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Exploring the microcirculatory effects of an exercise programme including aerobic and resistance training in people with limited cutaneous systemic sclerosis.

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Abstract

Purpose of the study: High intensity interval training (HIIT) is able to improve the endothelial-dependent microvascular function is people with limited cutaneous systemic sclerosis (lcSSc). Resistance training (RT) alone has shown significant improvements in the function of the vasculature; moreover, a combination of aerobic and RT have shown both in the past and recently to significantly improve the vascular function and the microcirculation. Therefore, the purpose of this study is to explore the effectiveness of a combined exercise protocol (aerobic and resistance training) on microvascular function in people with lcSSc.
Methods: Thirty-two lcSSc patients (66.5 ± 12 years old) were randomly allocated in two groups (exercise and control group). The exercise group underwent a 12-week exercise programme twice per week. All patients performed the baseline, three- and six-month follow up measurements where microvascular function, transcutaneous oxygen tension (ΔTcpO2) and body composition were assessed.
Results: The time to peak endothelial-dependent reactivity was significantly improved (91 ± 42 sec, d= 1.06, p = 0.007) when compared to control group after the exercise intervention. Endothelial-independent function was also significantly improved (3.16 ± 2, d=1.17, p = 0.005) when compared to the control group. Baseline (5.71 ± 4.4, $p < 0.05$)) and peak (15.4 ± 7.5, $p < 0.05$) transcutaneous oxygen pressure were also significantly improved compared to the control group

Conclusions: Our results suggest that a combined exercise protocol (aerobic and RT) was effective in improving endothelial-dependent reactivity in people with lcSSc. The next step would be to explore its clinical- and cost- effectiveness. Therefore, we recommend a large, community-based intervention against standard pharmacotherapy only, which would assess these important factors and support a change in therapeutic protocols and guidelines for this clinical population.

Trial registration: ClinicalTrials.gov (NCT number): NCT03058887, February 23, 2017, https://clinicaltrials.gov/ct2/show/NCT03058887?term=NCT03058887&rank=1

Key words: High intensity interval training, resistance training, vascular function, LDF, TcPO2.

Introduction

Raynaud's phenomenon (RP) is one of the first clinical manifestations observed in systemic sclerosis (SSc). This microvasculature disorder (Koch & Distler, 2007) 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.

A recent study from our research team (Mitropoulos et al., 2018a), 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., 2001) 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.

In particular for people with SSc, exploring the mechanisms behind the exercise-induced changes of an intervention combining HIIT and RT could lead to a better understanding of the pathophysiology of the condition, as well as offering adjunct therapies, which could be implemented in a wider scale, across the community.

To fill this knowledge gap, we implemented an intervention combining HIIT, RT and standard pharmacotherapy. 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 ($TcpO_2$).

Methods

Participants

We recruited thirty-two people (29 women, 3 men) with lcSSc, defined as per the American College of Rheumatology and European league against rheumatism criteria (Hoogen et al., 2013). Eligible patients were recruited from the Rheumatology Department of the Royal Hallamshire Hospital in Sheffield. All patients provided written consent to participate. The London - West London & GTAC NHS Research Ethics Committee approved the study protocol and the study complies with the Declaration of Helsinki. Patients were randomly allocated (block randomisation) between the exercise (n = 16), and control (n = 16) groups. The randomisation was performed by an independent statistician. The allocated group was announced to both the principal investigator and the participant only after the completion of the baseline measurements. All the pre- and post-intervention tests were performed at the same time of the day to minimize intra-day variability. An extensive methods section for our

study has been published elsewhere alongside the full study protocol (Mitropoulos et al., 2018b).

Exercise programme

The exercise group undertook twice-weekly supervised exercise sessions at three different Sheffield sport venues: a) Centre of Sport and Exercise Science of Sheffield Hallam University, b) Graves sport centre and c) Concord sport centre. The HIIT protocol has been presented previously (Mitropoulos et al., 2018a). 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) 6-20 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. No adverse effects were reported.

Procedures

Baseline assessments, undertaken at first visit, included peak oxygen uptake ($\dot{V}O_{2peak}$), anthropometry and microvascular reactivity. $\dot{V}O_{2peak}$ 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 whereas the control group did not perform the exercise programme. 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. 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; Mitropoulos 2018a).

Study 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 2.

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). Heart rate (HR) and electrocardiogram were continuously monitored using a Polar HR monitor (Polar FS1, Polar Electro, Kemple, Finland) and a 12-lead ECG device (Case, GE Healthcare, USA). 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 6-20 point scale. 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.

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 $\geq$ 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 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).

Overall data 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 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 $p \leq 0.05$. Data analysis was conducted at the end of the data collection.

Results

Oxygen uptake and pressure
$\dot{V}O_{2peak}$ was significantly greater in the exercise group (25.6 ± 7.2 ml kg^{-1} min^{-1}) compared to the control group after the exercise intervention (Table 3).
ΔTcpO2 (5.71 ± 4.4) and $\Delta TcpO2_{max}$ (15.4 ± 7.5) were also significantly improved compared to the control group (Table 3).

Cutaneous vascular conductance
As shown in Table 3 the endothelial-dependent function did not demonstrate any significant improvement in regard to microvascular reactivity. However, a statistically-significant improvement in the time to peak endothelial-dependent reactivity (91 ± 42 sec, d= 1.06, p = 0.007) was observed in comparison to the control group following the exercise intervention. Moreover, ACh (e.g., endothelial-dependent) Tmax 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 ± 2.0, p = 0.005, d= 1.17) compared to the control group. Four participants out of sixteen in the control group were presented digitals ulcers during the study and required hospitalisation.

Discussion

This is the first study to explore the microcirculatory effects of an exercise intervention, combining HIIT and RT, being used as an adjunct therapy to standard pharmacotherapy in people with lcSSc. The current study builds upon our previous work in this population (Mitropoulos et al., 2018a), aiming to provide important evidence, which would ultimately lead to a change in the therapeutic pathway for this clinical population.

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 (3). 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. 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 et al., 2018). 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 et al., 2018).

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., 2001) 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 (Thijssen et al., 2011). 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.

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 (Silva 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.

Clinical outcome

In our study four out of sixteen patients in the control group developed digital ulcers and required hospitalization for iloprost infusion (Vitielo et al., 2012; 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 mid- dle finger in one hand. Hospitalization is a psychologic- ally-stressful procedure for the patient, which directly affects QoL. 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 ischaemia in 20.4% of patients with SSc, 9.2% of which have multiple digit loss (Wigley et al., 1992). QoL in patients with SSc is adversely affected due to digital ischaemia. Consequently, our protocol has demonstrated that is capable of improving digital ischae- mia and preventing disease progression and digital ul- cers and thus, improving QoL.

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 4 (Byrne, 1984). The systemic factors can be improved through aerobic fitness and this could lead to an improvement of subcutaneous oxygen pressure (Hoppeler et al., 1985; Andersen & Henriksson, 1977). From the local factors, oxygen consumption of skin and capillary formation and density can also be significantly improved through aerobic fitness (Hoppeler et al., 1985; Andersen & Henriksson, 1977) which will lead to an improvement of TcpO2. However, skin thickness and skin inflammation or oedema are mainly affected by the disease severity in SSc and worsening of these two factors lead to a decrease in TcpO2. The analysis between the interactions of the different factors presented is beyond the scope of the manuscript and would require further data collection and data analysis, which couldn't be undertaken during the course of this study. It is, however, our intention, should the study progress in a multi-centre phase, to undertake the necessary data collection, which would support a clinically-meaningful analysis.

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., 2018a). 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 fiber and in the number and size of mitochondria in skeletal muscles (Holloszy, 2008; Laughlin & Roseguini, 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 & Henriksson, 1977). Interval exercise is able to induce increases in the expression of several angiogenesis-related mRNAs, including VEGF expression (34).

An initial increase in $\dot{V}O_{2peak}$ is usually observed as early as 2-4 weeks after initiating training (Andersen & Henriksson, 1977, 35) but it can also increase after 1 week (Henriksson & Reitman, 1976). 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; Ekblom et al., 1968). 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 fiber hypertrophy, induces alterations in the mechanisms responsible for the transport and utilization of oxygen. These mechanisms involve an increase in capillary density per fiber and an increase in the oxidative capacity of the muscle cell, as presented by an elevated citrate synthase activity (Frontera et al., 1990). 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-Arenas 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) (Romero-Arenas et al., 2013). 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 $TcpO_2$.

Experimental considerations

The relatively small sample size may be considered as a limitation for this study: nevertheless, Systemic Sclerosis is a relatively rare disease, with approx. 20000 people living with the condition in the U.K. (Royle et al., 2018). Therefore, it was not possible to recruit more widely within our study period and with the limited funding that we had. Also

due to funding restrictions we were unable to follow-up participants for more than 6 months – however, this is longer than most published interventional studies that explore microvascular adaptations. Finally, as this was a non-invasive study, we were unable to obtain data exploring the mechanisms underpinning the observed differences and changes in microvascular responsiveness to ACh and SNP. Regarding the latter, further research is warranted to determine the extent to which vascular structural changes in venous disease influence the non-invasive estimates of microvascular function and their clinical significance.

Conclusion

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 resulted in greater results for microvascular function, assessed by both LDF and TcPO2. Therefore, the addition of RT to the overall training load seems physiologically beneficial. The next step would be to explore its clinical- and cost-effectiveness. Therefore, we recommend a large, community-based intervention against standard pharmacotherapy only, which would assess these important factors and support a change in therapeutic protocols and guidelines for this clinical population.

Abbreviations

ACh: Acetylcholine; ACR: American college of rheumatology; BMI: Body mass index; CG: Control group; dcSSc: diffuse cutaneous systemic sclerosis; DUs: Digital ulcers; EG: Exercise group; EPCs: Endothelial progenitor cells; EULAR: European league against rheumatism; HIF-1a: hypoxia-induced factor 1-alpha; HIIT: High intensity interval training; HR: Heart rate; KCI: Potassium chloride; lcSSc: limited cutaneous systemic sclerosis; LDF: Laser Doppler fluximetry; L-NAME: N^G-nitro-L-arginine-methyl-ester; NO: nitric oxide; PPO: Peak power output; RM: Repetition maximum; RP: Raynaud's phenomenon; RPE: Rate of perceived exertion; RT: Resistance training; SNP: sodium nitroprusside; SSc: Systemic sclerosis; $TcpO_2$: Transcutaneous oxygen pressure; T_{max}: Peak vascular response in relation to time; VEGF: Vascular endothelial growth factor; $\dot{V}O_{2peak}$: Peak oxygen uptake.

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Declaration of interest

None.

Authors' contributions

AM helped to draft the manuscript, designed the exercise intervention, contributed to the study design and critically reviewed and revised the manuscript for important intellectual content. HC developed the qualitative aspects of the study, contributed to the study design and critically reviewed and revised the manuscript for important intellectual content. MA is the study's clinical lead, contributed to the study design and critically reviewed and revised the manuscript for important intellectual content. MK is the project leader and helped to draft the manuscript, contributed to the study design and critically reviewed and revised the manuscript for important intellectual content. All authors read and approved the final manuscript for publication.

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Tables

Table 1 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

Table 2 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	0/16	0/32
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

*$p < 0.05$

Table 3 Physiological 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	-

Endothelial function presented as cutaneous vascular conductance (CVC). *$p < 0.05$.

ACh and SNP CVC present the blood flow in the digital microcirculation and an increase in these outcomes indicate an improvement in vascular function. Similarly, with ΔTcpO2 and V̇O2peak.

ACh and SNP Tmax depict the time taken to reach peak perfusion and a decrease in these values indicate an improvement in the vascular reactivity to external stimulus.

A. Systemic

1. Arterial oxygen content
a. Inspired oxygen concentrations
b. Ventilation
c. Lung function
d. Haemoglobin level
e. Haemoglobin saturation
f. Haemoglobin affinity for oxygen

B. Local

1. Skin thickness
2. Capillary formation and density
3. Oxygen consumption of skin
4. Inflammation, oedema, etc.

Table 4. Factors Affecting Tissue Oxygen Tension