European Journal of Applied Physiology (2019) 119:743–752
https://doi.org/10.1007/s00421-018-04065-7

ORIGINAL ARTICLE

Circulating angiogenic cell response to sprint interval and continuous exercise

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Received: 16 October 2018 / Accepted: 24 December 2018 / Published online: 23 January 2019

Abstract

Introduction Although commonly understood as immune cells, certain T lymphocyte and monocyte subsets have angiogenic potential, contributing to blood vessel growth and repair. These cells are highly exercise responsive and may contribute to the cardiovascular benefits seen with exercise.

Purpose To compare the effects of a single bout of continuous (CONTEX) and sprint interval exercise (SPRINT) on circulating angiogenic cells (CAC) in healthy recreationally active adults.

Methods Twelve participants (aged 29 ± 2 years, BMI 25.5 ± 0.9 kg m^{-2}, $\dot{V}O_2$peak 44.3 ± 1.8 ml kg^{-1} min^{-1}; mean ± SEM) participated in the study. Participants completed a 45-min bout of CONTEX at 70% peak oxygen uptake and 6 × 20 s sprints on a cycle ergometer, in a counterbalanced design. Blood was sampled pre-, post-, 2 h and 24 h post-exercise for quantification of CAC subsets by whole blood flow cytometric analysis. Angiogenic T lymphocytes (T_{ANG}) and angiogenic Tie2-expressing monocytes (TEM) were identified by the expression of CD31 and Tie2, respectively.

Results Circulating (cells μL^{-1}) CD3$^+$CD31$^+$ T_{ANG} increased immediately post-exercise in both trials ($p < 0.05$), with a significantly greater increase ($p < 0.05$) following SPRINT (+ 57%) compared to CONTEX (+ 14%). Exercise increased ($p < 0.05$) the expression of the chemokine receptor CXCR4 on T_{ANG} at 24 h. Tie2-expressing classical (CD14^{++}CD16$^-$), intermediate (CD14^{++}CD16$^+$) and non-classical (CD14$^+$CD16^{++}) monocytes and circulating CD34$^+$CD45^{dim} progenitor cells were higher post-exercise in SPRINT, but unchanged in CONTEX. All post-exercise increases in SPRINT were back to pre-exercise levels at 2 h and 24 h.

Conclusion Acute exercise transiently increases circulating T_{ANG}, TEM and progenitor cells with greater increases evident following very high intensity sprint exercise than following prolonged continuous paced endurance exercise.

Keywords Angiogenic T cells · Tie2 expressing monocytes · Endothelial progenitor cells · High intensity exercise

Introduction

Since the isolation of putative endothelial progenitor cells (EPC) 20 years ago (Asahara et al. 1997), other circulating mononuclear cell subsets have been identified that have the capacity to influence vascular growth and repair (Capoccia et al. 2006; Hur et al. 2007). In the interim, these CD34$^+$ putative endothelial progenitors have been the most extensively studied. Less recognised is the role of circulating lymphocyte and monocyte subsets in vascular development. The term circulating angiogenic cell (CAC) should be used when referring to any peripheral blood mononuclear cell (PBMC) that supports vascular growth, repair and re-endothelialisation (Witkowski et al. 2011). CAC are typically defined with cell surface markers and enumerated by flow cytometry.

Angiogenic T lymphocytes (T_{ANG}) are characterised by the presence of platelet endothelial cell adhesion molecule-1 (CD31) on CD3$^+$ T cells (Hur et al. 2007; Kushner et al. 2010a). Evidence from cell culture, animal and human studies support a role for CD31$^+$ T lymphocytes in vascular development (Hur et al. 2007; Weil et al. 2011). Compared

Communicated by I. Mark Olfert.

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to $CD31^-$ cells, the $CD31^+$ subset secretes higher levels of angiogenic cytokines including VEGF, IL-8, MMP-9, G-CSF and IL-17, demonstrates greater migratory capacity towards SDF-1α and enhances capillary tube formation in vitro (Hur et al. 2007; Kushner et al. 2010a; Weil et al. 2011). In a hindlimb injury model of tissue ischemia, $CD31^+$ but not $CD31^-$ T cells restored tissue perfusion in ($CD3^+$ deficient) nude mice (Hur et al. 2007). Additionally, circulating T_{ANG} correlate with endothelial-dependent dilation (Weil et al. 2011) but inversely with age and Framingham risk score (Hur et al. 2007). T_{ANG} are considerably lower in older adults (Kushner et al. 2010b; Ross et al. 2018b), individuals with atherosclerotic aortic aneurysms (Caligiuri et al. 2006) and hypertensives with cerebral small vessel disease (Rouhl et al. 2012).

Monocytes can be classified into classical ($CD14^{++}CD16^-$), intermediate ($CD14^{++}CD16^+$) and non-classical ($CD14^+CD16^{++}$) subsets, though more recently these subsets have been termed Mon1, Mon2 and Mon3, respectively (Weber et al. 2016). Of these, Mon2 are considered to have the greatest angiogenic potential with higher expression of pro-angiogenic molecules, growth factors and chemokine receptors such as Tie2 and CCR2 (Jaipersad et al. 2014). Tie2 is an angiopoietin receptor and when expressed, allows monocytes to migrate along an angiopoietin gradient towards ischemic tissue. Tie2 expressing monocytes (TEM) are more frequently studied in relation to tumour angiogenesis, where they are the target of anti-cancer therapy (De Palma et al. 2007) but have also been studied in critical limb ischemia (Patel et al. 2013) and peripheral arterial disease (Dopheide et al. 2016). Indeed, Tie2 knockdown in TEM impairs restoration of blood flow in a mouse hindlimb model of ischemia, whereas Tie2 overexpression in macrophages rescues ischemia (Patel et al. 2013). TEM may therefore play a significant role in vascular growth and repair, both in physiological and pathological conditions, and may be a novel target of exercise training.

Whereas the effects of acute exercise on EPC have been extensively studied and reviewed (De Biase et al. 2013; Witkowski et al. 2011), only a limited number of studies have been conducted examining the effects of different modes of exercise on T_{ANG}. A 10 km treadmill time trial increased circulating $CD3^+CD31^+$ counts immediately post-exercise in recreationally active men with a return to baseline levels at 1-h post exercise (Ross et al. 2016). Increases have also been demonstrated in older men following 30 min of continuous moderate to vigorous exercise (Ross et al. 2018a, b). Continuous exercise may also alter CAC surface marker and gene expression (Lansford et al. 2016). We are not aware of studies that have examined the influence of acute exercise on TEM, though intermediate monocytes expressing the chemokine receptor CCR2, are increased following a maximal treadmill test in patient groups (Van Craenenbroeck et al. 2014). Changes in CAC number may have implications for vascular development, as these cells ingress from the marginal pools into the circulation during a short post-exercise window.

Sprint interval exercise (SPRINT) is characterised by brief periods of "all out" anaerobic exercise at very high intensities, separated by recovery periods of lower intensity aerobic exercise or rest. A considerable body of evidence has emerged to support maximal high intensity and supramaximal sprint exercise, as a time efficient means of achieving the same if not greater physiological benefits than continuous aerobic exercise (Gibala et al. 2012). Both exercise modes can clearly influence vascular growth and development (Jensen et al. 2004; Murias et al. 2011). However, the comparative effects of continuous and repeated sprint exercise on circulating angiogenic cells have not previously been examined. There is some evidence, however, in the immunology field, that high intensity and continuous exercise can exert certain differential effects on T cell subsets (Kruger et al. 2016). The primary purpose of this study was therefore to compare the effects of a single bout of CONTEX and a single bout of SPRINT on circulating T_{ANG}, TEM, progenitor cells and their subsets, in recreationally active adults. We hypothesised that exercise would stimulate an increase in circulating angiogenic cells, and this response would be significantly greater after SPRINT exercise compared to CONTEX.

Methods

Study design

In this crossover acute exercise study, participants completed two trials on a cycle ergometer, one 45-min bout of continuous exercise at 70% $\dot{V}O_2$ peak and one bout of sprint interval exercise involving six maximum effort sprints. Blood was sampled pre-exercise, post-exercise, at 2 h and 24 h post-exercise in each trial. The order of the trials was counterbalanced and separated by one week approximately.

Participants

Twelve active healthy individuals (8 men and 4 women, aged 29 ± 2 years, weight 79 ± 3 kg, BMI 25.5 ± 0.9 kg m^{-2}, $\dot{V}O_2$ peak 44.3 ± 1.8 ml kg^{-1} min^{-1}) (mean ± SEM) participated in the study. All had been participating twice weekly for two years or more in personal fitness or recreation—related physical activity. Competitive endurance athletes were excluded from the study. The study was approved by the Waterford Institute of Technology Research Ethics Committee and written informed consent was obtained from each participant.

Preliminary visit

On a preliminary visit to the laboratory, participants completed a $\dot{V}O_2$ peak test on a Wattbike cycle ergometer (Wattbike Ltd, Nottingham, UK). The Wattbike is an air-braked cycle ergometer that calculates power output via a load cell next to the chain. Participants completed a 5-min warm up on the Wattbike after which the resistance was increased by 30 W every 3 min, starting at 120 W, until the participant reached their maximum. Expired air and heart rate were monitored throughout the test.

Trials

All trials commenced in the morning with participants reporting to the laboratory at circa 9 am on consecutive mornings for the pre- and 24 h samples. Participants were only permitted a light breakfast (cereal /toast) without tea or coffee before their first visit and repeated this intake prior to subsequent visits. During CONTEX, expired air was collected for the first 10 min during which the intensity was adjusted if necessary to keep the participant close to 70% $\dot{V}O_2$ peak. Expired air was again collected between 25 and 30 min and between 40 and 45 min. The need to keep the effort continuous was emphasised and participants were not allowed to undertake a "sprint finish". SPRINT consisted of 6 maximum effort sprints of 20 s duration, with 2 min between each, during which the participant rested on the bike or pedalled at very low intensity. Some initial pilot work was undertaken prior to the study commencing, with respect to the sprint interval protocol. The sprint interval regime was chosen to ensure that the intensity of exercise remained very high throughout each sprint, that the blood lactate response was maximised via multiple sprints and that the bout was tolerable for the population in question via 2 min recoveries.

Blood sampling and analysis

Peripheral blood samples were obtained from a prominent forearm vein by separate venepunctures, with participants in a semi-recumbent position. Samples were collected into serum and EDTA plasma tubes, centrifuged at 1500*g* for 15 min at 4 °C, divided into ~500 µl aliquots and stored at −80 °C for subsequent analysis. Leukocyte counts from the EDTA sample were determined using a haematology analyzer (AcT Diff2, Beckman Coulter, USA), checked with appropriate cell controls (4C-ES Cell Control, Beckman Coulter, USA). Blood lactate was determined immediately post-exercise from a finger-tip capillary sample (LactatePro, H/P/Cosmos, Germany). Serum cortisol was determined using a commercially available competitive enzyme-linked immunosorbent assay (R&D Systems, UK) with samples analysed in duplicate.

Circulating leukocytes and angiogenic cells were enumerated and analysed using multi-parametric flow cytometry (FC500, Beckman Coulter, USA). Three separate assays were undertaken for T_{ANG}, TEM and progenitor cells, each with the corresponding negative control samples. The following antibodies and isotype controls were employed, CD3-FITC, CXCR4-PE, CD8-PECF594, CD4-PECy5, CD31-PECy7, CD14-FITC, CD16-PECy7, CD34-FITC, CD45-FITC, IgG1-PE (all BD Biosciences, UK), Tie2-PE, VEGFR2-PE, IgG2a-PE and IgG1-PE (all RnD Systems, UK). T_{ANG} and T_{ANG} subsets were enumerated using CD3, CD8, CD4, CD31 and CXCR4. TEM were identified using CD14, CD16 and Tie2. The gating strategy to identify classical, non-classical and intermediate monocytes was adopted from the European Society of Cardiology Working Groups Consensus Document (Weber et al. 2016). The flow cytometric gating strategy for T_{ANG} and TEM is presented in Fig. 1. Progenitor cells were defined as $CD34^{+}CD45^{dim}$ based on the International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol (Sutherland et al. 1996). EPC were defined as $CD34^{+}CD45^{dim}VEGFR2^{+}$ and enumerated as described previously (Ross et al. 2014). Matched isotype control antibodies were used to distinguish CXCR4, Tie2 and VEGFR2 positive and negative regions. The influence of exercise on T_{ANG}, TEM and progenitor cells are presented in terms of cell counts (cells μL^{-1} or cells mL^{-1}) with CXCR4 expression on T_{ANG} presented as the mean fluorescent intensity (MFI) ratio between positive and negative control samples. Flow cytometric events were converted to cell counts using flow count beads (AccuCount, Spherotech, USA).

Statistical analysis

The influence of both exercise modes on circulating leukocyte and angiogenic cell counts and on marker expression was determined using a two-way, repeated measures Analysis of Variance (ANOVA). Where a significant trial × timepoint interaction was observed, post-hoc Least Significant Difference (LSD) pairwise comparisons were undertaken in each trial separately between the pre-exercise value and subsequent timepoints. In the absence of a trial × timepoint interaction, the significance of the main effect for timepoint was determined, with post-hoc LSD pairwise comparisons as appropriate. Cell ingress and egress data (% change from pre- to post-exercise and % change from post-exercise to 2 h post-exercise, respectively) were compared between the $Tie2^{+}$ monocyte subsets using a one-way repeated measures Analysis of Variance with post-hoc LSD pairwise comparisons as appropriate. Cell ingress and egress data

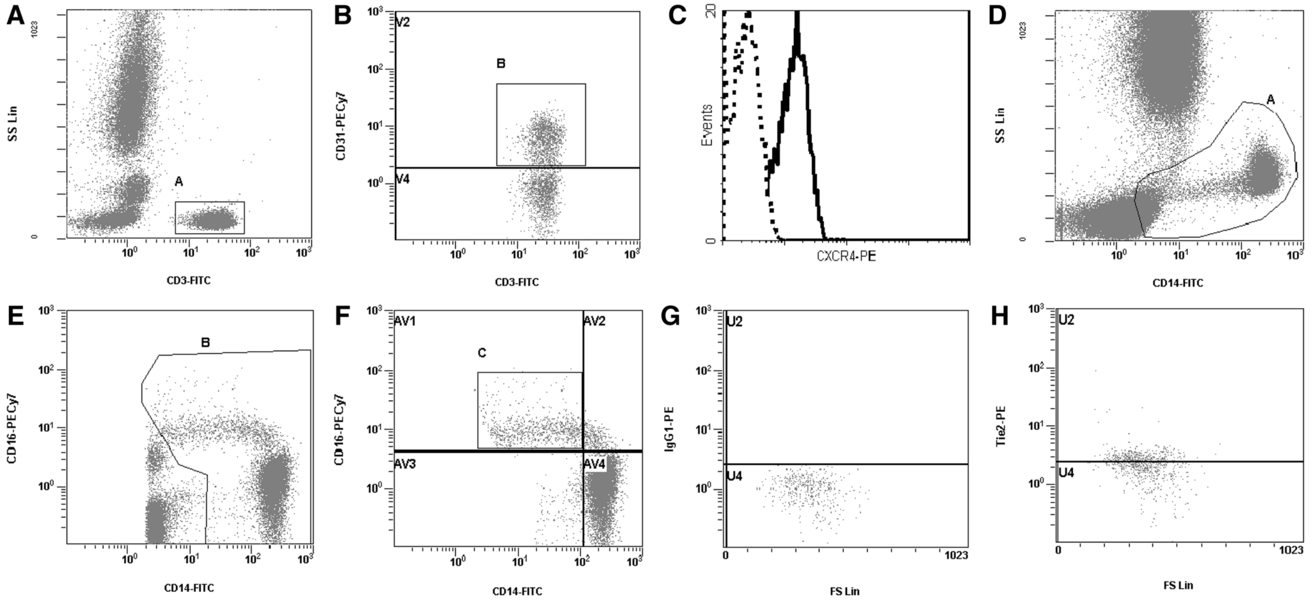

Fig. 1 Representative profile of the flow cytometric gating strategy for angiogenic T cell (**a**–**c**) and Tie2-expressing monocyte (**d**–**h**) analysis. **a** Initial T cell gate (A) on CD3 vs SS. **b** CD3 vs CD31 gated on gate (A) from previous plot **a**. **c** Overlay histogram of CXCR4 (solid line) and isotype control sample (dotted line) events, both gated on gate (B) from previous plot **b**. CD4 and CD8 subset analysis followed the approach in **a**–**c** with initial gating on gate (A) in CD3 vs SS. **d** Initial monocyte gate on CD14 vs SS with initial gate (A) encompassing all CD14 positive events and a portion of lymphocyte events. **e** CD14 vs CD16 events gated on (A) from previous plot **d** with exclusion of CD16 positive and negative lymphocytes outside of gate (B). **f** CD14 vs CD16 monocytes with Mon1, Mon2 and Mon3 subsets in the lower right, top right and top left quadrants, respectively. Additional gate (C) on Mon3 for subsequent analysis. **g**, **h** Tie2 positive Mon3 events (upper portion of plot **h**), with threshold determined using isotype control sample (plot **g**), both gated on (C) from plot **f**. Determination of Tie2 positive Mon1 and Mon2 events followed the approach in **d**–**h** with movement of gate (C) to the appropriate quadrant in plot **f**

were compared between the $CD4^+$ and $CD8^+$ T_{ANG} subsets and between CONTEX and SPRINT using paired t tests. In our laboratory, test–retest correlations for $CD3^+CD31^+$ cell counts are high (ICC > 0.83). Based on this preliminary data, we estimated that 9 participants would give 80% power to detect a 20% change in $CD3^+CD31^+$ counts (moderate effect size) in a repeated measures analysis, assuming $p < 0.05$. Values are reported as mean ± SEM. Significance was set at $p < 0.05$.

Results

$\dot{V}O_2$ during CONTEX was 30.5 ± 1.6 ml kg^{-1} min^{-1} representing 68 ± 2% of $\dot{V}O_2$ peak. The respiratory exchange ratio was 0.92 ± 0.01. The mean value for average power outputs across the 6 × 20 s sprints was 533 ± 30 W, representing 223 ± 6% of the power output corresponding to $\dot{V}O_2$ peak. The highest and lowest average power outputs during a 20-s sprint was 614 ± 53 W and 459 ± 34 W, representing 257 ± 12% and 192 ± 5% of the power output corresponding to $\dot{V}O_2$ peak, respectively. Blood lactate was 5.8 ± 0.7 mmol.L^{-1} and 13.5 ± 0.5 mmol.L^{-1} at the end of CONTEX and SPRINT, respectively.

There was a trial by timepoint interaction ($p < 0.05$) for circulating leukocytes, lymphocytes, $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, Mon1, Mon2 and Mon3 (Table 1). When compared to pre-exercise values, circulating leukocytes, lymphocytes, $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, Mon1, Mon2 and Mon3 were considerably higher ($p < 0.05$) post-exercise following SPRINT with leukocytes and Mon 1 still elevated at 2 h post-exercise (Table 1). There was a smaller increase ($p < 0.05$) in circulating leukocytes, lymphocytes, $CD3^+$ T cells, $CD8^+$ T cells and Mon1 post-exercise in CONTEX, with leukocytes and Mon1 still elevated at 2 h post-exercise (Table 1). There were no changes in circulating Mon2 or Mon3 in CONTEX (Table 1).

There was a trial by timepoint interaction ($p < 0.05$) for circulating $CD3^+CD31^+$ T_{ANG} and $CD8^+CD31^+$ T_{ANG}, with the interaction value for $CD4^+CD31^+$ T_{ANG} approaching significance ($p = 0.051$). When compared to pre-exercise values, $CD3^+CD31^+$ T_{ANG}, along with the $CD4^+CD31^+$ T_{ANG} and $CD8^+CD31^+$ T_{ANG} subsets, were considerably higher ($p < 0.05$) post-exercise following SPRINT (Fig. 2). Smaller increases ($p < 0.05$) were evident post-exercise in $CD3^+CD31^+$ T_{ANG} and the $CD8^+CD31^+$ T_{ANG} subset following CONTEX (Fig. 2). The percentage ingress and egress data (Fig. 3) show a greater ($p < 0.05$) ingress and

Table 1 Influence of continuous endurance exercise (CONTEX) and sprint interval exercise (SPRINT) on circulating mononuclear cells ($n = 12$)

Cell subset	Trial	Pre-Ex	Post-Ex	2 h post	24 h post	Trial × time-point interaction
Leukocytes (cells × 10^6.mL^{-1})	CONTEX	5.7 ± 0.5	7.1 ± 0.5*	8.0 ± 0.5*	5.5 ± 0.4	0.01
	SPRINT	5.9 ± 0.5	9.2 ± 0.6*	8.6 ± 0.7*	5.7 ± 0.4	
Lymphocytes (cells × 10^6.mL^{-1})	CONTEX	1.81 ± 0.10	2.25 ± 0.10*	1.87 ± 0.15	1.79 ± 0.14	< 0.001
	SPRINT	1.76 ± 0.14	3.58 ± 0.19*	1.65 ± 0.17	1.70 ± 0.13	
CD3$^+$ (cells µL^{-1})	CONTEX	1132 ± 91	1285 ± 83*	1138 ± 91	1115 ± 114	< 0.001
	SPRINT	1092 ± 94	1831 ± 163*	1040 ± 143	1094 ± 114	
CD3$^+$CD4$^+$ (cells µL^{-1})	CONTEX	698 ± 67	765 ± 68[a]	716 ± 66	686 ± 82	0.001
	SPRINT	675 ± 54	944 ± 83*	653 ± 86	651 ± 0.68	
CD3$^+$CD8$^+$ (cells µL^{-1})	CONTEX	354 ± 41	416 ± 38*	344 ± 40	329 ± 45	< 0.001
	SPRINT	334 ± 37	677 ± 77*	311 ± 48	324 ± 32	
Mon1 (cells µL^{-1})	CONTEX	488 ± 65	599 ± 62*	638 ± 59*	487 ± 33	0.013
	SPRINT	531 ± 71	833 ± 110*	676 ± 97*	512 ± 42	
Mon2 (cells µL^{-1})	CONTEX	23.0 ± 3.0	21.6 ± 2.8	18.2 ± 1.3	23.8 ± 4.6	0.002
	SPRINT	31.0 ± 7.9	72.9 ± 23.0*	23.7 ± 4.6	29.2 ± 7.1	
Mon3 (cells µL^{-1})	CONTEX	28.2 ± 5.0	31.5 ± 4.3	20.8 ± 3.2	29.2 ± 6.8	0.03
	SPRINT	32.2 ± 6.7	76.2 ± 19.0*	26.3 ± 5.0	32.1 ± 5.0	
CD34$^+$CD45^{dim} progenitors (cells mL^{-1})	CONTEX	1703 ± 225	1974 ± 314	1550 ± 226	1756 ± 279	0.03
	SPRINT	1515 ± 206	2496 ± 443*	1393 ± 204	1334 ± 130	
CD34$^+$CD45^{dim} VEGFR2$^+$ endothelial progenitors (cells mL^{-1})‡	CONTEX	245 ± 55	331 ± 83	267 ± 65	231 ± 34	0.88
	SPRINT	193 ± 37	260 ± 35	193 ± 36	114 ± 23	

Values shown are mean ± SEM. Mon1, Mon2 and Mon3 correspond to the classical (CD14^{++}CD16$^-$), intermediate (CD14^{++}CD16$^+$) and non-classical (CD14$^+$CD16^{++}) monocyte subsets, respectively. Analysis based on two-way (trial × timepoint) repeated measures Analysis of Variance with pairwise comparisons following significant interaction or main effect

*$p < 0.05$ compared to pre-exercise in same trial

‡Significant main effect for timepoint, Pre-Ex vs Post-Ex, $p < 0.05$

[a] $p = 0.06$ compared to pre-exercise

subsequent egress of CD3$^+$CD31$^+$ T$_{ANG}$, CD4$^+$CD31$^+$ T$_{ANG}$ and CD8$^+$CD31$^+$ T$_{ANG}$ in SPRINT compared to CONTEX. There was a greater ($p < 0.05$) ingress of CD8$^+$ T$_{ANG}$ compared to CD4$^+$ T$_{ANG}$ (Fig. 3). There was also a greater ingress of CD31$^-$ compared to CD31$^+$ cells in the CD3$^+$, CD4$^+$ and CD8$^+$ pools (data not shown). There was no trial x timepoint interaction for CXCR4 expression on any T cell subset. There were significant ($p < 0.05$) main effects for timepoint, however, for CXCR4 expression (MFI ratio) on CD3$^+$CD31$^+$, CD4$^+$CD31$^+$ and CD8$^+$CD31$^+$ cells with the MFI ratio increased at 24 h post-exercise compared to pre-exercise (Fig. 4).

There was a trial by timepoint interaction ($p < 0.05$) for circulating Tie2$^+$Mon1, Tie2$^+$Mon2, and Tie2$^+$Mon3. When compared to pre-exercise values, these subsets of angiogenic monocytes were considerably higher ($p < 0.05$) post-exercise following SPRINT, with no changes evident following CONTEX (Fig. 2). The ingress data and egress data (Fig. 3) show a greater ($p < 0.05$) ingress and subsequent egress of Tie2$^+$Mon2, and Tie2$^+$Mon3 cells in SPRINT compared to CONTEX. There was a greater ($p < 0.05$) ingress and subsequent egress of Tie2$^+$Mon2 and Tie2$^+$Mon3 cells compared to Tie2$^+$Mon1 cells following SPRINT (Fig. 3). Tie2 positivity was greater ($p < 0.05$) on Mon2 and Mon3 compared to Mon 1 (data not shown).

There was a trial by timepoint interaction ($p < 0.05$) for circulating CD34$^+$CD45^{dim} cells (total progenitor cells), but not for circulating CD34$^+$CD45^{dim}VEGFR2$^+$ endothelial progenitor cells (EPC). CD34$^+$CD45^{dim} counts were higher post-exercise in SPRINT but not in CONTEX (Table 1). There was a main effect for timepoint for EPC with values higher ($p < 0.05$) post-exercise and back to pre-exercise values at the 2 h post-timepoint (Table 1).

There was no trial × timepoint interaction for serum cortisol but a main effect for timepoint ($p < 0.05$) was observed. Timepoint (trial averaged) cortisol concentrations were 39.8 ± 2.9 ng mL^{-1}, 41.5 ± 5.0 ng mL^{-1}, 29.3 ± 3.0 ng mL^{-1} and 32.6 ± 2.9 ng mL^{-1} at pre-exercise, post-exercise 2 h and 24 h post-exercise, respectively, with values lower ($p < 0.05$) at 2 h and 24 h post-exercise compared to pre-exercise.

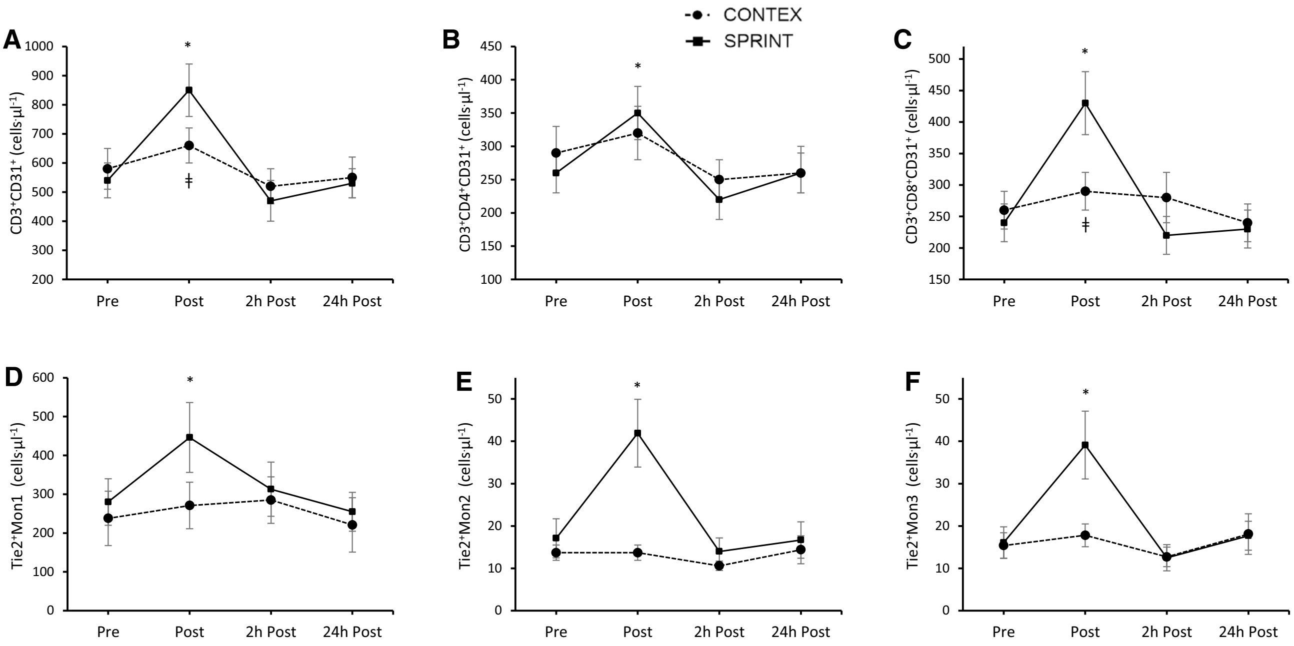

Fig. 2 Circulating angiogenic T cell (**a**–**c**) and Tie2-expressing monocyte (**d**–**f**) subsets at pre-, post-, 2 h and 24 h post-exercise following continuous exercise (CONTEX) and sprint interval exercise (SPRINT) ($n = 12$). Values shown are mean ± SEM. Mon1, Mon2 and Mon3 correspond to the classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocyte subsets, respectively. Analysis based on two-way (trial × timepoint) repeated measures Analysis of Variance with pairwise comparisons following significant interaction. Trial × timepoint interaction, $p < 0.05$ in **a**, **c**–**f**, $p = 0.51$ in **b**. $^{*}p < 0.05$ compared to pre-exercise in SPRINT. ǂ$p < 0.05$ compared to pre-exercise in CONTEX

Discussion

This study compared the effects of continuous endurance exercise and sprint interval exercise on circulating angiogenic T cell and monocyte subsets. Both exercise modes led to increases in angiogenic cell subsets, though the increase was of considerably greater magnitude following SPRINT. Increases in CAC were evident immediately post-exercise with counts typically back to pre-exercise values at the 2 h timepoint. Exercise also elicited a qualitative change in CAC, evidenced by the increase in CXCR4 expression on T_{ANG} at the 24 h timepoint.

To date, only a limited number of studies (Lansford et al. 2016; Ross et al. 2016, 2018a, b; Shill et al. 2016) examining the effects of acute exercise on circulating T_{ANG} have been undertaken. The results of the present study are similar to those observed following a 10-km treadmill time-trial (Ross et al. 2016), where increases in circulating $CD3^{+}CD31^{+}$, $CD8^{+}CD31^{+}$ and $CD4^{+}CD31^{+}$ cells were reported immediately post-exercise with values back to pre-exercise levels at 1 h post-exercise. Two other studies (Ross et al. 2018a, b) have reported that the response of these T_{ANG} cells to continuous exercise is age-dependent, with an impaired ingress and egress of these cells in older participants. The 45 min duration of exercise in CONTEX is similar to that in the 10 km time-trial. The percentage change following the time trial (+ 100%) was greater, however, than that observed following CONTEX (+ 14%). The relative exercise intensity during the self-paced time-trial is likely to have been higher than during CONTEX, particularly in the last minutes where a 1 km to completion notice was given to participants. "Sprint finishes" were not permitted in our study. Protocol differences may also be relevant as the 10 km trial analysis was based on PBMC. Direct comparisons with the other two works (Lansford et al. 2016; Shill et al. 2016) are more difficult as these reported increased CAC levels after exercise as percentage CAC changes within PBMC, compared to our absolute cell count changes. Of relevance to the present study, however, were the changes observed in surface marker and gene expression (Lansford et al. 2016), underlining the need to track both quantitative and qualitative cell changes in the post-exercise period.

Tie2 expression was higher on Mon2 and Mon3 than on Mon1, consistent with a greater pro-angiogenic role for these subsets (Shantsila et al. 2011). There were changes of 58–159% evident following SPRINT in all TEM subsets. The increases in $Tie2^{+}Mon2$ and $Tie2^{+}Mon3$ subsets were particularly large, the largest observed of any circulating angiogenic subset. The only comparable angiogenic monocyte ($CD14^{++}CD16^{+}CCR2^{+}$) study also reported increases

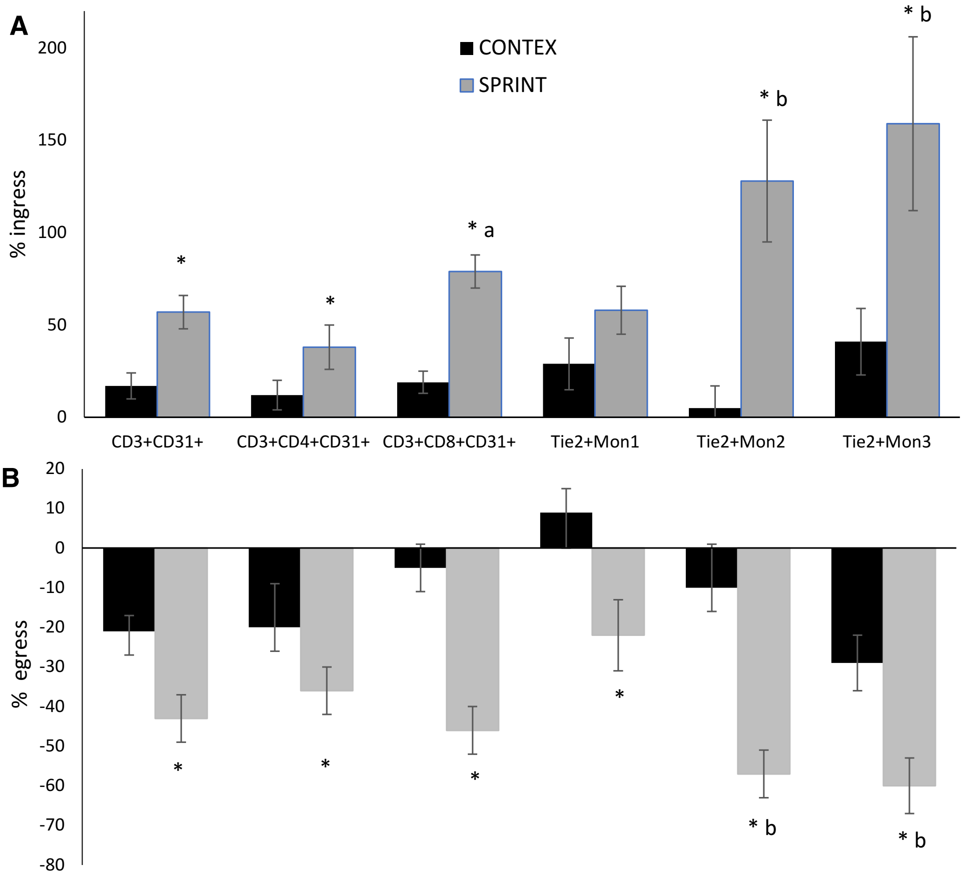

Fig. 3 Percentage ingress (**a**) and egress (**b**) of angiogenic T lymphocyte (T_{ANG}) and Tie2-expressing monocyte subsets following continuous exercise (CONTEX) and sprint interval exercise (SPRINT) ($n = 12$). Values shown are mean ± SEM. Mon1, Mon2 and Mon3 correspond to the classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocyte subsets, respectively. $^{a}p < 0.05$ compared to $CD4^{+}$ T_{ANG} SPRINT trial change, paired t test. $^{b}p < 0.05$ compared to $Tie2^{+}$Mon1 SPRINT trial change, one-way repeated measures Analysis of Variance with post-hoc pairwise comparisons. $^{*}p < 0.05$ compared to the corresponding CONTEX change, paired t tests

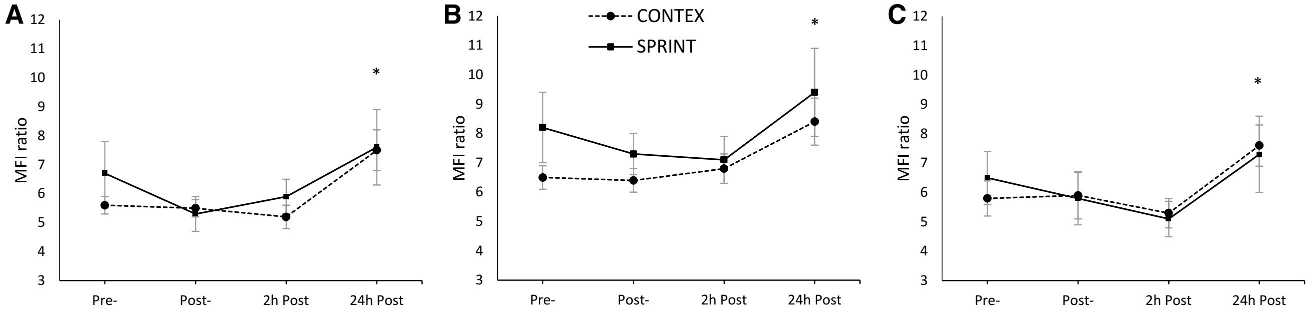

Fig. 4 CXCR4 mean fluorescence intensity (MFI) ratio on (**a**) $CD3^{+}CD31^{+}$ (**b**) $CD4^{+}CD31^{+}$ and (**c**) $CD8^{+}CD31^{+}$ angiogenic T cells at pre-, post-, 2 h and 24 h post-exercise following continuous exercise (CONTEX) and sprint interval exercise (SPRINT) ($n = 12$). Values shown are mean ± SEM. Analysis based on two-way (trial × timepoint) repeated measures Analysis of Variance. $^{*}p < 0.05$ compared to Pre-Ex, pairwise comparison following significant main effect for timepoint, no significant trial x timepoint interaction

following a maximal exercise test (Van Craenenbroeck et al. 2014). Unlike T_{ANG}, circulating Mon2, Mon3 and all TEM subsets were unchanged post-exercise in CONTEX. The $Tie2^{+}$Mon2 and $Tie2^{+}$Mon3 ingress data following SPRINT would indicate that these subsets are particularly responsive to acute exercise. The absence of any exercise effect on these TEM subsets following CONTEX deserves attention from a methodological perspective. The enumeration of low frequency events using flow cytometry is challenging. Unlike previous studies which analysed PBMC (Ross et al. 2016), our analysis was undertaken on fresh whole blood. Whereas this may decrease the signal to noise ratio in certain assays, whole blood has been recommended for monocyte assays (Weber et al. 2016) as monocytes are sensitive to sample processing. The use of additional angiogenic markers, such as CCR2 (Weber et al. 2016), can improve subset delineation and biological relevance. As technological advances in cytometry allow for the simultaneous detection of a greater number of markers, there is a trade-off, however, between the desire to enumerate novel CAC subsets of interest and the need for reproducible data.

In line with other subsets measured, $CD34^+CD45^{dim}$ circulating progenitor cells were increased post-exercise in the present study following SPRINT but not following CONTEX. The absence of a clear experimental effect on EPC, the original CAC, is noteworthy in the context of the difficulties in enumerating rare events but also in the context of the overlap and interaction between the various CAC populations. There is evidence that endothelial cell colony forming units (CFU-EC), formed from EPC in culture, are primarily composed of monocytes, with some T cells present (Yoder et al. 2007). The $CD31^+$ but not $CD31^-$ T cell fraction appears necessary for the formation of these CFU-EC (Hur et al. 2007). Future research efforts may be better directed at CAC subsets with high frequency in the circulation.

The increase in CAC was not uniform across all T lymphocyte and monocyte subsets; the increase in $CD31^-$ T lymphocytes was greater than that of $CD31^+$ T lymphocytes, the increase in CD8 T_{ANG} was greater than that of CD4 T_{ANG} and the increases in $Tie2^+Mon2$ and $Tie2^+Mon3$ were greater than that of $Tie2^+Mon1$. The T lymphocyte ingress data observed, with greater increases in the $CD8^+$ and $CD31^-$ subsets, mirror those of Ross et al. (2016) following the 10 km treadmill time-trial, also in recreationally active men. The preferential mobilisation of exercise responsive lymphocyte and monocyte subsets appears to be dependent on β2 adrenergic signalling (Graff et al. 2018), with preferential mobilisation of cytotoxic $CD8^+$ over $CD4^+$ T lymphocytes and pro-inflammatory $CD16^+$ over $CD14^+$ monocytes (Dimitrov et al. 2010). Less clear are the factors explaining the preferential mobilisation of the non-angiogenic $CD31^-$ cells but this may relate to T cell differentiation status (Ross et al. 2018a; Simpson et al. 2007). Regardless of these preferential release patterns, it should be noted that all angiogenic subsets were increased post-exercise in SPRINT by between 38 and 145%.

Sprint interval exercise has greater effects than continuous moderate intensity exercise on a number of vascular outcomes including flow-mediated dilatation (FMD) (Sawyer et al. 2016), vascular eNOS content (Cocks et al. 2013) and muscle capillarisation (Jensen et al. 2004). Sprint exercise may also have a greater effect on $\dot{V}O_2max$ (Milanovic et al. 2015). In an eloquent study, Tsai et al. (2016) demonstrated concomitant post-training increases in circulating angiogenic cells, vastus lateralis perfusion and the preservation of vascular endothelial integrity, that were greater following 6 weeks of high intensity interval training than following moderate intensity continuous training. Exercise, in part via adrenergic mechanisms, serves to ensure a re-distribution of T lymphocytes from the spleen through the circulation to target organs including the lungs, bone marrow and Peyer's patch (Kruger et al. 2008). Just as this redistribution of T cells to target organs is likely to play a role in immune surveillance, the regular redistribution of angiogenic cells from marginal pools with high intensity exercise training may be an important stimulus for angiogenesis and vascular development. The greater post-exercise increases in CAC following SPRINT may explain, at least in part, the enhanced vascular adaptations observed in other studies. Although back to pre-exercise levels at the 2 h timepoint, some of the liberated cells are likely to be redistributed through the circulation where they home to ischemic tissue via CXCR4, Tie2 and VEGFR2, adhere to an activated endothelium via CD31 and stimulate vascular growth via multiple secreted angiogenic factors. The factors known to be secreted by stimulated $CD31^+$ T cells include VEGF, IL-8, G-CSF, IL-17 and MMP-9 (Hur et al. 2007; Kushner et al. 2010a; Weil et al. 2011). An important issue not addressed in the present study is the fate of the mobilised angiogenic T cells and monocytes as they egress from the circulation following SPRINT. Direct evidence of their accumulation in exercised muscle following sprint and/or continuous exercise should be a consideration in future CAC research studies.

Exercise increased the expression of the chemokine receptor CXCR4 on $CD3^+$ T_{ANG}, $CD4^+$ T_{ANG} and $CD8^+$ T_{ANG} at 24 h post exercise without any differences between trials. The CXCR4/SDF-1α axis is essential for T lymphocyte and EPC migration along an SDF-1α gradient to ischaemic tissue (Mao et al. 2014) where they stimulate endothelial cell proliferation and vascular repair in a paracrine fashion. A strength of this study is that participants were followed for 24 h post-exercise, necessary to reveal this increase in CXCR4 expression. As circulating $CD3^+CD31^+$ cells were back to pre-exercise levels by 24 h, the change in MFI is less likely to be due to preferential mobilisation of $CXCR4^+$ cells and more likely to reflect increased protein expression. SDF-1α is also increased following continuous moderate to vigorous intensity exercise (Chang et al. 2015). The stimulus for the increases in CXCR4 expression is unclear. Cortisol has been shown to increase CXCR4 and CCR2 expression on T cells and monocytes, respectively, in vitro (Okutsu et al. 2005, 2008) but the cortisol data in this study do not support such a role, where values were reduced at the 2 h and 24 h timepoint. The present results do justify short-term training studies involving multiple exercise bouts utilising both exercise modes to identify late changes in CXCR4 expression on T_{ANG}, beyond the 24 h timepoint. The results also justify T cell functional studies to determine if changes in CXCR4 expression impact on T_{ANG} migration and angiogenic function.

This study had a number of strengths which add to knowledge in this field but also a number of limitations.

One strength is that participants were sampled up to 24 h post-exercise, allowing the increase in CXCR4 expression to be detected. During CONTEX, the participants were monitored so that a sprint finish did not occur in the concluding stages, which could have blurred any differences between trials. The study included men and women without reference to menstrual cycle stage. This must be considered a limitation, given the potential impact on angiogenic cells of menstrual cycle stage and contraceptive usage, identified recently (Shill et al. 2016). The small sample size is another limitation. Although adequate to identify differences in the key outcome measures between trials, a significant effect for timepoint was observed in some outcome measures, without sufficient statistical power to detect differences between trials.

In summary, anaerobic sprint interval exercise has a considerably greater effect on circulating angiogenic cell counts compared to continuous endurance exercise, suggestive of intensity dependent mobilisation. Angiogenic subsets of lymphocytes and monocytes are mobilised from exercise, but the effects are transient. Acute exercise also exerts changes on CXCR4 expression on T_{ANG} with the potential to increase migratory capacity of these novel vascular cells. The inclusion of some high intensity sprint interval exercise sessions in training regimes may therefore be beneficial to vascular development and repair.

Acknowledgements This study was supported by Technological Sector Research Strand I funding to Waterford Institute of Technology.

Author contributions MH, MDR and RM conceived and designed the research. LOC and BW conducted the experiments. LOC, BW, MDR and MH analysed the data. MH and LOC wrote the initial manuscript draft. All authors contributed to amendments and approved the manuscript.

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