Aerobically trained older adults show impaired resting, but preserved exercise-induced circulating progenitor cell count, which was not improved by sprint interval training

Zerbu Yasar[1*], Mark D Ross[2], Christopher J Gaffney[3], Ruth D Postlethwaite[4], Russell Wilson[2], Lawrence D Hayes[5]

[1]Active Ageing Research Group, Institute of Health, University of Cumbria, Lancaster, UK; [2]School of Applied Sciences, Edinburgh Napier University, Edinburgh, UK; [3]Lancaster Medical School, Faculty of Health and Medicine, Lancaster University, Lancaster, UK; [4]Faculty of Health and Life Sciences, Coventry University, Coventry, UK; [5]Institute of Clinical Exercise and Health Science, School of Health and Life Sciences, University of the West of Scotland, Glasgow, UK

***Correspondence:**
Zerbu@hotmail.co.uk

Keywords: Ageing, endothelial, endothelial progenitor cells, HIIT, sprint, vascular

ABBREVIATIONS

ANOVA: analysis of variance

BLa: blood lactate

BMI: body mass index

CPC: circulating progenitor cell

CV: cardiovascular

EPC: endothelial progenitor cell

HIIT: high-intensity interval training

N_2: nitrogen

O_2: oxygen

PPO: peak power output

RER: respiratory exchange ratio

RPE: rating of perceived exertion

SD: standard deviation

SIT: sprint interval training

VO_2: oxygen uptake

VO_{2peak}: peak oxygen uptake

ABSTRACT

Purpose: Older adults exhibit reduced number and function of CD34+ circulating progenitor cells (CPC), a known risk factor for cardiovascular disease. Exercise promotes mobilisation of CPCs from bone marrow so whether aging *per se* or physical inactivity in older age reduces CPCs is unknown. Thus, this study examined the effect of age on resting and exercise-induced changes in CPCs in aerobically trained adults, and the effect of 8-weeks of sprint interval training (SIT) on resting and exercise-induced CPCs in older adults.

Methods: Twelve young (22-34 years) and nine older (63-70 years) adults participated in the study. Blood was sampled pre, and immediately post a graded exercise test to exhaustion in both groups. Older participants repeated the process after 8 weeks of SIT (3 x 20 s 'all-out' sprints, 2 x a week). Total CPCs ($CD34^+$) and endothelial progenitor cells (EPCs: $CD34^+KDR^+$) were determined by flow cytometry.

Results: Older adults exhibited lower basal total $CD34^+$ CPCs (828 ± 314 vs. 1186 ± 272 cells·mL^{-1}, $p = 0.0149$) and $CD34^+KDR^+$ EPCs (177 ± 128 vs. 335 ± 92 cells·mL^{-1}, $p = 0.007$) than younger adults. The maximal exercise test increased CPCs in young ($CD34^+$: $p = 0.004$; $CD34^+KDR^+$: $p = 0.017$) and older adults ($CD34^+$: $p < 0.001$; $CD34^+KDR^+$: $p = 0.008$), without difference between groups ($p = 0.211$). SIT did not alter resting or exercise-induced changes in CPCs in the older cohort ($p > 0.232$).

Conclusion: This study suggests age *per se* does not impair exercise-induced CPC counts, but does lower resting CPC counts.

1 INTRODUCTION

Advancing age is associated with increased risk of onset and progression of cardiovascular disease (CVD) (Lozano et al., 2012), often attributed to cormorbidities such as hypertension (Rapsomaniki et al., 2014; Tran et al., 2018), diabetes and hyperlipidemia (Tran et al., 2018). Advancing age is also associated with reduced endothelial function (Black et al., 2008)and vascular reparative capacity, indicated by reduced circulating progenitor cell (CPC) count and function (Heiss et al., 2005; Thum et al., 2007; Hao Xia et al., 2012). These CPCs, defined as $CD34^+$ progenitors, offer regenerative benefit to the vascular endothelium, by taking part in endothelial repair by paracrine means (Landers-Ramos et al., 2015). Studies have demonstrated that individuals with lower resting number of these cells are at a greater risk of cardiovascular and all-cause mortality (Patel et al., 2015; Muggeridge et al., 2021), therefore, increasing CPC number and function may be of clinical significance.

Exercise acutely mobilises CPCs into the peripheral blood compartment in the recovery period post-exercise (van Craenenbroeck et al., 2008; Ross et al., 2014) and is intensity- and duration-dependent (Laufs et al., 2005). This is thought to be due to mobilisation from bone marrow, promoting CPC migration from the bone marrow niche, and into circulation, where these cells exert their vaso-reparative function. Interestingly, the extent to which CPCs are increased in response to an exercise stressor is associated with future cardiovascular (CV) risk in CVD patients (Moazzami et al., 2020), with a blunted response associated with increased risk of adverse events. Previous work has also demonstrated older adults display attenuated CPC response to submaximal exercise compared to younger individuals (Ross et al., 2018), therefore interventions may be required to not only promote the resting number of CPCs, but also the exercise-responsiveness, which may be related to bone marrow resident number, and capillarity of the bone marrow to allow for more CPCs to enter the circulation.

Sprint interval training (SIT) is a novel, time-efficient, mode of exercise which is known to promote markers of cardiometabolic health, such as aerobic capacity (Gillen et al., 2016; Martin-Smith et al., 2019; Yasar et al., 2021), leanness (Hazell et al., 2014; Naves et al., 2018), and lowered fasting blood glucose (Adams, 2013). One study has shown that SIT in young, healthy women was effective at increasing $CD34^+$ CPC resting number, but not function (Harris et al., 2014). Therefore, SIT may be an effective intervention for promoting changes in CPC counts in older adults who demonstrate lower resting numbers, which may subsequently improve vascular repair capacity, and reduce future CVD risk. However, one difficulty is discerning the effect of age specifically on CPCs (or any physiological parameter) is the age-associated reduction in physical activity (Milanović et al., 2013; Takagi et al., 2015; Trombetti et al., 2015). As such, it is important to differentiate the effect of age, rather than age *in addition* to years of reduced physical activity on physiological parameters, and in this case CPCs. In this context, we believe it important to match participants for fitness or physical activity to truly examine the effect of age on CPCs.

The present investigation aimed to examine the effect of age on resting and exercise-induced changes in CPCs in aerobically trained young and older individuals. A secondary aim was to examine the effect of a novel SIT stimuli in the older group on CPCs (both basal, and exercise-induced changes). It was hypothesised *a priori* that older adults would display a lower number of resting CPCs, an attenuated CPC rise in response to a maximal exercise stressor, and that an 8-week SIT protocol would recover resting and exercise-induced changes in CPCs to that similar of the younger cohort.

2 MATERIALS AND METHODS

2.1 Participants

Two cohorts were recruited for this study, younger (n = 12, 28 ± 5 years of age, body mass index [BMI]: 24.5 ± 2.2 $kg \cdot m^2$) and older (n = 9, 67 ± 3 years of age, BMI: 22.5 ± 2.0 $kg \cdot m^2$) adults, who regularly participated in a weekly minimum of 150 $min \cdot wk^{-1}$ of moderate or high intensity exercise for at least 6 months prior to participating in the study and continued habitual physical activity for the duration of the study. The older females in the study were post-menopausal. Participants were free of exercise contraindicating disease (metabolic, cardiovascular, and renal) or injury as determined by a Physical Activity Readiness Questionnaire (PAR-Q) and American College of Sports Medicine (ACSM) pre-exercise participation screening, without any requests for medical clearance submitted within the cohort (Riebe et al., 2015). This study was carried out in accordance with the Declaration of Helsinki and approved by the University of Cumbria Research Ethics Committee. Written informed consent was obtained from all participants prior to study commencement and subjects were excluded if they presented with atrial fibrillation. Descriptive statistics for participants are shown in **Table 1**, and further

described in the results section. Participants attended all sessions with exercise suitable clothing and footwear. The younger cohort attended a single test session whilst the older cohort attended two separate testing sessions; before (pre) and five days after the final training session of the 8-week SIT intervention (post) (**Fig 1**). Participants were fasted overnight before all testing sessions, breaking their fast only after the testing session. As this study was a secondary analysis (primary outcome: muscle power), no *a priori* power calculation was performed specific to CPCs.

Table 1. Participant characteristics with t-test alpha values for baseline comparisons between young and older participants, and pre- to post-sprint interval training comparisons.

	Young	**Older pre-SIT**	**Older post-SIT**	**Young vs Older pre-SIT**	**Older pre-SIT vs post-SIT**
Age (years)	28 ± 5	67 ± 3			
Sex (% female)	8%	22%			
Height (cm)	179 ± 7	174 ± 11			
Body Mass (kg)	78.5 ± 7.6	68.3 ± 10.4	67.9 ± 9.1	p = 0.008	p = 0.082
BMI ($kg \cdot m^2$)	24.4 ± 2.2	22.5 ± 2.0	22.4 ± 1.7	p = 0.052	p = 0.107
Systolic Blood Pressure (mmHg)	123 ± 7	125 ± 13	125 ± 16	p = 0.299	p = 0.465
Diastolic Blood Pressure (mmHg)	72 ± 7	73 ± 6	72 ± 6	p = 0.286	p = 0.085
Mean Arterial Pressure (mmHg)	89 ± 6	91 ± 7	89 ± 7	p = 0.246	p = 0.299
$\dot{V}O_{2max}$ ($ml \cdot kg \cdot min^{-1}$)	51.6 ± 12.6	37.4 ± 7.6	39.5 ± 8.7	p = 0.003	p = 0.113

Values shown are mean ± SD. BMI=Body Mass Index, $\dot{V}O_2max$=Maximum Oxygen Uptake.

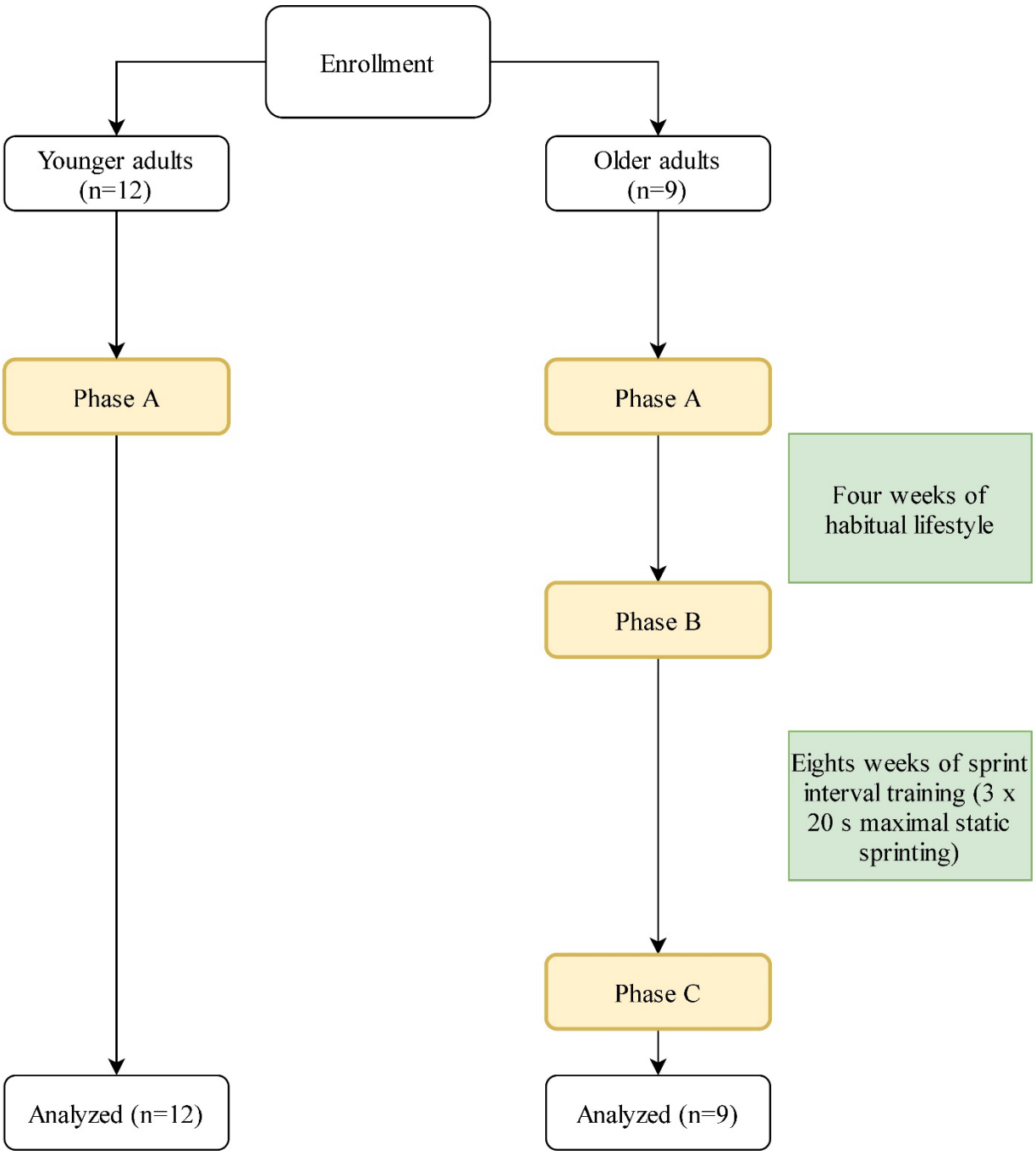

Fig 1 Schematic representation of the methodological flow. PPO = peak power output. $\dot{V}O_{2max}$ = maximal oxygen uptake.

2.2 Blood draws and analysis

Participants arrived at the exercise physiology laboratory between 08.00–11.00 h, following an overnight fast and having abstained from strenuous physical activity for a minimum of 48 h. Participants were reminded to maintain standardised conditions prior to each assessment point which included arriving in a hydrated state having abstained from caffeine and alcohol consumption for 24 h. Following 20 min supine rest, blood was sampled from the antecubital vein using standard venepuncture method into sterile TransFix® K3EDTA vacutainer tubes (TransFix, Cytomark Ltd, UK). These tubes contained Tranfix® solution which preserved cell antigens on mononuclear cell subsets for delayed flow cytometric analysis. The use of this preserving solution for progenitor cell analysis has been validated for flow cytometric analysis of

samples for up to 7 days post-collection (Hoymans et al., 2012). Blood samples were collected at the same time of day for each participant to control for biological variation and minimise within-participant variation (Hayes et al., 2014). Resting blood draws were completed prior to any exercise testing.

2.3 Anthropometry

Height was measured to the nearest 0.1 cm, and mass to the nearest 0.01 kg using a Seca 286 measuring station (Birmingham, UK), from which body mass index (BMI) was derived by dividing mass by the square of height ($kg \cdot m^2$).

2.4 Peak power output (PPO)

PPO was established using the 6 s Herbert test (Herbert et al., 2015) on an air-braked cycle ergometer (Wattbike Ltd., Nottingham, UK), which consisted of a maximal 6 s sprint from a standing start.

2.5 Maximal oxygen uptake ($\dot{V}O_{2max}$)

At least five min after PPO determination, $\dot{V}O_{2max}$ was determined using a Cortex II Metalyser 3B-R2 (Cortex, Biophysik, Leipzig, Germany). Expiratory airflow was achieved using a volume transducer (Triple V® turbine, digital) connected to an oxygen (O_2) analyser. Expired gases were analysed for O_2 with electrochemical cells and for carbon dioxide (CO_2) output with an infrared analyser. The Metalyser was calibrated according to manufacturer's guidelines prior to each test. After a 60 min warm-up period, the O_2 and CO_2 sensors were calibrated against environmental air in addition to reference gas of known composition (5% CO_2, 15% O_2, and 80% N_2) with volume calibrated by five inspiratory and expiratory strokes using a 3 L pump. Prior to determination of $\dot{V}O_{2max}$, a chest strap heart rate monitor was attached to participants' chests, with heart rate measured continuously throughout the test (Polar F1, Polar, Finland). The cycle ergometer (Wattbike Pro, Wattbike, UK) was adjusted to manufacturer's guidance. Saddle height was adjusted relative to the crank position and the foot was secured to a pedal with straps with participants' knee at almost full extension (~170°). Participants mounted the cycle ergometer, and a rubber face mask was fitted (Hans Rudolph Inc, USA), which was attached to the Cortex II Metalyser 3B-R2. $\dot{V}O_2$ and $\dot{V}CO_2$ were recorded continuously throughout the test.

Prior to the graded exercise test to exhaustion, participants completed a 3 min warm-up at an intensity equivalent to ~10% of PPO. Subsequently, participants cycled at increasing intensity with 25 $W \cdot min^{-1}$ increments until they reached volitional exhaustion, with rating of perceived exertion (RPE; 0-10 scale) (Foster et al., 2001) recorded in the last 10 s of each stage. Immediately following volitional exhaustion, participants had their index finger cleaned using a disinfectant wipe, and then a lancet lacerated a fingertip to obtain a blood sample for to measure blood lactate concentration [BLa] (Lactate Pro 2, Arkray, Japan). $\dot{V}O_{2max}$ was confirmed when participants achieved a minimum of any four of the following criteria; $\dot{V}O_2$ plateau, RER ≥1.10, peak heart rate within 10 beats of age predicted maximum, [BLa] ≥8 $mmol \cdot L^{-1}$, and final RPE of ≥9.

2.6 Flow cytometry

Flow cytometric analyses were performed on Tranfix® (Cytomark Ltd, UK) within 7 days post-blood sample collection. Briefly, 100 µL of whole blood was incubated with fluorescent antibodies against known cell surface antigens for determining CPCs. These included anti-CD34 BV650, anti-CD45 BV786, and anti-KDR PE (BD Biosciences, USA). 7-AAD (BD Biosciences, USA) staining was also performed to remove non-viable cells from analysis. After 45 min incubation, erythrocyte lysis was performed using lysis buffer (BD FACS™ Lysing Solution, BD Biosciences, USA). Quantification of CPC counts was then performed on a 12-colour flow cytometer (BD FACS Celesta, BD Biosciences, USA). 500,000 $CD45^+$ events were collected for each sample to ensure sufficient data for rare cell populations. After gating for $CD45^+$ events, non-viable $7AAD^+$ events were excluded, with subsequent gating for $CD34^+$ events, and lastly for KDR^+ events. Appropriate negative tubes were used to determine positive and negative events for each targeted antibody. Percentage events were collected as % mononuclear events, in addition to calculation of $cells \cdot mL^{-1}$ using dual platform analysis. To do so, lymphocytes were enumerated using differential hematology analysis (XS1000i, Sysmex, UK) and %

of lymphocyte events were used with lymphocyte number to determine CPCs as cells·mL^{-1}. Analyses of flow cytometric data were performed using BD FACSDiva™ software (BD Biosciences, USA). Gating parameters can be found in supplementary figure 1.

For pre- to post-exercise comparisons, changes in blood volume due to hemoconcentration were accounted for using measured hematocrit and hemoglobin obtained from automated hematology analysis using equations by Dill and Costill (1974).

2.7 Exercise training

In the present study the older adults underwent an 8-week SIT intervention involving 3 x 20 s 'all-out' sprints, twice per week. The two SIT sessions per week were ≥, 72 h apart, as our pilot work suggested older adults would be suitably recovered from SIT in this timeframe (Yasar et al., 2019). Participants avoided strenuous physical activity 24 h prior to SIT sessions whilst maintaining habitual physical activity according to self-reporting. Participants warmed up for a period of 3 min at a self-paced intensity by performing static running. Participants then performed three 20 s static sprints at an 'all-out' intensity, interspersed by 3 min self-paced recovery phases. Following the final sprint, a 3 min self-paced cool down was performed (**Fig 2**). During all sprints, participants were instructed to raise their feet to approximately knee height, with loud verbal encouragement throughout each sprint. No dietary guidance or monitoring was provided on during the training, excepting for the fasted testing sessions.

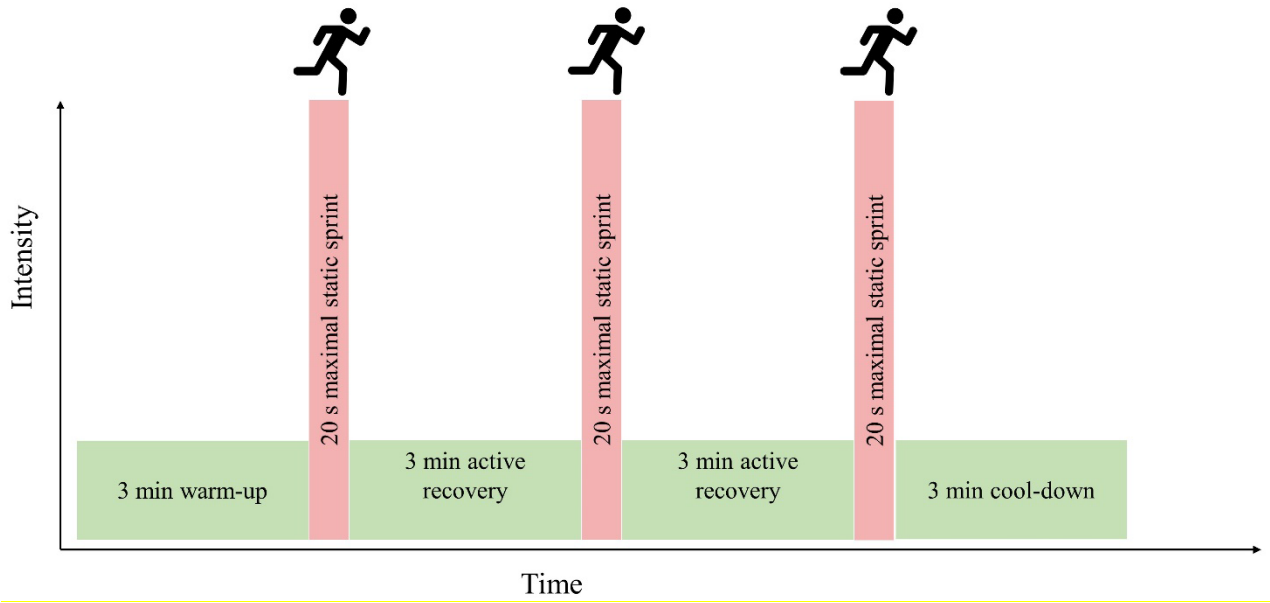

Fig 2 Schematic representation of the sprint interval session. Participants performed this session twice weekly for eight weeks

2.8 Statistical Analysis

All data were assessed for normal distribution using the Kolmogorov-Smirnov test for normality. All data were normally distributed. To assess the differences in resting and exercise-induced changes in $CD34^+$ and $CD34^+KDR^+$ CPCs between young and older adults, 2 x 2 mixed factorial analyses of variance (ANOVA) were performed with Tukey's multiple comparison post-hoc tests performed where necessary. Resting CPC counts were compared as both % MNCs and as cell·mL^{-1}, whereas exercise-induced changes (pre- to post-exercise) were compared for main effects of exercise, age, and intervention were compared as cells·mL^{-1}. The delta (Δ) change in cells·mL^{-1} were compared between young and older

adults by means of independent t-test. To compare the effect of the SIT intervention in the older adults, a mixed effects model was performed to compare resting and exercise-induced CPCs between pre- and post-SIT, and between these data with the young cohort. Data were analysed using GraphPad Prism (GraphPad Prism 9.1.0, GraphPad Software Inc, USA). Data are presented as mean ± SD (95% confidence intervals [CI]) without subjective terminology and alpha levels are reported as exact P values, without dichotomous interpretation of 'significant' or 'non-significant' as advised by the American Statistical Association (Hurlbert et al., 2019). Effect sizes are reported using Cohen's *d* (difference in means ÷ pooled standard deviation [SD]), and interpreted using guidelines for gerontology (Brydges, 2019), which are $d \geq 0.15$ = small, $d \geq 0.40$ = moderate, and $d \geq 0.75$ = large.

3 RESULTS

3.1 Influence of age on resting and exercise-induced changes in CPC counts

Older and younger adults circulating number of $CD34^+$ CPCs as a percentage of MNCs were 0.0159 ± 0.0073% [0.0103-0.0216% 95% CI] and 0.0233 ± 0.0060% [0.0195-0.0271% 95% CI] respectively (old vs young; $p = 0.026$, $d = 1.10$). Older and younger adults circulating number of $CD34^+$ CPCs were 828 ± 314 [587-1070 95% CI] cells·mL^{-1} and 1186 ± 272 [1012-1359 95% CI] cells·mL^{-1} respectively (old vs young; $p = 0.015$ $d = 1.22$). Older and younger adults circulating $CD34^+KDR^+$ EPCs as a percentage of MNCs were 0.0034 ± 0.026% [0.0014-0.0054% 95% CI] and 0.067 ± 0.023% [0.0052-0.0082% 95% CI] of MNCs respectively (old vs young; $p = 0.008$, $d = 2.59$). Older and younger adults' number of circulating $CD34^+KDR^+$ EPCs were 177 ± 128 [79-275 95% CI] cells·mL^{-1} and 335 ± 92 [227-394 95% CI] cells·mL^{-1} respectively (old vs young; $p = 0.007$ $d = 1.42$; **Fig 3**).

The number of $CD34^+$ progenitor cells in older adults before and after the graded exercise test to exhaustion were 828 ± 314 [587-1070 95% CI] cells·mL^{-1} and 1582 ± 381 [1290-1878 95% CI] cells·mL^{-1} respectively (pre- to post-exercise; $p < 0.001$, $d = 2.16$). The number of $CD34^+$ progenitor cells in the young adults before and after the graded exercise test to exhaustion were 1186 ± 272 [1012-1359 95% CI] cells·mL^{-1} and 2134 ± 1049 [1467-2800 95% CI] cells·mL^{-1} respectively (pre- to post-exercise; $p = 0.004$, $d = 1.23$). $CD34^+KDR^+$ EPCs in the older adults before and after the graded exercise test to exhaustion were 177 ± 128 [79-275 95% CI] cells·mL^{-1} and 280 ± 176 [145-416 95% CI] cells·mL^{-1} respectively (pre- to post-exercise; $p = 0.008$, $d = 0.67$). $CD34^+KDR^+$ EPCs in the young adults before and after the graded exercise test to exhaustion were 225 ± 92 [277-394 95% CI] cells·mL^{-1} and 717 ± 493 [403-1030 95% CI] cells·mL^{-1} respectively (pre- to post-exercise; $p = 0.017$, $d = 1.39$). The older cohort's $\Delta CD34^+$ from pre- to post-graded exercise test to exhaustion was 754 ± 430 [424-1084 95% CI] cells·mL^{-1}, whilst the young cohort's $\Delta CD34^+$ from pre- to post-graded exercise test to exhaustion was 948 ± 907 [372-1524 95% CI] cells·mL^{-1} (old vs young; $p = 0.775$, $d = 0.27$). The older cohort's $\Delta CD34^+KDR^+$ CPCs from pre- to post-graded exercise test to exhaustion was 103 ± 157 [-18-224 95% CI] cells·mL^{-1}, whilst the young cohort's Δ $CD34^+KDR^+$ CPCs from pre- to post-graded exercise test to exhaustion was 299 ± 365 [66-531 95% CI] cells·mL^{-1} (old vs young; $p = 0.212$, $d = 0.70$).

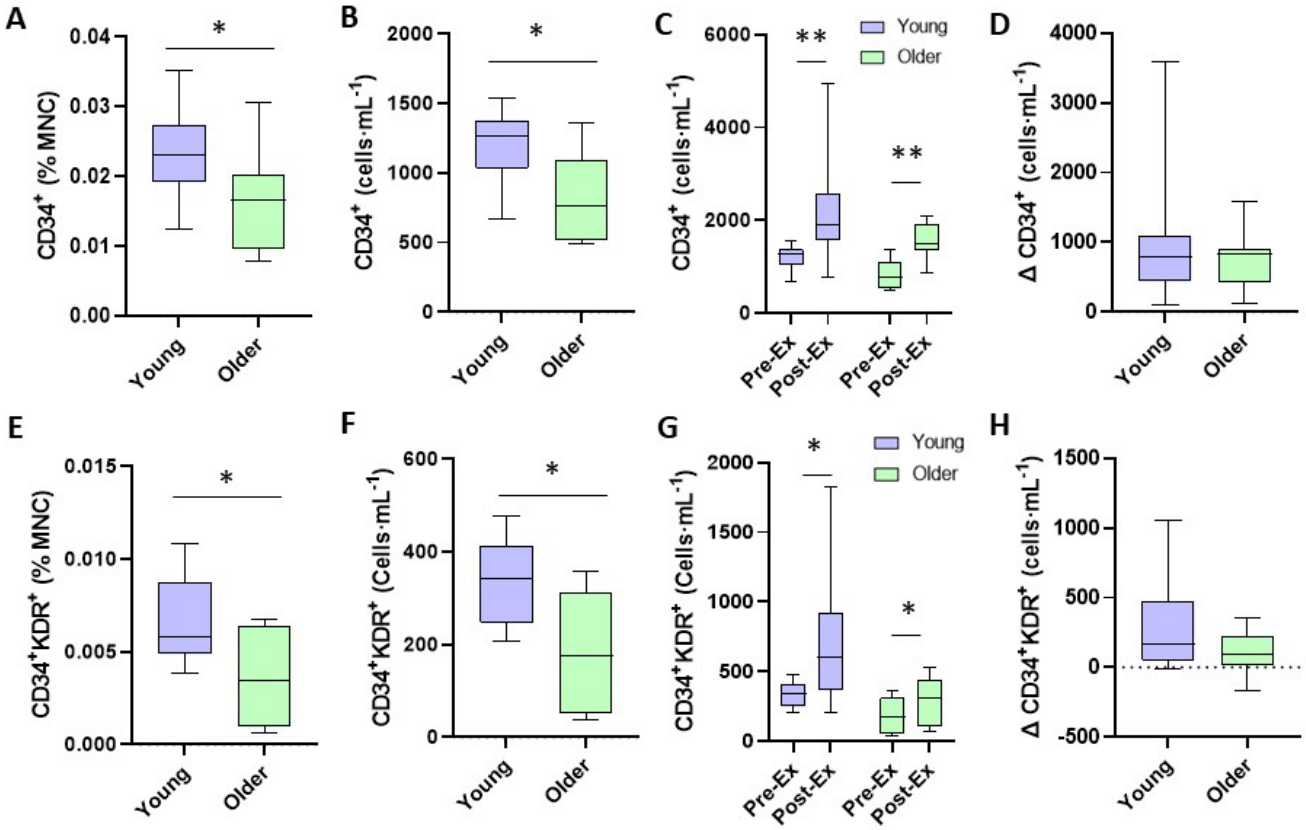

Fig 3 CD34$^+$ and CD34$^+$KDR$^+$ CPC Resting and Exercise-Induced Changes in CPC Counts in Young (n=12) and Older (n=9) Trained Adults. *A, B and E, F display differences between young and older adults in CD34$^+$ (A: % MNC, B: cells·mL^{-1}), CD34$^+$KDR$^+$ (E: % MNC, F: cells·mL^{-1}). C, D and G, H display changes in CPCs from pre- to post-exercise between young and older adults (C: CD34$^+$ changes from pre- to post-exercise, D: Δ change in CD34$^+$ CPCs; G: CD34$^+$KDR$^+$ changes from pre- to post-exercise, H: Δ change in CD34$^+$KDR$^+$ CPCs). * $p < 0.05$, ** $p < 0.005$*

3.2 Effect of 8-weeks of sprint interval training on resting and exercise-induced CPC changes in trained older adults

In terms of basal concentrations in the older group, CD34$^+$ CPC as a percentage of MNCs was 0.0159 ± 0.0073% MNC [0.0103-0.0216% 95% CI] and 0.0148 ± 0.0055% MNC [0.0106-0.0190% 95% CI] pre and post training respectively (p = 0.694, d = 0.17). CD34$^+$ CPC in cells·mL^{-1} was 828 ± 314 [587-1070 cells·mL^{-1} 95% CI] and 765 ± 299 cells·mL^{-1} [535-995 cells·mL^{-1} 95% CI] at pre and post training respectively (p = 0.602, d = 0.20). CD34$^+$KDR$^+$ CPC as a percentage of MNCs pre and post training was 0.0034 ± 0.0026% MNC [0.0014-0.0054% 95% CI] and 0.0030 ± 0.0008 % MNC [0.0024-0.0036% 95% CI] (p = 0.568, d =0.21). In cells·mL^{-1} this equated to 177 ± 128 [79-275 cells·mL^{-1} 95% CI] and 153 ± 38 cells·mL^{-1} [123-182 cells·mL^{-1} 95% CI] pre and post training respectively (p = 0.545, d = 0.25).

In terms of graded exercise test to exhaustion-induced changes in CD34$^+$ or CD34$^+$KDR$^+$ CPCs following SIT, the ANOVA resulted in an exercise (i.e., pre- to post-graded exercise test to exhaustion) x phase (i.e., pre and post) interaction of p = 0.233 for CD34$^+$ CPCs and p = 0.921 for CD34$^+$KDR$^+$ CPCs. The graded exercise test to exhaustion post-SIT resulted in CD34$^+$ CPCs of 765 ± 299 [535-995 95% CI] cells·mL^{-1} and 1266 ± 337 [1006-1525 95% CI] cells·mL^{-1} (pre- to post-exercise; $p < 0.001$, d = 1.57) and CD34$^+$KDR$^+$ CPCs of 153 ± 38 [123-182 95% CI] cells·mL^{-1} and 249 ± 121 [156-342 95% CI] cells·mL^{-1} (pre- to post-exercise; p = 0.035, d = 1.07) and the mean response was not different to that of pre-intervention, however there was a smaller spread of data, suggesting a more uniform response (**Fig 4)**.

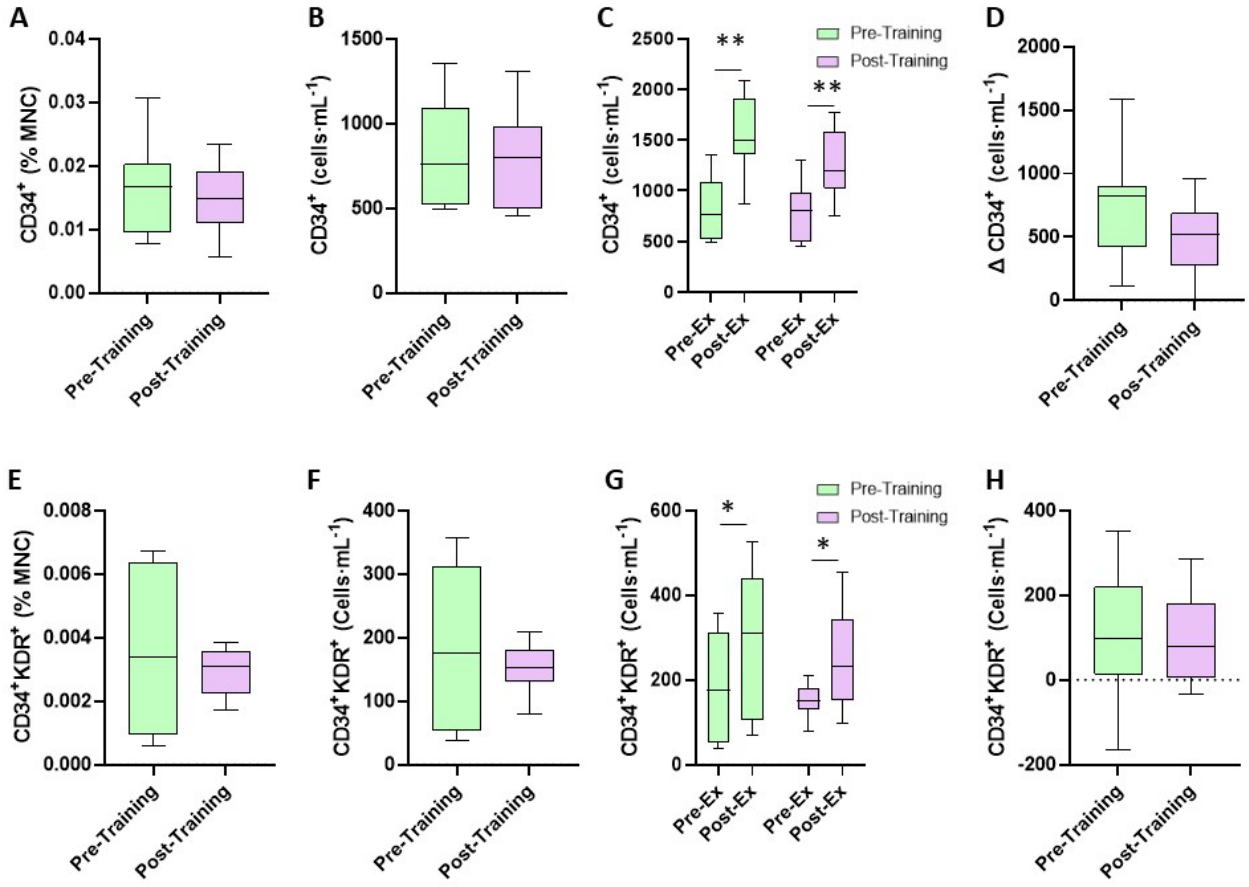

Fig 4 CD34$^+$ and CD34$^+$KDR$^+$ CPC Resting and Exercise-Induced Changes in CPC Counts in Older (n=9) Trained Adults before and after 8-Week SIT Intervention. *A, B and E, F display differences between pre- and post-intervention in CD34$^+$ (A: % MNC, B: cells·mL^{-1}), CD34$^+$KDR$^+$ (E: % MNC, F: cells·mL^{-1}). C, D and G, H display exercise-induced changes in CPCs from pre- to post-SIT intervention (C: CD34$^+$ changes from pre- to post-exercise, D: Δ change in CD34$^+$ CPCs; G: CD34$^+$KDR$^+$ changes from pre- to post-exercise, H: Δ change in CD34$^+$KDR$^+$ CPCs). * $p < 0.05$, ** $p < 0.005$.*

4 DICUSSION

The main findings of this study are that aerobically trained older adults display a reduced resting number of CPCs compared to younger trained adults but have a preserved ability to mobilise these cells in response to a graded exercise test to exhaustion. Moreover, 8 weeks of SIT did not increase basal CPC counts in already well-trained older adults.

Previous work has shown that advancing age is associated with lower CPC counts (Thijssen et al., 2006; Ross et al., 2018) which is apparently unaffected by cardiorespiratory fitness (Ross et al., 2018). These cells play an important role in vascular repair, via promoting endothelial proliferation by paracrine means (Hur et al., 2004) or by differentiating into mature endothelial cells at the site of repair (Xia et al., 2012). Studies report that such CPCs are associated with endothelial function (Hill et al., 2003; Bruyndonckx et al., 2014) and as such, promote endothelial integrity and health. The loss of such cells with ageing therefore results in reduced endothelial repair, and loss of endothelial function, which is itself linked heavily with future cardiovascular risk (Green et al., 2011). The mechanism for such reduction in CPCs with advancing age in humans is unknown but purported to be due to increases in oxidative stress, resulting in impairments in CPC number and/or function (Mandraffino et al., 2012), and increased CPC susceptibility to apoptotic stimuli (Kushner et al., 2011). There is no evidence for changes in bone marrow resident progenitor cell count with ageing (Povsic et al., 2010), but a stressor-induced CPC mobilisation from bone marrow may be impaired, with evidence from burn wound model of CPC mobilisation (Zhang et al., 2011) and exercise-induced mobilisation both displaying impaired mobilisation in older populations (Ross et al., 2018) . However, the present investigation observed a preserved exercise mobilisation of CPCs in older adults. There

are several key differences which are likely to explain divergent findings. Firstly, participants in this study were a very physically active, trained group of older adults, evidenced by their $\dot{V}O_{2max}$ of 37.4 ml·kg·min^{-1}. Previous work by Ross and colleagues (2018) which demonstrated impaired mobilisation following exercise in older adults included participants who were not highly physically active, and therefore the observed reduction in exercise induced-CPC mobilisation may not be a result of age *per se*, but a result of inactive ageing. Thus, high levels of physical activity throughout the lifespan may be required to preserve this process. In addition to divergent participant characteristics, the exercise stimuli in the work of Ross et al. (2018) study was a submaximal cycling protocol at 70% $\dot{V}O_{2max}$, whereas the stressor in the present investigation was a maximal graded exercise test to exhaustion, and CPC mobilisation is intensity dependent (Laufs et al., 2005).

Exercise training has shown promise to promote CPC number and function in both healthy (Tsai et al., 2016; Niemiro et al., 2018) and diseased states (Adams et al., 2004; Steiner et al., 2005; Sarto et al., 2007; Schlager et al., 2011; Gatta et al., 2012; Cesari et al., 2013). However, some studies report no changes in CPC counts after a short-term training programme (Luk et al., 2012; van Craenenbroeck et al., 2015), likely due to high intra- and inter-group variation associated with quantifying rare cells by flow cytometry. In the present study we aimed to investigate whether a short-term novel, time efficient SIT programme could improve the age-related reduction in resting CPC count, and therefore the trained older adults underwent an 8-week SIT intervention (3 x 20s 'all-out' sprints, 2 x a week). Although SIT has not been well-researched in older adults, HIIT in older adults has been observed to improve cardiorespiratory fitness (Knowles et al., 2015; StØren et al., 2017), muscle power (Herbert et al., 2017; Sculthorpe et al., 2017), and is facilitative in improving body composition (Herbert et al., 2016). Whilst SIT in younger demographics has been observed to improve both aerobic (Sloth et al., 2013; Vollaard et al., 2017) and anaerobic (MacDougall et al., 1998; Kim et al., 2011) fitness with a considerable variation of approaches pertaining to interval duration, repetition, and training frequency being evidenced as efficacious, whilst remaining easy to administer i.e., no power or heart monitors required. We observed no change in either resting, or exercise-induced changes in CPCs in response to the SIT intervention. We propose that the highly trained status of the older adults was responsible for this, in that the ceiling effect was likely evident in this well-trained group. As such, whether SIT would be beneficial in a sedentary older adult group warrants investigation.

4.1 Limitations

There are several limitations of the current study, which we accept. Firstly, addition of a sedentary older group and sedentary younger group to assess the influence of sedentary ageing vs active ageing vs sedentary youth vs active youth more comprehensively would have been beneficial. This would permit us to determine whether the effect of SIT was greater in a physically inactive group, as our older trained group had a CPCs mobilisation capability similar to a young, trained group, contrary to our previous work (Ross et al., 2018). However, additional recruitment would require greater resource commitment which was outside the scope of the present investigation. Secondly, whole body metabolism is largely dependent on skeletal muscle mass, as increased skeletal muscle mass increases metabolic load during rest and exercise, if all other factors are equal. Moreover, as muscle mass ageing and gender have meaningful effects on the physiological stimulus that can be achieved by exercise, mostly attributable to differences in muscle mass between old vs young, and male vs female participants (Jannsen & Ross, 1993). It is likely older female participants in this study were less muscular than their younger, male counterparts, resulting in a relative dampening of the relation between exercise intensity and metabolic stress (Miller et al., 1993). Admittedly, this increased risk of bias in the study results, and consequently, any conclusions derived. Thirdly, we did not assess CPC paracrine function, which improves post-training (Landers-Ramos et al., 2015), and therefore this may be an avenue for future research. Finally, and importantly, this study was not powered to detect changes in CPCs and was secondary analysis of an investigation with the primary outcome as muscle power. We believe this justifies our statistical approach of avoidance of dichotomous 'significance' or otherwise labelling based on an alpha level inappropriate for this dataset. An *a posteriori* power calculation testing for differences between the young and old group at baseline using CD34+ CPCs (in cells·mL^{-1}) as the outcome variable, an alpha of 0.05, a one-sided test, and a sample size of nine, resulted in statistical power of 0.83. Similarly, when using the same information and determining sample size, the required n was 10 per group. However, to detect a SIT-induced change in graded exercise test ΔCD34+ (in cells·mL^{-1}), a sample size of n = 36 would have been required to detect a change at the p = 0.05 level, with a statistical power of 0.80 and a one-sided test. Thus, a larger confirmatory study is required to corroborate observations made here.

4.2 Conclusions

Physically trained older adults display reduced CPC counts, but preserved exercise-induced mobilisation of these cells, which could offer vasoprotection. However, an 8-week SIT intervention was unsuccessful at improving resting CPCs, and exercise-induced mobilisation of these cells.

AUTHOR CONTRIBUTIONS ACCORDING TO THE CREDIT TAXONOMY

Conceptualisation: Zerbu Yasar, Mark D Ross, Lawrence D Hayes; Methodology: Zerbu Yasar, Mark D Ross, Lawrence D Hayes; Formal analysis and investigation: Zerbu Yasar, Mark D Ross, Russell Wilson, Lawrence D Hayes; Investigation: Zerbu Yasar, Mark D Ross, Ruth D Postlethwaite, Christopher J Gaffney, Lawrence D Hayes; Resources: Mark D Ross, Lawrence D Hayes; Writing - original draft preparation: Zerbu Yasar, Mark D Ross, Lawrence D Hayes; Writing - review and editing: Zerbu Yasar, Mark D Ross, Christopher J Gaffney, Ruth D Postlethwaite, Russell Wilson, Lawrence D Hayes; Visualization: Mark D Ross, Lawrence D Hayes; Supervision: Mark D Ross, Lawrence D Hayes; Project administration: Zerbu Yasar, Mark D Ross, Lawrence D Hayes; Funding acquisition: Mark D Ross, Lawrence D Hayes.

ACKNOWLEDGMENTS

Zerbu Yasar received a PhD scholarship from the University of Cumbria. Lawrence D Hayes received research funding from the University of Cumbria for data generation.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5 REFERENCES

Adams, O.P., 2013. The impact of brief high-intensity exercise on blood glucose levels. Diabetes Metabolic Syndrome and Obesity 6, 113–122. https://doi.org/10.2147/DMSO.S29222

Adams, V., Lenk, K., Linke, A., Lenz, D., Erbs, S., Sandri, M., et al. (2004). Increase of Circulating Endothelial Progenitor Cells in Patients with Coronary Artery Disease after Exercise-Induced Ischemia. Arteriosclerosis, Thrombosis, and Vascular Biology 24, 684–690. doi:10.1161/01.ATV.0000124104.23702.a0.

Bruyndonckx, L., Hoymans, V. Y., Frederix, G., de Guchtenaere, A., Franckx, H., Vissers, D. K., et al. (2014). Endothelial progenitor cells and endothelial microparticles are independent predictors of endothelial function. Journal of Pediatrics 165, 300–305. doi:10.1016/j.jpeds.2014.04.015.

Cesari, F., Marcucci, R., Gori, A. M., Burgisser, C., Francini, S., Sofi, F., et al. (2013). Impact of a cardiac rehabilitation program and inflammatory state on endothelial progenitor cells in acute coronary syndrome patients. International Journal of Cardiology 167, 1854–1859. doi:10.1016/j.ijcard.2012.04.157.

Dill, D. B., and Costill, D. L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. Journal of Applied Physiology 37, 247–248.

Farzad, B., Gharakhanlou, R., Agha-Alinejad, H., Curby, D.G., Bayati, M., Bahraminejad, M., Mäestu, J., 2011. Physiological and performance changes from the addition of a sprint interval program to wrestling training. Journal of Strength Conditioning Research 25, 2392–2399. https://doi.org/10.1519/JSC.0b013e3181fb4a33.

Foster, C., Florhaug, J.A., Franklin, J., Gottschall, L., Hrovatin, L.A., Parker, S., Doleshal, P., Dodge, C., 2001. A new approach to monitoring exercise training. Journal of Strength Conditioning Research 15, 109–115.

412 Gatta, L., Armani, A., Iellamo, F., Consoli, C., Molinari, F., Caminiti, G., et al. (2012). Effects of a short-term exercise
413 training on serum factors involved in ventricular remodelling in chronic heart failure patients. International Journal of
414 Cardiology 155, 409–413. doi:10.1016/j.ijcard.2010.10.045.

415 Gillen, J.B., Martin, B.J., MacInnis, M.J., Skelly, L.E., Tarnopolsky, M.A., Gibala, M.J., 2016. Twelve Weeks of Sprint
416 Interval Training Improves Indices of Cardiometabolic Health Similar to Traditional Endurance Training despite a Five-
417 Fold Lower Exercise Volume and Time Commitment. PLoS One 11, e0154075.
418 https://doi.org/10.1371/journal.pone.0154075

419 Gist, N.H., Fedewa, M.V., Dishman, R.K., Cureton, K.J., 2014. Sprint interval training effects on aerobic capacity: a
420 systematic review and meta-analysis. Sports Med 44, 269–279. https://doi.org/10.1007/s40279-013-0115-0

421 Grace, F., Herbert, P., Elliott, A.D., Richards, J., Beaumont, A., Sculthorpe, N.F., 2018. High intensity interval training
422 (HIIT) improves resting blood pressure, metabolic (MET) capacity and heart rate reserve without compromising cardiac
423 function in sedentary aging men. Exp Gerontol 109, 75–81. https://doi.org/10.1016/j.exger.2017.05.010

424 Green, D. J., Jones, H., Thijssen, D., Cable, N. T., and Atkinson, G. (2011). Flow-mediated dilation and cardiovascular
425 event prediction: Does nitric oxide matter? Hypertension 57, 363–369. doi:10.1161/HYPERTENSIONAHA.110.167015.

426 Hazell, T.J., Hamilton, C.D., Olver, T.D., Lemon, P.W.R., 2014. Running sprint interval training induces fat loss in women.
427 Applied Physiology, Nutrition, and Metabolism 39, 944–950. https://doi.org/10.1139/apnm-2013-0503

428 Herbert, P., Hayes, L.D., Sculthorpe, N.F., Grace, F.M., 2017. HIIT produces increases in muscle power and free
429 testosterone in male masters athletes. Endocrinology Connect 6, 430–436. https://doi.org/10.1530/EC-17-0159

430 Herbert, Peter, Hayes, L.D., Sculthorpe, N., Grace, F.M., 2017. High-intensity interval training (HIIT) increases insulin-
431 like growth factor-I (IGF-I) in sedentary aging men but not masters' athletes: an observational study. Aging Male 20, 54–
432 59. https://doi.org/10.1080/13685538.2016.1260108

433 Hill, J. M., Zalos, G., Halcox, J. P. J., Schenke, W. H., Waclawiw, M. A., Quyyumi, A. A., et al. (2003). Circulating
434 Endothelial Progenitor Cells, Vascular Function, and Cardiovascular Risk. New England Journal of Medicine 348, 593–
435 600. doi:10.1056/NEJMoa022287.

436 Hoymans, V. Y., van Craenenbroeck, A. H., Bruyndonckx, L., van Ierssel, S. H., Vrints, C. J., Conraads, V. M., et al.
437 (2012). TransFix® for delayed flow cytometry of endothelial progenitor cells and angiogenic T cells. Microvascular
438 Research 84, 384–386. doi:10.1016/j.mvr.2012.08.007.

439 Hur, J., Yoon, C. H., Kim, H. S., Choi, J. H., Kang, H. J., Hwang, K. K., et al. (2004). Characterization of Two Types of
440 Endothelial Progenitor Cells and Their Different Contributions to Neovasculogenesis. Arteriosclerosis, Thrombosis, and
441 Vascular Biology 24, 288–293. doi:10.1161/01.ATV.0000114236.77009.06.

442 Hurst, C., Weston, K.L., Weston, M., 2019. The effect of 12 weeks of combined upper- and lower-body high-intensity
443 interval training on muscular and cardiorespiratory fitness in older adults. Aging Clinical and Experimental Research 31,
444 661–671. https://doi.org/10.1007/s40520-018-1015-9

445 Janssen, I., Ross, R., 2004. Linking age-related changes in skeletal muscle mass and composition with metabolism and
446 disease. The journal of nutrition, health & aging 9, 408–19.

447 Kim, H.-K., Hwang, C.-L., Yoo, J.-K., Hwang, M.-H., Handberg, E.M., Petersen, J.W., Nichols, W.W., Sofianos, S.,
448 Christou, D.D., 2017. All-Extremity Exercise Training Improves Arterial Stiffness in Older Adults. Med Sci Sports Exerc
449 49, 1404–1411. https://doi.org/10.1249/MSS.0000000000001229

450 Kim, J., Lee, N., Trilk, J., Kim, E.-J., Kim, S.-Y., Lee, M., Cho, H.-C., 2011. Effects of sprint interval training on elite
451 Judoists. Int J Sports Med 32, 929–934. https://doi.org/10.1055/s-0031-1283183

452 Knowles, A.-M., Herbert, P., Easton, C., Sculthorpe, N., Grace, F.M., 2015. Impact of low-volume, high-intensity interval
453 training on maximal aerobic capacity, health-related quality of life and motivation to exercise in ageing men. Age 37, 25.
454 https://doi.org/10.1007/s11357-015-9763-3

Kushner, E. J., MacEneaney, O. J., Weil, B. R., Greiner, J. J., Stauffer, B. L., and Desouza, C. A. (2011). Aging is associated with a proapoptotic endothelial progenitor cell phenotype. Journal of Vascular Research 48, 408–414. doi:10.1159/000324837.

Landers-Ramos, R. Q., Sapp, R. M., Jenkins, N. T., Murphy, A. E., Cancre, L., Chin, E. R., et al. (2015). Chronic endurance exercise affects paracrine action of CD31 and CD34 cells on endothelial tube formation. American Journal of Physiology: Heart and Circulatory Physiology 309, 407–420. doi:10.1152/ajpheart.00123.2015.-We.

Laufs, U., Urhausen, A., Werner, N., Scharhag, J., Heitz, A., Kissner, G., et al. (2005). Running exercise of different duration and intensity: Effect on endothelial progenitor cells in healthy subjects. European Journal of Preventive Cardiology 12, 407–414. doi:10.1097/01.hjr.0000174823.87269.2e.

Lepretre, P.-M., Vogel, T., Brechat, P.-H., Dufour, S., Richard, R., Kaltenbach, G., Berthel, M., Lonsdorfer, J., 2009. Impact of short-term aerobic interval training on maximal exercise in sedentary aged subjects. International Journal of Clinical Practice 63, 1472–1478. https://doi.org/10.1111/j.1742-1241.2009.02120.x

Luk, T. H., Dai, Y. L., Siu, C. W., Yiu, K. H., Chan, H. T., Lee, S. W., et al. (2012). Effect of exercise training on vascular endothelial function in patients with stable coronary artery disease: A randomized controlled trial. European Journal of Preventive Cardiology 19, 830–839. doi:10.1177/1741826711415679.

MacDougall, J.D., Hicks, A.L., MacDonald, J.R., McKelvie, R.S., Green, H.J., Smith, K.M., 1998. Muscle performance and enzymatic adaptations to sprint interval training. Journal of Applied Physiology (1985) 84, 2138–2142. https://doi.org/10.1152/jappl.1998.84.6.2138

Mandraffino, G., Sardo, M. A., Riggio, S., D'Ascola, A., Alibrandi, A., Saitta, C., et al. (2012). Circulating progenitor cells and the elderly: A seven-year observational study. Experimental Gerontology 47, 394–400. doi:10.1016/j.exger.2012.03.007.

Martin-Smith, R., Buchan, D.S., Baker, J.S., Macdonald, M.J., Sculthorpe, N.F., Easton, C., Knox, A., Grace, F.M., 2019. Sprint Interval Training and the School Curriculum: Benefits Upon Cardiorespiratory Fitness, Physical Activity Profiles, and Cardiometabolic Risk Profiles of Healthy Adolescents. Pediatric Exercise Science 31, 296–305. https://doi.org/10.1123/pes.2018-0155

Miller, A.E.J., MacDougall, J.D., Tarnopolsky, M.A., Sale, D.G., 1993. Gender differences in strength and muscle fiber characteristics. Europ. J. Appl. Physiol. 66, 254–262. https://doi.org/10.1007/BF00235103

Naves, J.P.A., Viana, R.B., Rebelo, A.C.S., de Lira, C.A.B., Pimentel, G.D., Lobo, P.C.B., de Oliveira, J.C., Ramirez-Campillo, R., Gentil, P., 2018. Effects of High-Intensity Interval Training vs. Sprint Interval Training on Anthropometric Measures and Cardiorespiratory Fitness in Healthy Young Women. Frontiers in Physiology 9, 1738. https://doi.org/10.3389/fphys.2018.01738

Niemiro, G. M., Allen, J. M., Mailing, L. J., Khan, N. A., Holscher, H. D., Woods, J. A., et al. (2018). Effects of endurance exercise training on inflammatory circulating progenitor cell content in lean and obese adults. Journal of Physiology 596, 2811–2822. doi:10.1113/JP276023.

Niño, O., Balague, N., Aragones, D., Blasi, J., Alamo, J. M., Corral, L., et al. (2014). CD34+ Circulating Progenitor Cells after Different Training Programs. International Journal of Sports Medicine 36, 292–296. doi:10.1055/S-0034-1394394.

Povsic, T. J., Zhou, J., Adams, S. D., Bolognesi, M. P., Attarian, D. E., and Peterson, E. D. (2010). Aging is not associated with bone marrow-resident progenitor cell depletion. Journals of Gerontology - Series A Biological Sciences and Medical Sciences 65 A, 1042–1050. doi:10.1093/gerona/glq110.

Riebe, D., Franklin, B. A., Thompson, P. D., Garber, C. E., Whitfield, G. P., Magal, M., et al. (2015). Updating ACSM's recommendations for exercise preparticipation health screening. Medicine and Science in Sports and Exercise 47, 2473–2479. doi:10.1249/MSS.0000000000000664.

Ross, M. D., Malone, E. M., Simpson, R., Cranston, I., Ingram, L., Wright, G. P., et al. (2018). Lower resting and exercise-induced circulating angiogenic progenitors and angiogenic T cells in older men. American Journal of Physiology - Heart and Circulatory Physiology 314, H392–H402. doi:10.1152/ajpheart.00592.2017.

Sarto, P., Balducci, E., Balconi, G., Fiordaliso, F., Merlo, L., Tuzzato, G., et al. (2007). Effects of Exercise Training on Endothelial Progenitor Cells in Patients with Chronic Heart Failure. Journal of Cardiac Failure 13, 701–708. doi: 10.1016/j.cardfail.2007.06.722.

Schlager, O., Giurgea, A., Schuhfried, O., Seidinger, D., Hammer, A., Gröger, M., et al. (2011). Exercise training increases endothelial progenitor cells and decreases asymmetric dimethylarginine in peripheral arterial disease: A randomized controlled trial. Atherosclerosis 217, 240–248. doi:10.1016/j.atherosclerosis.2011.03.018.

Sculthorpe, N.F., Herbert, P., Grace, F., 2017. One session of high-intensity interval training (HIIT) every 5 days, improves muscle power but not static balance in lifelong sedentary ageing men: A randomized controlled trial. Medicine (Baltimore) 96, e6040. https://doi.org/10.1097/MD.0000000000006040

Sloth, M., Sloth, D., Overgaard, K., Dalgas, U., 2013. Effects of sprint interval training on VO2max and aerobic exercise performance: A systematic review and meta-analysis. Scandinavian Journal of Medicine and Science in Sports 23, e341-352. https://doi.org/10.1111/sms.12092

Søgaard, D., Baranowski, M., Larsen, S., Taulo Lund, M., Munk Scheuer, C., Vestergaard Abildskov, C., Greve Dideriksen, S., Dela, F., Wulff Helge, J., 2019. Muscle-Saturated Bioactive Lipids Are Increased with Aging and Influenced by High-Intensity Interval Training. Int J Mol Sci 20, E1240. https://doi.org/10.3390/ijms20051240

Søgaard, D., Lund, M.T., Scheuer, C.M., Dehlbaek, M.S., Dideriksen, S.G., Abildskov, C.V., Christensen, K.K., Dohlmann, T.L., Larsen, S., Vigelsø, A.H., Dela, F., Helge, J.W., 2018. High-intensity interval training improves insulin sensitivity in older individuals. Acta Physiologica (Oxf) 222, e13009. https://doi.org/10.1111/apha.13009

Steiner, S., Niessner, A., Ziegler, S., Richter, B., Seidinger, D., Pleiner, J., et al. (2005). Endurance training increases the number of endothelial progenitor cells in patients with cardiovascular risk and coronary artery disease. Atherosclerosis 181, 305–310. doi: 10.1016/j.atherosclerosis.2005.01.006.

Støren, Ø., Helgerud, J., Sæbø, M., Støa, E.M., Bratland-Sanda, S., Unhjem, R.J., Hoff, J., Wang, E., 2017. The Effect of Age on the V˙O2max Response to High-Intensity Interval Training. Med Sci Sports Exerc 49, 78–85. https://doi.org/10.1249/MSS.0000000000001070

Thijssen, D. H. J., Vos, J. B., Verseyden, C., van Zonneveld, A. J., Smits, P., Sweep, F. C. G. J., et al. (2006). Haematopoietic stem cells and endothelial progenitor cells in healthy men: Effect of aging and training. Aging Cell 5, 495–503. doi:10.1111/j.1474-9726.2006.00242.x.

Tsai, H. H., Lin, C. P., Lin, Y. H., Hsu, C. C., and Wang, J. S. (2016). High-intensity Interval training enhances mobilization/functionality of endothelial progenitor cells and depressed shedding of vascular endothelial cells undergoing hypoxia. European Journal of Applied Physiology 116, 2375–2388. doi:10.1007/s00421-016-3490-z.

van Craenenbroeck, E. M., Frederix, G., Pattyn, N., Beckers, P., van Craenenbroeck, A. H., Gevaert, A., et al. (2015). Effects of aerobic interval training and continuous training on cellular markers of endothelial integrity in coronary artery disease: A SAINTEX-CAD substudy. American Journal of Physiology - Heart and Circulatory Physiology 309, H1876–H1882. doi:10.1152/ajpheart.00341.2015.

Vollaard, N.B.J., Metcalfe, R.S., Williams, S., 2017. Effect of Number of Sprints in an SIT Session on Change in V˙O2max: A Meta-analysis. Medicine and Science in Sports and Exercise 49, 1147–1156. https://doi.org/10.1249/MSS.0000000000001204

Whyte, L.J., Gill, J.M.R., Cathcart, A.J., 2010. Effect of 2 weeks of sprint interval training on health-related outcomes in sedentary overweight/obese men. Metabolism 59, 1421–1428. https://doi.org/10.1016/j.metabol.2010.01.002

539 Xia, W. H., Li, J., Su, C., Yang, Z., Chen, L., Wu, F., et al. (2012). Physical exercise attenuates age-associated reduction
540 in endothelium-reparative capacity of endothelial progenitor cells by increasing CXCR4/JAK-2 signaling in healthy men.
541 Aging Cell 11, 111–119. doi:10.1111/j.1474-9726.2011.00758.x.

542 Yasar, Z., Dewhurst, S., and Hayes, L. D. (2019). Peak Power Output Is Similarly Recovered After Three- and Five-Days'
543 Rest Following Sprint Interval Training in Young and Older Adults. *Sports* 7, 94-undefined. doi:10.3390/SPORTS7040094.

544 Zhang, X., Sarkar, K., Rey, S., Sebastian, R., Andrikopoulou, E., Marti, G. P., et al. (2011). Aging impairs the mobilization
545 and homing of bone marrow-derived angiogenic cells to burn wounds. Journal of Molecular Medicine 89, 985–995.
546 doi:10.1007/s00109-011-0754-2.

547