between 1997-2006 by Olesen et al., (2010) demonstrated 222 cases of cancer, following an SSc diagnosis, reporting a higher standardized incidence ratio for cancer of 1.5. The standardized incidence ratio for men was higher [2.2 (95% CI 1.7-2.8)] compared to women [1.3 (95% CI 1.1-1.6)]. Smoking- and alcohol-related cancers were reported to be the most frequent malignancies. The study by Siau et al., (2011) in southwest England examined the age-matched malignancies in people with SSc based on a regional healthcare database. The results showed 15 malignancies out of 68 people with SSc with a relative risk of 3.15 (95% CI 1.77-5.20) in overall cancers.

2.7 Therapeutic approach

The approach of treating digital ischemia can be complex, given the fact that it must commence rapidly and effectively, and there are several new therapeutic options available. A study that examined patients with digital ischemia, showed that cutaneous vascular complications of SSc are frequently undertreated or treated inappropriately (Herrgott et al., 2008). Furthermore, the treatment of digital ulcers is associated

with an improvement in functional status and quality of life (Wigley et al., 1994). However, medical treatment has many side effects and it will be useful in the future to standardise care for the management of the ischemic digit and explore adjunctive non-medical treatments, which would reduce dependency on medication.

2.7.1 Non-medical therapy

In SSc initial treatments are usually aimed at symptom control and improving tissue integrity and viability (Macmahan and Wigley, 2010). Exposure to cold temperatures and stress are factors inducing vasoconstriction and need to be avoided. This includes lifestyle modification to avoid extreme cold, shifting temperatures and proper clothing to keep the whole body warm (Macmahan and Wigley, 2010). Variable outcomes have been demonstrated from studies examining conditioning, biofeedback (biofeedback therapy is a technique that trains people to improve their health by controlling certain bodily processes that normally happen involuntarily, such as heart rate, blood pressure, muscle tension, and skin temperature; Frank et al., 2010) and relaxation techniques. A controlled trial in a large cohort found no benefit in the use of biofeedback in primary RP (Wigley & Wise, 2000), and its use is also not recommended for secondary RP. The use of gloves is helpful in protecting the skin from trauma and maintaining it warm in the cold. Smoking cessation is crucial as smoking contributes to the underlying vascular disease. Moreover, creams and lotions can be applied locally to keep the affected skin moist. More specifically, Vitamin E gel has been demonstrated to reducing time of healing of digital ulcers in people with SSc (Fiori et al., 2009). For more serious ulcers, compressive stockings serve to protect them from trauma and promote healing. Hydrocolloid dressing also promotes healing of digital ulcers (Milburn et al., 1989). Patients with severe digital ischemia should be put at rest and in a warm environment. This might mean hospitalisation or stopping work for home care. Blood flow and recovery can be improved by preventing trauma to the digits, such as typing or repetitive hand work.

2.7.2 Pharmacological agents

The agents used for the treatment of RP and SSc vasculopathy can be separated into agents that mainly work as vasodilators, those that are able to protect vessels from disease progression, and agents that prevent thrombosis. A prescribed agent may have more than one effect. For example, prostaglandins can be both vasodilators and protective of vessel damage. The following review will first outline currently used medications and then will focus on a certain approach to critical ischemia.

Vasodilatory therapy

Alpha adrenergic blockers: Alpha adrenergic blockers were the first medical treatment used with some success in treating RP. Alpha-2 adrenoreceptors are involved in the vascular system and they play a critical role in cutaneous thermoregulation (Wigley, 2009). Several studies examined Prazosin for treatment of RP (Wollersheim et al., 1986; Russell and Lessard, 1985). A subsequent Cochrane systematic review inferred that Prazosin can improve RP in a small degree, but that side effects can limit RP tolerability (Pope et al., 2000). Furthermore, various other medications of this similar class showed a clinical benefit (Paterna et al., 1997; Wollersheim et al., 1986): Notably, the alpha 2c receptor, as subtype of the alpha-adrenergic receptor, is especially upregulated in cold exposure (Chotani et al., 2000). That led Wise and colleagues (2004) to study the efficacy and tolerability of a selective alpha 2c-adrenergic receptor blocker in people with SSc with vasospasm. The results demonstrated that the time to rewarm SSc patient's digit after a cold challenge was reduced after drug ingestion, thus proposing potential for therapeutic efficacy. Although these results are promising, these agents are not yet available, and more studies are required to validate the clinical efficacy.

Calcium channel blockers: Calcium channel blockers are commonly used for RP and act on vascular smooth muscle to promote arterial dilation. Nifedipine and nicardipine are deemed as the first-line therapy for RP according to EULAR recommendations for the treatment of SSc (van den Hoogen et al., 2013). These agents have the potential to decrease the risk of ulcers developing. Nevertheless, there is lack of data to support the efficacy of calcium channel blockers in the treatment of digital ulcers once

they have developed (Kowal-Bielecka et al., 2009). On the other hand, a meta-analysis reviewed the use of calcium channel blockers in RP and reported moderate efficacy at best (Thompson, 2001). In addition, the extend of the effect of calcium channel blockers for secondary RP is much less than in primary RP, although the reports indicated a 35% improvement in attack severity and a mean decrease of about 8 attacks per 2-week period when compared to placebo. Furthermore, appropriate dosing is not always reached: For example, Herrgott and colleagues demonstrated that 92% of the German centres did not aim for the recommended dose of 360 mg of diltiazem, or 10 mg of amlodipine, and 80% did not target for at least 40 mg of nifedipine (Herrgott et al., 2008; Riemekasten and Sunderkotter, 2006). Longer acting prescriptions can be utilised to minimise side effects of the medication and enhance tolerability.

Nitrates: Glyceryl trinitrate (GTN) has been assessed in several forms: Initially the intravenous form was examined only to discover that while there was an initial response, the effect was eventually blunted with disease progression (Matucci-Cerinic et al., 1990). A few years later the GTN patches (0.2 mg/hour) were examined in patients with primary RP and with secondary RP. Both groups showed an improved by the treatment; however, the side effects, specifically the headaches, were intolerable. Nevertheless, the use of GTN in the topical ointment formulation found to be effective with minimal side effects, even in patients with very thick skin (Anderson et al., 2002). Although topical nitrates can improve digital microcirculation, the practical issues of these agents such as difficulty with repeated applications and side effects limit their use. Topical, sublingual, or oral formulations of nitrates are sometimes used as complementary therapy in the treatment of RP and digital ulcers; however, there is lack of randomised controlled studies evaluating the effects of nitrates on digital ulcers healing (Gholam et al., 2009).

Phosphodiesterase Inhibitors: Phosphodiesterase inhibitors (PDE-I's) act by increasing levels of cyclic guanosine monophosphate (cGMP), causing intracellular calcium level to decrease and leading to vascular smooth muscle relaxation. Through this mechanism PDE-I's cause vasodilation and enhance perfusion to distal tissues (Rybalkin et al., 2003). This drug category has showed significant effects in patients with digital ischemia (Levien, 2006; Boin and Wigley, 2005; Fries et al., 2005; Lichtenstein,

2003). The five drugs available in this category of medications consist of sildenafil, tadalafil, vardenafil, pentoxifylline, and cilostazol, with the first two having been better studied: A double-blind, placebo controlled fixed dose crossover study was conducted to examine the effects of sildenafil on symptoms of capillary perfusion in 16 patients with primary RP (Fries et al., 2005). The results indicated that sildenafil was correlated to a reduced incidence and duration of RP as well as reduced Raynaud's condition score. Capillary blood flow velocity enhanced in individual patients and the mean capillary blood flow velocity of all patients who received sildenafil more than quadrupled (Fries et al., 2005). Studies that examined the effects of vardenafil and tadalafil have demonstrated on one hand promising results (Caglayan et al., 2006; Baumhaekel et al., 2005) and on the other no benefit of tadalafil over placebo (Schiopu et al., 2009). An important limitation of those studies was the small sample size. Large randomised controlled trials are still required to validate the use of PDE-I's in secondary Raynaud's. A double-blind, placebo-controlled study that examined 57 people with SSc with RP compared sildenafil 200 mg/day versus placebo. The findings showed a reduction in frequency of RP attacks in the sildenafil group, although the result was not statistically significant (Herrick et al., 2011). There is also a necessity for more prospective studies on this category of medications in digital ulcers (Steen et al., 2009).

Prostacyclins: Prostanoids are beneficial to microvasculature as they induce vasodilation, enhance intracellular cyclic adenosine monophosphate (cAMP), and prevent smooth muscle proliferation (Engel and Rockson, 2005). They also improve digital ulcers by acting as a potent vasodilator and actively prevent platelet aggregation. Prostacyclins and more specifically, iloprost has become the standard care for people with SSc with severe digital vasculopathy and digital ulcers and it should be deemed as the first-line therapy in the treatment of digital ulcers (Kowal-Bielecka et al., 2009). Intravenous iloprost is a popular medication for the treatment of severe RP in SSc as it reduces the frequency and severity of attacks and prevents and heals digital ulcers (Pope et al., 1998; Wigley et al., 1994). There are various protocols for intravenous iloprost therapy to treat severe RP and digital ulcers (Caramaschi et al., 2009; Rehberger et al., 2009; Caramaschi et al., 2006; Bettoni et al., 2002; Wigley et al., 1994; Wigley et al.,

1992). It is recommended by these reports that using prostacyclin by intravenous delivery intermittently can prevent digital ischemic events. Low dose (0.5 ng/kg compared to 2 ng/kg body weight per minute) iloprost was demonstrated to be equally effective (Kawald et al., 2008). Several studies reported that subcutaneous treprostanil is also potent in the treatment of severe digital ulcers (Chung and Fiorentino, 2006; Engel and Rockson, 2005). The efficacy of oral prostacyclins has been examined for treatment of severe RP and digital ulcers; however various studies utilising oral iloprost, beraprost, and cicaprost demonstrated no significant benefit compared to placebo (Wigley et al., 1998; Vayssairat, 1996; Lau et al., 1993).

Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Blockers: Angiotensin converting enzyme inhibitors and angiotensin receptor blockers were also examined in SSc in association with digital ischemia. The first trials demonstrated promising results as captopril produced a significant improvement in cutaneous microcirculation; however, it was not demonstrated to change the frequency or severity of RP attacks (Rustin et al., 1987). The findings from a subsequent trial demonstrated promising results in reducing the frequency of primary RP (Janini et al., 1988). Nevertheless, a review paper that aggregated the subsequent clinical trials on this drug category concluded to controversial findings (Challenor, 1994). A multicentre, randomised, double-blind, placebo-controlled study examined the dosage of quinapril 80 mg/day, or the maximum tolerated dosage, for 2-3 years in over 200 people with SSc. The findings did not demonstrate any benefit in limiting the incidence of digital ulcers or reducing the frequency or severity of RP episodes (Gliddon et al., 2007), thus this category of medications is not recommended for the treatment of RP or digital ulcers.

Vasoprotective agents

Anti-platelet agents: Several studies have reported increased platelet activity in people with SSc (Pamuk et al., 2007; Lau et al., 1993; Cuenca et al., 1990; Kahaleh et al., 1982). In a similar study platelet activation marker correlated with disease activity and severity in SSc (Agache et al., 2007). Moreover, a combination therapy with aspirin and dipyramidole significantly reduced the circulating platelet and beta-

thromboglobulin levels (Kahaleh et al., 1982). A double-blind placebo-controlled study reported no benefit with combination therapy with aspirin and dipyramidole versus placebo; however, the trial was too short (two years) with a small sample size (n = 28), thus conclusions for long term benefit cannot be drawn (Beckett et al., 1984).

Endothelial Receptor antagonists: The endothelial receptor antagonists have demonstrated promising results in preventing digital ulcers and have vasculoprotective effects. A small preliminary study with 122 people with SSc examined the effect of dosentan on preventing digital ulcers and the results were promising as indicated a 48% reduction in developing new ulcers during the treatment period (Korn et al., 2004). Another study observed similar results in the prevention of new ulcers, especially in patients with a high number of digital ulcers at baseline. On the other hand, in a 24-week duration study with 198 subjects, greater rates of healing of ulcers were observed with placebo compared to active drug. No difference between active treatment and placebo were found in net digital ulcer's burden, pain, measures of activities of daily living by the health assessment questionnaire or UK functional score or in hospitalisation rates (Matucci-Cerinic and Seibold, 2008). Larger studies will be required to define efficacy and long-term outcomes; however, these findings are promising.

Statins: Statins present vasculo-protective effects, by increasing high-density lipoprotein, decreasing low-density lipoprotein, decreasing coagulation, free radicals, and blood viscosity, increasing platelet function, and decreasing matrix metalloproteases (Wigley, 2009; Abou-Raya et al., 2007; Kuwana, 2006). The effects of statins on patients with RP and digital ulcers were examined in 84 people with SSc who were matched with 75 control subjects (Abou-Raya et al., 2008). The study demonstrated that the overall number of digital ulcers was significantly decreased in the stating group and that endothelial markers of activation were enhanced when comparing the statin to control groups (Abou-Raya et al., 2008). Results have been confirmed by other studies (i.e., Del Papa et al., 2008). Although these findings are promising, larger trials are still required.

Sympathectomies: Sympathetic nerve mediated vasospasm is suggested to be an important mechanism leading to digital ischemia. Therefore, sympathectomies are utilised aimed at blocking this mechanism. Uncontrolled series of case reports recommend beneficial effects for RP and the treatment of refractory digital ulcers. It has been demonstrated that local digital sympathectomy has long term benefits in people with SSc with digital ischemia (Kotsis et al., 2003; Yee et al., 1998). A follow up of 7.5 years revealed that sympathectomy resulted in complete ulcer healing and reduces in the total number of ulcers in 75% of the patients in this subgroup (Hartzell et al., 2009). Although the sample size was limited (n=20), these findings are promising for patients with refractory disease. Arterial revascularisation is sometimes performed at the same time and has also showed success (Taylor et al., 2002).

2.8 Quality of life in systemic sclerosis

2.8.1 Quality of life features in systemic sclerosis

There is lack of knowledge about the frequency and perceived impact of the various problems faced by people with SSc (Bassel et al., 2011; Valentini, 2003). Furthermore, it is important to define the difference between the assessment of disease severity and the health-related quality of life [HR-QoL (Ludici et al., 2013; McNearney et al., 2009)]. The patients' cognitive representations of the disease are the most important determinants of physical and mental health. It has been recommended that the fear of clinical consequences and the tendency to ascribe each physical complaint to SSc are key contributors to the physical health, while the emotional responses to personal representation of the disease contribute to mental health (Ludici et al., 2013; Arat et al., 2012). People with SSc are more dissatisfied with healthcare than other clinical populations, as suggested by the "Canadian Scleroderma Patient Survey of Health Concerns and Research Priorities" (Leite and Maia, 2013). This is because SSc complications are more visible such as digital disfigurement that tends to worse over time, leading to elevated psychological morbidity [e.g., more depressive symptoms and anxiety (van Lankveld et al., 2007)], regular use of healthcare and related increased costs. Moreover, clinicians may disregard or use unreliable assessments to evaluate psychological distress (Chularojanamontri et al., 2011). While physicians may highlight

objective indicators of disease status, patients may discern other aspects of their disease experience as more debilitating or distressing (Bassel et al., 2011; Arkachaisri et al., 2009; Suarez-Almazor et al., 2007), such as limited mobility and hand function, fatigue, pain, depression, sleep disturbance, sexual dysfunction and body image distress from disfiguring alterations in appearance [e.g., hand contractures, pigment changes and facial telangiectasias (Kwakkenbos et al., 2013)].

Fatigue, functional limitations, skin deformities, pain and disfigurement were reported to be the most annoying symptoms (van Lankveld et al., 2007), whereas physical pain, coping skills, social aspects of living with the disease, physical appearance and the relationship between patient and physician were identified to be particularly important to patients.

A large Canadian National Survey with people with SSc reported that fatigue, RP, stiff hands joint pain and sleeping disorders were the symptoms with the highest frequency and the most likely to have at least moderate impact on daily activities (Bassel et al., 2011). On the other hand, various symptoms with high frequency but low scored in terms of impact were dry mouth, itching and skin colour change (Bassel et al., 2011). Other studies (Razykov et al., 2013; Mouthon et al., 2010; Schieir et al., 2010; Thombs et al., 2009, 2008a, 2008b) report that difficulty breathing, gastrointestinal problems, depression, pain from various sources, fatigue and pruritis were correlated with disability and decreased HR-QoL.

Studies have demonstrated that HR-QoL is impaired in both lcSSc and dcSSc people but more in the latter sub-group (Ludici et al., 2013; Chularojanamontri et al., 2011; Khanna et al., 2007). Also, in both undifferentiated connective tissue disease and early people with SSc (Koenig et al., 2008), HR-QoL is impaired in physical and mental domains (Ludici et al., 2013). People with SSc have elevated levels of pain and fatigue compared to the general population (Thombs et al., 2008a). RP, digital ulcers, gastrointestinal symptoms and worsened synovitis were independently correlated with pain by multivariate analysis (Schieir et al., 2010).

Work Productivity

Adult life is remarkably affected by employment. The ability to work is a multifactorial phenomenon affected by determinants such as physical and psychological capacity, and by certain work requirements and factors outside the working life (Nguyen et al., 2010; Sandqvist et al., 2010). Extended periods of absence from work are often accompanied by loss of life roles and social status, with important financial consequences for the individual, the employer and the society (Calixto et al., 2014; Singh et al., 2012; Nguyen et al., 2010; Sandqvist et al., 2010).

Perceived symptoms such as general fatigue, pain and impaired hand function (related to RP, skin thickness, ulcers and pain) are frequent in SSc and they significantly affect both work ability and employment status (Sandqvist et al., 2010; Sandqvist et al., 2009; Sandusky et al., 2009; Sandqvist et al., 2005). Working ability is also affected by impaired grip force and dexterity (Sandqvist et al., 2008) that in turn influence the employment status, social insurance systems and perhaps economic situation for the patients (Sandqvist et al., 2010).

An important factor to take into account is that the occupational exposures to chemicals and pollutants (e.g., white spirit, crystalline silica, chlorinated and aromatic solvents, ketones and welding fumes) as well as the use of some drugs have been reported to be involved in SSc pathogenesis (Marie et al., 2014). Therefore, it is suggested that these patients avoid such occupational exposures.

Sleeping disorders

Effects of sleep deprivation on cytokines and immune dysfunction are familiar (Frech et al., 2011; Marshall and Born, 2002). According to polysomnographic evidence, people with SSc might have higher risk for sleep disturbances (Frech et al., 2011). It has been demonstrated that sleep efficiency was reduced by 70% in people with SSc compared with age adjusted norms (Prado et al., 2002) and that people with SSc have detrimental effects on their sleep over that of general population (Frech et al., 2011). Furthermore, the sleep duration of people with SSc was comparable with that of general population but

the sleep quality was poor (Frech et al., 2011). Some of the independent indicators for sleep disturbance were the reflux symptoms, worsening dyspnoea, depressed mood and pain (Abad et al., 2008).

Depression

Mild to severe psychological distress is present in half of the people with SSc (Nguyen et al., 2014; Chularojanamontri et al., 2011; Hyphantis et al., 2007) and this is occasionally underestimated by physicians (Chularojanamontri et al., 2011). Depression was correlated with the variables of age, symptom frequency and impact on mental health, anxiety and social phobia (Leite and Maia, 2013). Anxiety is frequent in people with SSc (Nguyen et al., 2014; Leite and Maia, 2013; Legendre et al., 2005) and is related to alterations in body image that results in predicting social phobia (Leite and Maia, 2013).

People with SSc have often difficulty accessing specialised services compared to other clinical population. Access to online information about physical, psychological and social causes as a result of the disease is considered as important by most people with SSc (Kwakkenbos et al., 2013), and that the rheumatologist is not often the preferred provider of information (Schouffoer et al., 2011). It was also observed that the need for more information regarding medical test results and treatment was correlated with worse physical functioning and having a partner (Schouffoer et al., 2011).

Raynaud's phenomenon and stiff hands

Contractures and deformities of the hand, consisting of reduced flexion, restricted extension, decreased thumb abduction, microvascular lesions, paroxysmal vasospasm or permanent ischemia and subsequently digital ulcers, tendons retractions, bone and articular involvement, skin sclerosis , and subcutaneous calcinosis contribute considerably to a large burden on social relationship and global disability in SSc (Granel et al., 2015; Poole, 2010). Disability is also attributed to RP and/or puffy hands and if there is also an increase erythrocyte sedimentation rate levels, they are adversely correlated with physical health status (Ludici et al., 2013). It has been demonstrated that HR-QoL might be sufficiently affected by the present of an autoimmune RP (Ludici et al., 2013).

Patients highlight that hand disability interfere with daily activities and work, and its treatment seems more important than of other internal organs (Jewett et al., 2012). Thus, healthcare professionals should take into account reversing disability, patient's satisfaction and social comfort as well as clinically-relevant objectives of therapy (Granel et al., 2015). RP occurs in over 90% of people with SSc, its sometimes severe and long lasting, and often is the heralding clinical manifestation in SSc preceding other manifestations (Silva et al., 2015). The functional outcomes and QoL may be improved by reducing the severity and complications of RP (Silva et al., 2015; Shenoy et al., 2010). To attain that, it is critical a wider patient recruitment at specialist referral centres in the early stages of disease, systematic use of diagnostic tools such as serological markers and capillaroscopic examination and early use of recent available treatments (Ferri et al., 2014). In a randomised trial, tadalafil reported to be quite beneficial for physical function, body pain and mental health compared to baseline in people with SSc (Shenoy et al., 2010). Another study demonstrated that two weeks of a daily home exercise programme was sufficient to improve hand mobility in people with SSc leading to improved QoL and is especially effective when combined with wearing gloves to prevent cold exposure triggering RP (Vannajak et al., 2014). It is also important to know the possible predictors of the development of digital ulcers (specifically autoantibodies; Villalta et al., 2012), which could aid in identifying patients with indication for target therapy (presence of anti-topoisomerase I autoantibodies, early first non-RP, great extent of skin fibrosis, late nailfold video-capillaroscopy scleroderma pattern and its worsening and decreased VEGF levels; Silva et al., 2015).

2.9 Exercise

Exercise reduces dramatically the risk of cardiovascular events, and the magnitude of this benefit can exceed that related with antihypertensive and lipid-lowering medical treatment.

In the past decade or so, the effects of exercise training on the vasculature in healthy humans and those with cardiovascular disease and risk factors have been explored (Green et al., 2014; Maiorana et al., 2003). Scientists have been particularly focused on the impact of exercise training on endothelium-

derived NO, a molecule that possesses several antiatherogenic properties. Despite consistently exploring alterations in artery function and remodelling in laboratory-based, closely monitored, and randomised controlled trials, changes in cardiovascular risk factors have rarely been found (Green et al., 2003). In recent years the focus has been on studying the direct effects of exercise, mediated through shear stress changes, on vascular adaptations in humans (Ramos et al., 2015; Green, 2009).

2.9.1 Impact of exercise on endothelial function

Increases in shear stress causes the release of vasodilator substances from the endothelium and, consequently, flow mediated dilatation (FMD). FMD has been used as a parameter of endothelial function in clinical protocols and is the support of therapies for improving cardiovascular performance through shear stress induced by exercise (Santos-García et al., 2011; Inoue et al., 2008). When referring to the discussion about the effect of exercise on shear stress and vascular health, it is important to establish that there is a large variability of flow patterns in response to different types of exercise. For instance, in incremental exercise of the lower limbs, significant increases of blood flow peaks have been observed, associated with a biphasic increase of blood flow in the brachial artery due to anterograde and retrograde flow which is correlated positively with the intensity of workloads (Birk et al., 2012; Gurovic and Braith, 2012). This retrograde flow observed in the radial artery (and perhaps in other vessels) may be due to the redistribution or the influence of retrograde diastolic flow, which is associated with lower limb exercise in the upright position (Green et al., 2002a, b). Meanwhile exercise of upper limbs induces anterograde flow proportionally to the workload (Green et al., 2005). In the same way, Tinken et al. (2009) compared the effects of blood flow modification and shear stress on FMD reporting that when the anterograde flow was increased by 30 min, the FMD increased. Also, they observed that when the anterograde flow was decreased (through a brachial cuff), the elevation in FMD is blocked, suggesting that FMD is modulated by differences in the magnitude of anterograde flow and shear stress (Tinken et al., 2009). Furthermore, it has been observed that low retrograde flow predisposes to NO dependent endothelial dysfunction, because it generates an altered FMD response, which is a hallmark of endothelial dysfunction (Thijssen

et al., 2009). However, it has been shown that aerobic exercise of moderate intensity (50% $\dot{V}O_{2max}$) increases the endothelium-dependent vasodilatation through stimulation of NO synthesis. Nonetheless, high intensity exercise could be an oxidative stress signal (Goto et al., 2003). Thus, these groups evaluated the response of brachial blood flow to different exercise intensities (25% $\dot{V}O_{2max}$, 50% $\dot{V}O_{2max}$, and 75% $\dot{V}O_{2max}$) in healthy subjects and they demonstrated that exercise at 50% $\dot{V}O_{2max}$ induces vasodilatation through high bioavailability of NO, whereas high intensity exercise was associated with an increase in the production of ROS (Goto et al., 2007).

2.9.2 Exercise and arterial diameter

Measures of vasodilator capacity, such as peak blood flow responses or peak diameter changes in response to ischaemia or ischaemic exercise (Naylor et al. 2006; Rakobowchuk et al. 2005), have been used to assess the extent of arterial remodelling of resistance vessel beds and conduit arteries *in vivo*, based on the assumption that it is necessary to dilate the vessel maximally in order to ascertain the real magnitude of remodelling, free of competing effects, such as sympathetic tone (Sinoway et al. 1987).

Differences in peak vasodilator capacity of peripheral arterial beds measured in this way have been consistently reported between athletes and control subjects, but such cross-sectional comparisons may be influenced by scaling issues (Hopkins *et al.* 2009; Thijssen *et al.* 2008). Longitudinal training studies involving small muscle groups have generally induced increases in peak vasodilator responses, suggesting collective luminal expansion of resistance arteries (Naylor *et al.* 2006; Rakobowchuk *et al.* 2005). These findings are intrinsic to the trained resistance arteries, do not involve sympathetic withdrawal and are unlikely to be due to increased capillary density (Hudlika *et al.* 1977). There are a few studies which suggest that lower limb exercise training may be capable of enhancing peak vasodilator capacity of the upper limbs (Maiorana *et al.* 2000; Silber *et al.* 1991), suggesting a systemic effect of exercise on arterial remodelling in keeping with the concept of enhanced vascular capacitance described by Clausen (1977).

As with the findings in resistance arteries, resting coronary and peripheral conduit arterial diameters have been reported to be larger in athletes than in control subjects (Thijssen *et al.* 2010; Huonker *et al.* 2003), although few of these studies corrected for scaling. Nonetheless, longitudinal training studies undertaken using within-subjects designs suggest that conduit artery diameter is enlarged in trained limbs (Naylor *et al.* 2006; Miyachi *et al.* 2001) and that the effect is regional, rather than central or reflex in nature. Studies in which training has involved one leg, which illustrate changes in the trained but not the contralateral untrained extremity, imply that localized effects on haemodynamic in the conduit arteries, rather than systemic arterial pressure effects (which would logically affect both limbs), may be responsible (Thijssen et al. 2010; Miyachi et al. 2001). In this context, it is well established that localized changes in shear stress can induce endothelium-dependent arterial remodelling (Langille & O'Donnell, 1986).

2.9.3 Exercise-induced arterial thickness

Research was originally focused on examining the arterial wall thickness in the carotid arteries, where increased intima-media thickening is present and indicates a preclinical atherosclerosis. Later on, studies assessed other large arteries of both the upper and lower limbs, to discover the impact of training on physiological remodelling (Seals et al., 2008). It seems that, although carotid artery or diameter does not alter through training (Moreau et al., 2006; Tanaka et al., 2002), it can influence the thickness of brachial and popliteal (Green et al., 2010) as well as the femoral arteries (Dinenno et al., 2001). Exercise training in older subjects induced remodelling of conduit arteries, leading to decreased wall thickness and increased lumen diameters, with consequent decrease in the wall-to-lumen ratio (Green et al. 2010). These findings indicate that aerobic endurance training might have a larger effect on wall thickness in 'muscular' arteries than in larger, more 'elastic' arteries (Moreau et al. 2002; Tanaka et al. 2002). Structural changes such as in wall thickness and arterial diameter are considered long term, since 8 weeks of training does not induce significant changes in wall thickness (Thijssen et al., 2007a).

Resistance training in healthy adults did not induce wall thickness changes (Rakobowchuk et al. 2005; Seals et al. 2008), whilst resistive training in patients with heart failure may induce some changes

(Maiorana et al. 2011). On the other hand, it is documented that vibration exercise is capable of preventing an increase in artery wall thickness (van Duijnhoven et al., 2010).

2.9.4 Endothelial-dependent function

Nitric oxide (NO) constitutes a unique signalling molecule that is largely responsible for the regulation of cardiovascular, nervous, renal, immune, and other system interactions. NO is released from the vascular endothelium and appears to be a powerful vasodilator signal to the underlying smooth muscle cells. NO release recurs continuously and is enhanced when membrane receptors on the endothelial cells are activated by soluble stimuli (including ACh, bradykinin, adenosine diphosphate, substance P, and serotonin), or when the increased shear stress triggers the calcium channels to open promoting the activation of the calcium-dependent endothelial NO synthase (eNOS; Kuo et al., 1992). In the vascular wall NO aims to bind on the soluble guanylate cyclase in smooth muscle cells. Activation of guanylate cyclase triggers smooth muscle relaxation through the accumulation of cyclic guanosine monophosphate (cGMP) and results in vasodilation (Wanstall et al., 2005).

Defective endothelial relaxation in SSc has been explained by impaired maximal responses to endothelial-dependent vasodilators with normal responses to endothelial-independent dilators (Anderson et al., 2003). This impairment is directly associated with a decrease in eNOS gene expression and NO release in SSc skin and microvascular endothelial cells found in involved and uninvolved skin biopsies in the steady state and after shear stress (Tmito et al., 1997). The effects of impaired endothelial NO release are associated not only to defective vascular tone control, but it could moderate other pathologic events, as NO impedes platelet aggregation and protects the endothelial cells from oxidation injury. NO also inhibits cytokine-induced endothelial activation and monocyte adhesion and limits the endothelial release of IL-6 and 8 (Berk et al., 2001). These biologic characteristics of NO render it a potent and vital regulator of inflammation processes within the vascular wall, a common process frequently seen in people with SSc. Moreover, smooth muscle cell proliferation is inhibited by NO through the accumulation of cGMP and prevention of the mitogenic peptides transforming growth factor beta and

platelet-derived growth factor. The pathogenesis of arteriolar intimal proliferation could also be associated to the impaired NO production in SSc; thus, NO may be of critical significance in the pathophysiology of the disease and namely in the commencement of intimal proliferation and structural vascular changes (Kahaleh et al., 2008).

Our results (Mitropoulos et al., 2018) indicate that exercise training may improve the microvascular function in people with SSc. This could be largely attributed to a shear-stress-related mechanism. Shear stress is a mechanical reaction of the blood vessel to accommodate the increased blood flow, which activates the potassium channels and facilitates the calcium influx into the endothelial cells. Endothelial nitric oxide synthase (eNOS) activation and expression are triggered by an increase in intracellular calcium (Laughlin et al., 2008), promoting NO production and thus vasodilation (Busse & Mülsch, 1990). It is possible that the recurring induction of NOS activity with exercise training decelerates the degradation of NO by free radicals in these conditions (Siegfried et al., 2000) or by reducing directly free radical production (Adams et al., 2004). A recent systematic review on exercise training and vascular function (Ramos et al., 2015) supports our findings indicating that the antioxidant status is enhanced after HIIT in patients with cardiometabolic disorders (Mitranun et al., 2014; Tjønna et al., 2008; Wisloff et al., 2007) and thus, the NO bioavailability is improved. Mitranum et al., (2014) assessed the effects of interval aerobic exercise training (3 times / week for 12 weeks) on endothelial-dependent vasodilation in patients with type 2 diabetes mellitus. The vascular outcomes demonstrated reductions in erythrocyte malondialdehyde and serum von Willebrand factor and increases in plasma glutathione peroxidase and nitric oxide (all $P < 0.05$). Therefore, HIIT seems to improve the microvascular function by reducing oxidative stress markers and enhance the antioxidants as well as the vasodilators in cardiometabolic conditions and potentially in connective tissue diseases such as SSc.

2.9.5 Vascular remodelling, shear stress and exercise training

It is now an accepted fact that exercise training is beneficial on impaired endothelial function at both resistance and conduit artery level (Green et al., 2004). A key stimulus for the improvement in endothelial

function has demonstrated to be shear stress, which is enhanced after aerobic interval training (Ribeiro et al., 2010). Shear stress and the adaptations in maximal blood flow or conductance responses with exercise training, induce resistance vessel remodelling in humans (Sinoway et al., 1986). Vascular remodelling potentially reflects alterations in the diameter or cross-sectional area of the resistance arteries, rather than an improved capillarity through angiogenesis, as capillary density is not the principal regulator of maximal muscle blood flow (Snell et al., 1987). These studies support the assertion that exercise training is strongly linked with resistance and conduit artery remodelling. Evidence suggests that chronic changes in shear stress are responsible for the arterial remodelling that is endothelium- and NO- dependent (Tronc et al., 1996).

Evidence for the time-course of functional or structural arterial adaptations to exercise training in humans is limited: Short-term effects of exercise improves NO bioavailability, whereas long term effects induce changes in vascular remodelling (Laughlin et al., 2003), an endothelium and NO-dependent outcome (Tronc et al., 1996). Tinken et al., (2008) assessed the effects of exercise training on vascular function and remodelling in brachial and popliteal arteries in healthy young men. The exercise program lasted 8 weeks with re-assessments of the artery function and structure every 2 weeks. The functional adaptations were immediate to exercise training, whereas the structural alterations adapted towards the end of the training period. Further evidence is required to assess the impact of exercise training on vascular remodelling in the arterial tree and the time course to adaptations.

The existing evidence supports that systemic effects occur after exercise training in the lower limbs (Ramos et al., 2015). Interestingly, the previously suggested systemic effect was not proved with our study (Mitropoulos et al., 2018) where the microvascular reactivity in the digital area was improved with arm cranking but not with cycling. Similar to our findings, Klonizakis & Winter, (2011) reported that arm exercise did not have any impact on lower limbs microcirculation in post-surgical varicose-vein patients. It seems that systemic effects of exercise training can only affect the vascular function in the large arteries (e.g. brachial artery) but not the conduit and resistance arteries. Moreover, the mass of

muscle engaged in exercise training could play an important role in the systemic effects as studies that utilized handgrip training have not demonstrated contralateral limb remodelling (Green et al., 1996; Green et al., 1994; Sinoway et al., 1986). The explanation probably relies on the magnitude and pattern of shear stress which in turn triggers the release of NO and acts as a main determinant for its bioavailability. It is possible that the induced-shear stress by lower limbs is not sufficient to improve the microcirculation in the acral body parts of the upper limbs. Therefore, the volume of blood flow and the magnitude of shear stress induced by HIIT could account for the local effects of exercise training in the smaller arteries (Liu et al., 2012; Green et al., 2004).

2.9.6 High Intensity Interval Training

Components and prescriptions of HIIT

Interval exercise comprises of five main components: peak workload intensity (P_{peak}), peak workload duration (t_{peak}), recovery load (P_{rec}), recovery duration (t_{rec}), and the mean load ($P_{mean),}$ of which the outcome of the latter four can be estimated accordingly or set as a separate determinant (Saltin et al., 1976). Moreover, the number of intervals which determines the total exercise duration could be considered a further variable of HIIT prescription. Bichheit and Laursen, (2013) also referred to the number of series, the duration and intensities in recovery phases between the series and the exercise modality as further determinants of interval exercise. Nevertheless, they did not contemplate P_{mean} as a relevant variable.

P_{peak} is usually set between the power output at the anaerobic threshold (Laursen and Jenkins, 2002) and "sprint" exercise, and t_{peak} varies from a few seconds up to several minutes. The P_{peak} phases are split by periods of low- or moderate-intensity exercise or passive recovery with a t_{rec} that can be shorter than, equal to, or longer than t_{peak}. Interestingly, limited information is available regarding settings of P_{mean}. Taking into account the several potential combinations of P_{peak}, t_{peak}, P_{rec}, and t_{rec}, it is not surprising that there is a wide range of diverse prescriptions for interval exercise utilised in scientific studies and exercise training. For example, Helgerud et al., (2017) utilised (among others) 4-minute work phases at 90% to

95% HR_{max} and 3-minute recovery phases at 70% HR_{max}, whereas Trapp et al., (2008) applied sprint exercise for 8 seconds during work phases and slow pedalling for 12 seconds during recovery phases.

Despite this diversity of applied HIIT protocols, beneficial effects could be attained in different HIIT studies and in several populations (highly trained, as well as healthy sedentary and diseased, individuals). Therefore, the variety of the different HIIT components constitute an important difficulty in defining the optimal exercise prescription. Nevertheless, the acute cardiometabolic and cardiopulmonary (and neuromuscular) responses during exercise, presumably can be directly affected by the isolated manipulation of each single variable of HIIT (Wisloff et al., 2009). If two or more components are handled at the same time, the impacts on the physiological responses are more complex and also more difficult to predict (Buchheit and Laursen, 2013). These acute physiological responses lead to specific medium- and long-term training adaptations on the one hand, and on the other hand they might represent certain health risks, particularly in diseased persons. Consequently, the understanding of the acute physiological mechanisms provoked by the manipulation of (interval) exercise variables is of high importance in exercise physiology research.

The wide range of interval training protocols implemented in different studies may indicate that the acute physiological responses during HIIT forced by specific exercise prescriptions are not clear in detail or not taken into consideration; a standardised and consistent approach to the prescription of HIIT is still missing.

HIIT VS. moderate intensity continuous training on vascular function

Both HIIT and moderate intensity continuous training (MICT) are able to improve vascular function in people with impaired vascular function. Both protocols improved flow mediated dilatation (FMD) in six out of the seven studies that compared these protocols and were included in the review by Ramos et al. (2015). Only one study did not find an effect; probably due to its short training period (2 weeks; Klonizakis et al., 2014). Four trials that utilised the 4 x 4 HIIT protocol (four intervals for 4 min at 85-

95% $HR_{max/peak}$) with 3 min active recovery (50-70% $HR_{max/peak}$) for 12-16 weeks (three times per week) were showed to significantly improve vascular-dependent function more than MICT (Molmen-Hansen et al., 2012; Tjonna et al., 2008; Schjerve et al., 2008; Wisloff et al., 2007). Studies that used HIIT protocols with a shorter interval duration but with a higher number of bouts (4-10 x 1 min at 80-85% peak oxygen uptake ($\dot{V}O_{2peak}$), 4 min active recovery at 50-60% $\dot{V}O_{2peak}$) showed either greater (Mitranun et al., 2014) or no significant difference (Currie et al., 2013) in brachial artery FMD relative to an isocaloric MICT after 12 weeks (three times per week). A recent meta-analysis revealed that post-intervention change in FMD was significantly greater following HIIT than following MICT [mean difference 2.26% $p < 0.05$, (Ramos et al., 2015)]. The average relative FMD value increased from 5.14 to 9.45% and from 5.12 to 7.27% after 2-16 weeks (three times per week) of HIIT and MICT, respectively. Exercise intensity also appears to affect exercise-induced improvements of endothelial function in hypertension (Ciolac, 2012). HIIT was more effective than MICT for improving endothelial function (9% vs 5% in HIIT and MICT, respectively - group difference, $p<0.001$) in metabolic syndrome patients (Tjonna et al., 2008). In the same study, there was an improvement in NO bioavailability after HIIT but not MICT, while various factors that affected NO availability (blood glucose, insulin sensitivity and oxidised low-density lipoprotein) were reached the healthy physiological values after HIIT only (Tjonna et al., 2008). Moreover, HIIT had obvious benefits in improving plasma NO, VEGF concentrations and controlling hypertension in postmenopausal women (Mona et al., 2016) The superiority of HIIT for improving endothelial function is not fully understood, but it is reasonably suggested that the low- and high-intensity training exercise protocols impact shear stress in the arterial wall differently during exercise training and that this may yield differences in molecular responses (Ciolac et al., 2010; Tjonna et al., 2008).

Effects of HIIT and MICT on cardiorespiratory fitness

Studies that compared the effects of HIIT (various protocols) vs MICT on cardiorespiratory fitness (CRF) in several clinical populations (Mitranum et al., 2014; Molmen-Hansen et al., 2012; Schjerve et al., 2008;

Tjonna et al., 2008; Wisloff et al., 2007), showed a greater improvement for the HIIT protocols compared to MICT (14-46 vs. 5-16 %, respectively). However, another study (Currie et al., 2013) that compared HIIT to MICT found no significant difference on CRF (24 vs. 19 %, respectively); the HIIT protocol in this study comprised of shorter intervals and recovery periods, however, the number of interval repetitions was greater when compared with other HIIT protocols (e.g., ten intervals for 1 min at 80-104% of PPO).

Effects of HIIT and MICT on cardiovascular risk factors

Blood pressure

Two studies that compared similar HIIT protocols (4 x 4 min; 12 weeks; three times per week) and MICT showed no change in SBP following both types of training (Schjerve et al., 2008; Wisloff et al., 2007). However, in the same studies, this HIIT protocol had a different effect on diastolic BP (DBP), demonstrating either no change (Wisloff et al., 2007) or lower significant reduction compared with MICT (7 vs. 9 %, respectively) (Schjerve et al., 2008). Moreover, a study that used a shorter interval duration but larger interval bout frequency (ten x 1 min HIIT; 12 weeks; three times per week) also found no change in SBP but revealed a significant reduction in DBP (HIIT vs. MICT 2 vs. 7 mmHg) after a 12-week program, although there were no significance between-group differences (Currie et al., 2013). Opposite results were illustrated in a study that utilised a similar HIIT protocol (4–6 x 1 min; 12 weeks, three times week), in which SBP reduced significantly only in the HIIT group (12 mmHg), with no change in DBP (Mitranum et al., 2014). In overall, as yet, there is no definitive evidence whether HIIT or MICT could induce better results on BP.

Lipid Profile

When iso-caloric exercise protocols were compared (4 x 4 HIIT protocol and an iso-caloric MICT, 12–16 weeks, three times per week) (Schjerve et al., 2008; Tjonna et al., 2008; Wisloff et al., 2007) no change in total cholesterol was found. Nevertheless, 4–6 x one HIIT for an intervention of similar

duration and frequency (12 weeks, three times per week) significantly reduced total cholesterol (Mitranum et al., 2014). In these studies (Mitranum et al., 2014; Tjonna et al., 2008; Wisloff et al., 2007) significant changes in plasma triglyceride levels were not found following either type of training. Moreover, high-density lipoprotein cholesterol (HDL-C) has only been demonstrated to significantly increase in people with metabolic syndrome (Tjonna et al., 2008) and type II diabetes mellitus (Mitranum et al., 2014). No significant changes in HDL- C concentrations were found in the other studies (Schjerve et al., 2008; Wisloff et al., 2007) following HIIT or MICT.

Oxidative stress

In the study by Wisloff et al. (2007), HIIT was demonstrated to induce a significant improvement in antioxidant status compared with an iso-caloric MICT in heart failure patients. In support to this finding, Mitranun et al. (2014) revealed an increase in glutathione peroxidase only following HIIT. These results were supported by studies that showed a significantly greater increase in NO bioavailability following HIIT compared with an MICT protocol (Mitranum et al., 2014; Tjonna et al., 2008). Moreover, in agreement with these findings, it has also been demonstrated a significantly greater reduction in plasma levels of oxidized low-density lipoprotein (LDL) following HIIT relative to an iso-caloric MICT (Wisloff et al., 2007; Tjonna et al., 2008). In contrast, Schjerve et al. (2008) demonstrated no change in antioxidant status following either type of training, however, found a significant decrease in oxidized LDL cholesterol following MICT in obese adults.

Insulin Sensitivity

Studies that assessed insulin sensitivity using an oral glucose tolerance test (Schjerve et al., 2008) and homeostatic model assessment–insulin resistance (Mitranum et al., 2014; Tjonna et al., 2008). Glucose and C-peptide concentrations were derived from the oral glucose tolerance test with no changes in obese individuals following either type of training (Schjerve et al., 2008). Nevertheless, following 12 weeks (three times per week) of 4–6 x 1 HIIT and 4 x 4 HIIT, insulin sensitivity assessed through homeostatic

model assessment–insulin resistance was demonstrated to improve, either similarly (Mitranum et al., 2014), or at a greater magnitude (Tjonna et al., 2008) relative to MICT in people with type II diabetes mellitus and metabolic syndrome, respectively. Interestingly, a study demonstrated that glycated haemoglobin levels were significantly decreased only in the HIIT group (Mitranum et al., 2014). In contrast, Schjerve et al. (2008) found no change in glycated haemoglobin following either type of training with a similar exercise program duration and frequency (12 weeks, three times per week) but with a different HIIT protocol (4 x 4 HIIT). Schjerve et al. (2008) utilized a HIIT protocol with much longer bouts of high- intensity exercise (4 min) than that of Mitranun et al. (2014), which only used 1-min bouts with similar recovery duration (4 min).

Inflammation

Inflammation assessed as the concentration of serum or plasma high-sensitivity C-reactive protein and found no change from baseline after 12 weeks of either HIIT or MICT in heart failure patients (Wisloff et al., 2007) and obese adults (Schjerve et al. 2008).

In overall, Ramos et al., (2015) suggests that a protocol based on 4 x 4 HIIT, three times per week for at least 12 weeks, can be a powerful form of exercise to enhance vascular function. However, a shorter HIIT protocol (e.g., 30s 100% PPO/ 30s passive recovery) has not been adequately assessed yet. Such a protocol may induce a greater stimulus on the acute effects of exercise on NO availability via shear stress. This is supported by Green et al., (2009) who conclude that greater shear stress patterns may result in a concomitantly greater NO availability. This evidence suggests that the efficacy of training interventions may depend upon the nature of the shear stress stimulus that is present in the endothelium during episodic exercise bouts (e.g., HIIT). Moreover, in clinical populations such as systemic sclerosis patients where arm cranking might be a better modality over cycling to improve the microvascular function in the digital area, the feasibility of longer exercise protocols (e.g., 4 x 4 HIIT) is questionable due to the weak upper body muscles especially in women. Moreover, a shorter HIIT protocol has been demonstrated to be more

enjoyable in an overweight/obese cohort (Smith-Ryan, 2017). Therefore, a short HIIT protocol seems to be a more appropriate exercise protocol to be implemented in SSc patients.

Evidence is sparse concerning the effects of different modalities on vascular function. Klonizakis et al., (2010) examined the effects of upper-limb HIIT on lower-limb cutaneous microvascular function in post-surgical varicose-vein patients. The findings indicated that upper-limb exercise was not effective on lower-limb microvascular reactivity potentially due to local effects of exercise for the resistance and conduit arteries (Green et al., 2009) and concomitant lack of limb specificity which appears to be an important factor in optimal exercise prescription for these patients. More studies are required to define and establish the potential benefits of arm crank exercise on vasculature.

2.9.7 Effects of resistance training on vascular function

Evidence supports the potential for resistance training (RT) to induce long-term anti-inflammatory effects in people with obesity (Phillips et al., 2012). However, evidence on the effects of RT on vascular function in populations with impaired vascular function is limited. Dias et al., (2015) examined the effects of a supervised RT regime on endothelial function and other cardiovascular outcomes in non-diabetic people with obesity. The RT regime consisted of exercise for all major muscle groups, the intensity increased progressively from 50-70% 10RM in the first two weeks to 70-85% 10RM the last five weeks. The findings indicated that 12-week of RT three times per week significantly improved the endothelial-dependent microvascular function measure by Laser Doppler Fluximetry (LDF) using iontophoresis (ACh and SNP). In a longer-term RT regime, Cohen et al., (2008) found that endothelial-dependent vasodilation was improved after 14 months of RT but not after 2 months in type 2 diabetes individuals. The improvement in vascular response was marked and was assessed by LDF using iontophoresis (ACh and SNP). The RT regime consisted of three sets of eight repetitions for all exercises in major muscle groups at 75-85% of 1RM and the training workload was increased regularly according to individual's tolerance. Another study assessed thirty overweight (BMI > 25 $kg{\cdot}m^{-2}$) premenopausal women aged 24-44 years (Olson et al., 2006). The findings indicate that 1-yr RT programme consisted of at least two

training sessions per week improved significantly the brachial artery endothelial function using the flow mediated dilatation (FMD) technique. Therefore, RT regimes appear to benefit vascular function in clinical population with impaired vascular function and future research should focus on combined protocols (aerobic and resistance training) that might induce further improvements in the vasculature.

There is lack of evidence regarding the effects of resistance exercise on endothelial-independent function. Animal research in N^G-nitro-L-arginine-methyl-ester (L-NAME)-induced hypertensive rats demonstrated that one resistance exercise session resulted in a reduction in the potassium chloride (KCI)-induced contracting mechanisms by enhancing the vasodilatory sensitivity of the mesenteric artery smooth muscle (Tharciano et al., 2015). Rats that underwent a resistance exercise session had a reduction in contraction in response to depolarising KCI solutions. This finding indicates that resistance exercise might change in a beneficial way the depolarisation of the vascular smooth muscle cells. Future research should focus on the identification of the physiological mechanisms underlying the endothelial-independent function after RT in humans.

2.9.8 Effects of combined exercise on vascular function

In clinical population, a combined exercise programme consisting of aerobic and resistance training is considered a complete approach to improve the individual's general fitness and thus the QoL. Other than the enhancement of QoL a combined exercise programme has demonstrated its ability to improve microvascular function in clinical populations such as patients with leg ulcers (Tew et al., 2018). Metsios et al., (2014) examined the effects of a combined exercise programme on endothelial function in rheumatoid arthritis patients. The findings demonstrate that an individualised aerobic and strength training programme for six months significantly improved both macro- and microvascular endothelial function in patients with rheumatoid arthritis. A research team explored the effects of a combined exercise programme (aerobic and resistance training) on the vascular function in heart failure (Maiorana et al., 2000) and type 2 diabetes (Maiorana et al., 2001) patients. In both studies the results demonstrated significant improvements in indices of endothelial function. Ramirez-Velez et al., (2013) assessed the

effects of a combined exercise programme during the second half of pregnancy on endothelial NO synthesis (eNOS) and NO production in human placenta. The findings showed that exercise training during pregnancy led to a 2-fold increase in eNOS and a 4-fold increase in NO production in placental cytosol. NO generated by NOS has been demonstrated to contribute to the regulation of vascular tone by counteracting the actions of vasoconstrictors (Rossmanith et al., 1999).

HIIT and RT alone or in combination have demonstrated significant improvements in both macro- and micro-vascular function in clinical populations with pathophysiological vascular impairment. Is also known that a holistic approach (aerobic and RT) is more beneficial from isolated exercise protocols as it offers different physiological adaptations to the human body. The existing evidence indicates that a combined exercise programme is capable to induce improvements in the microvasculature in clinical populations, however, evidence is limited, and further research is required.

2.9.9 Effects of exercise on quality of life in systemic sclerosis

Patients with SSc present a good exercise tolerance and studies demonstrate the efficacy of exercise regimes in increasing tolerance and aerobic capacity (Alexanderson et al., 2014; Schouffoer et al., 2011; de Oliveira et al., 2009), muscle strength (Alexanderson et al., 2014; Pinto et al., 2011), hand mobility (Antonioli et al., 2009; Maddali Bongi et al., 2009; Mancuso and Poole, 2009), function in daily activities (Maddali Bongi et al., 2009), and HR-QoL (Antonioli et al., 2009; Maddali Bongi et al., 2009), even in patients with some degree of lung involvement. These studies, however, present a lot of limitations in their design.

Namely, the pilot study from Alexanderson et al., (2014) recruited only four SSc patients (3 women, 1 man) with a mean age of 66.5 years old. They found an improvement in aerobic capacity and muscle strength after an 8-week aerobic exercise program on a stationary bike (2d/week) and muscular endurance training of the shoulder and hip flexors. Nevertheless, the very small sample size and the absence of a control group limit the validity of the study and the generalisation of the results. Similar sample size (n=7 interventional group) limitation was presented by de Oliveira et al., (2009) who found an improvement

in aerobic capacity after an 8-week program consisting of moderate intensity aerobic exercise. Schouffoer et al., (2011) did not report clearly the exercise protocol that was applied in SSc patients (n = 28) for 12 weeks (1d/week) and due to the multidisciplinary approach in this interventional program it is not clear which team care component contributed the most to the treatment effect. Moreover, conclusions from studies with no control group (Pinto et al., 2011), case studies with small sample size (n = 3) with potential for researcher bias, and studies that mainly applied physiotherapy (Maddali Bongi et al., 2009) cannot be generalised under the umbrella of the "effects of exercise on QoL in SSc patients".

Evidence regarding the effects of exercise in SSc is limited, with small participation in most studies, an absence of control groups, and information around the precise training dose not always being included. Only two randomised control trials have been identified (Rannou et al., 2016; Schouffoer et al., 2011) with one of these being a long-term study (Rannou et al., 2016). Study details such as differences in response to exercise regarding gender or other clinical manifestations are not included so far by the researchers. Moreover, when people with SSc are taking part in research studies examining the effects of exercise are often guided to maintain their pharmacological treatment (Alexanderson et al., 2014; Mugii et al., 2011; Pinto et al., 2011; Maddali Bongi et al., 2009; de Oliveira et al., 2009).

Deterioration in function in daily activities and the concomitant impact in people with SSc to engage in physical activity might be a result of chronic systemic inflammation. Exercise intolerance may also be attributed to pulmonary involvement which is common in people with SSc (Morelli et al., 2000). On the other hand, a sedentary lifestyle might contribute to inflammation, establishing a 'vicious circle' (Benatti and Pedersen, 2015).

Aerobic and resistance exercise may induce long-term benefits by enhancing fitness (aerobic capacity, muscle strength, functional ability and mobility) and body composition and decrease cardiovascular risk factors and fatigue, and improve HR-QoL (Mancuso and Poole, 2009). Regular exercise might be an important tool in improving overall clinical manifestations and course of SSc (Perandini et al., 2012).

2.10 Aims-rationale

The above evidence suggests that:

a) Vasculopathy is an important element of the SSc pathophysiology, which needs to be targeted to relieve symptoms and slow down/reverse disease progression.

b) People with SSc would benefit from an adjunct, non-medical therapy, which could potentially provide them with additional QoL benefits.

c) Exercise has been proven to offer significant clinical and QoL benefits (e.g., reduce disease severity, reverse clinical progression etc) in diseases and clinical conditions, with a similar microvascular profile to that of Systemic Sclerosis. Evidence is limited regarding QoL in SSc population, which necessitates further research to be conducted to establish the potential beneficial effects of exercise on QoL.

d) The mode of exercise that will induce microvascular improvements in the digital area needs to be defined. Currently, there is no evidence in the literature rendering it imperative need to be explored.

Considering all the evidence, it was decided that the main aim of this PhD programme was to examine the efficacy and feasibility of exercise in people with SSc. Practically, we wanted to compare upper and lower limb exercise in several physiological factors and peak oxygen uptake is one of those factors. Nevertheless, due to a significant difference in upper and lower limb muscle mass these two values ($\dot{V}O_{2peak}$) were not comparable. Thus, we validated a commonly used cycle ergometer protocol to an arm crank ergometer protocol producing an equation which can accurately predict the cycle ergometer $\dot{V}O_{2peak}$ from the physiological responses of an arm crank ergometer protocol (Study 1).

The predictive equation was used in Study 2 to compare upper and lower limb exercise as regards the peak oxygen uptake. Following the literature, we concluded that a HIIT protocol would induce better results in the microvasculature compared to continuous exercise protocols. The knowledge gap in the literature was the mode of exercise, upper or lower limb, which would induce greater results in the microcirculation in the digital area and that constituted a pilot study (Study 2).

In order to assess the feasibility of exercise to be implemented in systemic sclerosis patients we needed a complete exercise protocol which through the literature and the results of Study 2 would be a combined exercise protocol consisting of aerobic and resistance training (Study 3-Feasibility study). In the feasibility study (Study 3) we also assessed the feasibility of our exercise protocol through relevant questionnaires and face to face interviews.

Chapter 3: Theory of methods

3.1 Evaluating the microcirculation

Blood flow in the microcirculation is composed of the skin nutritional capillaries and thermoregulatory arteriovenous (AV) shunts. The proportional contribution of these two sources is different in glabrous and non-glabrous skin. Twenty-five percent of glabrous skin blood flow origins from nutritional capillaries and 75% from AV shunts, and it is therefore subject to wide fluctuations (Saad et al., 2001). Non-glabrous skin does not possess AV shunts, and blood flow is composed almost completely of nutritional capillaries.

The microcirculation was first evaluated in the coronary arteries, where it was shown that microcirculatory dysfunction was associated with an increased risk of coronary artery disease. Invasive techniques (i.e. Doppler velocity catheter) to measure the coronary microvasculature carry an increased risk associated with angiopathy and this led investigators to evaluate other arterial beds because endothelial dysfunction was thought to be a global process. FMD of the brachial artery (macrocirculation) due to occlusive hyperaemia has been demonstrated to relate with coronary vasoreactivity (Anderson et al., 1995). FMD does not specifically evaluate the end-resistance arteries of the microcirculation, requires an ultrasound technician, and can have up to 25% day-to-day variability (Verma et al., 2003). The importance of small-vessel resistance for ulcer formation led to the development of both invasive and non-invasive techniques (i.e. LDF) that could reliably quantify microcirculatory function.

3.1.1 Laser Doppler Fluximetry

Laser Doppler is based on the reflection of a beam of laser light. Light undergoes changes in wavelength when it hits moving blood cells. The magnitude and frequency distribution of these changes in wavelength are related to the number and velocity of blood cells. Several different signals can be recorded but the red blood cell flux (i.e. the product of the velocity and concentration of moving blood cells within the measuring volume) is frequently used.

Laser Doppler fluximetry enables the evaluation of cutaneous microvascular blood flow over time and its alterations following a given challenge (Table 3, chapter 3). The major advantage of this technique is its sensitivity at detecting and quantifying relative changes in skin blood flow in response to a given stimulus.

3.1 Table 3. Optimizing laser Doppler fluximetry

Standard procedures to minimise the variability of laser Doppler fluximetry
1. Room temperature should be neutral (22-24 °C). Local heating of the probes at 33 °C is also encouraged to maintain a standard skin temperature.
2. Subject position (sitting or supine) should be consistent throughout the study.
3. A stabilisation period in the experimental room is required before all measurements.
4. Given the site-to-site variability, the site of measurement should be accurately described for follow-up studies. Skin sites with dermatological lesions should be discarded.
5. The device, the wavelength and the type of probe used should be described.
6. Reproducibility of the technique should be assessed under its own experimental conditions by each investigator.
7. Raw flux data can be expressed as arbitrary perfusion units. However, conductance (flux divided by mean arterial pressure) should ideally be used to take into account potential variations in blood pressure.

8. Data can be best expressed as a percentage of a given vasodilation rather than as a percentage of baseline. Maximal dilatation can be obtained by local heating to 42-44 °C or by non-invasive intradermal infusion of vasodilatory drugs.
9. The report should state whether biological zero was subtracted from the raw data.

3.1.2 Laser Doppler Fluximetry Limitations

As LDF is non-invasive, it cannot measure absolute perfusion values (i.e. cutaneous blood flow in ml/min relative to the volume or weight of tissue). Therefore, measurements in most studies are expressed as arbitrary perfusion units (PU) or millivolts (1 PU = 10 mV) and are often referred to as "flux" rather than "flow". This is why data is usually expressed as cutaneous vascular conductance [i.e. flux divided by arterial pressure (in mV/mmHg)], taking into account differences and variations in blood pressure. However, this does not enable the comparison of absolute flux or conductance values across studies using different probes and/or brand of device and/or sites of measurement.

3.1.3 Standardisation of Laser Doppler Fluximetry

Vascular responses to most interventions are standardized to the baseline resting flux level, similar to the FMD of the brachial artery. However, variations in ambient and/or local temperature lead to huge differences in cutaneous vascular flow, because skin circulation is a vital aspect of normal thermoregulation in humans. Temperature variations are largest in the extremities, where AV shunts are present (Charkoudian, 2003). The problems associated with measuring basal flux using laser Doppler can partially be overcome by using a temperature-controlled room and recording skin temperature. A less physiological standardisation procedure is to use a probe heated to a thermoneutral temperature (33 °C). Even using such precautions, basal blood flux in the skin remains extremely variable and its use as the unique reference for a pharmacological test is not recommended. The optimal solution when considering the effect of a drug on flux is to relate its effect to the flux observed during a maximal vasodilatation. A

maximal vasodilatation can be achieved by either local warming of the skin to 44 °C or local sodium nitroprusside infusion and be used to normalize submaximal flux values (Charkoudian, 2003).

The biological zero

Flux does not reach the value of zero when perfusion is absent. Brownian motion of macromolecules arising from the interstitial space contributes to the remaining signal when red blood cell flow is absent. According to this phenomenon, the biological zero needs to be subtracted from flux values expressed as absolute values, however, it is less crucial when flux values are expressed as a percentage of a standard comparator.

Spatial variation

LDF refers to a single-probe technique in which the probe is located on the skin and is used to record velocities and concentrations of moving blood cells in a small volume of 1 mm^3 or smaller, depending on the incident wavelength. Because of the penetration of the signal, it records the velocities and concentrations of the subepidermal papillary loop in addition to those of the arterioles located in the superficial and median derma. Older and simpler probes relied on a single laser fibre and a single recorder. Newer, more sophisticated probes use a single laser Doppler fibre surrounded by several receiving fibres, or several transmitting and receiving fibres on the same probe. This enables the study of a larger volume, therefore minimising spatial variations. Our research studies utilized the newer probes with several receiving fibres (Probe 413, Perimed AB, Jarfalla, Sweden).

3.1.4 Reproducibility

LDF has often been considered poorly reproducible. The site of measurement seems to be the major source of the variation. When the recording site is standardised, the day-to-day reproducibility of post-occlusive hyperaemia, thermal hyperaemia and ACh iontophoresis (expressed as absolute values) compares well with that of flow-mediated dilatation of the brachial artery, with each having a coefficient of variation <10% (Boignard et al., 2005; Kubli et al., 2000). However, during baseline cutaneous blood flow the coefficient of variation is much higher (Bircher et al., 1994).

All the proceedings in our study complied to the reproducibility requirements to assure for valid and reliable results. Moreover, the leading researcher (Mr. Alexandros Mitropoulos) who performed all the LDF assessments for both of our studies, completed a 4-day LDF training programme in Stockholm, Sweden organised by Perimed AB. Perimed is the official provider of Perimed LDF devices used at Sheffield Hallam University laboratories. Therefore, the repeatability and reliability of the LDF technique in our study was adequately secured and controlled.

3.1.5 Iontophoresis

Iontophoresis is based on the principle that a charged drug in solution will migrate across the skin under the influence of a direct low-intensity electric current Kalia et al., 2004). The quantity of drug delivered depends on the magnitude and duration of the current applied and on the skin barrier. When combined with LDF, it detects the alterations in cutaneous blood flow in response to the time-controlled delivery of the vasoactive drug to a patch of skin.

ACh iontophoresis results in an early peak that is followed by a late prolonged vasodilatation (Durand et al., 2004). ACh and SNP are used to generate endothelium-dependent vasodilatation and endothelium-independent vasodilatation, respectively.

Dosage of ACh and SNP

The specific dosage and protocol that we utilised in our study has been validated for its reproducibility previously by Klonizakis et al., (2011; 2009a; 2009b). Microvascular assessments using LDF and iontophoresis were performed in a temperature-controlled room (22–24 ° C). LDF electrodes were attached to the dorsal aspect of the reference fingers for ACh and SNP administration. These were used as indicators of the changes occurring in the endothelial (dependent and independent) vasodilatory function. HR (Sports Tester, Polar, Finland) and blood pressure of the brachial artery (left arm; Dinamap Dash 2500, GE Health- care, USA) were monitored at 5-min intervals throughout the protocol. The two drug delivery electrodes (PF383; Perimed AB, Jarfalla, Sweden) were positioned over the healthy-looking skin, approximately 4 cm apart with one containing 100 μL of 1% ACh (Miochol-E, Novartis,

Stein) and the other 80 μL of 1% SNP (Nitroprussiat, Rotta- pharm). A battery-powered iontophoresis controller (Peri-Iont PF382b; Perimed AB) was used to provide the charge needed for ACh and SNP delivery. A 4 min stable recording of baseline flux was followed by administration of the two agents according to the following protocol: 0.2mA for 10s (i.e.2mC), 0.2mA for 15s (i.e.3mC), 0.2mA for 20s (i.e.4mC), and 0.3mA for 20s (i.e.6 mC), occurring between 4-min intervals (Klonizakis et al., 2009a; 2009b). To obtain an index of skin blood flow, cutaneous red cell flux was measured by placing an iontophoresis laser Doppler probe (PF481–1; Perimed AB), connected to a laser Doppler fluximeter (PF5001; Perimed AB).

3.2 Flow mediated dilatation

3.2.1 Physiology

FMD is entitled as an endothelium-dependent assessment that reflects the relaxation of a conduit artery when exposed to increased blood flow and therefore increased shear stress (Moens et al., 2005). Each blood vessel within the human body is lined by a single layer of cells that is called endothelium. As shear stress increases, a number of vasodilators are released by the endothelium including nitric oxide, prostaglandins (Okahara et al., 1998) and endothelium-derived hyperpolarizing factor (Busse et al., 2002); however, it is nitric oxide that is thought to be mainly responsible for the flow-mediated dilatation response (Joannides et al., 1995). As a result of the shear stress, specialized ion channels open that are hosted in the endothelial membrane. These calcium-activated potassium channels open to hyperpolarize the endothelial cell and increase the force for calcium entry which in turn activates endothelial eNOS. The subsequent production of NO then explains the flow-mediated dilatation (Pohl et al., 1986).

3.2.2 Assessment

In humans, FMD is usually assessed in large peripheral conduit arteries (brachial, radial, and femoral). The primary goal of this assessment is to form a shear stress stimulus that produces a NO-dependent response and thus, FMD can be utilised as a direct marker of NO bio-availability. For instance, a small FMD response is considered as an index of low NO bio-availability and a potential association to

increased cardiovascular disease risk (Pyke et al., 2005). The most well-known technique for the assessment of FMD is that first described by Celermajer et al., (1992). This technique uses an inflated blood pressure cuff above systolic pressure in order to hinder blood flow to the lower limb. This artery occlusion then produces an ischemia-induced reactive hyperaemia and therefore a concomitant increase in shear stress upon cuff release. In order to adequately perform this technique, a number of factors must be considered including subject preparation, equipment, image acquisition, image analysis, quality control, and staff training/experience.

Assessment prerequisites

It is critically important that any confounding variables that may affect vascular reactivity are controlled for. Therefore, fasting of at least 4 hours, especially the consumption of caffeine and fatty substances is compulsory before any testing take place. Physical and environmental factors also play an important role in vascular reactivity so the test should be performed in a temperature controlled, quiet, and relaxing space. Moreover, any vasoactive medications must be discontinued prior to the test (including ascorbic acid; Levine et al., 1996) and the participant must have refrained from intense physical exercise on the day of testing.

Limitations

Brachial and femoral arteries are commonly imaged as part of FMD assessment. The femoral artery is a larger vessel and as such it should be easier to locate and maintain the ultrasound beam for accurate flow-mediated dilatation assessment. However, previous research has suggested that arteries with a diameter >5 mm show a reduced flow-mediated dilatation response when compared to smaller vessels (Correti et al., 2002). Although the brachial artery is a smaller vessel (therefore more difficult to locate and hold the image in the ideal position for flow-mediated dilatation), it should provide a more pronounced response. It should also be noted that arteries with a diameter <2.5 mm are extremely difficult to image accurately with conventional vascular ultrasound equipment and therefore should be avoided. Moreover, the

assessment of endothelial independent vasodilation during FMD requires the administration of an exogenous NO donor (single dose of nitro-glycerine). Previous research has identified that the greater the cardiovascular risk, the more reduced the nitro-glycerine response (independent of endothelial dysfunction; Ducharme et al., 1999) and also patients with hypertension who exhibit similar impairment to vascular smooth muscle function (Gokce et al., 2001).

Based on the above, we chose LDF combined with iontophoresis was to assess both the endothelial-dependent and -independent microvascular vasodilation.

3.3 Transcutaneous oxygen pressure

The transcutaneous oxygen pressure ($TcpO_2$) technique, although not a primary care technique, has been used in clinical populations presenting cutaneous hypoxia such as in patients with claudication to argue for a vascular origin of pain, detect buttock ischemia or estimate the effect of rehabilitation programmes (Abraham et al., 2003; 2005; Caillard et al., 1990). The $TcpO_2$ technique is an old technique initially proposed in neonates to non-invasively estimate arterial PO_2. Although a complex and time-consuming technique as compared to pulse oxymetry (saturometry), it is expected of advantage as compared to saturometry to detect abnormal arterial oxygen changes during exercise. Indeed, keeping in mind the sigmoid relationship between oxygen pressure and oxygen saturation in human blood, arterial saturation may remain in normal limit despite a significant decrease in arterial PO_2, specifically in patients with normal arterial PO_2 at rest. Further intra-arterial blood sampling cannot be proposed as a screening technique of eventual abnormal PO_2 changes in all patients suffering exercise intolerance.

There is multiple evidence that chest $TcpO_2$ changes at rest ($\Delta tcpO_2$) mimic the changes in arterial PO_2 at rest and during mild or moderate exercise (Planes et al., 2001; Carter and Banham 2000) despite the presence of an unpredictable transcutaneous gradient and provided that the changes are relatively slow (90% time response of $TcpO_2$ being ~20 s). In our study, the use of $TcpO_2$ changes at rest and during

incremental exercise was aimed primarily to compare the effectiveness of our rehabilitation program on cutaneous oxygen pressure in SSc patients who present cutaneous hypoxia (Silverstein et al., 1988).

$TcpO_2$ measurements were performed during the cardiorespiratory tests using sensors that will be non-invasively attached to the skin and allow to heat. The sensors induce skin blood capillary dilatation through heat, which increases the blood flow and results in oxygen diffusion through the skin to the sensor. The sensor measures $TcpO_2$ values inwardly through an electrochemical process.

Measurements were performed using the TINA TCM400 $TcpO_2$ device (Radiometer, Copenhagen, Denmark). The temperature of the probe was set to 44.5 °C to allow maximal skin vasodilation, thereby decreasing the arterial-to-skin surface oxygen pressure gradient. Before the exercise test, 15–20 min was allowed with the probe attached to the skin for stabilisation of $TcpO_2$ value. Following the test, the $TcpO_2$ values were automatically corrected according to a temperature of 37 °C by the TINA device. The electrode was placed slightly below the right scapula on the back away from any bone.

Fixation rings were used to hold the probe attached to the skin and this was filled with two small drops of contact fluid before attachment to the sensor. The fluid was then heated causing the subsequent dilatation of the skin. The raw values of the patient's oxygen perfusion were defined as previously described in Wasilewski et al. (2016). The definitions are also presented in the methods section, chapter 5.

3.4 Bioelectrical impedance analysis

In our research studies we utilised the InBody 720 (InBody, Seoul, Korea) composition analyser for the direct segmental multi-frequency bioelectrical impedance (DSM-BIA). This equipment has previously been demonstrated to have high test-pre-test accuracy and reliability (Ling et al., 2011). Unlike conventional BIA devices which usually perform partial measurements and thus, rely upon formulas to estimate whole body composition, DSM-BIA technique employs the assumption that the human body consists of five interconnecting cylinders and takes direct impedance measurements from the various body compartments. A tetrapolar eight-point tactile electrode system is utilised, which separates the

measurements impedance of the subject's trunk, arms, and legs at six different frequencies (1 kHz, 5 kHz, 50 kHz, 250 kHz, 500 kHz, 1000 kHz) for each of the body segment. The spectrum of electrical frequencies is used to predict the intracellular water (ICW) and extracellular water (ECW) compartments of the total body water (TBW) in the various body segments. Low-level frequencies (e.g., 1-50 kHz) rely on the conductive properties of extracellular fluid, whereas, at high-level frequencies (e.g., 250 kHz), the conductive properties of both ICW and ECW are instrumental. Lower body mass (LBM) is estimated as TBW (ICW + ECW)/0.73. Fat mass is calculated as the difference between total body weight and LBM. The machine gives immediate and extensive quantitative values of various body composition parameters. The test in our research project was carried out by a trained researcher. The InBody (720) body composition analyser has in-built hands and feet electrodes. Our participants wore normal indoor clothing and advised to stand barefooted in upright position with their feet on the feet electrodes on the machine platform and their arms abducted with hands gripping on to the hand's electrodes on the handles. Participants were fasted (2hrs) before the test.

InBody 720 has been demonstrated to have a strong correlation with the dual energy X-ray absorptiometry (DEXA, Ling et al., 2011), which is considered to be the gold standard for body composition analysis. More specifically, this study compared the accuracy of DSM-BIA (InBody 720) against DEXA with 484 middle-aged participants. The study found that the InBody had a 99% correlation to DEXA when measuring lean mass in normal and overweight populations. This study shows DSM-BIA to be a valid tool for the assessment of whole-body composition and segmental lean mass measurements in middle-aged population when validated against DEXA.

3.5 Blood pressure

Blood pressure was measured using a manual aneroid sphygmomanometer (DuraShock DS54, Welch Allyn, USA) and a stethoscope (Littman Classic II, 3M, USA). Prior to the exercise tests, patients were seated on a chair with their back well supported, feet flat on the floor and the arm was placed on a table at the level of the heart with the palm facing up. For the selection of the right cuff size the brachial

circumference was measured using a tape. The cuff was placed as such that the lower edge was 2-3 cm above the point of brachial artery pulsation. The stethoscope was placed gently over the brachial artery at the point of maximal pulsation. The values were recorded according to Korotkoff sounds. When all sounds were disappeared, the SBP was recorded and the cuff was deflated rapidly and completely before repeating the next measurement after 2mins to prevent venous congestion of the arm.

3.6 Electrocardiogram

The areas for electrode application were first shaved (if required) and then rubbed with alcohol-saturated gauze. A 12-lead electrocardiogram (ECG) was applied before the commencement of the exercise tests. V1 and V2 were placed in the fourth intercostal space. V3 was placed halfway between V2 and V4. V4, 5 and 6 were placed along a horizontal line at the level of the fifth intercostal space with V4 being on the mid-clavicular line. The right and left arm leads were placed outwardly on the shoulders. The left and right leg leads were placed just below the umbilicus on the left and right side of the abdomen, respectively. The 12-lead display (Case, New York, USA) was monitored throughout the exercise tests observing for any contraindications prior or during the exercise tests.

3.7 Quality of life assessments

3.7.1 Functional ability test

To test the functional ability to perform daily activities for our patients we performed a six-minute walking test (6MWT). The 6MWT that we performed in our studies complied to the technical aspects (see below) according to ATS guidelines (ATS, 2002). 6MWT is a surrogate marker for disability and complaints in SSc patients (Deuschle et al., 2011). Therefore, 6MWT could provide a valuable outcome parameter although it lacks organ specificity in SSc.

Technical aspects

Location

The 6MWT was performed indoors (Sheffield Hallam University laboratories), along a 10 m, flat, straight, enclosed corridor with a hard surface that was stable. The length of the corridor was marked every 2 m. The turnaround points were marked with an orange traffic cone. A starting line, which marked on the beginning and end of each 20-m lap, was marked on a blue floor using a white tape. The test performed by the same trained researcher throughout the studies.

Required equipment

1. Countdown timer (or stopwatch)
2. Mechanical lap counter
3. Two small cones to mark the turnaround points
4. A chair that can be easily moved along the walking course
5. Worksheets on a clipboard
6. A source of oxygen
7. Sphygmomanometer
8. Telephone
9. Automated electronic defibrillator

Patient preparation

1. Comfortable clothing should be worn.
2. Appropriate shoes for walking should be worn.
3. Patients should use their usual walking aids during the test
4. The patient's usual medical regimen should be continued.
5. A light meal is acceptable before early morning or early afternoon tests.

6. Patients should not have exercised vigorously within 2 hours of beginning the test.

Measurements

1. The tests were performed about the same time before and after the exercise intervention so as to minimise intraday variability.

2. We did not perform any warm up and we checked for any contraindication prior to test such as elevated pulse and blood pressure.

3. Prior to the commencement of the test, patients instructed as follows (no verbal encouragement was given during the test):

"The object of this test is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. Six minutes is a long time to walk, so you will be exerting yourself. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting but resume walking as soon as you are able. You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I'm going to show you. Please watch the way I turn without hesitation." "Are you ready to do that? I am going to use this counter to keep track of the number of laps you complete. I will click it each time you turn around at this starting line. Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but don't run or jog. Start now, or whenever you are ready."

3.7.2 EQ-5D-5L Questionnaire

In our research studies we measured QoL through the EQ-5D-5L questionnaire which was administered prior and after the exercise intervention as well as in the following up assessment. The EQ-5D-5L questionnaire has been previously validated in SSc patients (Gualtierotti et al., 2016).

The 5-level EQ-5D version (EQ-5D-5L) was introduced by the EuroQoL Group in 2009 to improve the instrument's sensitivity and to reduce ceiling effects, as compared to the EQ-5D-3L. The EQ-5D-5L

essentially consists of 2 pages: the EQ-5D descriptive system and the EQ visual analogue scale (EQ VAS).

The descriptive system comprises five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems and extreme problems. The patient is asked to indicate his/her health state by ticking the box next to the most appropriate statement in each of the five dimensions. This decision results in a 1-digit number that expresses the level selected for that dimension. The digits for the five dimensions can be combined into a 5-digit number that describes the patient's health state.

3.7.3 Enjoyment level and exercise tolerance

The feasibility and the perceived enjoyment of HIIT and RT were assessed through measures that interpreted patients' perception regarding the A) exercise intensity, B) the affect, C) the exercise task self-efficacy, D) the intentions and E) the enjoyment. The above data was collected at the first and last exercise session each month in order to examine several time points during the exercise intervention. Specifically, the questionnaires were administered at the 1st, 8th, 9th, 16th, 17th and 24th exercise sessions.

A) Exercise intensity

Rate of perceived exertion (RPE) was measured during exercise through a 20-point Borg scale (Borg, 1998) at 2.5%, 8.2%, 42.5%, 48.2%, 92.5% and 98.2% of exercise completed. These time points were chosen to incorporate both interval and recovery periods during HIIT. In the RT the time points were straight after the completion of each exercise. The 20-point Borg scale is ranging from 6 to 20 with anchors ranging from "No exertion at all" (0) to "Maximal exertion" (20). Except the time points during exercise, the RPE was measured pre- and post-exercise as well as 10 minutes after the exercise session. Participant's heart rate was also recorded using Polar heart-rate monitor at the same time points as RPE.

B) Affective Valence

The one item Feeling scale (Hardy & Rejeski, 1989) was used to measure the general affective valence (e.g., pleasure and displeasure) during the exercise session at the same time points as RPE (Appendix 1). Patients were informed at the beginning of the first exercise session with the following instructions "Experiencing alterations in your mood is very common while performing exercise. The sense of pleasure or displeasure varies among individuals during the exercise; in addition, feelings may fluctuate across time. So, the answers might feel good and bad a number of times during exercise, when you will be asked to express your feelings using the scale below". The feeling scale is scored on an 11-point bipolar scale ranging from -5 to +5. Seven anchors are provided ranging from, "Very Good" (+5) to "Very Bad" (-5).

C) Exercise Task Self-Efficacy

Patient's confidence in their ability to repeat the exercise session that they will just have completed was assessed only after the first exercise session at 20-minutes post-exercise using a 3-item measure (Appendix 2). Each question included the same introductory theme, "How confident are you that you can…". The 3-items will be: 1) "perform one bout of exercise a week for the next 4 weeks that is just like the one you completed today?" 2) "Perform two bouts of exercise a week for the next 4 weeks that is just like the one you completed today?" 3) "Perform three bouts of exercise a week for the next 4 weeks that is just like the one you completed today?". The scale score varied from 0% (Not at all) to 100% (Extremely confident) in 10% increments. The specificity of the three items measure was formed and adapted according to Jung et al. (2014).

D) Intentions

Patient's intentions to engage in the exercise session over the next month was measured utilising a 2-item measure (Appendix 3) at the 1st, 9th and 17th exercise session, 20-minutes post-exercise (Jung et al., 2014). Particularly, patients were asked "Please rate the extent to which you agree with the following statements 1) "I intend to engage in the type of exercise I performed today at least 2 times per week during the next month" and 2) "I intend to engage in the type of exercise I performed today at least 3

times per week during the next month". Answers will be scored on a 7-point rating scale with anchors ranging from "Very unlikely" (1) to "Very likely" (7). The two items were analysed individually.

E) Enjoyment

Patient's enjoyment of the assessed exercise sessions was examined using a modified version of the physical activity enjoyment scale (PACES; Kendzierski & DeCarlo, 1991) 20-minutes post-exercise. This 18-item measure is scored on a 7-point bipolar scale (Appendix 4). Example items are "I find it energizing/I find it tiring" and "it's very pleasant/it's very unpleasant". The original measure is amended by erasing one of the 18 items that is irrelevant due to the time point that will be measured ("I am absorbed in the activity-I am not at all absorbed in the activity"). Moreover, the original PACES instructions were amended from "Please rate how you feel AT THE MOMENT about the physical activity you have been doing" to " Please rate how you feel about the exercise you just completed". Both modifications were made to reflect the correct time point that the questions/questionnaire will be administered (20-minutes post-exercise).

3.7.4 Interviews

Patients experiences for study procedures

We undertook an in-depth exploration of the patients' study experience, in a sub-sample of 6 and 6 patients of each group (exercise and control group). Interviews lasted between 15 to 20 minutes and took place three months after randomisation for both groups.

We aimed to explore the following topics:

1. Patients' experiences of RP.

2. Experiences of treatment and advice received pre FESS trial.

3. Participant's preference for trial allocation (exercise or control group).

4. Experiences of study participation -both exercise intervention group and control group. Participant's acceptability of the exercise intervention and study procedures.

A constructivist approach (Fosnot, 1996) was adopted which recognises the individual and personal nature of patients exercise experiences both before and during the trial. Semi-structured face to face in-depth interviews were conducted in the Centre for Sports and Exercise Science at Sheffield Hallam University in a comfortable and private room. Interviews were recorded and then transcribed verbatim, and then were analysed thematically by using framework analysis (familiarisation, identifying a thematic framework, indexing, charting and mapping).

Sample and recruitment**:** Six and six patients from exercise and control group, respectively, were recruited using purposive sampling (mixture of genders, younger and older patients from exercise and control group).

Chapter 4: Validation of an Arm Crank Ergometer Test for Use in Inactive Adults

4.1 Chapter overview: Chapter one and two explored the current literature on vascular disease which constitutes the touchstone of systemic sclerosis (SSc) pathophysiology. In addition, these chapters assessed the effects of exercise and more specifically, high intensity interval training and resistance training on the vascular function in several clinical population with macro and microvascular pathophysiological elements. It was concluded that further knowledge was required on the mode of exercise, lower or upper-limb, that would induce greater results in the digital microcirculation in people with SSc. To compare these two modes of exercise, a validated arm crank ergometer exercise protocol was required.

Chapter four examines the physiological differences of upper (arm cranking) and lower limb (cycling) exercise. The comparison of arm cranking and cycling exercise protocols will attempt to provide a

predictive equation for arm crank ergometer's peak oxygen uptake ($\dot{V}O_{2peak}$) that will allow the comparison between these two entirely physiologically different modes of exercise.

4.2 Abstract

Background: The maximal oxygen uptake ($\dot{V}O_{2peak}$) test is an approved pre-operative examination tool, in a clinical setting: Both $\dot{V}O_{2peak}$ and anaerobic threshold indicate a patient's physiological tolerance for major surgery and post-operative mortality, with cycle ergometry being routinely used for $\dot{V}O_{2peak}$ tests in clinical settings, in many European countries. Nevertheless, the opportunities to assess populations with restricted mobility of the lower limbs are limited, as alternative methods (such as an arm-crank test protocol) to assess $\dot{V}O_{2peak}$ are yet to be established.

Methods: Twelve inactive middle-aged adults (55.1 ± 5.0 years) per-formed two incremental protocols on an arm crank and cycle ergometer on separate occasions. During exercise, gas exchange was collected and analysed by an online breath-by-breath analysis system.

Results: Regression analysis showed that the model with dependent variable cycle ergometer $\dot{V}O_{2peak}$ ($CE\dot{V}O_{2peak}$) in ml·kg-1·min-1 and independent variables arm crank $\dot{V}O_{2peak}$ ($ACE\dot{V}O_{2peak}$) in ml·kg-1·min-1, lean body mass lower limbs (LBMLL) and total lean body mass (TLBM) fitted the population the best, with r2 = 0.87, adj. r2 = 0.82 and SEE = 3.14. The equation estimated with this model is: CE $\dot{V}O_{2peak}$ = 11.776 + 1.418 X $ACE\dot{V}O_{2peak}$(ml·kg-1·min-) – 1.454 x TLBM + 3.967 X LLLBM.

Conclusions: Our study suggests that arm cranking might be an alternative mode of exercise for inactive middle-aged adults (and potentially in clinical settings) to assess the cardiorespiratory fitness of people with restricted lower-limb mobility.

4.3 Introduction

The cardiovascular and respiratory systems support in-creased energy requirements of the musculature during physical activity. The functional limit of the cardiovascular system can be best assessed through the maximal oxygen uptake test ($\dot{V}O_{2max}$), which is commonly defined as an index of cardiorespiratory

fitness and typically reflects the upper limit of the body's ability to intake and consume oxygen (Åstrand and Saltin, 1961). Nevertheless, the term "peak oxygen uptake" ($\dot{V}O_{2peak}$) is used in the present paper, as it reflects more precisely a stress test in a clinical setting where the exercise test termination could be due to other than cardiorespiratory limitations. Recent research has explored how upper-limb aerobic exercise can be applied in clinical populations (Ilias et al., 2009). More specifically, this exercise modality seems to be appropriate for cardiorespiratory fitness assessments aimed at patients having limited functional capacity in the lower limbs. In clinical settings the cardiopulmonary exercise (VO_{2peak}) test has been established as an approved pre-operative examination (Weisman et al., 2003). More specifically, $\dot{V}O_{2peak}$ and anaerobic threshold have been demonstrated as an index of patients' physiological tolerance for major surgery (Davies and Danjoux, 2010). Anaerobic threshold has also been associated with post-operative mortality (Older et al., 1999) and its concomitant use for pre-operative risk stratification (Orr et al., 2013). Moreover, arm exercise has been demonstrated to predict clinical outcomes (Chan et al., 2011; Ilias et al., 2009) and researchers reported that the prognostic value of the clinical data obtained during arm exercise may be equivalent to that reported for treadmill or cycle ergometer exercise (Dutcher et al., 2007; Myers et al., 2002).

Arm crank ergometry (ACE) seems to constitute a reliable mode of exercise that is able to assess all the physiological responses that are elicited during physical activity. Several factors are considered to play a vital role in eliciting significant physiological responses during arm crank ergometry including crank rate (Schrieks et al., 2011; Smith et al., 2001), the type of incremental protocol (Sawka et al., 1983; Smith et al., 2004), and the ramp slope during an incremental ramp protocol (Castro et al., 2010). These studies have demonstrated that a crank rate of 70 revolutions per minute is considered to be the optimal ‘tempo’ during a $\dot{V}O_{2peak}$ test and that a continuous incremental ramp protocol induces higher values of oxygen uptake, ventilation and heart rate responses compared with slower crank rates. Furthermore, fast (increment: 2W/6 s) and slow (increment: 1W/6 s) ramp protocols seem equal in attaining peak oxygen uptake in healthy young individuals (Castro et al., 2010).

Cycle ergometry is routinely used in clinical settings in many European countries. In addition, cycle ergometry compared with treadmill testing is cost-effective, requires less space and is a feasible alternative in individuals who are obese or those presenting with orthopaedic, peripheral vascular, and/or neurological limitations (ACSM, 2014). Therefore, it is a widely-used exercise modality in clinical populations. Nevertheless, a validated arm crank ergometer protocol whose values are strongly associated with cycle ergometer measures for the prediction of $\dot{V}O_{2peak}$ has yet to be established.

Wasserman's cycle ergometer test ramp protocol (Wasserman, 1976) is a validated and widely used test in the clinical setting when patients are assessed for either cardiovascular or cardiorespiratory limitations. This protocol is practical and preferable for patients as they do not experience sudden increases in work rate, which is the case with graded test protocols (Wasserman et al., 2012). Nonetheless, some patients may not be able to pedal either due to lack of coordination and cycling experience and / or may experience seating discomfort during a long test.

Exercise tests are commonly used in clinical practice for both functional and diagnostic assessments (Arena et al., 2011). Interpretation of cardiopulmonary exercise test (CPET) for clinical purposes includes comparison of data from individual patients with those from healthy and disease populations. Substantial data are available characterising exercise responses of patients with certain common heart and lung diseases, providing a basis for using CPET to compare individual patients' impairment relative to others from the same populations. Diagnostic applications of CPET such as evaluating unexplained exertional dyspnoea or exercise intolerance, also rely on a comparison of patients; data with those of patients with known diagnoses. In clinical practice, in contrast to much of the research related to specific disorders, patients frequently have multiple medical problems, confounding the assessment of impairment or the attribution of symptoms to one or another condition. Although there are few systematic analyses of the effects of coexistent conditions on exercise responses, an advantage of CPET compared with other forms of testing is the potential for gaining insight into these interactions.

Therefore, the estimation of $\dot{V}O_{2peak}$ from an arm crank test would be of use for clinicians performing routine CPET in adults unable to stress maximally the lower limbs (e.g., severe leg ulcers or phlebitis) or feel more comfortable to use the upper limbs. During a CPET the clinician assesses the electrical signs of the heart through an electrocardiogram (ECG) and the cardiovascular and cardiorespiratory responses such as $\dot{V}O_{2peak}$, minute ventilation (VE) and BP that would be induced by an arm crank test. $\dot{V}O_{2peak}$ is a powerful tool for diagnosis and prognosis in the clinical setting (Arena et al., 2011). However, there is lack of evidence for cut-off values in ACE $\dot{V}O_{2peak}$ that would be of use for disease and/or mortality diagnosis and prognosis. Therefore, the application and usefulness of a predictive $\dot{V}O_{2peak}$ equation resulting from an arm-crank test seems warranted.

The purpose of the present study is to produce an equation that will be able to predict cycle ergometer $\dot{V}O_{2peak}$, using ACE physiological outcomes as equation elements. The study would also determine the differences in physiological responses in ACE and a cycle ergometer test protocol in middle-aged adults with low-to-moderate cardiovascular risk, following the most recent ACE test protocol recommendations (e.g., Castro et al., 2010; Wasserman et al., 2012).

4.4 Method

4.4.1 Participants

Twelve middle-aged adults (6 men and 6 women, mean age 55.1± 5) were recruited from the Sheffield Hallam University voluntary database. All participants lived a inactive lifestyle, had office-based employment, with no training history as athletes of any sport. Participants underwent health screening to confirm the absence of any cardiovascular and/or metabolic disease. Each participant received a study information sheet and became aware of any possible risks before signing the consent form. The research was approved by the Human Ethics Committee of Sheffield Hallam University and complied with the principles laid down in the Declaration of Helsinki.

4.4.2 Sample size

A post-hoc analysis was performed according to the multiple regression analysis with input parameters of error (error probability = 0.05), the total sample size (n = 12) and the number of predictors (e.g., ACE$\dot{V}O_{2peak}$, lean body mass lower limbs, total lean body mass). The result showed a statistical power of 0.99 which indicates that the total sample size was sufficient to predict any relationships between these two exercise modes.

4.4.3 Experimental approach

Apart from an inactive status, our inclusion criteria for participation consisted of ages ≥45 for men and ≥55 years for women, which are considered to be the cut-off age limits for each sex respectively, beyond which cardiovascular risk is increased according to American College of Sport Medicine (ACSM) guidelines (Pescatello et al., 2014). Participants were allowed ≥ 2 risk factors without symptomatic, or known cardiovascular, pulmonary, renal, or metabolic disease. Prior to each peak oxygen uptake test participants were requested to abstain from vigorous exercise, alcohol, caffeine and tobacco for a period of 24h and to have fasted for at least 3h prior to measurement. Moreover, resting ECG and blood pressure were assessed prior to the exercise tests to identify any contraindications to exercise. All the participants performed the exercise tests with the absence of any contraindications both at rest and during exercise. Each participant performed both the Wasserman's cycle ergometer and arm crank test in a randomly-assigned order separated by at least five days to assure for full recovery.

4.4.4 Pre-participation health screening

Participants were assessed for cardiovascular risk prior to participation. The health screening was consistent with the ACSM's guidelines for cardiovascular disease risk stratification (Pescatello et al., 2014). After health screening anthropometric measurements were performed [body mass (kg), stature (cm), body mass index (BMI) and upper- and lower-arm circumference (cm) according to guidelines (National Institutes of Health, 1998)] and seated blood pressure (mm Hg) was assessed. The participants

that were classified as “low and moderate risk”, after risk stratification, were eligible to take part in the study.

4.4.5 Arm crank test

The arm crank ergometer (Lode BV, Groningen, Nether-lands) was adjusted to ensure alignment between the ergometer's crankshaft and the centre of the participant's glenohumeral joint. Participants' sitting position was set up to ensure that the elbows were slightly bent when the arm was outstretched. Participants were instructed to maintain their feet flat on the floor at all times. Due to different power capabilities two different protocols were identified for men and women. Men commenced at a workload of 30W and women at 20W. In both protocols the crank rate was maintained at 70 rev min-1 (Smith et al., 2001; 2007) and power requirements increased as a linear ramp at a rate of 10W min^{-1} and 6W min^{-1} for men and women, respectively (Smith et al., 2007). The test commenced with 3 minutes rest and then 3 minutes of warm-up (unloaded cranking). Rating of perceived exertion (RPE) ≥ 18 and/or inability to maintain a crank rate above 60 rev min-1 resulted in the termination of the test. After exercise termination an unloaded bout of 2 - 3 minutes exercise at a crank rate below 50 rev min^{-1} allowed for an active recovery period.

4.4.6 Wasserman's cycle ergometer test

Wasserman's cycle ergometer test was performed on an electromagnetic cycle ergometer (Lode Excalibur, Groningen, Netherlands). The test commenced with a 3-minute rest period followed by 3 minutes of unloaded pedalling. Participants were requested to maintain a cycle rate around 60 rev min^{-1} during the exercise test. The rate of around 60 rev min^{-1} was requested for three reasons. A) very slow rate < 40 rev min^{-1} could make the pedalling harder, b) very high rate > 80 rev min^{-1} could make the pedalling easier and c) our experience indicate that a rate around 60 rev min^{-1} is a preferred pace for inactive populations with no experience in cycling on laboratory bikes. The initial load and the concomitant increments were individually calculated according to participants estimated physical fitness and Wasserman's equations (Wasserman et al., 2012, p. 141-2). The work increments increased every

minute until volitional exhaustion or until any exercise contraindications arose such as consecutive premature ventricular contractions observed on the ECG (no exercise contraindications were observed). Rating of perceived exertion (RPE) ≥ 18 and/or inability to maintain a crank rate above 40 to 45 rev min-1 resulted in test termination. Following the exercise test 2-3 min of unloaded pedalling allowed for an active recovery period.

4.4.7 Measurements during exercise tests

During cardiopulmonary tests gas exchange was analysed by an online breath-by-breath analysis system (UltimaTM, Medical Graphics, UK). The gas analyser was calibrated before each test according to the calibration guidelines of the manufacturer. Heart rate (HR) breathing frequency, tidal volume (VT), minute ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$) and volume of exhaled carbon dioxide ($\dot{V}CO_2$), as well as respiratory exchange ratio (RER) was displayed on a monitor (Breeze Suite, MGC Diagnostics, USA) on a breath-by-breath analysis. HR was continuously monitored using a Polar heart rate monitor (Polar FS1, Polar Electro, Kempele, Finland) and blood pressure was assessed using a manual sphygmomanometer (DuraShock DS54, Welch Allyn, USA) and stethoscope (Littman Classic II, 3M, USA). The electrical signs of the heart were continuously recorded (Case, New York, USA) throughout the test producing a 12-lead ECG on a screen monitor (see details in Chapter 3-Theory of Methods). RPE was recorded during the last 10s of every minute during the exercise test until volitional exhaustion using Borg's scale 6-20 point (Borg, 1973). Peak power output and test duration was measured in both tests. $\dot{V}O_{2peak}$ defined as the average oxygen uptake recorded from expired air during the final 30s of exercise.

4.4.8 Body composition analysis

The participant's stature was measured using a Hite-Rite Precision Mechanical Stadiometer. Body mass (kg), fat mass (kg), lean body mass (kg) segmented in upper- and lower-limbs were assessed by using bioelectrical impedance analysis (In Body 720, Seoul, Korea). Upper and lower arm circumferences were measured by a standard metric measuring tape (Seca 206, Hamburg, Deutschland). BMI was the derivative of body weight in kilograms divided by height in meters squared ($kg \cdot m^{-2}$).

4.4.9 Statistical analysis

Data analysis was performed using SPSS software (version 23, IBM SPSS, New York, USA) and presented as mean ± SD. Cardiorespiratory measures, peak power and duration of the exercise tests were compared using paired sample t-tests. Pearson's correlation coefficient was used to correlate $\dot{V}O_2$ in $L \cdot min^{-1}$ and in $ml \cdot kg^{-1} \cdot min^{-1}$ and HR. Correlation coefficients were calculated for all physiological and anthropometrical variables. The variables most closely associated with $\dot{V}O_2$ were included in a backward stepwise linear regression analysis and supported the development of an equation to estimate $\dot{V}O_2$ values based on ACE $\dot{V}O_2$ and other physiological and/or anthropometrical outcomes. The predictors for cycle ergometer $\dot{V}O_2$ (CE $\dot{V}O_2$) that were included into the regression analysis were arm crank $\dot{V}O_2$ (ACE $\dot{V}O_2$) in L·min-1 and ml·kg-1·min-1, lean body mass lower (LBMLL) and upper limbs (LBMUL), lean body mass in total (LBM), HR, $\dot{V}E$, RER and sex. Statistical significance was set at $p<0.05$.

4.5 Results

4.5.1 Anthropometric characteristics

Participants' anthropometric characteristics are shown in Table 4. Men were significantly younger compared to women and that can be attributed to the sex specific different cut-off age limit at which age is considered as a cardiovascular risk factor. Anthropometrically, men have a higher lean body mass than women which is usually evident as the percentage of lean body mass on the upper limbs and the total lean body mass.

4.5 Table 4 Anthropometric characteristics. Data are means (± SD).

	Men (n = 6)	Women (n=6)	Total (n = 12)
Age (years)	51.7 (4.7) **	58.5 (2.4)	55.1 (5.0)
Body weight (kg)	85.0 (12.3)	73.6 (13.4)	79.3 (13.6)
Height (m)	1.76 (.08) **	1.60 (.07)	1.68 (.10)
Body mass index (kg·m-2)	27.6 (4.4)	28.8 (5.9)	28.2 (5.0)
Upper arm circumference (cm)	31.8 (3.8)	29.2 (3.1)	30.5 (3.6)
Lower arm circumference (cm)	24.5 (2.4)	22.2 (1.5)	23.3 (2.2)
Lean body mass upper limbs (%)	8.8 (.6) ***	6.4 (.4)	7.6 (1.3)
Lean body mass lower limbs (%)	22.6 (3.6)	18.7 (3.3)	20.7 (3.8)
Total lean body mass (%)	70.6 (7.0) *	58.7 (8.7)	64.7 (9.8)

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to women.

4.5.2 Physiological responses

Table 5 presents the physiological responses from the arm crank test and Wasserman's cycle ergometer test. The Shapiro-Wilk test was performed to test the normality of the data and Levene's test, $p \geq 0.05$, to confirm the homogeneity of variances. Absolute $\dot{V}O_2$ with a mean difference of [0.41 (0.12, 0.70) $L \cdot min^{-1}$, $p < 0.05$, ES: 0.89) and relative $\dot{V}O_2$ (with a mean difference of [6.7 (3.6, 9.9) $ml \cdot kg^{-1} \cdot min^{-1}$, $p < 0.01$, ES: 1.34) were higher in cycle ergometry compared with arm crank, in all participants. HR_{peak} and $\dot{V}E_{peak}$ with mean differences of [8.3 (0.38, 16.12) $beats \cdot min^{-1}$, $p < 0.05$, ES: 0.67] and [14.8 (5.9, 23.6) $L \cdot min^{-1}$, $p < 0.01$, ES: 1.06], were also higher in cycle ergometry compared to arm crank. Whereas RER_{peak} was higher [-0.1 (-0.17, -0.03), $p < 0.01$, ES: 0.90] in arm crank compared to cycle ergometry, in all participants. Peak power was significantly higher in cycle ergometry compared to arm crank [82 (50, 114) W, $p < 0.001$, ES= 1.63].

4.5 Table 5 Physiological outcomes of the cycle ergometer and arm crank test. Data are means (±SD).

	Men (n = 6)		Women (n = 6)		Total (n = 12)	
	Cycle ergometer	Arm crank	Cycle ergometer	Arm crank	Cycle ergometer	Arm crank
$\dot{V}O_{2peak}$ (l min^{-1})	2.2 ± 0.7	1.8 ± 0.5	1.48 ± 0.24*	1.06 ± 0.24	1.84 ± 0.63*	1.43 ± 0.54
$\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1})	25.8 ± 9.5	19.0 ± 3.8	20.4 ± 4.0**	13.8 ± 2.5	23.1 ± 7.5**	16.4 ± 4.1
HR_{peak} (beats min^{-1})	147.5 ± 18.4	140.7 ± 18.4	153.8 ± 11.0	144.2 ± 13.9	150.7 ± 14.9*	142.4 ± 15.6
Peak $\dot{V}E$ (l min^{-1}, stpd)	78.6 ± 17.8	63.1 ± 7.0	53.9 ± 8.6*	40.0 ± 7.0	66.3 ± 18.6**	51.5 ± 13.8
Peak RER	1.25 ± 0.1	1.40 ± 0.1**	1.26 ± 0.1	1.31 ± 0.1	1.25 ± 0.1	1.35 ± 0.1**
Peak RPE	18.7 ± 1.0	19.2 ± 0.8	18.0 ± 1.9	18.7 ± 1.0	18.3 ± 1.5	18.9 ± 0.9
Test duration (min)	8.9 ± 2.7	8.0 ± 1.1	7.7 ± 1.0*	6.7 ± 0.5	8.3 ± 2.0	7.3 ± 1.0
Peak power (W)	203.3 ± 68.9*	100.0 ± 11.0	117.5 ± 21.4**	57.3 ± 6.7	160.4 ± 66.1***	78.7 ± 23.9

CE, Cycle ergometer; ACE, Arm crank; HR, heart rate; VE, minute ventilation; RER, respiratory exchange ratio; RPE, ratings of perceived exertion; STPD, standard temperature pressure dry. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to the cycle ergometer test.

4.5 Table 6 Linear regression analysis to estimate cycle ergometer V̇O2peak based on anthropometrics and arm crank physiological outcomes.

Model	Variables	r^2	Adj. r^2	SEE
1	$CE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), $ACE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), $ACE\dot{V}O_{2peak}$ (l min^{-1}), LBMLL (%), LBMUL (%), TLBM (%), $ACEHR_{peak}$ (beats min^{-1}), $ACEVE_{peak}$ (l min^{-1}), $ACERER_{peak}$, Gender	0.900	0.450	5.556
2	$CE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), $ACE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), $ACE\dot{V}O_{2peak}$ (l min^{-1}), LBMLL (%), LBMUL (%), TLBM (%), $ACEHR_{peak}$ (beats min^{-1}), $ACEVE_{peak}$ (l min^{-1}), $ACERER_{peak}$	0.900	0.633	4.536
3	$CE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), $ACE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), LBMLL (%), LBMUL (%), TLBM (%), $ACEHR_{peak}$ (beats min^{-1}), $ACEVE_{peak}$ (l min^{-1}), $ACERER_{peak}$	0.900	0.725	3.929
4	$CE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), $ACE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), LBMLL (%), LBMUL (%), TLBM (%), $ACEVE_{peak}$ (l min^{-1}), $ACERER_{peak}$	0.900	0.779	3.518
5	$CE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), $ACE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), LBMLL (%), LBMUL (%), TLBM (%), $ACERER_{peak}$	0.892	0.802	3.336
6	$CE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), $ACE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), LBMLL (%), TLBM (%), $ACERER_{peak}$	0.886	0.821	3.168
7	$CE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), $ACE\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1}), LBMLL (%), TLBM (%)	0.872	0.824	3.138

4.5.3 Regression analysis

Correlation coefficient analysis between the arm crank and cycle ergometer demonstrated a strong correlation between absolute $CE\dot{V}O_{2peak}$ and $ACE\dot{V}O_{2peak}$ ($r = 0.78$, $p < 0.01$) and between ACE HR_{peak} and CE HR_{peak} ($r = 0.67$, $p < 0.05$).

Regression analysis is illustrated in Table 6. The regression model with dependent variable $CE\dot{V}O_2$ in ml kg^{-1} min^{-1} and independent variables $ACE\dot{V}O_2$ in ml kg^{-1} min^{-1}, lean body mass lower limbs (LBMLL) and total lean body mass (TLBM) fitted the test population the best, with $r^2 = 0.87$, adj. $r^2 = 0.82$ and SEE = 3.14. The equation is: CE $\dot{V}O_{2peak}$ = 11.776 + 1.418 X ACE $\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) – 1.454 x TLBM + 3.967 X LLLBM.

4.6 Discussion

The current study is the first to demonstrate a significant correlation between an arm crank and cycle ergometer for $\dot{V}O_2$ and HR. Correlations coefficient test were performed for all the physiological and anthropometric characteristics resulting in the strong correlation of $\dot{V}O_2$ and HR. Between $\dot{V}O_2$ and HR, our study correlation demonstrated that the ACE $\dot{V}O_{2peak}$ was strongly correlated with CE $\dot{V}O_2$ ($r = 0.78$, $\dot{V}O_2$ in $ml \cdot kg^{-1} \cdot min^{-1}$) suggesting its role as a predictor. ACE and CE HR_{peak} demonstrated a significant correlation ($r = 0.67$, $p < 0.05$) but not as strong as between $\dot{V}O_{2peak}$. Having established the relationship between these two measures, we then performed a regression analysis to explore the role of the other physiological outcomes, which would allow us to most accurately estimate cycle ergometer $\dot{V}O_2$ from the physiological and anthropometrical variables of ACE. For this reason, a regression analysis was performed to examine the complementary physiological outcomes to $\dot{V}O_2$ that would most accurately predict cycle ergometer $\dot{V}O_2$. Lower limb lean body mass and the total lean body mass together with arm crank $\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$) constitute a valid estimation ($r^2 = 0.87$, SEE = 3.14) of cycle ergometer $\dot{V}O_2$. Moreover, Schrieks et al. (2011) compared treadmill to arm crank ergometer and presented a regression equation by which treadmill $\dot{V}O_2$ could be predicted by physiological parameters of ACE. Therefore, based on the findings of the current study arm cranking could be an alternative mode of exercise testing to be used in inactive middle-aged adults and potentially to clinical populations such as systemic sclerosis patients who match the age range (Alba et al., 2014) and activity levels and exercise tolerance profile (De Oliveira et al., 2017) with our participants.

ACE elicited a $\dot{V}O_2$ ($L \cdot min^{-1}$) approximately 22.3% less than cycling and 29% when adjusted for body weight ($ml \cdot kg^{-1} \cdot min^{-1}$), which was similar to findings from previous studies (Muraki et al., 2004; Orr et al., 2013). Moreover, it was observed in the current study that HR and $\dot{V}E$ were significantly greater in cycling than in arm cranking. These findings agree with previous studies (Muraki et al., 2004; Orr et al., 2013) and also with Schrieks et al. (2011) who utilised a comparable arm crank exercise protocol to compare it with a Bruce treadmill protocol.

The lower $\dot{V}O_2$ observed during arm exercise may be explained by the specificity of the muscle groups involved in that exercise mode. The primary working muscles during arm cranking, biceps and triceps brachii and the deltoid, are smaller and less conditioned compared with the leg muscles. These arm muscles have a greater amount of type II muscle fibres than the muscles of the legs (Turner et al., 1997) and consequently higher O_2 cost than slow-twitch (type I) fibres (Schneider, Wing, Morris, 2002). This leads to an increase in anaerobic metabolism in arm exercise which has been demonstrated to induce muscle deoxygenation in the triceps, peaking at only 50% of $\dot{V}O_2$ compared with above 80% in cycling (Muraki et al., 2004). Moreover, the exercise-induced metabolic responses differ between arm and leg muscles (Heldge, 2010). Evidence reports greater carbohydrate oxidation and lactate release for the arm musculature (Ahlborg and Jensen-Urstad, 1991) and lower oxygen extraction capacity, even in elite athletes who have intensively trained the upper body muscles over the years (Calbet, 2005). In addition, arm muscle has a lower oxidative capacity when compared to the vastus lateralis, despite the similarity in fibre type composition (Kiilerich et al., 2008) and capillarization (Heldge et al., 2008). The lower oxidative capacity in the human arm muscle is probably related to deconditioning due to the non-postural nature of upper body musculature.

Although anaerobic metabolism is the primary metabolic pathway in arm exercise compared to cycling, $\dot{V}E$ was significantly greater in cycling than arm cranking. This can be explained by the higher lactate accumulation during cycling than arm cranking at intensities exceeding 80% of $\dot{V}O_2$ which is proportionate to the muscle mass (Sawka et al., 1983). Consistent with our findings, other investigations have reported that $\dot{V}E$ is lower after arm cranking compared with cycling (Muraki et al., 2004, Schrieks et al., 2011).

A higher HR has been observed for cycling, as reported in previous studies (Muraki et al., 2004, Sanada et al., 2005, Schrieks et al., 2011) that compared leg with arm exercise. The higher HR could be explained by the greater muscle mass in the lower limbs that stresses the cardiovascular system more than the upper limb musculature. In contrast, RER values were significantly higher in the arm crank test in comparison

with the cycle ergometer test; this may be directly linked to the greater lactate accumulation per regional skeletal muscle mass and the lower oxidative capacity of the exercising muscles in arm cranking. All our participants stopped the arm crank test due to muscle fatigue and not for cardiorespiratory limitations. This is another indication for a higher anaerobic metabolism in arm cranking compared to cycling. Muraki et al. (2004) measured the muscle deoxygenation in both modes of exercise and found that anaerobic metabolism was higher in arm cranking compared to cycling.

The key difference in the physiological responses between these two modes of exercise is apparently the greater muscle mass that is utilised by the lower limbs during cycling. Concomitantly, this stresses the cardiovascular system more than upper limb exercise and thus, certain values such as $\dot{V}O_2$, $\dot{V}E$ and HR are higher in cycling. However, this is not always the case with older adults or patients who may stop an exercise test prematurely due to muscle fatigue or other systemic abnormalities such as high blood pressure and/or ECG contraindications.

An equation that estimates CE $\dot{V}O_2$ from the physiological responses of ACE in inactive middle-aged adults is a key finding of the current research study. The equation can be used by physicians in cases where middle-aged patients are required to perform a CPET for cardiovascular or mortality risk assessment before an operation. It is important to acknowledge that the average age of clinical populations for heart failure patients and/or chronic obstructive pulmonary disease patients is over 65 years old. Nevertheless, there are patients within those clinical populations below that age and other patients with obesity, diabetes and other cardiovascular risk factors with an average age of 55 ± 5 years old who present with mobility difficulties that would benefit from a CPET. A CPET determines the physical fitness of the individual and consists of both a cardiovascular and a mortality risk assessment. By converting the ACE$\dot{V}O_2$ to CE$\dot{V}O_2$ physicians obtain a comparable value of the patients' physical fitness which might be used for decision making. Therefore, the utility and the application of the equation could cover a broad spectrum of clinical and non-clinical populations of inactive middle-aged adults that are in need of clinical care and arm cranking might seem the preferable mode for these individuals.

4.7 Limitations of the study

The recruitment of different muscle masses and muscle fibre types between arm and leg exercise may be considered as a limitation. These differences could lead to exercise-induced exertion either due to cardiorespiratory or local muscle fatigue limitations. Nevertheless, in the current study we recorded incidents where the participants prematurely ended the cycle ergometer test due to local muscle fatigue which could be an indication of weak muscles in the lower limbs and poor physical conditioning. We also stress that the deliberate age restriction in our study intended to simulate the age and fitness of several clinical populations.

Our participants did not perform a maximal oxygen uptake test; instead they undertook a peak oxygen uptake test due to their age and low level of physical fitness. A peak oxygen uptake test warrants several test termination causes, other than cardiorespiratory limitations for many clinical populations (Pescatello et al., 2014).

Another limitation is that our participants were healthy adults and therefore, our results cannot be generalised for clinical populations and/ or patients with restricted lower limb mobility. However, our results could be related to lcSSc patients as this clinical population is deemed as normal in terms of exercise capacity and tolerance (De Oliveira et al., 2017), they are inactive as our population and the age range of disease onset is between 20 and 50 years (Alba et al., 2014) with our current participants having a mean age of 55 ± 5 years old.

4.8 Conclusions

The current study is the first to demonstrate a strong correlation between a routinely used cycle ergometer test (Wasserman's protocol) and an arm crank test to assess cardiorespiratory fitness in inactive middle-aged adults. The arm crank test could be used as an alternative to cycle ergometry by accurately predicting $\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) in inactive middle-aged adults. Future research should focus upon comparing these protocols in older patients, clinical populations, and/or younger people to examine test reproducibility.

4.9 Strengths of the research study

This study created a statistically adequate formula that can be used to predict accurately $\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) of a cycle ergometer test using the physiological responses of an arm crank test and specific anthropometric characteristics of the individuals. $\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) is a strong predictor of cardiovascular disease and mortality.

Moreover, our participants represent a large cohort of clinical populations with similar characteristics (mean age, inactivity and exercise tolerance) and therefore, the predictive formula could be utilised in these clinical and/or general populations in people unable to exercise with the lower limbs.

Chapter 5: The effects of upper and lower limb exercise on the microvascular reactivity in limited cutaneous systemic sclerosis patients

5.1 Chapter overview: Chapter four examined the physiological differences between upper and lower limb exercise and established that arm cranking could be an alternative mode of exercise for sedentary middle-aged adults to assess the cardiorespiratory fitness in people with restricted lower-limb mobility.

Chapter five will investigate the effects of upper (arm cranking) and lower (cycling) limb exercise on vascular function in people with SSc. As discussed in chapter two, the hallmark symptom in SSc is the vascular dysfunction in the resistance arteries in the digital area leading to RP. The main aim in this chapter will be to define the mode of exercise, which could induce vascular improvements compared to control group. QoL, functional capacity and cardiorespiratory fitness will also be assessed and reported.

5.2 Abstract

Background: Aerobic exercise in general and high intensity interval training (HIIT) specifically is known to improve vascular function in a range of clinical conditions. HIIT in particular has demonstrated improvements in clinical outcomes, in conditions that have a strong macroangiopathic component. Nevertheless, the effect of HIIT on microcirculation in limited cutaneous systemic sclerosis (lcSSc)

patients is yet to be investigated. Therefore, the purpose of the study was to compare the effects of two HIIT protocols (cycle and arm cranking) on the microcirculation of the digital area in people with SSc.

Methods: Thirty-four patients with lcSSc (65.3 ± 11.6 years old) were randomly allocated in three groups (cycling, arm cranking and control group). The exercise groups underwent a twelve-week exercise program twice per week. All patients performed the baseline and post-exercise intervention measurements where physical fitness, functional ability, transcutaneous oxygen tension ($\Delta tcpO_2$), body composition and quality of life were assessed. Endothelial-dependent and -independent vasodilation were assessed in the middle and index fingers using LDF and incremental doses of acetylcholine (ACh) and sodium nitroprusside (SNP). Cutaneous flux data were expressed as cutaneous vascular conductance (CVC).

Results: Peak oxygen uptake was increased in both exercise groups ($p<0.01$, $d=1.36$). $\Delta tcpO_2$ demonstrated an increase in the arm cranking group only, with a large effect, but it was not found statistically significant,($p=0.59$, $d=0.93$). Endothelial-dependent vasodilation improvement was greater in the arm cranking ($p<0.05$, $d=1.07$) in comparison to other groups. Both exercise groups improved life satisfaction ($p<0.001$) and showed a reduced discomfort and pain due to Raynaud's phenomenon ($p<0.05$). Arm cranking seems to be the preferred mode of exercise for study participants as compared to cycling ($p<0.05$). No changes were observed in the body composition or the functional ability in both exercise groups.

Conclusion: Our results suggest that arm cranking has the potential to improve the microvascular endothelial function in people with SSc. Notably, our recommended training dose (e.g., a 12-week HIIT program, twice per week), appeared to be sufficient and tolerable for this population.

5.3 Introduction

Systemic sclerosis (SSc) is an idiopathic systemic autoimmune disease characterized by an ongoing cutaneous and visceral fibrosis, fibroproliferative vasculopathy and immunologic abnormalities

(Gabrielli et al., 2009; Bolster, 2008; Varga & Abraham, 2007; Jimenez & Derk, 2004). The vascular element has an important role in the SSc pathophysiology from early onset to late complications (e.g., pulmonary arterial hypertension and kidney disease). SSc can be distinguished in either limited cutaneous scleroderma (lcSSc) with skin involvement mainly limited to the hands and face; or diffuse cutaneous scleroderma (dcSSc) with skin involvement proximal to the elbows and knees (Isenberg & Black, 1995). Blood vessels are directly affected by SSc, as manifested by the diverse clinical complications that take place from the initiation to the propagation of the disease and have important ramifications on the quality of life of patients.

Raynaud's phenomenon (RP) precedes other clinical manifestations and is observed in over 95% of people with SSc (Kavian & Batteux, 2015). Evidently, RP is triggered by endothelial injuries in association with dysregulations in the vascular tone (Kahaleh, 2004). In addition to the imbalance of vascular tone, RP is also associated with structural vascular alterations in small- and medium-sized arteries leading to luminal narrowing. As a result, the blood vessels are unable to compensate for the impairment of blood flow during severe RP attacks and this leads to the so-called ischaemia-reperfusion reactions. These vascular complications may progress to gangrene and digital amputation (Sunderkötter & Riemekasten, 2006). Notably, SSc has the highest case-specific mortality of any rheumatic disease being also associated with substantial morbidity (Denton & Khanna, 2017).

Pharmacological agents (e.g., nifedipine) are commonly used as first-line approach. Although it can be effective and provide pain-relief to patients, the short-term (e.g., oedema, headaches, heart palpitations, dizziness and constipation) and long-term (e.g., heart dysfunction, increased cardiovascular risk) side effects of the medical treatment should also be considered as well as the financial cost of treatment. Therefore, adjunct therapies with less side effects and cost implications are warranted (Prescription Cost Analysis for England 2015; Pope, 2007), with a view to reduce dependency on medication.

Exercise in general and high intensity interval training (HIIT) specifically could be a useful adjunct therapy for this population. HIIT has come to prominence over the last few years for its effectiveness in

inducing greater improvements in vascular function than moderate-intensity continuous training in a number of clinical populations (e.g., heart failure, metabolic syndrome, obesity; Ramos et al., 2015). Nevertheless, due to the variation in HIIT protocols, limited evidence exists to support which protocol would be the most effective in people with SSc, although the options are many, based on evidence from other patient populations. For example, a HIIT protocol with short intervals (30s exercise/30s passive recovery) may elicit more favourable patient reported satisfaction/enjoyment levels compared to other longer duration exercise protocols (Meyer et al., 2012). In chronic heart failure patients, a short duration HIIT protocol (30s exercise/30s passive recovery) has demonstrated to be well tolerated, preferred protocol with a low perception of effort, patient comfort and with a longer time spent at higher percentage of peak oxygen uptake ($\dot{V}O_{2peak}$) than a longer duration HIIT protocol with active recovery phases (Meyer et al., 2012). Recent evidence supports this notion; when enjoyment levels in an overweight/obese cohort were examined after a short HIIT protocol and demonstrated that performing a HIIT protocol on a cycle ergometer present on an average 4.5 rating on a seven-point scale (Smith-Ryan, 2017).

Although we know the potential of HIIT in improving both the micro-and the macro- vascular function in several clinical populations such as heart failure (Guiraud et al., 2012) and cardiometabolic disease (Kessler et al., 2012) by using the treadmill and cycle ergometer as modes of exercise, no evidence exists about the mode of exercise that would be more effective on digital microcirculation where the RP attacks are present, such as in people with SSc. Assumptions could be made that utilising an upper-body exercise would potentially be more beneficial for the digital microcirculation rather than lower-body exercise where the working muscles promote the blood flow in the lower limbs. Hence, the effects that may occur by the upper- and lower-limb exercise on digital microcirculation in people with SSc should be examined.

We will attempt to bridge the knowledge gap by assessing the effects of a supervised and individually-tailored exercise program based on arm cranking (ACE) and cycle ergometry (CE) on microvascular reactivity, aerobic capacity, exercise tolerance and enjoyment levels, as well as on quality of life in people with SSc.

5.4 Methods

5.4.1 Patients

We recruited thirty-four patients (31 women, 3 men) with lcSSc, defined as per the ACR and European league against rheumatism criteria (Hoogen et al., 2013), with disease duration between 1 to 10 years. All participants were able to undertake exercise. Patients with pulmonary arterial hypertension, interstitial lung disease, those diagnosed with another inflammatory condition and/ or presenting myositis with proximal muscle weakness were excluded. Moreover, patients with New York Heart Association class 3 or 4, smokers or people who stopped smoking within 4 weeks of screening and women who were pregnant were also not permitted to participate. Eligible patients were recruited from the Rheumatology Department of the Royal Hallamshire Hospital in Sheffield. All patients provided written consent to participate. The regional health research ethics committee for clinical studies approved the protocol. Patients were randomly allocated (block randomisation) between the ACE (n = 11), CE (n = 11) and control (n = 12) groups. All the pre- and post-intervention tests were performed at the same time of the day to minimize intra-day variability.

5.4.2 Procedures

Baseline assessments, undertaken at first visit, included $\dot{V}O_{2peak}$, anthropometry, functional ability, microvascular reactivity and quality of life. $\dot{V}O_{2peak}$ test was performed either on an arm crank ergometer (ACE group) or on a cycle ergometer (CE and control group). After the baseline assessments, participants were allocated to ACE (n=11), CE (n=11) and control group (n=12). The exercise groups underwent a three-month exercise intervention twice per week. Three months post baseline measurements all groups were repeated the assessments. During the intervention three participants dropped out (ACE=1, CE=1 and control group=1).

5.4.3 Anthropometry

The participant's stature was measured using a Hite-Rite Precision Mechanical Stadiometer. Body weight (kg), body mass index (BMI), fat mass (kg) and lean body mass (kg) segmented in upper and lower-limbs

were assessed by using bio-electrical impedance analysis (In Body 720, Seoul, Korea). Patients' demographic characteristics are illustrated in table 7.

5.4 Table 7 Demographic data (means ± SD)

	Baseline ACE	Baseline CE	Baseline Control
Age (years)	69.1 ± 9.7	65.1 ± 10	62.2 ± 14.3
Body weight (kg)	69 ± 15.8	66 ± 9.7	73.2 ± 14.8
Body mass index ($kg/m^{2)}$	25.6 ± 4.8	24.5 ± 3.6	27.3 ± 4.0
Stature (cm)	163.7 ± 9.1	164.4 ± 7.9	163.4 ± 6.7

5.4.4 Microvascular reactivity

Microvascular function was assessed by laser Doppler Fluximtery and Iontophoresis technique in a temperature-controlled room (22-24°C). LDF electrodes were attached to the dorsal aspect of the reference fingers for acetylcholine (ACh) and sodium nitroprusside (SNP) administration. These were used as indicators of the changes occurring in the endothelial –dependent and –independent vasodilatory function. Heart rate (Sports Tester, Polar, Finland) and blood pressure of the brachial artery (left arm; Dinamap Dash 2500, GE Healthcare, USA) were monitored at 5-minute intervals throughout the protocol. The two drug delivery electrodes (PF383; Perimed AB, Jarfalla, Sweden) were positioned over healthy looking skin, approximately 4 cm apart with one containing 100 μL of 1% ACh (Miochol-E, Novartis, Stein) and the other 80 μl of 1% SNP (Nitroprussiat, Rottapharm). ACh was placed over the middle finger between the distal and proximal interphalangeal joints and SNP was placed over the index finger between the metacarpophalangeal and carpometacarpal joints. A battery-powered iontophoresis controller (PeriIont PF382b; Perimed AB) was used to provide the charge needed for ACh and SNP delivery. A 4 minute stable recording of baseline flux was followed by administration of the two agents according to the following protocol: 0.2 mA for 10 s (i.e., 2 mC), 0.2 mA for 15 s (i.e., 3 mC), 0.2 mA for 20 s (i.e., 4 mC), and 0.3 mA for 20 s (i.e., 6 mC), occurring between 4-minute intervals (Klonizakis

et al., 2009a; 2009b). To obtain an index of skin blood flow, cutaneous red cell flux was measured by placing an iontophoresis LDF (PF481-1; Perimed AB), connected to a laser Doppler fluximeter (PF5001; Perimed AB).

Data is expressed as cutaneous vascular conductance (CVC) [i.e. flux divided by arterial pressure (in mV/mmHg)], taking into account differences and variations in blood pressure. The results are presented as CVC, CVC_{max} and CVC_{Tmax} both for ACh and SNP. CVC demonstrates the baseline blood flow values before the drug delivery (ACh and SNP) which indicates that the vascular function or dysfunction is similar among our participants. CVC_{max} is the highest value of blood flow during the test, which usually takes place after the delivery of the 4^{th} dose (highest dose) and demonstrates the vascular reactivity (endothelioum-ACh and smooth muscle cells-SNP). Finally, CVC_{Tmax} is the time from the point of drug delivery (regardless the dose) until the CVC_{max} and is an indication of the reaction time for the vasculature.

5.4.5 Quality of life

The EQ-5D-5L was the main outcome used to assess the patients' quality of life pre- and post-exercise intervention. The EQ-5D-5L is a generic measure of health state by considering five key dimensions of daily living (mobility, self-care, ability to undertake usual activities, pain, anxiety/depression) (Dolan, 1997). Participants were asked to describe their level of health on each dimension using one of five levels: no problems, slight problems, moderate problems, severe problems, extreme problems. EQ-5D-5L was integrated to a broader health-related questionnaire which also measured the physical activity and nutritional status of the patients, the effect of RP on their daily activities and any other existing long-term clinical conditions. Patients were also asked about to rate their life satisfaction on a scale of zero to ten as well as to rate the RP pain during the last couple of weeks on one to five ascending grading: not at all, slightly, moderately, severely, extremely. Digital ulcers and hospitalization for iloprost infusion and amputations were also recorded.

5.4.6 Functional ability test

The functional ability was assessed through a six-minute walking test (6MWT). Although the 6MWT lacks organ specificity in SSc, it can provide a valuable outcome parameter and thus, is suggested as a regular assessment in this clinical condition (Deuschle et al., 2011). Patients were instructed to walk as far as possible back and forth on a 10m corridor for six minutes. They were also instructed to slow down, stop and/or rest as necessary if they got out of breath or became exhausted, but to resume walking as soon as they felt able to. The laps and the total walking distance were recorded on a worksheet.

5.4.7 Peak oxygen uptake test

During the cardiopulmonary tests gas exchange was collected and analysed by an online breath-by-breath analysis system (Ultima^{TM}, Medical Graphics, UK). The gas analyser was calibrated before each test according to the calibration guidelines of the manufacturer. Heart rate (HR) breathing frequency, tidal volume (VT), minute ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$) and volume of exhaled carbon dioxide, and their ratios, as well as respiratory exchange ratio (RER) was displayed on a monitor (BreezeSuite, MGC Diagnostics, USA) on a breath by breath analysis. HR was continuously monitored using a Polar heart rate monitor (Polar FS1, Polar Electro, Kemple, Finland) and blood pressure was assessed by the researcher using a manual sphygmomanometer (DuraShock DS54, Welch Allyn, USA) and a stethoscope (Littman Classic II, 3M, USA). The electrical signs of the heart were continuously recorded (Case, New York, USA) throughout the test producing a 12-lead ECG on a screen monitor (see details in Chapter 3-Theory of Methods). Rating of perceived exertion (RPE) was recorded during the last 10s of every minute during the exercise test until volitional exhaustion using Borg's scale (Borg, 1973) 6-20 point. Peak power output (PPO) and test duration was measured in both tests. $\dot{V}O_{2peak}$ defined as the average oxygen consumption was recorded from expiratory samples during the final 30s of exercise.

5.4.8 Arm crank test

The arm crank ergometer (Lode BV, Groningen, Netherlands) was adjusted to ensure alignment between the ergometer's crankshaft and the centre of the patient's glenohumeral joint. Patients' sitting position was

set up to ensure that the elbows were slightly bent when the arm was outstretched. Patients were instructed to maintain their feet flat on the floor at all times. Due to differences in gender power capabilities two separate protocols were instructed for men and women. Men commenced at a workload of 30 W and women at 20 W. In both protocols the crank rate was maintained at 70 rev min^{-1} (Smith et al., 2007; 2001) and power requirements increased as a linear ramp at a rate of 10 W/min and 6 W/min for men and women, respectively (Smith et al., 2007). The test commenced with 3 minutes resting and then 3 minutes of warm-up (unloaded cranking). RPE ≥ 18 and/or inability to maintain a crank rate above 60 rev min^{-1} resulted in the termination of the test. After the exercise termination an unloaded bout of 2 - 3 minutes exercise at a crank rate below 50 rev min^{-1} followed allowing for an active recovery period.

5.4.9 Cycle ergometer test

The cycle ergometer test was performed on an electromagnetic cycle ergometer (Lode Excalibur, Groningen, Netherlands). The test commenced with a 3-minute resting period followed by 3 minutes of unloaded pedalling. Participants were requested to maintain a cycle rate of 60 rev min^{-1} during the exercise test. The starting load and the concomitant increments were individually calculated according to participants estimated physical fitness and Wasserman's equations (Wasserman, 2012). RPE ≥ 18 and/or inability to maintain a crank rate above 40 to 45 rev min^{-1} resulted in the termination of the test. Following the exercise test 2 - 3 min of unloaded pedalling was performed to allow for an active recovery period.

5.4.10 Transcutaneous oxygen pressure ($TcpO_2$)

$TcpO_2$ measurements were performed during the cardiorespiratory tests using sensors that were non-invasively attached onto the skin and allowed to heat. The sensors induce skin blood capillaries dilatation through heat, which increases the blood flow and results in oxygen diffusion through the skin to the sensor. The sensor measures $TcpO_2$ values inwardly through an electrochemical process.

Measurements were performed using the TINA TCM400 $TcpO_2$ device (Radiometer, Copenhagen, Denmark). The temperature of the probe was set to 44.5 °C to allow maximal skin vasodilation, thereby decreasing the arterial to skin surface oxygen pressure gradient. Before the exercise test 15-20 minutes were allowed with the probe attached on the skin for stabilisation of TcpO2 value. After the test the TcpO2 values were automatically corrected according to a temperature of 37 °C by the TINA device. The electrode was placed slightly below the right scapula on the back away from any bone.

Fixation rings were used to hold the probe attached to the skin and this was filled with two small drops of contact fluid before attachment to the sensor. The fluid was then heated causing the subsequent dilatation of the skin. The raw values of the patient's oxygen perfusion obtained directly from TcpO2 device were defined (Table 8) as previously described in Wasilewski et al., (2016).

5.4.11 Exercise program

Patients undertook twice-weekly supervised exercise sessions at the Centre of Sport and Exercise Science at Sheffield Hallam University. The weekly frequency of the training sessions (twice-weekly) was decided as a pragmatic frequency through the research team's experience in exercise interventions with clinical populations (e.g., Klonizakis et al., 2018). Each session started with a 5-minute warm-up on an arm crank or cycle ergometer depending on the group (involving light aerobic exercise and gentle range of motion exercises). This was followed by HIIT for 30 seconds at 100% of PPO interspersed by 30 seconds passive recovery for a total of 30 minutes. At the end of the session patients undertook a 5-minute cool-down period, involving lower- and upper-limb light intensity aerobic exercise and light stretching. Patients were wearing heart rate monitors throughout the exercise sessions. Heart rate and RPE and affect (see below) were assessed at regular intervals throughout the supervised exercise session.

5.4.12 Exercise tolerance

The exercise tolerance of HIIT was assessed through measures that were interpreted participants' perception regarding the A) exercise intensity, B) the affect, C) the exercise task self-efficacy, D) the intentions and E) the enjoyment. The above data was collected at the first and last exercise session each

month in order to examine several time points during the exercise intervention. Specifically, the questionnaires were repeated at the 1st, 8th, 16th, and 24th exercise sessions. Moreover, compliance to the exercise intervention was reported as the percentage of the attended sessions out of the total (24 sessions).

Exercise intensity

RPE was measured during the exercise session through a 20-point Borg scale (Borg, 1973) at 2.5%, 8.2%, 42.5%, 48.2%, 92.5% and 98.2% of exercise completed. These time points have been chosen to incorporate both interval and recovery periods during HIIT. The 20-point Borg scale ranges from 6 to 20 with anchors ranging from "No exertion at all" (0) to "Maximal exertion" (20). Except from the time points during exercise, the RPE was measured pre- and post-exercise as well as 10 minutes after the exercise session. Participant's heart rate was also recorded using Polar heart-rate monitors at the same time points as RPE.

Affect

The one-item Feeling scale (Hardy & Rejeski, 1989) was used to measure the general affective valence (e.g., pleasure and displeasure) during the exercise session at the same time points as RPE (Appendix A). Participants were informed at the beginning of the first exercise session with the following instructions "Experiencing alterations in your mood is very common while performing exercise. The sense of pleasure or displeasure varies among individuals during the exercise; in addition, feelings may fluctuate across time. So, the answers might be feel good and bad a number of times during exercise, when you will be asked to express your feelings using the scale below". The feeling scale is scored on an 11-point bipolar scale ranging from -5 to +5. Seven anchors are provided ranging from, "Very Good" (+5) to "Very Bad" (-5).

Exercise Task Self-Efficacy

Participant's confidence in their ability to repeat the exercise session that they had just completed was assessed only after the first exercise session at 20-minutes post-exercise using a 3-item measure (Appendix B). Each question included the same introductory theme, "How confident are you that you can…". The 3-items were: 1) "perform one bout of exercise a week for the next 4 weeks that is just like the one you completed today?" 2) "Perform two bouts of exercise a week for the next 4 weeks that is just like the one you completed today?" 3) "Perform three bouts of exercise a week for the next 4 weeks that is just like the one you completed today?". The scale score varied from 0% (Not at all) to 100% (Extremely confident) in 10% increments. The specificity of the three items measure was adapted from Jung et al. (2014) who followed the recommendations of Bandura, (1997).

Intentions

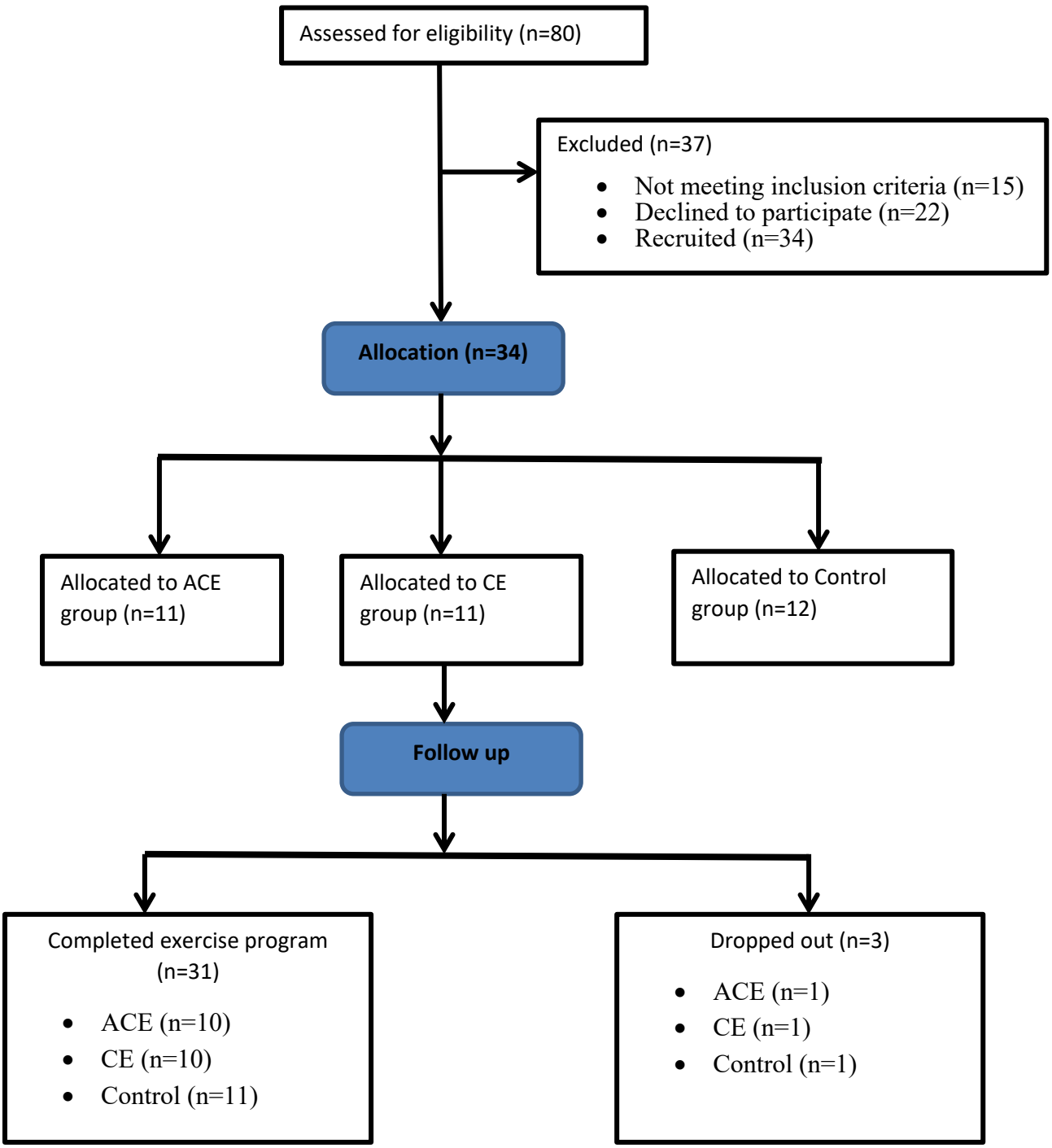
Participant's intentions to engage in the exercise session over the next month were measured utilising a 2-item measure (Appendix C) at the 1st, 9th and 17th exercise session, 20-minutes post exercise (Jung et al., 2014). Particularly, participants were asked "Please rate the extent to which you agree with the following statements 1) "I intend to engage in the type of exercise I performed today at least 2 times per week during the next month" and 2) "I intend to engage in the type of exercise I performed today at least 3 times per week during the next month". Answers were scored on a 7-point rating scale with anchors ranging from "Very unlikely" (1) to "Very likely" (7). The two items were analysed individually.

Enjoyment

Participant's enjoyment for the assessed exercise sessions was examined using a modified version of the physical activity enjoyment scale (PACES; Kendzierski & DeCarlo, 1991) 20-minutes post-exercise. This 18-item measure was scored on a 7-point bipolar scale (Appendix D). Example items are "I find it energizing/I find it tiring" and "it's very pleasant/it's very unpleasant". The original measure was amended by erasing one of the 18 items that is irrelevant due to the time point that was measured ("I am absorbed in the activity-I am not at all absorbed in the activity"). Moreover, the original PACES instructions were

amended from "Please rate how you feel AT THE MOMENT about the physical activity you have been doing" to " Please rate how you feel about the exercise you just completed". Both modifications were made to reflect the correct time point that the questions/questionnaire was administered (e.g., 20-minutes post-exercise).

5.4 Figure 6 CONSORT flow diagram



5.4 Table 8 Definitions of TcpO2 quantities

TcpO2 quantity	Definition
Baseline	The arithmetic means of maximum TcpO2 at rest.
$TcpO2_{max}$	The highest TcpO2 value recorded every minute of exercise or at rest.
Maximum change from baseline ($\Delta TcpO2_{max}$)	The outcome of the subtraction of baseline from $TcpO2_{max}$: e.g. $TcpO2_{max}$ - baseline
Changes in transcutaneous oxygen pressure (ΔTcpO2)	The average sum of the change from baseline at rest and exercise period: e.g. (Σ) $\Delta Y1 \ldots n) / n) =$ ΔTcpO2

5.4.13 Statistical analysis

Data analysis was performed using SPSS software (version 23, IBM SPSS, New York, USA) and is presented as mean ± SD. Normal distribution of the data and homogeneity of variances were tested using the Shapiro-Wilk and Levene's test, respectively. The comparison in the anthropometric, physiological and vascular characteristics among the three groups was done through a one-way ANOVA test. Independent t-tests were also used to identify the differences between two groups. Effect sizes (Cohen's d) were calculated wherever the results were statistically significant with 0.2, 0.5, and 0.8 representing small, medium, and large effects respectively (Mullineaux et al., 2001). To compare the between group differences using a one-way ANOVA we adjusted the ACE values according to the physiological and anthropometrical responses of CE (Mitropoulos et al., 2017). Correlation coefficient tests were performed to identify a potential relationship between vascular reactivity values (CVC, CVC_{max} and CVC_{Tmax}) and other variables. Statistical significance was set at $p \leq 0.05$.

5.5 Results

5.5.1 Compliance and exercise intensity

Compliance to the 12-week exercise program twice weekly was 92% and 88% for the ACE and CE group respectively, with one drop out for each exercise group. No exercise-related complications were reported. The average % peak heart rate ($\%HR_{peak}$) for each exercise session was 92.1% ± 6.0 for the ACE group and 90.8% ± 7.5 for the CE group. The average rate of perceived exertion (RPE) and affect were 13 ± 1 and + 3 (Good) ± 1, respectively, for both exercise groups.

5.5.2 Oxygen uptake and pressure

ACE $\dot{V}O_{2peak}$ (21.9 ± 7.1 ml kg^{-1} min^{-1} d=1.09) improved significantly in comparison to control but not compared to CE group (Table 9).

A tendency to improve was also observed in both ΔTcpO2 (p = 0.59, d= 0.93) and $\Delta TcpO2_{max}$ (p = 0.71, d= 0.80) in ACE group. Although this improvement is not statistically significant the Cohen's d reveals that the effect size of the change is large (> 0.8) both at rest and during provocation (exercise test).

5.5.3 Cutaneous vascular conductance (CVC)

No statistically significant differences were observed at baseline between the exercise and control groups (p >0.05). Post-exercise intervention improvements were observed in the ACE group, especially over the control group, while values in CE group were slightly decreased (Table 9).

5.5 Table 9 Vascular function, oxygen uptake and pressure results

	ACE (n=10)		CE (n=10)		Control (n=11)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
ACh CVC	0.14 ± 0.06	0.19 ± 0.08	0.20 ± 0.11	0.26 ± 0.1	0.20 ± 0.08	0.15 ± 0.08
ACh CVC_{max}	1.28 ± 0.78	1.56 ± 0.88*	1.49 ± 0.99	1.26 ± 0.52	1.40 ± 0.78	0.82 ± 0.47
ACh T_{max} (sec)	159.4 ± 83	104.1 ± 71.8	172 ±57.9	119.4 ± 82.9	127.9 ± 51.1	149.9 ± 70.3
SNP CVC	0.15 ± 0.08	0.24 ± 0.14	0.21 ± 0.11	0.25 ± 0.08	0.20 ± 0.09	0.20 ± 0.1
SNP CVC_{max}	1.73 ± 2.01	1.88 ± 1.52	1.61 ± 1.21	2.38 ± 1.8	1.70 ± 1.3	1.40 ± 0.56
SNP T_{max} (sec)	161.2 ± 88.5	131.3 ± 77.5	167.4 ± 66.3	138.8 ± 80.5	165.5 ± 56.5	166.9 ± 76.4
ΔTcpO2	6.5 ± 4.0*	9.2 ± 12.1	1.56 ± 4.8	1.56 ± 9.5	1.39 ± 3.4	0.89 ± 2.6
$\Delta TcpO2_{max}$	11.5 ± 3.9	18.4 ± 16.5	11.7 ± 3.6	13.6 ± 9.6	9.44 ± 7.7	8.0 ± 7.0
$\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1})	17.7 ± 4.7	21.9 ± 7.1*	14.6 ± 2.9	18.5 ± 2.8*	14.3 ± 6.9	14.7 ± 6.2

Endothelial function presented as cutaneous vascular conductance (CVC). T_{max} is the time taken to reach peak perfusion. *$p < 0.05$ compared to the other groups.

5.5 Table 10 Endothelial-dependent correlations in arm cranking

		Soft lean mass (kg)	Fat free mass (kg)	Skeletal muscle mass (kg)	$\dot{V}O_{2peak}$ (L min^{-1})	$\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1})
ACh	Pearson's r	0.529	0.520	0.530	0.120	0.220
CVC_{max}	Sig. (2-tailed)	0.116	0.123	0.115	0.740	0.569
	n=10	10	10	10	10	10

5.5.4 Feasibility and tolerance of exercise

ACE showed to be the mode of exercise that will more likely ($p<0.05$) engage people with SSc to physical activity twice per week (6.9 ± 0.3, d= 1.17) compared to the CE group (6.2 ± 0.79). Moreover, ACE demonstrated to be better ($p<0.05$) regarding participant's confidence to perform two bouts per week (95 ± 7%, d= 0.82) than CE (83 ± 19.5%) but not statistically significant. Both exercise modes aggregated a high score of enjoyment levels >94 out of 119 with an average affect before, during and after the exercise session of + 3 equals to "Good" (Table 11).

5.5 Table 11 Feasibility of exercise

	ACE (n=10)	CE (n=10)
Confidence one bout	95 ± 8.5	90 ± 18.9
Confidence two bouts	95 ± 7.1*	83 ± 19.5
Confidence three bouts	80 ± 19.4	72 ± 23.9
PA enjoyment scale	100.6 ± 12.9	94.8 ± 14.1
Intentions two bouts	6.9 ± 0.3*	6.2 ± 0.8
Intentions three bouts	5.9 ± 1.8	5.6 ± 1.1
$\%HR_{max}$ average	92.1 ± 5.9	90.8 ± 7.5
RPE average	13.0 ± 0.8	12.6 ± 0.7
Affect average	2.9 ± 0.9	3.1 ± 1.4

*$p < 0.05$.

5.5.5 Quality of life and clinical outcomes

The EQ-5D-5L questionnaire did not demonstrate any significant difference between the groups neither at baseline nor after the completion of the exercise intervention, in any of its 5 elements. However, both exercise groups reported improved life satisfaction ($p<0.000$) as well as reduced discomfort and pain of Raynaud's phenomenon ($p<0.05$) after the exercise intervention compared to the control group (Table

12). To evaluate the EQ-5D-5L outcome in overall pre- and post-exercise intervention we calculated (Table 13) the quality-adjusted life years (QUALY; a metric used in cost-utility analysis that combines survival and health related QoL; Devlin et al., 2017). We also recorded digital ulcers and hospitalization for iloprost infusion for four out of eleven patients (36.3%) in the control group. One of them proceeded to amputation of the distal phalange of the middle finger in one hand.

5.5 Table 12 Quality of life

	ACE (n=10)		**CE (n=10)**		**Control (n=11)**	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Life satisfaction	6.5 ± 1.6	8.1 ± 1.7***	8.4 ± 1.4*	8.8 ± 1.1***	7.5 ± 1.6	4.9 ± 1.5
Mobility	2.4 ± 1.0	2.3 ± 0.8	1.9 ± 0.9	1.7 ± 1.0	1.9 ± 0.9	2.3 ± 1.2
Self-care	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.4	1.0 ± 0.0	1.4 ± 0.9	1.7 ± 1.4
Usual activity	2.3 ± 1.3	1.9 ± 1.1	1.9 ± 1.0	1.6 ± 0.7	1.8 ± 1.0	2.4 ± 1.2
Pain/ discomfort	2.4 ± 1.0	2.3 ± 1.1	2.8 ± 1.1	1.8 ± 0.9	2.4 ± 0.7	2.8 ± 1.2
Anxiety/ depression	1.7 ± 0.8	1.5 ± 0.7	1.6 ± 0.7	1.2 ± 0.4	1.6 ± 0.7	1.9 ± 1.4
Raynaud's pain	2.4 ± 1.4	1.8 ± 0.6*	2.6 ± 1.5	1.9 ± 1.2*	2.4 ± 0.9	3.1 ± 1.1

*$p < 0.05$ and ***$p < 0.000$ compared to the other groups.

5.5 Table 13 Health related quality of life calculation

	ACE (n=10)		CE (n=10)		Control (n=10)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
QUALY	0.77 ± 0.15	0.81 ± 0.16	0.78 ± 0.16	0.86 ± 0.16	0.82 ± 0.14	0.69 ± 0.33

5.6 Discussion

This study is the first to demonstrate that upper-limb, aerobic exercise may improve microvascular endothelial-dependent function in the digital area in patients with systemic sclerosis experiencing RP. Similarly, cycling showed that it has the potential to decelerate the disease progression in the vasculature (ACh) whereas patients in the control group showed a disease worsening (Table 9). An assessment using Pearson's correlation coefficient (Table 10) indicated that ACE-induced, endothelial improvement has a correlating trend with the soft lean and fat-free mass as well as with skeletal muscle mass. Interestingly, ACh (baseline, max and Tmax) was not correlated with ACE $\dot{V}O_{2peak}$ which is in contrast to previous findings that have shown association of endothelial-dependent function with the improvement in aerobic capacity in patients with rheumatoid arthritis (Metsios et al., 2014). However, the two studies differ in clinical populations (SSc vs. rheumatoid arthritis), exercise protocols (aerobic vs. combined exercise) as well as site of microvascular measurement (digital area vs. brachial artery) which might explain the different results. The correlation between the endothelial-dependent function and the lean muscle might be a vital evidence for future exercise prescription for this population. Resistance training is capable to increase muscle mass and to improve microcirculation in people with obesity (Dias et al., 2015) Thus a combination of the current HIIT protocol with resistance training might increase the chances for further improvement in the endothelial function.

HIIT has been demonstrated to enhance the antioxidant status indicating decreased oxidative stress and increased NO bioavailability in patients with cardiometabolic disorders (Molmen-Hansen et al., 2012; Tjonna et al., 2008; Wisloff et al., 2007). Increased oxidative stress adversely affect NO bioavailability.

A rapid inactivation of NO into peroxynitrate is the result of the increased presence of ROS, which could in turn aggravate vascular oxidative stress by stimulating the 'uncoupling' of eNOS (Ramos et al., 2015). Uncoupling of eNOS changes its normal function from a NO generator to a superoxide anion-generating enzyme, which further aggravates vascular dysfunction (Forstermann, 2010). The enhanced flow of electrons via the electron transport chain in the mitochondria is considered to be a critical contributor to increased ROS production (i.e. superoxide anions and hydrogen peroxide) (Korshunov et al., 1997). Conversely, the overexpression of PGC-1a in endothelial cells has been demonstrated to neutralise an increased presence of ROS (Valle et al., 2005). HIIT has been reported to increase PGC-1a abundance in the vastus lateralis of patients with cardiometabolic disorders (Schjerve et al., 2008; Tjonna et al., 2008; Wisloff et al., 2007). This comes in agreement with studies utilising an essentially smaller HIIT programme (10 x 1 min HIIT at ~ 80% HR reserve, 1 min recovery, three times per week for 2 weeks) in sedentary (Hood et al., 2011) and T2DM patients (Little et al., 2011), indicating a critical increase in PGC-1a by 56% following the training intervention. Assuming that the mechanism of up-regulating the expression of PGC-1a responds in a similar manner after HIIT regardless of the tissue involved, the increased vascular function could partly be explained by a reduction in ROS and thus enhanced NO availability.

5.6.1 Clinical outcome

Inadequate blood flow to living tissue is often a painful experience, threatening the life of the tissue involved. Digital tissue loss not only results in disfigurement and functional disability, it is also the clinical manifestation of an underlying systemic disease process (Mcmahan & Wigley, 2010). One of the direct consequences of digital ischemia are the persistent digital ulcers developing irreversible tissue loss in 30% of patients (Ingraham, 2006). In our study 4 out of 11 patients in the control group developed digital ulcers and required hospitalization for iloprost infusion (Pope, 2007; Pope et al., 2000) for a period of 1 to 3 weeks and one patient proceeded to digital amputation of the distal phalange in the middle finger in one hand. Hospitalization is a psychologically stressful procedure for the patient which directly affects

quality of life. The most common side effects of iloprost infusion could be headache, flushing of the skin, nausea, vomiting and sweating. Amputation has been reported to occur in one or more digits due to ischemia in 20.4% of patients with SSc, 9.2% of which have multiple digit loss (Wigley et al., 1992). Quality of life in patients with SSc is adversely affected due to digital ischemia. Consequently, our protocol has demonstrated that is capable of improving digital ischemia and potentially preventing disease progression and digital ulcers and thus, improving quality of life.

5.6.2 Transcutaneous oxygen pressure

In an attempt to depict a complete picture of the HIIT effects on microvasculature we assessed TcpO2 changes during an incremental exercise test. It is known that patients with SSc due to vasculopathy are at a high risk of developing interstitial lung disease, cardiac fibrosis, and pulmonary arterial hypertension (Matucci-Cerinic et al., 2013; Steen, 2008). Vascular involvement is the initial event and the leading cause for further disease-related comorbidities in SSc. Although the improvement in oxygen pressure at rest and under provocation (exercise test) was not significant in our study, the effect size of this change was large. This indicates that ACE is able to induce systemic changes in oxygen pressure and vascular function in people with SSc, while the control group showed a slight decrease. It is probable that a higher training load or a larger cohort would have revealed a statistically significant difference between ACE and control group. Our TcpO2 protocol (site of measurement, exercise intervention, ramp incremental exercise protocol, arm crank and cycle ergometer) is not similar to other studies and therefore, our results could not be linked and /or compared to other studies. Recently, Abraham et al., (2018) reviewed the current methodology for a validated use of TcpO2 during exercise and highlighted the most important gaps in knowledge about exercise TcpO2. It should be emphasised that the use of TcpO2 during exercise (baseline and post exercise intervention) was performed for evaluating the exercise intervention and not to accurately depict the use of TcpO2 (see Limitations). Evidently, further research is needed to substantiate our findings and explore other training protocols which will reveal the effects of exercise on skin oxygen pressure, when oxygen demand is higher.

5.6.3 Quality of life

Both modes of exercise have shown improvement in life satisfaction and reduction in pain or discomfort induced by RP attacks after the exercise training. However, further research is required to confirm the improvement in RP by applying more qualitative measures (e.g. case-specific questionnaires and face to face interviews). Exercise tolerance, cardiorespiratory fitness, walking distance, muscle strength and function as well as health related QoL have been demonstrated to be improved in people with SSc after participation in exercise programs involving aerobic exercise and aerobic exercise combined with resistance training (De Oliveira et al., 2017). People with SSc encountering progressive and limiting impairments such as skin deformities and disfigurement, pain, chronic fatigue and dyspnoea which are elements strongly related to a poor health-related QoL (Almeida et al., 2015). Patients with SSc are likely to have joint involvement (Avouac et al., 2010) and develop enthesopathy (Kilic et al., 2015). Proximal muscle weakness in the shoulder and hip-pelvic region (Akesson et al., 2003) as well as a decreased strength in quadriceps (Lima et al., 2015) are common hallmarks of SSc. Aerobic capacity has also been found to be decreased compared to healthy individuals (Pettersson et al., 2017; Rosato et al., 2014) which is probably attributed to physical inactivity observed in patients with limited cutaneous SSc with pulmonary arterial hypertension (PAH) compared with patients without PAH (Mainguy et al., 2011). Muscle weakness (Turesson et al., 2007) and limited oxygen transport and consumption (Blom-Bülow et al., 1983) are developed due to physical inactivity and may contribute to a poor QoL (Lima et al., 2015; Hudson et al., 2009). Therefore, promoting physical activity for the improvement of QoL in people with SSc should be deemed as one of the priorities for the future research.

5.6.4 Feasibility of HIIT in people with limited cutaneous SSc

Our findings demonstrate that HIIT (30s 100% PPO/ 30s passive recovery) maintained an average affect of +3 ("Good") throughout the exercise training for both modes of exercise. This result indicates that the exercise protocols did not affect the mood and /or enjoyment levels of the participants. It is also noteworthy that the patient's affect was similar before, during and after the exercise session which could

be explained by the moderate cardiorespiratory stress induced by this protocol. Supportive to this finding is the RPE for both groups which averaged to 13 ("Somewhat hard"), a value which is strongly correlated to anaerobic threshold and low to moderate exercise intensity in a large cohort of adults (Scherr et al., 2013). Exercise intensity and affective response have presented a negative relationship in inactive and overweight adults and it has been reported that as incremental exercise progresses above the ventilatory threshold, the affective response to exercise becomes more negative (Parfitt & Hughes, 2009; Blanchard et al., 2001). Therefore, a short protocol of HIIT seems to not induce great cardiovascular responses in patients with SSc and that might explain the affect's stability throughout the session.

In several studies in which the intensity of exercise was gradually increased to levels that approached the participants' physical limits, valence (Term in psychology: Means the intrinsic attractiveness/ 'good'-ness, positive valence) ratings showed a progressive decline with each increase in intensity (Parfitt et al., 1996; Parfitt & Eston, 1995; Acevedo et al., 1994). Furthermore, as exercise intensity increased, the negative correlations between valence and various indices of metabolic strain (e.g., heart rate, ventilation, respiratory rate, oxygen consumption, blood lactate) increased in magnitude (Acevedo et al., 1994; Hardy & Rejeski, 1989), suggesting an increasingly stronger link between valence and interoceptive afferents.

The negative shift in affective valence is specifically linked to an important metabolic event, namely the transition from aerobic to anaerobic metabolism (Ekkekakis, 2003). Analyses of the metabolic responses across the entire range of exercise intensity reveal three distinct stages of intensity (Gaesser & Poole, 1996). The first stage includes aerobic metabolism, and the second entails the lactate threshold through anaerobic metabolism. However, if the rate of accumulation is not too rapid, over time there may be a new stabilisation of lactate, but this time at increased levels of concentration. The intensity that corresponds to this ``maximal lactate steady-state'' is the upper limit of this domain. The final stage of intensity extends from the higher level at which lactate can be stabilised to the point of maximal exercise capacity. In this range, neither oxygen uptake nor lactate can be stabilised. Both rise continuously until exercise is terminated due to exhaustion.

The adaptational implications in these three different physiological stages are fairly clear. In the low range of intensity, activity can be continued for a long time while in a physiological steady-state. This situation poses no threat to homeostasis and the physiological adjustments that occur remain largely outside awareness. Then, there is a range of intensity in which the maintenance of a steady-state is threatened. In this range, the amount and intensity of interoceptive information increases exponentially, as the accumulating lactate stimulates free nerve endings, respiration becomes quicker and deeper, and additional (nonoxidative) muscle fibres are recruited disrupting coordination patterns. Since this situation presents a potential challenge, good adaptational sense dictates that the possibility of a critical homeostatic perturbation should enter consciousness. As several investigators have noted, affect provides the primary means by which information about critical disruptions of homeostasis enters consciousness (Panksepp, 1998; Schulze, 1995). Finally, above the maximal lactate steady-state, the energy supply system is overwhelmed, and the maintenance of a steady-state is impossible. At this point, if the intensity of the activity is not reduced or the activity is not stopped, the available energy stores will soon be depleted, and the muscles will go into rigor. What prevents this from happening is a strong and unambiguously negative affective ``message'' from the body (Ekkekakis, 2003).

The engagement intentions to exercise and the task self-efficacy questionnaires as well as the enjoyment levels of the patients could further substantiate whether HIIT is a feasible mode of exercise in people with SSc. Both modes of exercise demonstrated a strong patient's confidence to perform two and three bouts of exercise with arm cranking being slightly higher than cycling. Both modes of exercise were enjoyable for the patients, however, arm cranking was found to be significantly higher in the intentions for engagement in two bouts of exercise per week compared to cycling. HIIT is a feasible protocol to be implemented in patients with SSc and ACE is considered more acceptable than CE potentially because it is a new mode of exercise for this population and that might increase their interest to perform an alternative type of exercise.

Compliance to the 12-week exercise programme twice weekly was 92% and 88% for the ACE and CE group respectively, with one drop out for each exercise group. Compliance rates have been reported in adults with mild-moderate asthma to be at 80% after a 12-week walking programme (three times per week) exercising at 60-75% HR_{max} (Boyd et al., 2012). Moreover, a study that examined the feasibility of exercise in people with leg ulcers performed a 12-week exercise programme (three times per week) and reported a compliance rate of 79% (Klonizakis et al., 2018). In another study, older adults (≥ 65yrs) performed a 6-week aerobic training (two days per week) with a compliance rate of 83.3% (Falck et al., 2017). All of the studies performed their exercise programme in a single facility and none of them reported the exact available hours of training.

The high compliance rates for both groups (ACE and CE) for our study compared to other studies could be explained through four main factors. A) The HIIT has been reported as a more enjoyable exercise protocol to continuous moderate intensity (Bartlett et al., 2011). B) We assume that the training frequency of twice per week might have improved the compliance rates in our study. C) People with SSc have been reported (interviews) that some barriers to exercise participation are the access (transport) to the sport venue and the travelling time (see Chapter 6, Theme 3). Our study offered one-to-one exercise sessions in three different sport venues in Sheffield (Graves, Concord and Sheffield Hallam sport centres) selected to cover two opposite sites in the outskirts of Sheffield as well as the city centre reducing significantly the travelling time for our participants. Moreover, the training sessions were available six days per week (Monday-Saturday) from 8am to 8pm. D) Finally, the trainer (Mr. Alexandros Mitropoulos) who supervised the exercise sessions has an extensive working experience as a personal trainer which might have increased the individual's motivation. He was also considered as an expert by our participants (see Chapter 6, Theme 2) which increased their motivation during the exercise session and potentially the enjoyment levels and concomitantly the compliance to the exercise programme.

5.7 Limitations

The ratio between women and men is uneven, however, SSc women to men ratio is estimated to 5.2:1 in northeast England (Allcock et al., 2004).

TcpO2 is a direct value of vascular function as changes at rest mimic the changes in arterial pO_2 during mild or moderate exercise (Planès et al., 2001; Brudin et al., 1994). However, the time response of these changes is relatively slow (90% time response of TcpO2 being ~ 20s). Carter and Banham (Carter & Banham, 2000) demonstrated that TcpO2 values closely followed those assessed by direct arterial sampling during cardiopulmonary exercise testing with 2 mins interval. We acknowledge that our protocol utilized 1 min intervals until symptomatic limitation of exercise which might affect the accuracy of TcpO2, however, we need to stress that the utilization of TcpO2 measurement in our study was more of a research interest aiming to evaluate the improvement in vascular function after an exercise program rather than accurately depicting hypoxemia levels in the arterial wall for this clinical population.

5.8 Conclusions

Aerobic exercise in general, and HIIT (30s 100% PPO/ 30s passive recovery) specifically, involving the upper limbs may improve the microvascular reactivity through an enhancement of the endothelial-dependent function. Our study did not assess resistance training, however, the improvement in microvascular function correlated well with the lean muscle mass, which might indicate that resistance training could potentially be a complementary training element in inducing further improvements in microcirculation. Moreover, our protocol appears to reduce digital ischemia risk, which can be the leading cause for further systemic complications and a major factor affecting the quality of life. Exercise is a non-invasive, adjunct treatment with no adverse effects that is well-tolerable by patients with SSc. There is a need for a study to assess the feasibility of ACE in people with SSc. This will then allow us to proceed with a large multi-centre, randomised-controlled study to further establish the effectiveness and cost-effectiveness of exercise on people with SSc.

The main impact of the current findings is that our HIIT protocol performed on an ACE it seems to be effective (prevention of DUs, improvement of microvascular function) and feasible (compliance rates, enjoyment levels, affect, RPE, no adverse events) in people with SSc and thus able to improve QoL in this population. If our results, could be established through further research and larger cohorts, the specific protocol could be included as an adjunct therapy to medical treatment in the NHS guidelines.

Chapter 6: The feasibility of a combined exercise protocol including aerobic and resistance training in people with limited cutaneous systemic sclerosis: a randomised controlled feasibility trial

6.1 Chapter overview: Chapter five examined the effects of two different modes of exercise (arm cranking and cycling) on the vascular reactivity in people with systemic sclerosis. The findings support the original assumption that was made prior the study that upper limb exercise would induce greater results in the microcirculation compared to lower limb exercise and as a result to control group.

Chapter six will investigate the feasibility of a combined exercise protocol in people with systemic sclerosis. More specifically, the recruitment, attrition and adherence rates will be recorded, and individual's experiences will be explored through interviews. Enjoyment levels, task self-efficacy and intentions to engage to exercise will also be assessed and reported to support a well-rounded examination of the feasibility of exercise.

6.2 Abstract

Background: Vasculopathy is the main clinical feature presented in systemic sclerosis (SSc) resulting in further clinical manifestations and a concomitant reduction in quality of life (QoL) in people with SSc. Exercise has shown to be able to improve the vascular function in people with SSc. Therefore, the purpose of this study is to examine the feasibility of a combined exercise protocol (aerobic and resistance training) in people with SSc.

Methods: Thirty-two limited cutaneous People with SSc (66.5 ± 12 years old) were randomly allocated in two groups (exercise and control group). The exercise group underwent a 12- week exercise program twice per week. All patients performed the baseline, three- and six-month follow up measurements where physical fitness, functional ability, transcutaneous oxygen tension (ΔTcpO2), body composition and QoL were assessed. Participants' experiences were assessed through interviews.

Results: Compliance to the twice weekly, twelve-week exercise program was 92.6% with no drop outs. The individuals' confidence to participate in the study's exercise protocol for once and twice per week was 95% and 80% for three times per week out of 100%. No exercise-related complications were reported. The average value for the physical activity enjoyment scale was 103 ± 10 out of 119 (highest score). The mean values for the intention to engage in exercise twice and thrice per week were 6.4 ± 1 (likely) and 5.3 ± 2 (slightly likely) out of 7 (very likely). Regarding QoL the exercise group showed to have a better life satisfaction (9.25 ± 0.9), less difficulty to perform the usual activities (1.63 ± 0.7), less anxiety (1.06 ± 0.3), and less Raynaud's phenomenon-accompanied pain (2.19 ± 1).

Conclusion: Our results suggest that a combined exercise (HIIT and RT) protocol was feasible for people with SSc, resulting in high adherence and low attrition rates, high enjoyment levels and intentions for future engagement to this type of exercise. People with SSc feel comfortable and capable of performing our protocol with no adverse events in this population. The specific protocol is defined as a safe adjunct therapy for people with SSc.

6.3 Introduction

Vascular involvement is a direct implication in the pathogenesis of systemic sclerosis (SSc), representing an acute manifestation in this clinical condition. Vasculopathy is targeted the blood vessels and skin involvement distinguishing the disease into two clinical forms (Koch & Distler, 2007). People with limited SSc (lcSSc) display a skin involvement that is limited to the face, neck, and areas distal to elbows and knees. However, in people with diffuse SSc (dcSSc) the skin involvement expands proximally to involve upper arms, thighs and/or trunk.

Raynaud's phenomenon (RP) is one of the first clinical manifestations observed in SSc. This microvasculature disorder affects mostly the fingers and toes but can also affect other extremities. Over 95% of people with SSc present evidence of RP that can commence many years before any other clinical symptoms of SSc. Evidence suggests that RP is triggered by endothelial injuries in association with dysregulations in the production of nitric oxide and vasoactive factors (Kahaleh, 2004). RP can lead to the formation of digital ulcers that is also one of the earliest complications of the disease. Healing of digital ulcers is often difficult, and the most threatening complication is the loss of digits that is secondary to infections. RP and digital ulcers have an important effect on the quality of life (QoL) of the patients.

Pharmacological agents (e.g., nifedipine) are commonly used as first-line approach. Nevertheless, their efficacy is debatable and the short-term (e.g., oedema, headaches, heart palpitations, dizziness and constipation) and long-term (e.g., heart dysfunction, increased cardiovascular risk) side effects of the medical treatment should also be considered as well as the financial cost of treatment. Therefore, adjunct therapies such as physical activity, with less side effects and cost implications should be explored.

A recent study from our research team (Mitropoulos et al., 2018), revealed that exercise and more specifically high intensity interval training (HIIT) is able to improve the endothelial-dependent microvascular function is people with SSc. The study explored the effects of a HIIT protocol (30s 100 peak power output (PPO)/ 30s passive recovery) using two different modes of exercise, arm-cranking and cycling, on the digital microvascular function in SS patients. The results showed that the HIIT upper-limb exercise induced significant improvements in the endothelial-dependent function in the digital area compared to lower-limb exercise and/or physical inactivity (control group).

Resistance training (RT) alone has shown significant improvements in the function of the vasculature (Dias et al., 2015); moreover, a combination of aerobic and RT have shown both in the past (Maiorana et al., 2000) and recently (Metsios et al., 2014) to significantly improve the vascular function and the microcirculation. However, the limited number of studies that have investigated the effects of RT on

vasculature indicates a lack of robust evidence. Moreover, to our knowledge the feasibility of combined exercise (RT and HIIT) on digital microcirculation has yet to be examined in people with SSc.

In an era of limited financial and human resources, such a study will be important prior to the assessment of the clinical- and cost-effectiveness of a combined intervention in a large cohort. At the same it would be important to explore the mechanisms behind the exercise-induced changes, as an understanding of how these happen could lead to a better understanding of the pathophysiology of the condition.

Therefore, the first part of this manuscript aimed to investigate the feasibility of exercise to be performed by people with SSc using an established upper-limb HIIT protocol (arm cranking) and RT. This was assessed through adherence, compliance and attrition rates, exploration of enjoyment levels, assessment of exercise tolerance, number of adverse events and exploration of individual experiences. We also reported on rates of screening, eligibility, and recruitment. The second part of this manuscript reports on the mechanisms behind the observed, exercise-induced microcirculatory changes, using two established, non-invasive techniques: Laser Doppler Fluximetry (LDF) and Trancutaneous Oxygen Pressure ($TcpO_2$).

6.4 Methods

6.4.1 Participants

We recruited thirty-two people (29 women, 3 men) with lcSSc, defined as per the ACR and European league against rheumatism criteria (Hoogen et al., 2013). Eligible patients (Table 14) were recruited from the Rheumatology Department of the Royal Hallamshire Hospital in Sheffield. All patients provided written consent to participate. The regional health research ethics committee for clinical studies approved the protocol. Patients were randomly allocated (block randomisation) between the exercise (n = 16), and control (n = 16) groups. All the pre- and post-intervention tests were performed at the same time of the day to minimize intra-day variability.

6.4 Table 14 Eligibility criteria

Inclusion criteria	Exclusion criteria
Patients diagnosed with Limited Cutaneous Systemic Sclerosis according to the 2013 ACR/EULAR criteria experiencing Raynaud's phenomenon.	Patients with advanced pulmonary arterial hypertension or interstitial lung disease.
Men or women aged <18 years old.	Patients who are diagnosed with another inflammatory condition.
Disease duration between 1 to 10 years.	Patients presenting myositis with proximal muscle weakness.
Patients should be able to perform exercise.	Patients with New York Heart Association class 3 or 4.
	Current smokers or people who stopped smoking within 4 weeks of health screening.
	Women who are currently pregnant.

ACR, American College of Rheumatology; EULAR, European League Against Rheumatism

6.4.2 Exercise program

Participants undertook twice-weekly supervised exercise sessions at the Centre of Sport and Exercise Science at Sheffield Hallam University, Graves and Concord sport centres in Sheffield. The HIIT protocol has been presented previously (Mitropoulos et al., 2018). With respect to the RT, patients performed five upper body exercises in a circuit row for three circles interspersed by 2-3 minutes. In between the exercises 10s to 20s were allowed for a safe movement from one exercise to the other. The intensity was kept to 10 maximum repetitions and weights adjustments were done to compensate for any strength improvements during the exercise intervention. The intensity was monitored using Borg's scale

(Borg, 1973)-20 point. The 10 repetitions maximum limit for each exercise was assessed at the first exercise session. The five RT exercises were as follows 1) chest press with dumbbells on a 30° inclined bench, 2) arms lateral raise with dumbbells in a seated position, 3) biceps curl with dumbbells, 4) triceps extension on the pulley from a standing position and, 5) handgrip dynamometer.

6.4.3 Procedures

Baseline assessments undertaken at first visit included peak oxygen uptake ($\dot{V}O_{2peak}$), anthropometry, functional ability, microvascular reactivity and quality of life. $\dot{V}O_{2peak}$ test was performed on an arm crank ergometer for both groups. Thereafter, patients were randomly allocated to two groups (exercise and control group). The exercise group (HIIT and RT) performed a 12-week exercise programme and the control group did not perform any type of physical activity. Both groups were followed up after a 12-week (3 months) and 24-week (6 months) period performing the same measurements as conducted at baseline.

6.4.4 Feasibility and acceptability outcomes

Recruitment rates were measured as rate of invited participants who were eligible and consenting. Acceptability of allocation was assessed by assessing the attrition rates and comparing the two groups and examining reasons for dropout. Suitability of measurement procedures was assessed by outcome completion rates and reasons for missing data. Attrition rate was defined as discontinuation of intervention and loss to follow-up measurement for all conditions. The acceptability of the exercise programme was evaluated by using session attendance and compliance data and participants' feedback via one -to-one semi-structured interviews conducted with a subgroup of participants after the 3-month follow-up visit. Moreover, we assessed, as measures for the acceptability of exercise, the participants' enjoyment levels, intentions of engagement to exercise and task-self efficacy after the end of the exercise session at several time points during the exercise intervention. We also monitored the rate of perceived exertion and affect throughout each exercise session so as to document important information about the acceptability of exercise. The safety of exercise was also assessed by exploring reasons for dropout from

the exercise programme and the number and type of adverse events that occurred during the exercise intervention.

6.4.5 Quality of life

The EQ-5D-5L was the main outcome used to assess the patients' quality of life pre- and post-exercise intervention. The EQ-5D-5L is a generic measure of health state by considering five key dimensions of daily living (mobility, self-care, ability to undertake usual activities, pain, anxiety/depression) (Dolan 1997). Participants were asked to describe their level of health on each dimension using one of five levels: no problems, slight problems, moderate problems, severe problems, extreme problems. Patients were also asked to rate their life satisfaction on a scale of zero to ten as well as to rate the RP pain during the last couple of weeks on a one to five ascending grading: not at all, slightly, moderately, severely, extremely. Digital ulcers and hospitalization for iloprost infusion and amputations were also recorded.

6.4.6 Functional ability test

The functional ability was assessed through a six-minute walking test (6MWT). Although the 6MWT lacks organ specificity in SSc, it can provide a valuable outcome parameter and thus, is suggested as a regular assessment in this clinical condition (Deuschle et al., 2011). Patients were instructed to walk as far as possible back and forth on a 10m corridor for six minutes. They were also instructed to slow down, stop and/or rest as necessary if they got out of breath or became exhausted, but to resume walking as soon as they felt able to. The laps and the total walking distance were recorded on a worksheet.

6.4.7 Exercise tolerance

The exercise tolerance of HIIT was assessed through measures that interpreted participants' perception regarding the exercise intensity (Borg, 1973), the affect (Appendix A), the exercise task self-efficacy (Appendix B), the intentions (Appendix C) and the enjoyment (Appendix D). The above data was collected at the first and last exercise session each month in order to examine several time points during the exercise intervention. Specifically, the questionnaires were repeated at the 1st, 8th, 16th, and 24th

exercise sessions. The individual questionnaires and the time points that were incorporated during the exercise session are described in Jung et al., (2014).

6.4.8 Interviews

Semi-structured interviews were conducted by AM in a purposive sample of 12 patients from exercise (n=6) and control (n=6) group. The interviews were all held at the Sheffield Hallam University. HC and AM developed a semi-structured interview guide, which acted as a trigger and a motivation for further conversation. The interview guide was piloted in interview 1 and only minor changes were subsequently made. The guide is presented in Table 15. Each interview ended with the interviewer asking the patient if they wanted to make any additional comments not explored via the interview guide. The interviews lasted between 15 and 20 min and were digitally recorded. Inter-rater reliability and intercoder agreement were monitored by ML to ensure that our results are reliable.

6.4 Table 15 Interview guide

Focus topic: Patients' experiences of RP	Please, describe your feelings when RP attacks take place, symptoms, thoughts, when and how much do they occur.
Focus topic: Treatment of RP	Advice given by clinicians, how efficient is it, how satisfied are you, side effects from medical treatment.
Focus topic: Exercise intervention-study procedures	Please, describe your experiences regarding the study procedures, exercise intervention, effects upon RP and QoL, motivations from supervised exercise training, lifestyle changes. Your thoughts about exercise training and its potential benefits.

6.4.9 Physiological outcomes

Anthropometry

The participant's stature was measured using a Hite-Rite Precision Mechanical Stadiometer. Body weight (kg), body mass index (BMI), fat mass (kg) and lean body mass (kg) segmented in upper and lower-limbs

were assessed by using bio-electrical impedance analysis (In Body 720, Seoul, Korea). Participants' demographic characteristics are illustrated in Table 16.

Peak oxygen uptake test

During the cardiopulmonary tests gas exchange was collected and analysed by an online breath-by-breath analysis system (UltimaTM, Medical Graphics, UK). HR was continuously monitored using a Polar HR monitor (Polar FS1, Polar Electro, Kemple, Finland) and blood pressure was assessed by the researcher using a manual sphygmomanometer (DuraShock DS54, Welch Allyn, USA) and a stethoscope (Littman Classic II, 3M, USA). Rating of perceived exertion (RPE) was recorded during the last 10s of every minute during the exercise test until volitional exhaustion using Borg's scale (Borg, 1973) 6-20 point. PPO and test duration were also recorded. $\dot{V}O_{2peak}$ defined as the average oxygen consumption was recorded from expiratory samples during the final 30s of exercise.

6.4 Table 16 Demographic data (means ± SD).

	Baseline Exercise (n=16)	Baseline Control (n=16)	Baseline Total (n=32)
Age (years)	69.6 ± 11.4	63.6 ± 12.2	66.5 ± 12
Body weight (kg)	64.7 ± 10.2	72.2 ± 14.2	68.4 ± 12.7
Body mass index (kg/m^2)	24.8 ± 3.1	26.6 ± 4.6	25.7 ± 4
Stature (cm)	161.5 ± 9	164.5 ± 6.1	163 ± 7.7
Disease duration (years)	8 ± 2	8 ± 2	8 ± 2
Digital ulcers (Treatment iloprost infusion)	0/16	4/16	4/32
Raynaud's treatment	9/16	13/16	22/32
Nifedipine	7/9	7/13	14/32
Sildenafil	2/9	6/13	8/32
Blood pressure treatment	8/16	7/16	15/32
Candesartan	3/8	3/7	6/32
Ramipril	5/8	4/7	9/32

Arm crank test

The arm crank ergometer (Lode BV, Groningen, Netherlands) was adjusted to ensure alignment between the ergometer's crankshaft and the centre of the patient's glenohumeral joint. Patients' sitting position was set up to ensure that the elbows were slightly bent when the arm was outstretched. Patients were instructed to maintain their feet flat on the floor at all times. Due to differences in gender power capabilities two separate protocols were instructed for men and women. Men commenced at a workload of 30 W and women at 20 W. In both protocols the crank rate was maintained at 70 rev min^{-1} (Smith et al., 2001; 2007) and power requirements increased as a linear ramp at a rate of 10 W/min and 6 W/min for men and women, respectively (Smith et al., 2007). The test commenced with 3 minutes resting and then 3 minutes of warm-up (unloaded cranking). RPE ≥ 18 and/or inability to maintain a crank rate above 60 rev min^{-1} resulted in the termination of the test. After the exercise termination an unloaded bout of 2 - 3 minutes exercise at a crank rate below 50 rev min^{-1} followed allowing for an active recovery period.

Microvascular reactivity

Microvascular function was assessed by laser Doppler Fluximtery and Iontophoresis technique in a temperature-controlled room (22-24°C). LDF electrodes were attached to the dorsal aspect of the reference fingers for acetylcholine (ACh) and sodium nitroprusside (SNP) administration. These were used as indicators of the changes occurring in the endothelial –dependent and –independent vasodilatory function. HR (Sports Tester, Polar, Finland) and blood pressure of the brachial artery (left arm; Dinamap Dash 2500, GE Healthcare, USA) were monitored at 5-minute intervals throughout the protocol. The two drug delivery electrodes (PF383; Perimed AB, Jarfalla, Sweden) were positioned over healthy looking skin, approximately 4 cm apart with one containing 100 μL of 1% ACh (Miochol-E, Novartis, Stein) and the other 80 μl of 1% SNP (Nitroprussiat, Rottapharm). ACh was placed over the middle finger between the distal and proximal interphalangeal joints and SNP was placed over the index finger between the metacarpophalangeal and carpometacarpal joints. The incremental iontophoresis protocol for ACh and SNP delivery is described in Klonizakis et al., (Klonizakis et al., 2009a; 2009b). The results are presented

as cutaneous vascular conductance (CVC) to account for the variability of blood pressure during the assessment. The peak vascular response in relation to time (T_{max}) is also reported. T_{max} has been proved to be a reproducible measurement using LDF combined with the iontophoresis method (Klonizakis et al., 2011), assessing the microvascular reactivity.

Transcutaneous oxygen pressure ($TcpO_2$)

$TcpO_2$ measurements were performed during the cardiorespiratory tests using sensors that were non-invasively attached onto the skin and allowed to heat. The sensors induce skin blood capillaries dilatation through heat, which increases the blood flow and results in oxygen diffusion through the skin to the sensor. The sensor measures $TcpO_2$ values inwardly through an electrochemical process.

Measurements were performed using the TINA TCM400 $TcpO_2$ device (Radiometer, Copenhagen, Denmark). The temperature of the probe was set to 44.5 °C to allow maximal skin vasodilation, thereby decreasing the arterial to skin surface oxygen pressure gradient. Before the exercise test 15-20 minutes were allowed with the probe attached on the skin for stabilisation of $TcpO_2$ value. After the test the $TcpO_2$ values were automatically corrected according to a temperature of 37 °C by the TINA device. The electrode was placed slightly below the right scapula on the back away from any bone.

Fixation rings were used to hold the probe attached to the skin and this was filled with two small drops of contact fluid before attachment to the sensor. The fluid was then heated causing the subsequent dilatation of the skin. The raw values of the patient's oxygen perfusion obtained directly from $TcpO_2$ device were defined as previously described in Wasilewski et al., (2016).

6.4.10 Data analysis

All analyses were conducted on an intention-to-treat basis, using SPSS software (version 23, IBM SPSS, New York, USA).

Feasibility and acceptability

Outcomes used to assess the feasibility and acceptability of important parameters were rates of eligibility, recruitment, attrition, outcome completion, exercise adherence and adverse events. Enjoyment levels and intentions for exercise, as well as task self-efficacy of exercise are also presented. Individual's experiences relative to the feasibility and acceptability of exercise are reported. Continuous variables were summarized with descriptive statistics. Frequency counts and percentages were provided for categorical data. Interviews were analysed using framework analysis (Gale et al., 2013). Analysis was aimed at describing the individual's experience of exercise, searching for common, recurrent patterns but also identifying an understanding of participant experiences that might explain the feasibility and acceptability of exercise. The coding framework that was used for the interview analysis is of a deductive approach, framing the analysis within a-priori topic guide, yet data were borne out of original transcripts from the interviews (Pope et al., 2000).

Physiological and fitness outcomes

Data analysis was performed using SPSS software (version 23, IBM SPSS, New York, USA) and is presented as mean ± SD. Normal distribution of the data and homogeneity of variances were tested using the Shapiro-Wilk and Levene's test, respectively. The comparison in the anthropometric, physiological and vascular characteristics between the two groups was performed through independent t-tests and Chi-squared tests. Mixed model ANOVA were also performed to test the differences both within and between subject effects across time (three measurements). Effect sizes (Cohen's d) were calculated wherever the results were statistically significant with 0.2, 0.5, and 0.8 representing small, medium, and large effects respectively (Mullineaux et al., 2001). Statistical significance was set at $p \leq 0.05$.

6.5 Results

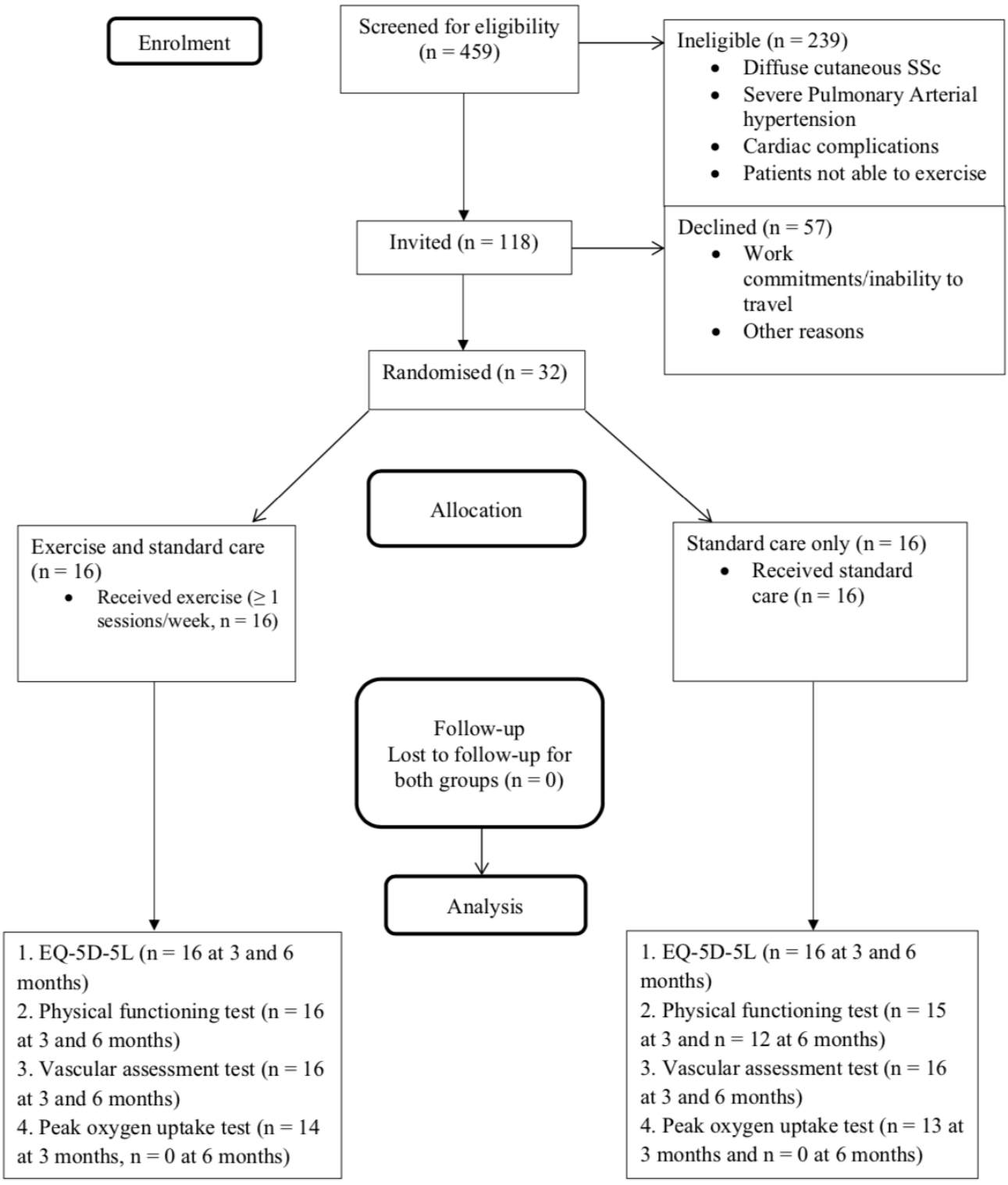
6.5.1 Feasibility outcomes

Figure 7 shows the flow of participants through the trial. Recruitment took place between January 2016 and December 2017. Control group did not perform the exercise intervention and continued their standard care (medical treatment).

Feasibility of exercise

Compliance to the twice weekly, 12-week exercise program was 92.6% with no drop outs. The average percentage of peak HR ($\%HR_{peak}$) for the aerobic part of the training was 89.6% ± 4.5. The average RPE and affect during both the aerobic and resistance exercise was 13 ± 1 ("Somewhat hard") and 3 ± 1 ("Good"), respectively. The average value for the physical activity enjoyment scale was 103 ± 10 out of 119 (highest score). The mean values for the intention to engage in exercise twice and thrice per week were 6.4 ± 1 (likely) and 5.3 ± 2 (slightly likely) out of 7 (very likely). The individuals' confidence to participate in the study's exercise protocol for once and twice per week was 95% and 80% for three times per week out of 100%. No exercise-related complications were reported.

6.5 Figure 7 Flow of participants through the trial



Quality of life

QoL improved significantly in several domains after the exercise intervention in favour of exercise group compared to control group. More specifically, the exercise group showed to have a better life satisfaction (9.25 ± 0.9), less difficulty to perform the usual activities (1.63 ± 0.7), less anxiety (1.06 ± 0.3), and less RP-accompanied pain (2.19 ± 1). Similarly, the exercise group maintained these significant improvements in these QoL domains six months after the baseline measurements as shown in Table 17.

Digital ulcers

The exercise group did not present any digital ulcers (DUs) throughout the six-month period of the study whereas the control group presented five incidents (32% of the control group) of DUs and four hospitalisations for iloprost infusion.

Participant's qualitative feedback on acceptability of the study

The findings are presented based on three key themes, which contextualise the participant's experiences of the study. Direct quotes, with references to participant number are presented to illustrate three key themes 1) Experiences of Raynaud's phenomenon, 2) Participants positive exercise (HIIT intervention) experiences enabling benefits in health, 3) Barriers to exercise.

Theme 1: Experiences of Raynaud's phenomenon

Most of the patients commented that they did not get adequate information from the clinicians about SSc, its symptoms and the progression of the condition *"I didn't know what it was I didn't know anything about it. The consultant didn't much explain to me what it was and I found most of the information about it on the internet"* [SSc002-Exercise group (EG)] *"I didn't know that it could affect me later in life."* (SSc007-EG).

RP attacks were reported to affect participants daily activities and quality of life *"Sometimes you're in the middle of doing something and your feeling on your fingers just goes and you have to stop doing it"*

(SSc002-Exercise group) *"It's affected my daily living dramatically. And I'm finding now that I'm even less able to go outside unless it's really really warm."* (SSc005-EG).

Moreover, during an RP attack which may last up to several hours the individuals are unable to continue their work the most of the time *" When I do have an attack of the Raynaud's my fingers do go numb so it restricts me doing things until the blood flow comes back in because there is very painful as well when they're warming back up so I can't do anything until this comes back to normal."* [SSc009-Control group (CG)]. Therefore, activities of daily living are restricted and as such quality of life is adversely affected particularly in the winter *"Usually my finger ends can become quite painful and particularly in cold weather and even if I go out to put something in the bin I would put a jacket and gloves just to go from the backdoor to the bin. So everything has to be carefully though about before I do any jobs."* (SSc013-CG).

RP attacks were reported to last from minutes to hours or even days, *"It can last anything from 20-30' to..I've had a couple of days. Where I had no feeling to these couple of days."* (SSc016-CG). One participant explained how RP created additional psychosocial burden *"And there is the social side like people "oh your hands look awful, are you alright?" and then people start thinking that you're ill when you're not necessarily that ill with that. And then stupid things like my grandchildren wouldn't hold my hands, because my hands are so cold"* (SSc002-EG).

Theme 2: Participants positive experiences of exercise (HIIT study intervention) enabling benefits in health

Those individuals that took part in the HIIT exercise felt that exercise improved their fitness *"I think that it helped me, my body, it's always very welcoming coming here, you know, you actually feel quite good about it and nice about it. I valued it I think."* (SSc002-EG), *"I think my breathing has got a little bit better."* (SSc018-EG) and their overall QoL *"I think exercise helped my overall wellbeing and mental state."* (SSc019-EG).

Furthermore, participants in the exercise group appeared to have enjoyed the HIIT exercise training and this fostered intention to engage in exercise after the end of the study "I quite enjoyed the regime and doing it", (SSc005-EG). *"Had I the facilities and the opportunity I would continue to do that"*, (SSc002-EG),

Supervision in the exercise sessions gave participants reassurance about the safety of engaging in exercise and it also proved to be one of the key factors that participants enjoyed and valued about the exercise intervention *"I know people who go to gyms and have a personal trainer and I was though "how pretentious" they're just trying to be clever. But actually, really does encourage you and it's not only encourages you it pushes you, And it also makes you more confident because you know you're doing it right and you're not worried that you're gonna drop something and damage yourself or strain something."*. (SSc002-EG). Moreover, supervision helped the participants to both adhere to the exercise programme "*Oh that was good. It definitely did because as I said it was like having your own personal trainer."*, (SSc010-EG), as well as the exercise protocol *" On your own devices you don't do things the same do you? But if you know that there is someone there to say "did you do it?" and you can't say no then you do it, don't you? Because you were there all the time I couldn't stop doing it could I?"* (SSc018-EG).

Participants reflected that exercise improved their QoL through specific mechanisms relating to physical, mental and social well-being *"Exercise improved my fitness and socialising."* (SSc005-EG), *"After exercise I feel more happy and more energetic, I feel stronger"* (SSc010-EG). However, participants did not report any direct beneficial effects upon RP symptoms *"I can't say. I don't think I can say. I think my hands, arms and shoulders all feel stronger and I would imagine that it's got an effect but I can't say this is a direct one."* (SSc002-EG).

Theme 3: Barriers to exercise

One of the main barriers mentioned by participants was access to the exercise venue; the transport and the travelling time that it involved *"The main thing for me is transport. Where I live a couple of years ago we had a descent bus service now Sheffield it's just changed all its buses and it is just helpless. And I don't drive and my husband doesn't drive so getting here is difficult. So it's fine for a 12-week programme but long term getting somewhere 2 or 3 times a week is not easy. Mainly the time involved."* (SSc002-EG). When the travelling time was minimised participants found it was more feasible to attend the sessions *"To come here (Sheffield Hallam University-City centre) I find it a problem but when I went to Graves sport centre (outskirts of Sheffield) I find it easier."* (SSc011). Attendance depended not only on the distance and travelling time but also on the time slot that the exercise sessions could be performed *"Certain hours trying to get in Sheffield on a rush hour takes twice as long as normal so purely depends on the time. On my days off it really doesn't make that much difference, I mean if it was in the evenings then it might be a little more like time travel or whatever."* (SSc016-CG). Another important barrier for this clinical population was the impact of bad weather. Participants did not feel confident to go out in cold weather *"No in winter. I think I would struggle due to the cold."* (SSc011-CG) *"if I am standing any length of a time at the bus stop I can get very cold indeed well I wouldn't like rely on coming on buses in winter time."* (SSc013).

6.5.2 Microcirculatory measures

Oxygen uptake and pressure

$\dot{V}O_{2peak}$ was significantly greater in the exercise group (25.6 ± 7.2 ml kg^{-1} min^{-1}, $p < 0.01$, $d = 1.30$) compared to the control group after the exercise intervention (Table 17).

ΔTcpO2 (5.71 ± 4.4, $p < 0.05$, $d = 1.36$) and $\Delta TcpO2_{max}$ (15.4 ± 7.5, $p < 0.05$, $d = 1.16$) were also significantly improved compared to the control group (Table 17).

Cutaneous vascular conductance

As shown in Table 17 the endothelial-dependent function did not demonstrate any significant improvement as regards the microcirculation, however, it did demonstrate a significant improvement in the time to peak endothelial-dependent reaction (91 ± 42 sec, d= 1.06, p = 0.007) compared to control group after the exercise intervention. ACh T_{max} was also significantly improved at 12-weeks compared to baseline when we controlled disease duration as a covariate. The endothelial-independent function was also improved in the exercise (3.16 ± 2.01, p = 0.005, d= 1.16) compared to the control group. There were no significant differences between groups at baseline and six months after the baseline.

6.5 Table 17 Physiological and quality of life outcomes

	Exercise (n=16)			**Control (n=16)**		
	Baseline	**12 weeks**	**24 weeks**	**Baseline**	**12 weeks**	**24 weeks**
ACh CVC	0.2 ± 0.1	0.22 ± 0.1	0.24 ± 0.2	0.23 ± 0.1	0.17 ± 0.1	0.2 ± 0.1
ACh CVCmax	1.59 ± 1.4	2.62 ± 2	1.7 ± 1.5	1.72 ± 0.8	1.59 ± 2.3	1.6 ± 2.2
ACh Tmax (sec)	122 ± 71	91 ± 42*	111 ± 68	126 ± 55	147 ± 65	143 ± 64
SNP CVC	0.21 ± 0.1	0.24 ± 0.1	0.25 ± 0.1	0.25 ± 0.1	0.21 ± 0.1	0.22 ± 0.1
SNP CVCmax	1.52 ± 1.4	3.16 ± 2*	2.95 ± 2.8	1.72 ± 1.1	1.52 ± 0.8	1.69 ± 0.7
SNP Tmax (sec)	157 ± 67	133 ± 63	129 ± 44	161 ± 60	154 ± 79	148 ± 69
$\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1})	20.6 ± 5.6	25.6 ± 7.2*	-	15.7 ± 7.3	16.0 ± 7.6	-
ΔTcpO2	9.75 ± 6.5	5.71 ± 4.4*	-	1.32 ± 3.6	0.82 ± 2.8	-
$\Delta TcpO2_{max}$	18.3 ± 9.7	15.4 ± 7.5*	-	8.09 ± 6.9	7.27 ± 6.5	-
Life satisfaction	8.13 ± 2.2	9.25 ± 0.9*	9.38 ± 0.9**	7.31 ± 1.4	7.33 ± 1.8	6.83 ± 2
Mobility	2.0 ± 1	1.63 ± 1	1.75 ± 0.7	1.81 ± 0.8	2.07 ± 1	2.17 ± 0.9
Self-care	1 ± 0	1.19 ± 0.5	1.06 ± 0.3	1.25 ± 0.8	1.6 ± 1.1	1.42 ± 1.2
Usual activities	1.69 ± 0.8	1.5 ± 0.7*	1.63 ± 0.7*	1.88 ± 1	2.33 ± 1	2.42 ± 0.9
Pain	2.44 ± 1	1.81 ± 1	1.75 ± 0.7*	2.19 ± 0.8	2.47 ± 1	2.42 ± 0.9
Anxiety	1.38 ± 0.6	1.06 ± 0.3*	1.13 ± 0.3*	1.69 ± 0.7	1.8 ± 1.2	1.83 ± 0.9
Raynaud's pain	2.19 ± 1.2	2.19 ± 1*	2 ± 0.9*	2.5 ± 1.1	3.07 ± 1	2.83 ± 0.9

Endothelial function presented as cutaneous vascular conductance (CVC). T_{max} is the time taken to reach peak perfusion. *p < 0.05 and **p < 0.001 compared to the other groups.

6.6 Discussion

6.6.1 Feasibility outcomes and individuals' experiences of exercise intervention

Evidently, the high rate of compliance to the HIIT exercise program (92.6%), with no dropouts, is an encouraging sign of the acceptability of a novel combined HIIT training approach in people with SSc. Participants enjoyed the exercise sessions and felt that the supervised component of the program, and particularly the fact that the supervision was performed by an exercise specialist (exercise physiologist), was important for developing confidence towards exercise via an increased awareness of their capabilities and a feeling of safety. Supervision also increased their motivation to remain consistent in performing the whole exercise protocol in every session and also to adhere to the exercise programme in overall (Theme 2). Supervised exercise is also considered a safe method to perform exercise in people with SSc (Mitropoulos et al., 2018) and it also educates the participants to self-manage in the future. Therefore, it could be suggested that supervised exercise might be a key element for a complete exercise program. Supportive to this conclusion, a study that examined the feasibility of a supervised exercise in people with leg venous ulcers reported a high exercise attendance (79%; Klonizakis et al., 2018). Furthermore, unsupervised home-based exercise programmes in people with idiopathic pulmonary fibrosis demonstrated significantly low levels of exercise attendance and limited improvement (Kenney et al., 2012; Rammaert et al., 2011; Jordan et al., 2010; Ozalevli et al., 2010).

Although the average HR_{peak} was 89.6% the exercise program stressed the cardiovascular system moderately and thus, the RPE was also relatively low (13 "somewhat hard", Borg scale) and the mean affect was reported as good throughout the whole exercise session (+ 3 "Good"). The average HR_{peak} does not represent the average HR throughout each sprint as it was only measured at peak HR levels. The average enjoyment score of the exercise sessions was also high (103 ± 10). From a physiological perspective, the enjoyment of the exercise and moderate RPE (13) could be explained by the low levels of lactate production that a short HIIT protocol is able to induce (Scherr et al., 2013; Parfitt & Hughes, 2009; Blanchard et al., 2001). Moreover, the participants did state that they enjoyed the exercise sessions

attributing this feeling to the supervised training, to the welcoming environment and to the tangible improvements in their breathing and fitness status (Theme 2). Another important finding is the high score in the task-self efficacy questionnaire of 95% and 80% for two and three bouts per week, respectively. This shows the feasibility of our exercise protocol and the possibility to increase the training dose (three times per week) giving that it might induce greater improvements.

Participants' intentions towards engaging in our exercise protocol twice and thrice per week were positive throughout the exercise programme. Participants responded they would likely engage in our exercise protocol at least twice per week (6.4 ± 1) and slight likely to engage at least three times per week (5.3 ± 2). Moreover, participants' perspective for exercise is that it contributes to the overall wellbeing by improving the fitness and social status, mental health and forming a positive approach towards life in general (Theme 2).

It is important to mention that none of the participants mentioned the duration of the exercise sessions as a barrier which further highlights the feasibility of our exercise protocol to be implemented in people with SSc. The main two barriers were the access to the venue and the weather (Theme 3). Participants can find it very challenging to travel to Sheffield's city centre from the outskirts of the city by public transport or via their own means of transport. This is a significant barrier which requires strong motivation to be overcome and thus sustain study participation. Therefore, a community-based programme offered across a number of localities which minimises travelling time for participants seems an important feature for future intervention studies. Weather constitutes another key barrier for our participants, it was mentioned however that if the travelling time was short, participation would be more possible (Theme 3). The long waiting time at the bus stops during participants' transportation seems to be the main difficulty as it is associated with cold weather during winter which exacerbates RP symptoms. The wide range of available time slots was key to accommodate participants' preferences and fit with their daily schedule. Our study provided exercise sessions six days per week (Monday-Saturday) from 8am to 9pm. Therefore, we believe that the high rates in attendance, engagement, and in recruitment were

a result of the short travelling time to the study venue and the wide range of the available time slots where participants could perform their supervised exercise sessions.

6.6.2 Quality of life

People with SSc' QoL is adversely affected by RP attacks which induce numbness, pain and restrict individuals from performing their daily activities (Almeida et al., 2015). An RP attack may last up to several hours and the most people with SSc in our study reported that they are unable to perform their activities unless they perceive blood flow comes back to normal (Theme 1). The RP symptoms such as very cold hands or hand disfigurement are able to affect the social life in people with SSc acting as a psychosocial burden/anxiety (Theme 1).

The current QoL findings indicate that life satisfaction and RP related pain were improved significantly in the exercise HIIT group compared to the control group, which aligns with the findings of our previous research study (Mitropoulos et al., 2018). Moreover, people with SSc that took part in the current exercise HIIT intervention had less anxiety and were more readily able to perform their usual activities compared to the control group. Noticeably, these positive findings for the exercise group were maintained three months after the completion of the exercise intervention compared to the control group. Therefore, our exercise protocol seems capable of improving QoL in this patient group.

6.6.3 Clinical outcome

Our study demonstrated that a combined exercise programme is feasible to be implemented in people with SSc. In addition to that, we observed a beneficial effect of exercise on DUs which negatively affect QoL in people with SSc. Specifically, 32% of the control group developed DUs and most of them required hospitalisation to heal, whereas the exercise group had no incidents of DUs even three months after the cessation of the exercise program.

DUs are common in SSc and approximately half of patients reporting a history of DUs (Khimdas et al., 2011; Steen et al., 2009; Tiev et al., 2009; Hachulla et al., 2007) and ~ 10% presenting current DU (Ennis

et al., 2013; Khimdas et al., 2011). Often DUs are presented early in the disease (Hachulla et al., 2007). Patients with a shorter duration between the first and the second DUs (especially if the second is within 2 years) have an increased (yearly) DU burden (Hachulla et al., 2007). About one- and two-thirds of people with SSc develop recurrent DUs (Steen et al., 2009; Nihtyanova et al., 2008; Hachulla et al., 2007). DUs often involve both hands with multiple fingers (Mouthon et al., 2014; Hachulla et al., 2007) and DUs per episode (Nihtyanova et al., 2008; Hachulla et al., 2007). The healing of DUs is often slow, specifically if there is underlying calcinosis, and can be related to underlying bone infection (Zhou et al., 2014).

Therefore, knowing that a combined exercise is feasible in people with SSc and that might also offer some clinical meaningful outcomes, there is a need for a multicentre clinical trial to establish its effectiveness on DUs and other clinical components.

6.6.4 Mechanistic exploration of exercise-induced microcirculatory changes

Endothelial-dependent function

Although the endothelial-dependent vasodilation was not statistically significant after the intervention for the exercise group compared to baseline, the size of the improvement ($p = 0.071$, ES= 0.6) indicates that a potentially larger sample size might have demonstrated a significant improvement in the endothelium. This hypothesis is supported by our previous findings where the endothelial-dependent improvement was found to be statistically significant after a HIIT protocol in arm cranking after three months intervention (Mitropoulos et al., 2018). Nevertheless, we found a statistically-significant difference in the endothelial-dependent time to peak flow both between groups and across the baseline and follow-up measurements. CVC_{Tmax} is the time from the point of drug delivery (regardless the dose) until the CVC_{max} and is an indication of the reaction time for the vasculature. T_{max} has been proved to be a reproducible measurement using LDF combined with the iontophoresis method (Klonizakis et al., 2011). This parameter indicates that the vasodilatory mechanisms of the endothelium respond quicker to external stimulus (pharmacological agent) after the exercise intervention. However, the physiological

explanation for this improvement is unclear. We know that the increased blood flow from aerobic exercise is able to increase the nitric oxide (NO) bioavailability through shear stress stimulus and improve the vasodilatory capacity (Green, 2009). Moreover, improvements can be found in the arterial compliance and long-term training protocols are even able to induce arterial remodelling that will further improve the endothelial-dependent vasodilation (Green, 2009).

Little is known about the effects of RT on endothelial-dependent function in clinical populations. Evidence from an acute bout of exercise in thirty-eight healthy women demonstrated significant improvements in the endothelial progenitor cells (EPCs), vascular endothelia growth factor (VEGF), hypoxia-inducible factor 1-alpha (HIF-1a) and erythropoietin (Ribeiro et al., 2017). Moreover, the data revealed that resistance protocols with high intensity [80% of 1-Repetition Maximum (RM)] resistance exercise induced the greatest increase in circulating EPCs compared to lower intensity protocols (Ribeiro et al., 2017). It seems that there might be a dose-relationship between resistance exercise intensity and the circulating levels of EPCs in women. Supportive to these findings, Guzel et al., (2007) examined the effects of different resistance exercise protocols in sedentary males and demonstrated that the increase in NO production found to be in the high resistance group (80-95%). Therefore, our high intensity resistance protocol (> 80% of 1-RM) could explain the endothelial-improvement in those physiological upregulations of EPCs and NO production. Nevertheless, further research is required to establish these beneficial effects of high resistance exercise in clinical populations with vascular pathology.

Endothelial-independent function

We found significant improvements in the exercise group for the endothelial-independent function. Acute alterations in shear stress that regulate endothelial function do not typically have an impact on the magnitude of the endothelium-independent vasodilation (Tinken et al., 2009). Likewise, chronic alterations in shear stress with exercise (Tronc et al., 1996) or heating (Naylor et al., 2011) do not regulate smooth muscle sensitivity to NO, thus it is likely that the changes are limited to the endothelial layer. These human data generally propose that exercise training induces alterations in endothelial, but not

smooth muscle, vasodilator function (Thijssen et al., 2010; Green et al., 2004). Nevertheless, it should be noted that the assessment of vascular smooth muscle function in humans has classically been restricted to administration of NO contributors and measurement of peak vasodilatory responses. Therefore, it is possible that exercise-induced adaptations to smooth muscle might occur, but they have not been detected due to the limitations related to in vivo human research. For example, animal data suggest that exercise training may change gene expression and the phenotype of smooth muscle cells, which might lead to a greater affinity to NO and/or more rapid responses (Newcomer et al., 2011). If similar alterations also occur in humans, then the "kinetics" of response to smooth muscle vasodilators (e.g., time to peak blood flow) could provide important additional information regarding adaptation (Tharciano et al., 2015). This theory explains why we included the T_{max} parameters in our study with the endothelial-dependent T_{max} being significantly higher in the exercise group after the intervention.

Transcutaneous oxygen pressure and oxygen uptake

In an attempt to explore the effects of our exercise protocol on microvascular and systemic oxygen transport function, we assessed $TcpO_2$ changes during maximal exercise. Tissue oxygen tension is a direct quantitative measure of O_2 availability to tissue. Tissue O_2 data are used in clinical decision making by several medical specialties, including wound care and hyperbaric medicine (Sheffield, 1998). The main factors affecting $TcpO_2$ are presented in Table 18 (Byrne, 1984).

The improvements in $\Delta TcpO_2$ and $\Delta TcpO_{2max}$ were significant in the exercise group. Our previous findings indicated an incline of oxygen pressure to improve but it did not reveal a statistically significant difference probably due to small sample size or training load (Mitropoulos et al., 2018). In this study the sample size was larger and the training load greater, thus these factors potentially contributed to the significant difference. It seems our training protocol is sufficient to improve peak oxygen uptake ($p < 0.05$) in the exercise group. The increased oxygen uptake mainly due to metabolic and cardiovascular adaptations to exercise contributed to the improvement of oxygen transport to the internal organs and tissue and resulted in an increase of the skin oxygen pressure.

More specifically, aerobic exercise is able to induce an increase in the number of capillaries per muscle fibre and in the number and size of mitochondria in skeletal muscles (Holloszy et al., 2008; Laughlin et al., 2008). As a result of the new capillaries formed in trained muscles, there is an increase in blood flow to active muscles and provide a greater surface area for the exchange of gases during exercise. Skeletal muscle capillarization is manifested after weeks to months in response to exercise training (Hoppeler et al., 1985; Andersen et al., 1977). Interval exercise is able to induce increases in the expression of several angiogenesis-related mRNAs, including VEGF expression (Hoier et al., 2013).

An initial increase in $\dot{V}O_{2peak}$ is usually observed as early as 2-4 weeks after initiating training (Andersen et al., 1977, Henriksson et al., 1976) but it can also increase after 1 week (Hickson et al., 1977). The main mechanism underlying $\dot{V}O_{2peak}$ improvement is attributed to an increase in stroke volume (and cardiac output) as opposed to the arteriovenous O_2 difference (Montero et al., 2015; Bassett, 2000). The increase in maximum cardiac output is also related to exercise-induced haematological adaptations (Ekblom et al., 1968). It is logical that both the metabolic and cardiovascular adaptations are responsible for the increase in $\dot{V}O_{2peak}$ and thus in oxygen tension.

RT besides producing fibre hypertrophy, induces alterations in the mechanisms responsible for the transport and utilization of oxygen. These mechanisms involve an increase in capillary density per fibre and an increase in the oxidative capacity of the muscle cell, as presented by an elevated citrate synthase activity (Frontera et al., 1990). Cytrate synthase is an enzyme active in muscle cells where it is most often responsible for catalysing the first reaction of the citric acid cycle (the condensation of acetyl-CoA and oxaloacetate to form citrate). Its function has been varied in post-training adaptations (Porter et al., 2015) and further research needs to be done. RT might also be able to induce alterations in the way in which muscles use energy, such as the enhancement in phosphagen and glycogen degradation and utilization of intramuscular triglycerides (Romero-Arena et al., 2013). It is possible that all these adaptations may account for the improvements in oxygen consumption in people who perform resistance circuit training, including older adults.

Cardiovascular adaptations have been demonstrated after resistance circuit training with increases from 15% to 18.6% in $\dot{V}O_{2peak}$, utilising a programme with 8-12 stations performed three days per week (Brentano et al., 2008; Takeshima et al., 2004). The resting intervals between the exercises in a circuit programme vary between no rest (Brentano et al., 2008) to 30s (Takeshima et al., 2004) with work intervals of 30 s. These intervals can be used as exercise prescription guidelines of a programme for improving $\dot{V}O_{2peak}$ (Waller et al., 2011). There is not a standard work to rest ratio, however, the most frequently used ratios are 1:1 (30:30 s) or 2:1 (30:15 s) (Frontera et al., 1990). Our circuit protocol utilised a 2:1 work to rest ratio between exercises with a larger resting interval (60-90 s) at the end of each circle (six exercises). Therefore, a short rest period during resistance circuit training it seems able to augment improvements in $\dot{V}O_{2peak}$ and concomitantly in $TcpO_2$.

6.6 Table 18 Factors affecting tissue oxygen tension

A. Systemic	
1.	Arterial oxygen content
a.	Inspired oxygen concentrations
b.	Ventilation
c.	Lung function
d.	Hemoglobin level
e.	Hemoglobin saturation
f.	Hemoglobin affinity for oxygen
B. Local	
1.	Skin thickness
2.	Capillary formation and density
3.	Oxygen consumption of skin
4.	Inflammation, edema, etc.

6.7 Conclusions

A combined exercise (HIIT and RT) was feasible for people with SSc, resulting in high adherence and low attrition rates, high enjoyment levels and intentions for future engagement to this type of exercise. People with SSc feel comfortable and capable of performing our protocol with no adverse events in this population. The specific protocol is defined as a safe adjunct therapy for people with SSc. Moreover, a combined exercise approach appears to improve QoL in people with SSc and to prevent clinical manifestations such as digital ulcers. A multi-centre exercise programmes are more feasible to be implemented in people with SSc as they tend to eliminate one of the main barriers to exercise participation which is the travelling time and transportation. Our HIIT protocol has previously been demonstrated capable to improve microvascular function in people with SSc (Mitropoulos et al., 2018). The current addition of a circuit RT for the upper limb seems to induce greater results both for the microvascular function and QoL, however, a direct comparison between aerobic and combined exercise was not performed. Therefore, the addition of RT to the overall training load seems beneficial.

We believe that greater training loads, by the addition of another exercise session per week (three times/ week), would induce greater results. However, a community-based programme needs to be pragmatic and thus, two times per week is a feasible training frequency as our study indicated.

There is a need for large multi-centre, randomised controlled studies to explore the effectiveness and cost-effectiveness of a community-based supervised combined exercise protocol (HIIT and RT) in people with SSc.

Chapter 7: General Discussion

7.1 General Background

Microvascular dysfunction in the digital area that induces RP, is the heralding symptom for SSc. RP leads to the occlusion of resistance arteries, can be triggered with cold temperatures or severe emotional stress and can last between a few seconds and several hours. During RP the main sensations are either pain or numbness preventing people with SSc to continue their daily activities (e.g., writing, gardening etc).

Another SSc symptom is the disfiguration of the digits, which further compromises the functional ability of the hands even in the absence of RP. In addition, people with SSc present DUs as a result of the digital ischemia caused by RP. Despite medical treatment DUs are difficult to heal and thus, hospital treatment is required (e.g., iloprost infusion) lasting from a few days to several weeks. Hospitalisation is not always effective for the treatment of DUs, with digital amputation to one or several digits, being a common outcome for many people in this group. Evidently, the QoL of people with SSc is dramatically affected by RP's symptoms and its complications despite medical treatment. Therefore, this research programme aimed to explore the effects of exercise on digital microvascular function (Study 2), identifying the most appropriate exercise regime for this clinical population (Study 2), exploring the feasibility of using it as an adjunct therapy to pharmacotherapy (Study 3).

It is well known that exercise in general, and HIIT in specific, may improve vascular function in several clinical conditions (e.g., heart failure, obesity). Prior to the commencement of this work, the evidence indicated that HIIT can induce systemic effects by improving the vascular function in the brachial artery in patients that performed cycling training. However, it was unknown whether this improvement could be observed in smaller arteries (e.g., resistance arteries) in the digital area. Therefore, we proceeded in comparing two modes of exercise (cycling vs arm cranking) to explore the differences on microvascular function in the digital area in people with SSc (Study 2). Study 2 results showed that cycling is not able to induce systemic improvements in digital microvasculature, in contrast to arm cranking which improved digital microvascular function via shear stress-induced mechanism. Moreover, arm cranking turned out to be the preferable mode of exercise by our participants, demonstrating also a preventative mechanism in relation to the formation of digital ulcers.

Previous research had shown that $\dot{V}O_{2peak}$ was strongly correlated with an improvement in endothelial-dependent and independent function in rheumatoid arthritis patients after a three-month exercise intervention. Thus, in Study 2 our aim was to compare $\dot{V}O_{2peak}$ between cycling and arm cranking. However, these two modes of exercise are not comparable in terms of oxygen uptake as they utilise

different muscle groups which induce different physiological and cardiorespiratory responses. Therefore, before conducting Study 2, we validated an ACE protocol (Study 1) and proposed an equation, which would accurately predict CE$\dot{V}O_{2peak}$ by using the physiological responses of ACE protocol.

Another important Study 2 finding was the strong correlation between soft lean muscle mass and the endothelial-dependent improvement in the digital microvasculature. Thus, in Study 3 we combined a circuit RT for the upper limbs with the aerobic part forming the basis of our exercise protocol. The main outcome of Study 3 was the feasibility of exercise in people with lcSSc. Study 3 strongly suggests that the proposed exercise protocol (combining HIIT and RT) is feasible to be performed in people with lcSSc twice per week for at least twelve weeks. Nevertheless, our protocol needs to be tested in larger cohorts of patients, before its wider adoption

7.2 Key Findings

Study 1: Validation of an Arm Crank Ergometer Test for Use in Inactive Adults

Literature review as well as the discussion section of chapter 5 illustrated that $\dot{V}O_{2peak}$ has been strongly associated with improvement in microvascular function in rheumatoid arthritis patients (Metsios et al., 2014). Therefore, during the development of Study 2 protocol, it was considered necessary to perform a CPET both on an arm crank and cycle ergometer at baseline measurements and following the exercise intervention, for all the groups. The control group performed a cycle ergometer test and thus $\dot{V}O_{2peak}$ was comparable to the cycle ergometer group but not with the arm crank ergometer group. It is well known that these two modes of exercise are not comparable in terms of oxygen uptake as they utilise different muscle groups which induce different physiological and cardiorespiratory responses. Moreover, a thorough review of the literature revealed that a validated arm crank ergometer test that could compare $\dot{V}O_{2peak}$ between these two different modalities was not existed. Therefore, the aim of the first study was to create an arm crank ergometer test based on the current recommendations and compare it to a validated and established cycle ergometer test that is routinely used in the clinical setting.

The main Study 1 finding is the development of a predictive equation that can accurately predict cycle ergometer's $\dot{V}O_{2peak,}$ based on the physiological responses of the arm crank ergometer test and anthropometrical characteristics. The applicability of the arm crank test could be in a clinical setting in people unable to perform lower body exercise and may provide a valuable tool for exercise testing to individuals with vascular, orthopaedic and neurological conditions. A pragmatic case study in a clinical setting would be a 45-year old (male) inactive patient unable to perform lower body exercise and in request to perform a stress exercise test. It is well established that a CPET could reveal symptoms not visible at rest, assess parameters that would lead to a more appropriate decision making about future treatment by the clinicians, and it can also predict disease severity and mortality.

A similar study by Schrieks et al., (2011) compared treadmill versus arm crank ergometry in thirty subjects (16 men and 14 women) with a mean age of 31 ± 11.3 years. They demonstrated a similar predictive equation [$T\dot{V}O_{2peak}$ = 0.852 + 0.8 x $A\dot{V}O_{2peak}$ + 0.019 x weight + 2.025 x gender – 0.038 x gender x weight)] to ours [$CE\dot{V}O_{2peak}$ = 11.776 + 1.418 x $ACE\dot{V}O_{2peak}$ (ml kg min^{-1}) – 1.454 x total lean body mass + 3.967 x lower limb lean body mass] for treadmill comparing the two modalities. Interestingly, in our study the lean body mass demonstrated to play a key role in predicting $CE\dot{V}O_{2peak.}$ This could potentially be explained by the different populations that were assessed in these two studies. Schrieks et al., (2011) examined healthy adults with a mean age of 31 years compared to 55 years in our study in inactive adults. That means that the lean body mass between these two groups of people could be a significant anthropometric difference and thus regression analysis revealed its significance in the derived formula. Future research should try to replicate our exercise test in clinical populations and test its reproducibility and accuracy.

The effects of upper and lower limb exercise on the microvascular reactivity in limited cutaneous systemic sclerosis patients

Study 2 aimed to identify the best mode of exercise between arm crank and cycle ergometer that would induce significant improvements in microvascular function in people with lcSSc. Cycle ergometer has

been demonstrated to improve macrovascular function in the upper limb (brachial artery) using a HIIT protocol in several clinical populations (Ramos et al., 2015), however, it was unknown whether these improvements were obvious at the smaller arteries in the digital area (microcirculation). Moreover, no study had assessed the effects of an arm crank ergometer in microvascular function and more specifically in people with lcSSc. It was hypothesised that arm crank would be able to induce more favourable local vascular effects (digital microcirculation) compared to cycling. Therefore, our study is the first to assess the effects exercise on digital microvascular function in people with lcSSc.

One of the key Study 2 findings is that aerobic exercise in general, and HIIT (30 s 100% PPO/30 s passive recovery) involving the upper limb in specific, may improve digital microvascular reactivity through an enhancement of the endothelial-dependent function. Moreover, our protocol appears to reduce digital ischaemia risk, which is the leading cause for further systemic complications and a major factor affecting the QoL.

An important highlight of our research is that upper limb exercise (arm cranking) is able to induce local effects in the microvasculature (resistance arteries) in the digital area, an outcome that lower limb exercise (cycling) was unable to induce. Moreover, our exercise protocol (30 s 100% PPO/30 s passive recovery) showed to be safe, tolerable and enjoyable for people with SSc presenting no exercise-related adverse events. Therefore, upper limb HIIT exercise is an appropriate mode of exercise for this clinical population when targeting improvements in the microvasculature in the digital area.

The observed, exercise-induced microvascular benefits will probably not be obvious to people with lcSSc as tangible results. In plain words, people with lcSSc will not observe large differences in RP frequency and/or severity during or after the exercise intervention. Nonetheless, the improvement in the microvasculature will prevent digital ischemia and thus the formation of digital ulcers. The prevention of digital ulcers improves QoL in people with lcSSc substantially as hospitalisations and the risk for amputations will be significantly reduced. Moreover, the patients will be able to perform daily activities that were not able to do due to pain and discomfort associated with digital ulcers (Mouthon et al. 2014).

The feasibility of a combined exercise protocol including aerobic and resistance training in people with limited cutaneous systemic sclerosis: a randomised controlled feasibility trial

Following the demonstration of the effects of upper limb HIIT protocol on microvascular function in Study 2, we aimed to investigate the feasibility of a combined exercise protocol in people with lcSSc. Our key findings demonstrated that a combined exercise (HIIT and RT) is feasible for people with lcSSc, resulting in high adherence and low attrition rates, high enjoyment levels and intentions for future engagement to this type of exercise. People with lcSSc feel comfortable and capable of performing our protocol with no adverse events in this population. The specific protocol is defined as a safe adjunct therapy for people with lcSSc. Moreover, as demonstrated with aerobic exercise (Study 2), a combined exercise approach is also able to improve QoL in people with lcSSc and to prevent clinical manifestations such as digital ulcers which constitutes the most important clinical outcome for both studies. Our HIIT protocol has previously been demonstrated the capability to improve microvascular function in people with lcSSc (Mitropoulos et al., 2018). The current addition of a circuit RT for the upper limb might be able to induce greater results both for the microvascular function and QoL. Therefore, the addition of RT to the overall training load seems beneficial and future research should compare an aerobic and a combined exercise protocol for use in lcSSc. Our study demonstrated that an exercise programme including two times per week is the recommended feasible training frequency as our findings indicate. There is a need for large multi-centre randomised controlled studies to explore the effectiveness and cost-effectiveness of a community-based supervised combined exercise protocol (HIIT and RT) in people with lcSSc.

7.3 Strengths of Research Project

The main strength of the research project is the Studies' 2 and 3 design.; We developed study protocols which were pragmatic and realistic to be completed as part of a PhD programme. This is supported by the high recruitment and retention rates, which were than other, similar studies in clinical populations. We employed a patient-centred and patient-friendly strategy, which paid off for the final outcome. The

principal researcher (PI) of the study (Mr. Alexandros Mitropoulos) was responsible for the identification of the eligible patients via medical screening notes when clinics were performed (every Tuesday and Thursday from 9am to 2pm). After the identification of the eligible patients the PI was providing an informative pack (PIS and consent form) within the medical notes to the consultant rheumatologist (Dr. Mohammed Akil). At the end of each patients visit, those interested to know more about the study, were introduced briefly to the PI, in a private room at the clinic, with the study's procedures being described and the opportunity to ask study-related questions being given. The aim of this session was to build a rapport with the patient and directly answer to any concerns about the study by the PI, who would be responsible for the delivery of the assessments and training intervention. Patients were given sufficient amount of time to think about the study (14 days). Therefore, we believe that this recruiting strategy constitute an important part of the completion of recruitment earlier (e.g., 3 months) than it was expected.

Another strength in the study's design was the rapid amendment in expanding the sports venues. The reasoning behind this action was that the first two patients that were approached in the clinics expressed their concerns about their ability to drive into Sheffield city centre (Collegiate Campus) twice per week for their exercise sessions regarding the increased travelling time (40-50 minutes) due to city's traffic jams. Consequently, Graves and Concord sports centres, in the opposite sides in the outskirts of Sheffield, were chose as additional studies. Both of these centres hold "state of the art" facilities and equipment. In this way, patients had the opportunity to select one of the three sports venues according to their preferences and this led to higher-than-expected recruiting rates.

The excellent retention and completions rates for Study 2 and 3 can be partly explained to the strategy our research team implemented. More specifically, to support the successful participation of our participants, we used our 'six pillars of adherence' strategy (based upon 'social support', 'education', 'reachability', 'small groups intervention implementation', 'reminders', and 'simplicity'), which we have used previously with excellent results in lifestyle interventions (i.e., over 90% of retention and 79% of completion; Klonizakis et al., 2018; Wasilewski et al., 2016).

The strength in the delivery of the exercise intervention as well as the baseline measurements was that the PI had previous experience in supervising exercise (7 years of gym instructor experience and supervisor in pulmonary rehabilitation programmes for COPD patients within the hospital facilities) and performing CPETs with clinical populations (e.g., chronic obstructive pulmonary disease). It can be safely assumed that this was the main reason of the high retention and adherence to the exercise intervention. Moreover, the fact that the same person (PI) was discussing in person during the clinics with the patients, arranging the baseline assessments and the exercise sessions, performing the baseline assessments as well as supervising the exercise sessions created a solid rapport with the participants and thus the drop outs were minimal (high retention rates 12 months post follow up).

7.4 Research Project Limitations

A programme limitation is the different environmental conditions under which participants took part in studies 2 and 3. It is well known that RP is triggered primarily by cold temperatures and secondary under severe emotional stress. Therefore, we assume that those recruited during the winter months would have more frequently RP attacks than those recruited during the summer months. For that reason, the RP diary that patients could record the severity, number and duration of RP attacks was recalled after we realised that there was a huge variability between cold and warm days as well as in some patients that were doing outdoor occupations (e.g., farmer). RP was another qualitative measurement of the effects of exercise intervention in reducing the frequency and severity of RP attacks, but it was not practically possible to be implemented. This weakness could only be overcome, if the whole cohort of patients were to be recruited at the same time period, which was practically impossible.

Another limitation is that, due to our limited resources, we did not report the cost-effectiveness of our study so as to compare it with the costs per patient when hospitalisations are required due to the formation of digital ulcers and other treatment related costs. However, since our results are very promising and lead to a larger clinical trial to test the feasibility of our exercise protocol to be implemented within the NHS

guidelines and be part of the rehabilitation programmes, there is another chance to improve the design and incorporate a detailed expenses diary for any disease-related expenses the patients have.

Finally, a detailed medical treatment diary was missing during studies 2 and 3. We did report the basic medical treatment such as blood pressure (e.g., Ramipril) and RP treatment (e.g., sildenafil) medication. It might be possible that medication could affect the results of our study however statistical analysis did not reveal any significant differences between those receiving medication and those not receiving any medical therapy.

7.5 Impact of Findings and Future Research Recommendations

The novelty of all three studies as part of the wider PhD research project cannot be questioned: Study 1 has provided a unique evidence that might change clinical practice. If our findings prove to be reproducible and accurate in clinical population it could be used as a standard clinical practice. More specifically, the main findings of Study 1 is the exercise test protocol on arm crank ergometer and the predictive equation of CE$\dot{V}O_{2peak}$. It is demonstrated that CE$\dot{V}O_{2peak}$ is the gold standard assessment of fitness which is strongly linked to disease severity and prognosis as well as post-operation survival and mortality rates. Our participants match in age and fitness status the people with lcSSc recruited in our concomitant studies, however, our protocol needs to be tested in older adults with more severe conditions such as heart failure and or COPD.

The main Study 2 finding was the significant microvascular improvement in arm crank ergometer group against control group and not statistically significant improvement against cycler ergometer group. Physiologically, the improvement via arm crank versus cycle ergometer exercise performing a HIIT protocol (30 s 100% PPO/30 s passive recovery) on the digital microcirculation can be explained through the intensified local response potentially because of a higher shear stress on vascular walls (acute effects) and the vascular remodelling that this stimulus induces (long term effects). The main clinical impact of this finding is that arm crank could induce better improvement on the digital microcirculation in people with lcSSc and thus it is the preferred mode of exercise when performing our HIIT protocol.

Both modes of exercise revealed to be effective in terms of digital ulcer prevention compared to control group which indicates that feasibility of exercise should be tested before included to NHS guidelines as an adjunct therapy to medical treatment. Taking this into consideration, we proceeded to Study 3, which tested the feasibility of a combined exercise protocol (HIIT and RT) through a 12-week exercise programme twice per week in people with lcSSc. The results demonstrate that our exercise programme is feasible to be implemented to this population with no adverse events, high adherence and engagement rates as well as enjoyment levels. The feasibility of our study design needs to be tested in a larger cohort of patients in a multi-centre UK feasibility pragmatic clinical trial. If the results of the larger clinical trial are considered successful, according to a pre-determined set of success criteria, then our exercise programme will have the opportunity to influence clinical practice through the use of our regime as an adjunct therapy to pharmacotherapy in people with lcSSc. Our research team has already compiled a research protocol and has secured academic and clinical collaborations with other sites in UK (Sheffield, Leeds, Manchester, London) to apply for a research grant that will support this large clinical trial.

7.6 Conclusions

Reflecting briefly to the PhD journey, the current PhD research project can be considered as successful. Three publications and two more currently under review reflect the scientific knowledge that this project produced. I strongly believe that this PhD journey provided me with sufficient knowledge and skills to overcome any difficulties that may emerge during my future academic career.

In overall, the PhD project provided a plethora of evidence that may induce changes in clinical practice. Study 1 is the first to demonstrate a strong correlation between a routinely used cycle ergometer test (Wasserman's protocol) and an arm crank test to assess cardiorespiratory fitness in inactive middle-aged adults. The arm crank test could be used as an alternative to cycle ergometry by accurately predicting $\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) in inactive middle-aged adults. Future research should focus upon comparing these protocols in older patients, clinical populations, and/or younger people to examine test reproducibility.

Study 2 and 3 demonstrated that aerobic exercise in general, and HIIT (30s 100% PPO/ 30s passive recovery) specifically, involving the upper limbs may improve the microvascular reactivity through an enhancement of the endothelial-dependent function. This will then allow us to proceed with a large multi-centre, randomised-controlled study to further establish the effectiveness and cost-effectiveness of exercise on people with SSc.

The main impact of the current findings is that our HIIT protocol performed on an ACE it seems to be effective (prevention of DUs, improvement of microvascular function) and feasible when RT was added to the overall exercise protocol (compliance rates, enjoyment levels, affect, RPE, no adverse events) in people with SSc and thus able to improve QoL in this population. If our results could be established through further research and larger cohorts, the specific protocol could be included as an adjunct therapy to medical treatment in the NHS guidelines. Future research should assess the feasibility, efficacy, and cost-effectiveness of our exercise protocol in larger cohorts of people with SSc.

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Appendices in thesis

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Appendix A

Feeling Scale (FS)

While participating in exercise, it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasant. Additionally, feeling may fluctuate across time. That is, one might feel good and bad a number of times during exercise. Scientists have developed this scale to measure such responses.

+5 Very good

+4

+3 Good

+2

+1 Fairly good

0 Neutral

-1 Fairly bad

-2

-3 Bad

Appendix B

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Exercise task self-efficacy

Please circle the percentage that best describes you in the questions below.

How confident are you that you can….

1) perform one bout of exercise a week for the next 4 weeks that is just like the one you completed today?

10% Not at all	20%	30%	40%	50%	60%	70%	80%	90%	100% Extremely confident

2) perform two bouts of exercise a week for the next 4 weeks that is just like the one you completed today?

10% Not at all	20%	30%	40%	50%	60%	70%	80%	90%	100% Extremely confident

3) perform three bouts of exercise a week for the next 4 weeks that is just like the one you completed today?

10% Not at all	20%	30%	40%	50%	60%	70%	80%	90%	100% Extremely confident

Appendix C

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Intentions for engagement to exercise

Please rate the extent to which you agree with the following statements.

1) I intend to engage in the type of exercise I performed today at least 2 times per week during the next month.

1	2	3	4	5	6	7
Very unlikely	Unlikely	Slight unlikely	Neutral	Slight likely	Likely	Very likely

2) I intend to engage in the type of exercise I performed today at least 3 times per week during the next month.

1	2	3	4	5	6	7
Very unlikely	Unlikely	Slight unlikely	Neutral	Slight likely	Likely	Very likely

Appendix D

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Physical Activity Enjoyment Scale

Please rate how you feel about the exercise you just completed.

Look at the statement, choose the left or right hand side of the statement that **most** represents how you felt about participating in your recent exercise session, then choose a number on your chosen side to show how much you agree with that statement.

If you don't agree with either side of the statement choose neutral (4).

Only circle one number per statement.

Example: I enjoyed it or I hated it.

If you mainly enjoyed the exercise session you would circle 2.

	Absolutely agree	Mainly agree	Somewhat agree	Neutral	Somewhat agree	Mainly agree	Absolutely agree	
	1	2	3	4	5	6	7	
enjoyed it								**I hated it**
	1	2	3	4	5	6	7	
felt bored								**I felt interested**
	1	2	3	4	5	6	7	
disliked it								**I liked it**
	1	2	3	4	5	6	7	
found it leasurable								**I found it unpleasurable**
	1	2	3	4	5	6	7	
t was not fun at ll								**It was a lot of fun**
	1	2	3	4	5	6	7	
found it nergizing								**I found it tiring**
	1	2	3	4	5	6	7	

t made me lepressed								**It made me happy**
	1	2	3	4	5	6	7	
t was very leasant								**It was very unpleasant**

	Absolutely agree	**Mainly agree**	**Somewhat agree**	**Neutral**	**Somewhat agree**	**Mainly agree**	**Absolutely agree**	
	1	2	3	4	5	6	7	
felt good hysically while loing it								**I felt bad physically while doing it**
	1	2	3	4	5	6	7	
t was very nvigorating								**It was not at all invigorating**
	1	2	3	4	5	6	7	
was very rustrated by it								**I was not at all frustrated by it**
	1	2	3	4	5	6	7	
t was very ratifying								**It was not at all gratifying**
	1	2	3	4	5	6	7	
t was very xhilarating								**It was not at all exhilarating**
	1	2	3	4	5	6	7	
t was not at all timulating								**It was very stimulating**
	1	2	3	4	5	6	7	
t gave me a trong sense of ccomplishment								**It did not give any sense of accomplishment**
	1	2	3	4	5	6	7	
t was very efreshing								**It was not at all refreshing**
	1	2	3	4	5	6	7	

felt as though I would rather be doing something else	**I felt as though there was nothing else I would rather doing**

Appendices for Research Study

Appendix 1

Information for participants

Title of the study:

Comparison between a validated cycle ergometer test and an arm crank ergometer test in assessing maximal oxygen uptake in healthy adults.

Introduction

We are inviting you to participate in a research study. Before you decide whether you would like to take part, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with us and/or friends if you wish. If you require more information or any further clarification on the information given to you we will gladly be at your disposal to answer at any relative inquiry. Please take your time to decide whether or not you wish to take part.

Background and purpose of the study

Maximal oxygen uptake test is a validated measurement that is used to assess the capacity of the human body to intake and consume oxygen while performing physical activity. This test is essential when is implemented in the clinical settings not only in assessing the health status of the patients but also in prescribing exercise that will be an important aid for a further improvement to their disease state.

The purpose of this research is to add to our knowledge of a validated arm crank ergometer test which will be able to measure accurately the peak oxygen uptake in any patients. Multiple disease states present limited lower limbs mobility and thus, are considered to be a major contraindication to perform a peak oxygen uptake test on a treadmill or a cycle ergometer. Therefore, we aim to compare a validated commonly used cycle ergometer test to an arm crank ergometer test that will further enlighten the current knowledge about the clinical utility of arm ergometry.

Am I suitable participant for the study?

We are recruiting men and women ≥ 45 and ≥ 55 years old, respectively which are apparently healthy. The study can be divided in three phases. Only the apparently healthy will be eligible to participate in Phase B and C. During the study proceedings you have the right to withdraw at any time you wish to.

What will happen if I take part?

All the assessments will take place at the facilities of Sheffield Hallam University in the Collegiate Hall laboratories. Phase A consists of the baseline measurements and a health risk stratification. After the assessment, participants presenting a cardiovascular disease, cardiovascular risk or are suspected to have a cardiovascular risk will be excluded from the study. The apparently healthy participants will take part in Phase B (second visit) soon after their first visit probably within 1-4 days where they will randomly perform one of the maximal oxygen uptake tests after the baseline measurements. Finally, Phase C will require the participants to a third visit in our labs after 6-7 days of the first assessment to perform the other maximal oxygen uptake test. Table 1 outlines what will happen in each visit.

Table 1: Overview of the study

Visit number	Purpose of visit	Duration of visit
1	**Pre-testing procedure: Phase A** Screening questionnaire, blood pressure assessment.	15 minutes
2	**Phase B:** Maximal exercise test (cycle or arm ergometer), Body composition: % Body Fat, height, body weight	35-40 minutes
3	**Phase C:** Maximal exercise test (cycle or arm ergometer)	35-40 minutes

Screening (1st visit)

Before testing and once you have given consent, you will be screened by the researchers by use of a questionnaire. You will be asked to disclose lifestyle information (alcohol, smoking, physical activity – through a physical activity recall questionnaire), diagnosed conditions and medications, plus family history of medical conditions. You will also have your height, weight and blood pressure (BP) measured.

Maximal exercise test (2nd and 3rd visit)

If you are eligible and willing to participate in our study you will be requested to visit us again to perform the exercise test. This test will last 8-12 minutes. The intensity will increase progressively up to a maximal level which will only be sustained for a few seconds. Heart rate, blood pressure as well as the electrical activity of your heart may also be monitored (ECG) and expired air will be collected via a mouthpiece. The sequence of the two tests will be determined through the randomisation process.

What do I have to do?

Before each exercise test you will be requested to abstain from vigorous exercise, alcohol, caffeine and tobacco for a period of 24h but also to be at least 2h fasting prior to the assessment as these parameters could affect your responses. Moreover, we encourage you to wear sport clothing that will allow for a more comfortable movement during exercise test. Prior to the test we will place you to an appropriate position on the cycle ergometer or arm ergometer and you will be required to breath via a mouthpiece while your nose will be kept sealed with a nose clip to ensure no air leak occurs. The test will commence with an unloaded light pace exercise for a period of 2-3 minutes and afterwards the intensity will progressively increasing accordingly to your estimated physical performance and you will be requested to maintain a specific pace. The test will be terminated at the point you reach volitional exhaustion, the point that you cannot longer maintain the required pace or if any other contraindication sign/s are present during the test which will be evaluated by the researcher. Directly after the termination of the test you will be requested to continue exercising in an unloaded light pace for 2-3 minutes to allow for an active cool-down and recovery of the physiological responses close to the resting levels.

What are the possible benefits of taking part in this study?

This study is being undertaken for research purposes and to advance our knowledge in the use of arm ergometry as an exercise test in the clinical settings by comparing it to a validated clinical exercise test on a cycle ergometer (Wasserman's protocol). The main benefit to you will be the opportunity to have a state of the art tests to determine your current fitness status and to assess your risk factors for cardio-metabolic disease. Moreover, this information will give further data to the researcher to make any recommendations upon your lifestyle activities if you wish for it.

What happens if something goes wrong?

All of the experimental procedures that will be used in this study have been rigorously tested to ensure that they meet health and safety standards. These tests are all routinely and regularly performed on patients and healthy volunteers alike. The researchers who perform the tests are all trained and skilled to do so. If we show any signs, as regards your health status, that may cause you harm by participating, you will be informed and withdrawn immediately from the study.

Will taking in this study be kept confidential?

All information collected about you will be kept strictly confidential, other than to those of us who are involved directly with the study. Any information that leaves Sheffield Hallam University has our name and address removed so that you cannot be recognised from it. As a group of participants you will receive feedback, but all names will be removed from the individual data set. A participant's ID number will be your identification for us instead of your name.

Who will be working on the study?

The researcher in charge is Mr. Alexandros Mitropoulos (PhD student in Clinical Exercise Physiology) supervised by Dr. Markos Klonizakis (Senior Research Fellow in Clinical Physiology).

What will happen to the results of the study?

Once the study has been completed all data will be anonymised and stored as per current data protection laws. The results will be written up for publication in academic journals and possibly used at academic conferences. Anything with your personal details (name, DOB, contact details etc.) will be kept securely in a locked filing cabinet by the Principal Investigator. Results will also be made available to you (the participants) on request at any time throughout the study. Moreover, information provided by the participant will be stored at Sheffield Hallam Research Facilities in Sheffield for further analyses until the end of the project. After the completion of the project, the samples will be disposed according to the guidance on disposal provided by the HTA Code of Practice on the Removal, Storage and Disposal of Human Organs and Tissue (see http://www.hta.gov.uk/guidance/codes_of_practice.cfm).

Contact for further information

If you require further advice about this study, at any time during participation, you may contact Mr Alexandros Mitropoulos, Dr. Markos Klonizakis, Dr. Rob Copeland or Dr. Anil Gumbler at Sheffield Hallam University.

Study Team Contact Details

Health and Wellbeing Faculty, Centre of Sport and Exercise Science,

Collegiate Hall, Collegiate Campus, Sheffield, S10 2BP.

Principal Investigator:

Mr Alexandros Mitropoulos

E-mail: alexandros.mitropoulos@student.shu.ac.uk

Sheffield Hallam University

Appendix 2

PARTICIPANT CONSENT FORM

Study 1: Consent form_version_2.0_12/10/2016

Comparison between a validated cycle ergometer test and an arm crank ergometer test.

Participant's Identification Number:	**YES**	**NO**
1. I have read the Information Sheet for this study and have had details of the study explained to me.	☐	☐
2. My questions about the study have been answered to my satisfaction and I understand that I may ask further questions at any point.	☐	☐
3. I understand that I am free to withdraw from the study within the time limits outlined in the Information Sheet, without giving a reason for my withdrawal or to decline to answer any particular questions in the study without any consequences to my future treatment by the researcher.	☐	☐
4. I agree to provide information to the researchers under the conditions of confidentiality set out in the Information Sheet.	☐	☐
5. I wish to participate in the study under the conditions set out in the Information Sheet.	☐	☐
6. I consent to the information collected for the purposes of this research study, once anonymised (so that I cannot be identified), to be used for any other research purposes.	☐	☐
7. I agree to this consent form and other data collected as part of this research study to be kept at Sheffield Hallam University.	☐	☐
8. I understand that records relating to me will be kept confidential. No information will be released or printed that would identify me without my permission unless required by law.	☐	☐
9. I agree to take part in the above study.	☐	☐

Participant's signature:_____ **Date:**_____

Participant's Name (Printed):_____

Contact details:_____

_____

Researcher's Name (Printed): Alexandros Mitropoulos

Researcher's Signature:_____

Researcher's contact details:

Alexandros Mitropoulos. Sheffield Hallam University, Faculty of Health and Wellbeing/ The Centre of Sport and Exercise Science/ Collegiate Crescent/ Chestnut court/ Room S002/ Sheffield S10 2BP. e-mail: hwbam13@exchange, Tel: 07926126426.

Appendix 3

Pre-participation Health Screening Questionnaire

Participant's ID:

Assess your health needs by marking all true statements.

History

You have had:

A heart attack_____

Heart surgery_____

Cardiac catheterization_____

Coronary angioplasty (PTCA)_____

Pacemaker/ implantable cardiac defribrillator/ rhythm disturbance_____

Heart valve disease_____

Heart failure_____

Heart transplantation_____

Congenital heart disease_____

Symptoms

You experience chest discomfort with exertion_____

You experience unreasonable breathlessness_____

You experience dizziness, fainting, blackouts_____

You take heart medications_____

Other health issues

You have musculoskeletal problems_____

You have concerns about safety of exercise_____

You take prescription medication (s)_____

You are pregnant_____

Cardiovascular risk factors

You are a man older than 45 years_____

You are a woman older than 55 years or you have had a hysterectomy or you are postmenopausal

_____

You smoke_____

Your blood pressure is >140/90_____

You take blood pressure medication_____

Your blood cholesterol level is >240 mg/dL_____

You don't know your cholesterol level_____

You have a close blood relative who had a heart attack before age 55 (father or brother) or age 65 (mother or sister)_____

You are diabetic or take medicine to control your blood sugar_____

You are physically inactive (ie, you get < 30 minutes of physical activity on at least 3 days per week) _____

You are overweight_____

Appendix 4 Study 1: Maximal oxygen uptake test-Data collection sheet

Participants ID:		**Mode**		Date:		
Height (cm):		ACE	CE	DOB:		
Weight (kg):						
Upper arm circumference (cm):						
Lower arm circumference (cm):						
Resting period	Time	Watts	SBP	DBP	HR	RPE
	0-1					
	1-2					
	2-3					
Warm-up	0-1	0				
	1-2	0				
	2-3	0				
Exercise	0-1					
	1-2					
	2-3					
	3-4					
	4-5					
	5-6					
	6-7					
	7-8					
	8-9					
	9-10					
	10-11					
	11-12					
	12-13					
	13-14					
Recovery period	0-1					
	1-2					
	2-3					
Termination cause:						
Total exercise time:						

Appendix 5

Study 2 & 3: Invitation Letter Version 3.0 26/04/2016

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Sheffield Hallam University

Dear .....

We are undertaking a research study to investigate the practicality and effectiveness of supervised exercise training in people diagnosed with Systemic Sclerosis experiencing Raynaud's phenomenon.

You may have been identified as being a potentially suitable from attending the Rheumatology Department of Royal Hallamshire Hospital about an undue digital pain.

Please find enclosed a participant information sheet, which describes the study in detail and answers the most frequently asked questions.

If you are interested in participating in this study, or would like to obtain further information, please phone Mr. Alexandros Mitropoulos at Sheffield Hallam University on 07926126426 or email him at hwbam13@exchange.shu.ac.uk. Alternatively, you can complete the tear-off slip below and return it in the pre-paid envelope provided. It is important to note that there is no pressure to participate in this study and your standard care will not be affected in any way by your decision to take part in this study.

Yours sincerely,

Dr. Mohammed Akil

Consultant Rheumatologist

Department of Rheumatology - Sheffield Teaching Hospitals NHS Foundation Trust

Glossop Road, S10 2JF, Sheffield

Email: m.akil@sheffield.ac.uk

Tel: 01142711932

SYSTEMIC SCLEROSIS & EXERCISE STUDY

☐ Yes. I am interested in taking part in the above named study. I understand that a member of staff will be contacting me, regarding this study.

☐ No, I am not interested in taking part in the study

Name: _____

Telephone Number: _____

Appendix 6

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Sheffield Hallam University

Study 2: Information sheet Part 1 (Version 3.0 26-04-2016)-Project ID: 68096

Information for participants Part 1 (Pilot study)

The effects of different modes of exercise on microcirculatory parameters in patients with Systemic Sclerosis.

Introduction

We are inviting you to participate in a research study. Before you decide whether you would like to take part, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with us and/or friends if you wish. If you require more information or any further clarification on the information given to you we will gladly be at your disposal to answer at any relative inquiry. Please take your time to decide whether or not you wish to take part.

Background and purpose of the study

Approximately 50% of patients with Systemic Sclerosis develop decreased blood flow in the fingers and/or wound which seem to be painful, difficult to heal, susceptible to infections and heavily influences quality of life. Raynaud's phenomenon is a common condition with vasospasm in the small veins and arteries of the fingers causing pallor with cyanosis and/or rubor. It usually consists of painful vascular spasms of the fingers stressed by cold or emotional changes.

Although we know the capability of exercise to improve vascular function (blood flow) in the large arteries in several clinical populations, we still do not know about the effectiveness of exercise in the small arteries in Systemic Sclerosis patients. The mode of exercise (cycling or arm-cranking) may play an important role in improving the blood flow in the small arteries of the fingers in these patients. Hence, the purpose of the study is to examine the effectiveness of exercise through two different modes (cycling and arm-cranking) in Systemic Sclerosis patients.

Am I suitable participant for the study?

We are recruiting men and women aged 18-80 years old, who are diagnosed with SSc experiencing RP for a period between 1 to 10 years being able to perform the planned exercise programme. Patients will be selected according to their medical profile by our collaborator physician. Those with cardiovascular disease, other inflammatory condition than Systemic Sclerosis, current smokers or women in pregnancy

will not be able to participate. The study can be divided in three phases (Table 1). During the study proceedings you have the right to withdraw at any time you wish to.

What will happen if I take part?

If you are eligible for the study and are happy to take part we will arrange for you to attend the Centre for Sport and Exercise Science at Sheffield Hallam University where the baseline measurements will take place (See table 1 below). You will be asked to sign a consent form agreeing to take part in the study. You will be given a copy of your signed consent form and this information sheet to keep.

The study design is a randomised controlled trial which means that you will be allocated to either the exercise groups (cycling or arm-cranking) or to the control group (no exercise) by chance (random).

Exercise group: If you are randomised to the exercise groups you will be asked to attend the Centre for Sport and Exercise Science at Sheffield Hallam University twice for the baseline measurements. We will provide you with instructions on how to get to the centre and where you can park for free. Unfortunately we are unable to pay other travel costs. Afterwards, a training period of 12 weeks will commence where you will be required to attend the gym located at the Centre for Sport and Exercise Science at Sheffield Hallam University two times per week to perform a supervised exercise session. Training hours and dates will be fixed according to your eligibility and in agreement with the personal trainer. Straight after the 12-week training period you will be asked to visit our laboratories at the Centre for Sport and Exercise Science at Sheffield Hallam University twice in order to be assessed in the same tests as prior the exercise intervention (post-exercise intervention measurements-see Table 1).

1st visit: will include questionnaires that will estimate your quality of life, examination of the small arteries of the fingers (non-invasively), body weight and height and a 6 minute walking test. Here you will be asked to walk up and down a corridor and cover as far a distance as possible in 6 minutes. The 6 minute walking test will give us an essential indication about your functional capacity to perform daily activities. The assessment of the small blood vessels in the skin of your fingers function will be performed using a non-invasive test called laser Doppler fluximetry. Two small sticky patches will be applied to the skin of your reference finger. Tiny quantities of two drugs will be administered through these patches (no injections), which will cause local relaxation of the small blood vessels in your finger. Special probes and computer software will be used to measure changes in skin blood flow. This procedure will be performed when you are lying down and relaxed.

2nd visit: The second visit will take place the next or the next few days after the first visit. It involves an examination of the small arteries of the fingers (non-invasively). Moreover, both groups (exercise and control) will be required to perform a maximal oxygen uptake test where it will be performed either

on a cycle ergometer or on an arm crank ergometer. The maximal oxygen uptake test will help us assess your physical fitness, identify reasons related to your disease that might impair your ability to perform exercise and examine the differences before and after the exercise programme on several outcomes.

The maximal oxygen uptake test: involves a procedure where a blood pressure cuff will be attached at your arm during the exercise test on a cycle or arm-crank ergometer to assess the blood pressure at various intervals, a mouthpiece will be placed to measure the inhaled and exhaled oxygen breath by breath. We will also place to your chest some pads connected with the electrocardiogram to assess the electrical signs of your heart during exercise. While you exercise the intensity will increase progressively up to a maximal level which will only be sustained for a few minutes. The whole visit will last roughly 30 to 40 minutes and the actual exercise test will last between 8 to 12 minutes.

Exercise session: Participants assigned to the exercise groups will be invited to undertake 2 sessions of supervised exercise training each week for 12 consecutive weeks at The Centre for Sport and Exercise Science at Sheffield Hallam University. Each session will last approximately 30-40 minutes and will involve 30 minutes of an aerobic individualized exercise protocol performed either on a cycle ergometer or on arm-crank ergometer accompanied by the warm-up (5 minutes) and cool down (5 minutes) period. Patients assigned to the control group will receive basic advice about exercise but no supervised training.

During some of the exercise sessions you will be required to fill in questionnaires relating to your affect and enjoyment of the each protocol and type of exercise. Moreover, a sub-sample of six participants will be randomly chosen from each group in order to be interviewed. The interview will refer to your experience of Raynaud's phenomenon, treatment and advice given, your preference for trial allocation (exercise or control group) as well as your experiences of study participation in both the exercise groups and the control group. You will meet with the researcher face to face at the end of the exercise intervention for about 30-35 minutes.

Before the baseline measurements participants will be randomly allocated into three groups (Group A-exercise group, Group B-exercise group and Group C-control group).

Some participants will be randomly invited to take part in the interview after completing the final exercise session so we can explore your experiences of the exercise programme and study.

Control Group: If you are randomised to the control group you will be asked to attend the Centre for Sport and Exercise Science at Sheffield Hallam University for all the measurements but will not take

part in the exercise intervention. Between the baseline measurements and those after 12 weeks you will be receiving regular calls, approximately once a week, to obtain information about your condition.

Whichever group you are in you will keep taking your normal medical treatment that has been prescribed by your physician for the digital pain you experience.

All the visits will take place at the Centre for Sport and Exercise Science at Sheffield Hallam University.

Table 1: Overview of the study

Visit number	Purpose of visit	Duration of visit
1	**Baseline measurements** Quality of life questionnaires, laser-doppler fluximetry assessments, body weight and height, 6min walking test	60 minutes
2	**Baseline measurements** Laser-doppler fluximetry assessment, maximal oxygen uptake test on cycle or arm ergometer **(8-12 minutes)**.	60 minutes
3-26	**Training sessions (exercise groups only)** • 2 sessions per week • 30 minutes aerobic exercise either on cycle or arm-crank ergometer	35-40 minutes
27	**Post-exercise intervention measurements** Quality of life questionnaires, laser-doppler fluximetry assessments, body composition, 6-MWT.	90 minutes
28	**Post-exercise intervention measurements** Maximal oxygen uptake test on cycle or arm ergometer **(8-12 minutes)**, interview.	60 minutes

What do I have to do?

Before the baseline and post-exercise intervention measurements you will be requested to abstain from vigorous exercise, alcohol, caffeine and tobacco for a period of 24h but also to be at least 2h fasting prior to the assessment as these parameters could affect your responses. We encourage you to wear sport clothing that will allow for a more comfortable movement during the exercise test.

What are the possible benefits of taking part in this study?

This study is being undertaken for research purposes and to advance our knowledge in the mode of exercise that will potentially improve the blood flow in the small arteries of the fingers. It is not known whether exercise will make the condition better, however, people who undertake regular exercise training often become fitter and healthier but also previous research has shown that exercise can improve blood flow in the larger arteries of the body, so you might experience this if you are allocated to the exercise group.

What happens if something goes wrong?

All of the experimental procedures that will be used in this study have been rigorously tested to ensure that they meet health and safety standards. These tests are all routinely and regularly performed on patients and healthy volunteers alike. The researchers who perform the tests are all trained and skilled to do so. If we notice any signs, as regards your health status, that may cause you harm by participating, you will be informed and withdrawn immediately from the study.

Overall the risks of the procedures included in this study are low. The potential risks associated with the microvascular assessments include skin irritation and infection and will be minimised through strict adherence to established protocols, using sterile procedures and carefully prepared pharmacological agents. These sessions will be conducted by appropriately-trained staff.

What if I change my mind during the study?

You are free to withdraw from the study at any time. If you decide to withdraw, we may ask you to consider attending one final assessment, but this is entirely optional. You can choose to leave the study at any time without having any further assessments. We would like to use all of your data up to the point of withdrawal as this will help with our analysis. However, if you would prefer us not to use any of your data you may request for all of your data to be removed from the study. A decision not to carry on with the study will not affect the quality of care you receive in any way.

Will taking in this study be kept confidential?

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held

securely on paper and electronically at Sheffield Hallam University under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the sponsor, who is not involved in the trial. You will be allocated a trial number, which will be used as a code to identify you on all trial forms.

The information collected about you may also be shown to authorised people from the UK Regulatory Authority, the local NHS Trust and Independent Ethics Committee; this is to ensure that the study is carried out to the highest possible scientific standards. All the study research team will have a duty of confidentiality to you as a research participant.

If you withdraw consent from further study involvement, unless you object, your data will remain on file and will be included in the final study analysis.

In line with Good Clinical Practice guidelines, at the end of the study, your data will be securely archived for a minimum of 7 years from the end of the study (the end of the study is defined as the last visit of the last patient in the study). Arrangements for confidential destruction will then be made.

Coded results from the study may be stored indefinitely for subsequent analyses in the future. Any identifying information is kept strictly confidential, and access will be limited strictly to the original study team and database team. Researchers analysing the clinical data in the future will be unable to identify you.

With your permission, your GP, and other doctors who may be treating you, will be notified that you are taking part in this study.

Who will be working on the study?

The researcher in charge is Mr. Alexandros Mitropoulos (PhD student in Clinical Exercise Physiology), supervised by Dr. Markos Klonizakis (Senior Research Fellow in Clinical Physiology) and the leading NHS clinician collaborating to the study is Dr. Mohammed Akil (Consultant Rheumatologist).

What will happen to the results of the study?

Once the study has been completed all data will be anonymised and stored as per current data protection laws. The results will be written up for publication in academic journals and possibly used at academic conferences and will also contribute to a Doctor of Philosophy degree (PhD) completion. Anything with your personal details (name, DOB, contact details etc.) will be kept securely in a locked filing cabinet by the Principal Investigator. Overall study results will also be made available to you on request at the end of the study. Moreover, information provided by the participant will be stored at Sheffield Hallam Research Facilities in Sheffield for further analyses until the end of the project.

What if I have further questions or would like more information about the study?

If you would like more information about the study you are invited to contact:-

Mr. Alexandros Mitropoulos, Sheffield Hallam University tel. 07926126426.

What happens if I have a complaint?

If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study. If you have complaints on concerns please contact the project co-ordinator Dr. Markos Klonizakis Tel 0114 225 5697. Or alternately you can use the normal Trust complaints procedure and contact PALS Advisor, Social Care NHS Foundation Trust, Tel 0114 275 8956. If you require an independent individual to complain about this study through Sheffield Hallam University, you may contact Dr. Donna Woodhouse (Senior Lecturer) Chair Sport Exercise Research Ethics Group and Vice Chair Faculty Research Ethics Committee via email d.woodhouse@shu.ac.uk or by telephone on 0114 225 5670 or by letter Academy of Sport and Physical Activity, Faculty of Health & Wellbeing, Sheffield Hallam University, A225 Collegiate Hall, Collegiate Crescent, Sheffield, South Yorkshire, S10 2BP.

What if I am harmed?

In the event that something does go wrong and you are harmed during the research study, there are no special compensation arrangements. If you are harmed as a result of someone's negligence then you may have grounds for legal action for compensation, but you may have to pay your legal costs.

Who is organizing and funding the research?

This study is being funded by the Sheffield Hallam University and supported by the Sheffield Teaching Hospitals NHS Trsut. The investigators of this study will not receive any payment for conducting this research.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed by London - West London & GTAC Research Ethics Committee, NHS.

Further information/independent advice

If you require any further information or independent advice about this study, please contact the Patient Advice And Liaison Service (PALS) Team Monday-Friday 9am-5pm by telephone on 0114 271 8956, via email on complaints@shsc.nhs.uk or in person at the Patient Advice And Liaison Service (PALS), Fulwood House, Old Fulwood Road, Sheffield, South Yorkshire (by appointment).

Thank you for taking the time to read this information sheet and to consider this study.

Study Team Contact Details

Health and Wellbeing Faculty, Centre of Sport and Exercise Science,

Collegiate Hall, Collegiate Campus, Sheffield, S10 2BP.

Researcher:

Mr Alexandros Mitropoulos

E-mail: alexandros.mitropoulos@student.shu.ac.uk

Tel. 07926126426.

Sheffield Hallam University

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Appendix 7

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ☐

I have slight problems in walking about ☐

I have moderate problems in walking about ☐

I have severe problems in walking about ☐

I am unable to walk about ☐

SELF-CARE

I have no problems washing or dressing myself ☐

I have slight problems washing or dressing myself ☐

I have moderate problems washing or dressing myself ☐

I have severe problems washing or dressing myself ☐

I am unable to wash or dress myself ☐

USUAL ACTIVITIES (e.g. work, study, housework, family or leisure activities)

I have no problems doing my usual activities ☐

I have slight problems doing my usual activities ☐

I have moderate problems doing my usual activities ☐

I have severe problems doing my usual activities ☐

I am unable to do my usual activities ☐

PAIN / DISCOMFORT

I have no pain or discomfort ☐

I have slight pain or discomfort ☐

I have moderate pain or discomfort ☐

I have severe pain or discomfort ☐

I have extreme pain or discomfort ☐

ANXIETY / DEPRESSION

I am not anxious or depressed ☐

I am slightly anxious or depressed ☐

I am moderately anxious or depressed ☐

I am severely anxious or depressed ☐

I am extremely anxious or depressed ☐

Appendix 8

Information for participants Part 2

The feasibility of a combined exercise in people with Systemic Sclerosis.

Introduction

We are inviting you to participate in a research study. Before you decide whether you would like to take part, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with us and/or friends if you wish. If you require more information or any further clarification please do not hesitate to contact us via the details provided. Please take your time to decide whether or not you wish to take part.

Background and purpose of the study

Approximately 50% of patients with Systemic Sclerosis develop decreased blood flow in the fingers and/or wound which can be painful, difficult to heal, susceptible to infections and negatively affect quality of life. Raynaud's phenomenon is a common condition with vasospasm in the small veins and arteries of the fingers causing pallor with cyanosis and/or rubor. It usually consists of painful vascular spasms of the fingers stressed by cold or emotional changes.

It is known that exercise can improve vascular function in the large arteries in several clinical populations, but it is unknown about the effectiveness of exercise to improve vascular function in the small arteries in Systemic Sclerosis patients. The purpose of this study is to examine the effectiveness of a combined exercise (aerobic and weight training) on the potential improvement of blood flow in the fingers in Systemic Sclerosis patients.

Am I suitable participant for the study?

If you have received a letter from us then a Rheumatologist at Royal Hallamshire Hospital on who is part of our research team has considered you eligible to participate in the study. We are recruiting men and women aged 18-80 years old, who have been diagnosed with Systemic Sclerosis experiencing Raynaud's phenomenon for a period between 1 to 10 years. Patients will be selected according to their medical profile by our collaborator physician. Those with cardiovascular disease, other inflammatory

conditions than Systemic Sclerosis, current smokers or pregnant women will not be eligible to participate. The study can be divided in three phases (Table 1). During the study proceedings you have the right to withdraw at any time you wish to.

What will happen if I take part?

If you are eligible for the study and are happy to take part we will arrange for you to attend the Centre for Sport and Exercise Science at Sheffield Hallam University where the baseline measurements will take place (See table 1 below). You will be asked to sign a consent form agreeing to take part in the study. You will be given a copy of your signed consent form and this information sheet to keep.

The study design is a randomised controlled trial which means that you will be allocated to either the exercise or control group by chance (random).

Exercise group: If you are randomised to the exercise group you will be asked to attend the Centre for Sport and Exercise Science at Sheffield Hallam University twice for the baseline measurements. We will provide you with instructions on how to get to the centre and where you can park for free. Unfortunately we are unable to pay other travel costs. Afterwards, a training period of 12 weeks will commence where you will be required to attend the gym located at the Centre for Sport and Exercise Science at Sheffield Hallam University two times per week to perform a supervised exercise session. Training hours and dates will be fixed according to your eligibility and in agreement with the personal trainer. Straight after the 12-week training period you will be asked to visit our laboratories twice in order to be assessed in the same tests as prior the exercise intervention (post-exercise intervention measurements-See Table 1). Following these assessments two more visits will take place at the Centre for Sport and Exercise Science at Sheffield Hallam University 3 and 6 months after the end of the exercise training period where we will re-assess you on tests as in the baseline measurements (see Table 1).

1st visit: will include questionnaires that will estimate your quality of life, examination of the small arteries of the fingers (non-invasively), body weight and height and a 6 minute walking test. Here you will be asked to walk up and down a corridor and cover as far a distance as possible in 6 minutes. The 6 minute walking test will give us an essential indication about your functional capacity to perform daily activities. The assessment of the small blood vessels in the skin of your fingers function will be performed using a non-invasive test called laser Doppler fluximetry. Two small sticky patches will be applied to the skin of your reference finger. Tiny quantities of two drugs will be administered through these patches (no injections), which will cause local relaxation of the small blood vessels in your

finger. Special probes and computer software will be used to measure changes in skin blood flow. This procedure will be performed when you are lying down and relaxed.

2nd visit: The second visit will take place the next or the next few days after the first visit. It involves an examination of the small arteries of the fingers (non-invasively). Moreover, both groups (exercise and control) will be required to perform a maximal oxygen uptake test where it will be performed either on a cycle ergometer or on an arm crank ergometer. The maximal oxygen uptake test will help us assess your physical fitness, identify reasons related to your disease that might impair your ability to perform exercise and examine the differences before and after the exercise programme on several outcomes.

The maximal oxygen uptake test: involves a procedure where a blood pressure cuff will be attached at your arm during the exercise test on a cycle or arm-crank ergometer to assess the blood pressure at various intervals, a mouthpiece will be placed to measure the inhaled and exhaled oxygen breath by breath. We will also place to your chest some pads connected with the electrocardiogram to assess the electrical signs of your heart during exercise. While you exercise the intensity will increase progressively up to a maximal level which will only be sustained for a few minutes. The whole visit will last roughly 30 to 40 minutes and the actual exercise test will last between 8 to 12 minutes.

Exercise session: Participants assigned to the exercise groups will be invited to undertake 2 sessions of supervised exercise training each week for 12 consecutive weeks at The Centre for Sport and Exercise Science at Sheffield Hallam University. Each session will last approximately 60-70 minutes and will involve 30 minutes of an aerobic individualized exercise protocol and 30 minutes of weight training accompanied by the warm-up and cool down period. Patients assigned to the control group will receive basic advice about exercise but no supervised training.

During some of the exercise sessions you will be required to fill in questionnaires relating to your affect and enjoyment of each protocol and type of exercise. Moreover, a sub-sample of seven participants will be randomly chosen from each group in order to be interviewed regarding your experience of Raynaud's phenomenon, treatment and advice given, your preference for trial allocation (exercise or control group) as well as your experiences of study participation in both the exercise groups and the control group. You will meet with the researcher face to face at the end of the exercise intervention for about 30-35 minutes.

Before the baseline measurements participants will be randomly allocated into two groups (Group A- exercise group and Group B- control group).

Some participants will be randomly invited to take part in the interview after completing the final exercise session so we can explore your experiences of the exercise programme and study.

Control Group: If you are randomised to the control group you will be asked to attend the Centre for Sport and Exercise Science at Sheffield Hallam University for all the measurements but will not take part in the exercise intervention. Between the baseline measurements and those after 12 weeks you will be receiving regular calls, approximately once a week, to obtain information about your condition.

Whichever group you are in you will keep taking your normal medical treatment that has been prescribed by your physician for the digital pain you experience.

All the visits will take place at the Centre for Sport and Exercise Science at Sheffield Hallam University.

Table 1: Overview of the study

Visit number	Purpose of visit	Duration of visit
1	Baseline measurements Quality of life questionnaires, laser-doppler fluximetry assessments, body weight and height, 6min walking test	60 minutes
2	Baseline measurements Laser-doppler fluximetry assessment, maximal oxygen uptake test on cycle or arm ergometer (8-12 minutes).	60 minutes
3-26	Training sessions (exercise group only) 2 sessions per week 30 minutes of aerobic exercise and 30 minutes of weight training.	60 minutes
27	Post-exercise intervention measurements Quality of life questionnaires, laser-doppler fluximetry assessments, body weight and height, 6min walking test.	90 minutes

28	Maximal oxygen uptake test on cycle or arm ergometer (8-12 minutes), interview.	60 minutes
29	3 months post-exercise intervention Quality of life questionnaires, laser-doppler fluximetry assessments, 6min walking test, interview.	90 minutes
30	6 months post-exercise intervention Quality of life questionnaires, laser-doppler fluximetry assessments, 6min walking test, interview.	90 minutes

What do I have to do?

Before the baseline and post-exercise intervention measurements you will be requested to abstain from vigorous exercise, alcohol, caffeine and tobacco for a period of 24h but also to be at least 2h fasting prior to the assessment as these parameters could affect your responses. We encourage you to wear sport clothing that will allow for a more comfortable movement during the exercise test.

What are the possible benefits of taking part in this study?

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All of the experimental procedures that will be used in this study have been rigorously tested to ensure that they meet health and safety standards. These tests are all routinely and regularly performed on

patients and healthy volunteers alike. The researchers who perform the tests are all trained and skilled to do so. If we notice any signs, as regards your health status, that may cause you harm by participating, you will be informed and withdrawn immediately from the study.

Overall the risks of the procedures included in this study are low. The potential risks associated with the microvascular assessments include skin irritation and infection and will be minimised through strict adherence to established protocols, using sterile procedures and carefully prepared pharmacological agents. These sessions will be conducted by appropriately-trained staff.

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Once the study has been completed all data will be anonymised and stored as per current data protection laws. The results will be written up for publication in academic journals and possibly used at academic conferences and will also contribute to a Doctor of Philosophy degree (PhD) completion. Anything with your personal details (name, DOB, contact details etc.) will be kept securely in a locked filing cabinet by the Principal Investigator. Overall study results will also be made available to you on request at the end of the study. Moreover, information provided by the participant will be stored at Sheffield Hallam Research Facilities in Sheffield for further analyses until the end of the project.

What if I have further questions or would like more information about the study?

If you would like more information about the study you are invited to contact:-

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What happens if I have a complaint?

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Faculty Research Ethics Committee via email d.woodhouse@shu.ac.uk or by telephone on 0114 225 5670 or by letter Academy of Sport and Physical Activity, Faculty of Health & Wellbeing, Sheffield Hallam University, A225 Collegiate Hall, Collegiate Crescent, Sheffield, South Yorkshire, S10 2BP.

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Further information/independent advice

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Thank you for taking the time to read this information sheet and to consider this study.

Study Team Contact Details

Health and Wellbeing Faculty, Centre of Sport and Exercise Science,

Collegiate Hall, Collegiate Campus, Sheffield, S10 2BP.

Researcher: Mr Alexandros Mitropoulos

E-mail: alexandros.mitropoulos@student.shu.ac.uk Tel. 07926126426.

Health Research Authority

Appendix 9

Mr Alexandros Mitropoulos

PhD student in Health and Well Being Faculty Email: hra.approval@nhs.net Sheffield Hallam University

Collegiate Cresent, Sheffield, South Yorkshire
Chestnut court

S10 2BP

2nd June 2016

Dear Mr Mitropoulos,

Letter of HRA Approval

Study title:	**Investigating the effectiveness and feasibility of exercise on microcirculatory parameters and quality of life in systemic sclerosis patients.**
IRAS project ID:	**68096**
REC reference:	**16/LO/0811**
Sponsor	**Sheffield Hallam University**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

1. *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities

2. *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.

3. *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm

4. capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

A – List of documents reviewed during HRA assessment

B – Summary of HRA assessment

After HRA Approval

The document *"After Ethical Review – guidance for sponsors and investigators",* issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

Registration of research

Notifying amendments

Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.

Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.

The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please email the HRA at hra.approval@nhs.net.

Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **68096**. Please quote this on all correspondence.

Yours sincerely

Miss Lauren Allen

Assessor

Email: hra.approval@nhs.net

Copy to: *Mr Brian Littlejohn (Sponsor contact)*

Mrs Aimee Card, Sheffield Teaching Hospitals NHS Foundation Trust (Lead NHS R&D contact)

IRAS project ID	68096

HRA Approval - List of Documents

The final document set assessed and approved by HRA Approval is listed below.

Document	*Version*	*Date*
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [PL confirmation of cover]	v1	11 April 2016
GP/consultant information sheets or letters [letter for GP_part 1]	v1	25 February 2016
GP/consultant information sheets or letters [letter for GP_part 2]	v1	26 February 2016
Instructions for use of medical device [Laser Doppler Probes-perimed]	v1	14 April 2016
Instructions for use of medical device [Iontophoresis manual]	v1	14 April 2016
Letters of invitation to participant [Invitation letter]	version 3	26 April 2016
Other [LDF safety cover letter]	v1	14 April 2016
Other [Amendments_NHS ethics review]	v1	24 April 2016
Other [Amendments_NHS ethics review]	version 2.0	26 April 2016
Other [Statement of Activities]	1.0	19 May 2016
Other [PI signature]		
Other [Schedule of Events]	2	27 May 2016
Participant consent form	3.0	28 April 2016
Participant information sheet (PIS) [Part 1]	3.0	26 April 2016
Participant information sheet (PIS) [Part 2]	3.0	26 April 2016

REC Application Form [REC_Form_13042016]		13 April 2016
Referee's report or other scientific critique report [research ethics review 1]	v1	09 February 2016
Referee's report or other scientific critique report	v1	11 February 2016
Referee's report or other scientific critique report [letter of confirmation of ethics review]	v1	08 April 2016
Research protocol or project proposal [systemic sclerosis protocol]	v1	11 February 2016
Summary CV for Chief Investigator (CI) [MITROPOULOS CV]	v1	12 April 2016
Summary CV for student [MITROPOULOS CV]	v1	11 April 2016
Summary CV for supervisor (student research) [short-cv-Klonizakis]	v1	12 February 2016
Summary, synopsis or diagram (flowchart) of protocol in non technical language [diagram of research protocol]	v1	11 April 2016
Validated questionnaire [intentions for engagement to exercise]	v1	11 April 2016
Validated questionnaire [exercise task self efficacy]	v1	11 April 2016
Validated questionnaire [physical activity enjoyment scale]	v1	11 April 2016
Validated questionnaire [EQ-5D-5L]		24 April 2016