1 **Title Page**

2 **Manuscript Title**: Circulating Angiogenic Cell Response to Sprint Interval and Continuous
3 Exercise

4 **Authors:** Louis O'Carroll[1], Bruce Wardrop[1], Ronan P. Murphy[2], Mark D. Ross[3], Michael
5 Harrison[1]

6 **Affiliations**

7 [1] Department of Sport and Exercise Science, Waterford Institute of Technology, Waterford,
8 Ireland.

9 [2] School of Health and Human Performance, Dublin City University, Dublin, Ireland.

10 [3] School of Applied Sciences, Edinburgh Napier University, Edinburgh, United Kingdom

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12 **Corresponding author**

13 Michael Harrison
14 Department of Sport and Exercise Science
15 Waterford Institute of Technology,
16 Waterford,
17 Ireland.
18 Telephone: +353-51-302161
19 Email: mharrison@wit.ie
20

21

22 **Abstract**


23 Although commonly understood as immune cells, certain T lymphocyte and monocyte subsets
24 have angiogenic potential, contributing to blood vessel growth and repair. These cells are
25 highly exercise responsive and may contribute to the cardiovascular benefits seen with
26 exercise. **Purpose:** To compare the effects of a single bout of continuous (CONTEX) and
27 sprint interval exercise (SPRINT) on circulating angiogenic cells (CAC) in healthy
28 recreationally active adults. **Methods**: Twelve participants (aged 29 ±2y, BMI 25.5±0.9 $kg \cdot m^{-2}$,
29 $\dot{V}O_2$peak 44.3±1.8 $ml \cdot kg^{-1} \cdot min^{-1}$; mean±SEM) participated in the study. Participants
30 completed a 45 min bout of CONTEX at 70% peak oxygen uptake and 6x20 sec sprints on a
31 cycle ergometer, in a counterbalanced design. Blood was sampled pre-, post-, 2h and 24h post-
32 exercise for quantification of CAC subsets by whole blood flow cytometric analysis.
33 Angiogenic T lymphocytes ($T_{ANG}$) and angiogenic Tie2-expressing monocytes (TEM) were
34 identified by the expression of CD31 and Tie2 respectively. **Results**: Circulating ($cells \cdot \mu L^{-1}$)
35 $CD3^+CD31^+T_{ANG}$ increased immediately post-exercise in both trials ($p<0.05$), with a
36 significantly greater increase ($p<0.05$) following SPRINT (+57%) compared to CONTEX
37 (+14%). Exercise increased ($p<0.05$) the expression of the chemokine receptor CXCR4 on
38 $T_{ANG}$ at 24h. Tie2-expressing classical ($CD14^{++}CD16^-$), intermediate ($CD14^{++}CD16^+$) and
39 non-classical ($CD14^+CD16^{++}$) monocytes and circulating $CD34^+CD45^{dim}$ progenitor cells were
40 higher post-exercise in SPRINT, but unchanged in CONTEX. All post-exercise increases in
41 SPRINT were back to pre-exercise levels at 2h and 24h. **Conclusion:** Acute exercise
42 transiently increases circulating $T_{ANG}$, TEM and progenitor cells with greater increases evident
43 following very high intensity sprint exercise than following prolonged continuous paced
44 endurance exercise.



45 **Keywords:** angiogenic T cells; Tie2 expressing monocytes, endothelial progenitor cells; high
46 intensity exercise

47 **Introduction**

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

57 Angiogenic T lymphocytes ($T_{ANG}$) are characterized by the presence of platelet endothelial cell
58 adhesion molecule-1 (CD31) on $CD3^+$ T cells (Hur et al. 2007; Kushner et al. 2010a).
59 Evidence from cell culture, animal and human studies support a role for $CD31^+$ T lymphocytes
60 in vascular development (Hur et al. 2007; Weil et al. 2011). Compared to $CD31^-$ cells, the
61 $CD31^+$ subset secretes higher levels of angiogenic cytokines including VEGF, IL-8, MMP-9,
62 G-CSF and IL-17, demonstrates greater migratory capacity towards SDF-1α and enhances
63 capillary tube formation *in vitro* (Hur et al. 2007; Kushner et al. 2010a; Weil et al. 2011). In a
64 hindlimb injury model of tissue ischemia, $CD31^+$ but not $CD31^-$ T cells restored tissue
65 perfusion in ($CD3^+$ deficient) nude mice (Hur et al. 2007). Additionally, circulating $T_{ANG}$
66 correlate with endothelial-dependent dilation (Weil et al. 2011) but inversely with age and
67 Framingham risk score (Hur et al. 2007). $T_{ANG}$ are considerably lower in older adults (Kushner
68 et al. 2010b; Ross et al. 2018b), individuals with atherosclerotic aortic aneurysms (Caligiuri et
69 al. 2006) and hypertensives with cerebral small vessel disease (Rouhl et al. 2012).

70 Monocytes can be classified into classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and
71 non-classical ($CD14^{+}CD16^{++}$) subsets, though more recently these subsets have been termed
72 Mon1, Mon2 and Mon3 respectively (Weber et al. 2016). Of these, Mon2 are considered to
73 have the greatest angiogenic potential with higher expression of pro-angiogenic molecules,
74 growth factors and chemokine receptors such as Tie2 and CCR2 (Jaipersad et al. 2014). Tie2
75 is an angiopoietin receptor and when expressed, allows monocytes to migrate along an
76 angiopoietin gradient towards ischemic tissue. Tie2 expressing monocytes (TEM) are more
77 frequently studied in relation to tumour angiogenesis, where they are the target of anti-cancer
78 therapy (De Palma et al. 2007) but have also been studied in critical limb ischemia (Patel et al.
79 2013) and peripheral arterial disease (Dopheide et al. 2016). Indeed, Tie2 knockdown in TEM
80 impairs restoration of blood flow in a mouse hindlimb model of ischemia whereas Tie2
81 overexpression in macrophages rescues ischemia (Patel et al. 2013). TEM may therefore play
82 a significant role in vascular growth and repair, both in physiological and pathological
83 conditions, and may be a novel target of exercise training.

84 Whereas the effects of acute exercise on EPC has been extensively studied and reviewed (De
85 Biase et al. 2013; Witkowski et al. 2011), only a limited number of studies have been conducted
86 examining the effects of different modes of exercise on $T_{ANG}$. A 10 km treadmill time trial
87 increased circulating $CD3^{+}CD31^{+}$ counts immediately post-exercise in recreationally active
88 men with a return to baseline levels at 1-hour post exercise (Ross et al. 2016). Increases have
89 also been demonstrated in older men following 30 min of continuous moderate to vigorous
90 exercise (Ross et al. 2018a; Ross et al. 2018b). Continuous exercise may also alter CAC
91 surface marker and gene expression (Lansford et al. 2016). We are not aware of studies that
92 have examined the influence of acute exercise on TEM, though intermediate monocytes
93 expressing the chemokine receptor CCR2, are increased following a maximal treadmill test in
94 patient groups (Van Craenenbroeck et al. 2014). Changes in CAC number may have

95 implications for vascular development, as these cells ingress from the marginal pools into the
96 circulation during a short post-exercise window.

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

112

113 **Methods**

114 *Study design*

115 In this crossover acute exercise study, participants completed two trials on a cycle ergometer,
116 one 45 min bout of continuous exercise at 70% $\dot{V}O_2$ peak and one bout of sprint interval
117 exercise involving six maximum effort sprints. Blood was sampled pre-exercise, post-exercise,

118 at 2 h and 24 h post-exercise in each trial. The order of the trials was counterbalanced and
119 separated by one week approximately.
120
121 *Participants*
122 Twelve active healthy individuals (8 men and 4 women, aged 29 ± 2 y, weight 79 ± 3 kg, BMI
123 25.5 ± 0.9 $kg{\cdot}m^{-2}$, $\dot{V}O_2$peak 44.3 ± 1.8 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) (mean ± SEM) participated in the study.
124 All had been participating twice weekly for two years or more in personal fitness or recreation
125 - related physical activity. Competitive endurance athletes were excluded from the study. The
126 study was approved by the Waterford Institute of Technology Research Ethics Committee and
127 written informed consent was obtained from each participant.
128
129 *Preliminary visit*
130 On a preliminary visit to the laboratory, participants completed a $\dot{V}O_2$ peak test on a Wattbike
131 cycle ergometer (Wattbike Ltd, Nottingham, UK). The Wattbike is an air-braked cycle
132 ergometer that calculates power output via a load cell next to the chain. Participants completed
133 a 5-minute warm up on the Wattbike after which the resistance was increased by 30W every 3
134 min, starting at 120W, until the participant reached their maximum. Expired air and heart rate
135 were monitored throughout the test.
136
137 *Trials*
138 All trials commenced in the morning with participants reporting to the laboratory at circa 9 am
139 on consecutive mornings for the pre- and 24 h samples. Participants were only permitted a
140 light breakfast (cereal /toast) without tea or coffee before their first visit and repeated this intake
141 prior to subsequent visits. During CONTEX, expired air was collected for the first 10 min
142 during which the intensity was adjusted if necessary to keep the participant close to 70% $\dot{V}O_2$

143 peak. Expired air was again collected between 25 and 30 min and between 40 and 45 min. The
144 need to keep the effort continuous was emphasised and participants were not allowed to
145 undertake a "sprint finish". SPRINT consisted of 6 maximum effort sprints of 20 sec duration,
146 with 2 min between each, during which the participant rested on the bike or pedalled at very
147 low intensity. Some initial pilot work was undertaken prior to the study commencing with
148 respect to the sprint interval protocol. The sprint interval regime was chosen to ensure that the
149 intensity of exercise remained very high throughout each sprint, that the blood lactate response
150 was maximised via multiple sprints and that the bout was tolerable for the population in
151 question via 2 min recoveries.
152
153 *Blood sampling and analysis*
154 Peripheral blood samples were obtained from a prominent forearm vein by separate
155 venepunctures, with participants in a semi-recumbent position. Samples were collected into
156 serum and EDTA plasma tubes, centrifuged at 1500g for 15 min at 4ºC, divided into ~500 µl
157 aliquots stored at -80ºC for subsequent analysis. Leukocyte counts from the EDTA sample
158 were determined using a haematology analyzer (AcT Diff2, Beckman Coulter, USA), checked
159 with appropriate cell controls (4C-ES Cell Control, Beckman Coulter, USA). Blood lactate was
160 determined immediately post-exercise from a finger-tip capillary sample (LactatePro,
161 H/P/Cosmos, Germany). Serum cortisol was determined using a commercially available
162 competitive enzyme-linked immunosorbent assay (R&D Systems, UK) with samples analysed
163 in duplicate.
164
165 Circulating leukocytes and angiogenic cells were enumerated and analysed using multi-
166 parametric flow cytometry (FC500, Beckman Coulter, USA). Three separate assays were
167 undertaken for $T_{ANG}$, TEM and progenitor cells, each with corresponding negative control

168 samples. The following antibodies and isotype controls were employed, CD3-FITC, CXCR4-
169 PE, CD8-PECF594, CD4-PECy5, CD31-PECy7, CD14-FITC, CD16-PECy7, CD34-FITC,
170 CD45-FITC, IgG1-PE (all BD Biosciences, UK), Tie2-PE, VEGFR2-PE, IgG2a-PE and IgG1-
171 PE (all RnD Systems, UK). $T_{ANG}$ and $T_{ANG}$ subsets were enumerated using CD3, CD8, CD4,
172 CD31 and CXCR4. TEM were identified using CD14, CD16 and Tie2. The gating strategy to
173 identify classical, non-classical and intermediate monocytes was adopted from the European
174 Society of Cardiology Working Groups Consensus Document (Weber et al. 2016). The flow
175 cytometric gating strategy for $T_{ANG}$ and TEM is presented in figure 1. Progenitor cells were
176 defined as $CD34^{+}CD45^{dim}$ based on the International Society of Hematotherapy and Graft
177 Engineering (ISHAGE) protocol (Sutherland et al. 1996). EPC were defined as
178 $CD34^{+}CD45^{dim}VEGFR2^{+}$ and enumerated as described previously (Ross et al. 2014). Matched
179 isotype control antibodies were used to distinguish CXCR4, Tie2 and VEGFR2 positive and
180 negative regions. The influence of exercise on $T_{ANG}$, TEM and progenitor cells are presented
181 in terms of cell counts ($cells \cdot \mu L^{-1}$ or $cells \cdot mL^{-1}$) with CXCR4 expression on $T_{ANG}$ presented as
182 the mean fluorescent intensity (MFI) ratio between positive and negative control samples. Flow
183 cytometric events were converted to cell counts using flow count beads (AccuCount,
184 Spherotech, USA).
185
186 *Statistical analysis*
187 The influence of both exercise modes on circulating leukocyte and angiogenic cell counts and
188 on marker expression was determined using a two-way, repeated measures Analysis of
189 Variance (ANOVA). Where a significant trial x timepoint interaction was observed, post-hoc
190 Least Significant Difference (LSD) pairwise comparisons were undertaken in each trial
191 separately between the pre-exercise value and subsequent timepoints. In the absence of a trial
192 x timepoint interaction, the significance of the main effect for timepoint was determined, with

193 post-hoc LSD pairwise comparisons as appropriate. Cell ingress and egress data (% change
194 from pre- to post-exercise and % change from post-exercise to 2 h post-exercise respectively)
195 were compared between the $Tie2^+$ monocyte subsets using a one-way repeated measures
196 Analysis of Variance with post-hoc LSD pairwise comparisons as appropriate. Cell ingress
197 and egress data were compared between the $CD4^+$ and $CD8^+$ $T_{ANG}$ subsets and between
198 CONTEX and SPRINT using paired t-tests. In our laboratory, test-retest correlations for
199 $CD3^+CD31^+$ cell counts are high (ICC>0.83). Based on this preliminary data, we estimated that
200 9 participants would give 80% power to detect a 20% change in $CD3^+CD31^+$ counts (moderate
201 effect size) in a repeated measures analysis, assuming $p<0.05$. Values are reported as mean ±
202 SEM. Significance was set at $p<0.05$.

203

204 **Results**

205 $\dot{V}O_2$ during CONTEX was 30.5 ± 1.6 $ml \cdot kg^{-1} \cdot min^{-1}$ representing 68 ± 2% of $\dot{V}O_2$ peak. The
206 respiratory exchange ratio was 0.92 ± 0.01. The mean value for average power outputs across
207 the 6 x 20 sec sprints was 533 ± 30 W, representing 223 ± 6% of the power output
208 corresponding to $\dot{V}O_2$ peak. The highest and lowest average power outputs during a 20 sec
209 sprint was 614 ± 53 W and 459 ± 34 W, representing 257 ± 12% and 192 ± 5% of the power
210 output corresponding to $\dot{V}O_2$peak respectively. Blood lactate was and 5.8 ± 0.7 $mmol \cdot L^{-1}$ and
211 13.5 ± 0.5 $mmol \cdot L^{-1}$ at the end of CONTEX and SPRINT respectively.

212 There was a trial by timepoint interaction ($p<0.05$) for circulating leukocytes, lymphocytes,
213 $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, Mon1, Mon2 and Mon3 (table 1). When compared
214 to pre-exercise values, circulating leukocytes, lymphocytes, $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$
215 T cells, Mon1, Mon2 and Mon3 were considerably higher ($p<0.05$) post-exercise following
216 SPRINT with leukocytes and Mon 1 still elevated at 2 h post-exercise (table 1). There was a

217 smaller increase ($p<0.05$) in circulating leukocytes, lymphocytes, $CD3^+$ T cells, $CD8^+$ T cells
218 and Mon1 post-exercise in CONTEX, with leukocytes and Mon1 still elevated at 2 h post-
219 exercise (table 1). There were no changes in circulating Mon2 or Mon3 in CONTEX (table 1).

220 There was a trial by timepoint interaction ($p<0.05$) for circulating $CD3^+CD31^+$ $T_{ANG}$ and
221 $CD8^+CD31^+$ $T_{ANG}$, with the interaction value for $CD4^+CD31^+$ $T_{ANG}$ approaching significance
222 ($p=0.051$). When compared to pre-exercise values, $CD3^+CD31^+$ $T_{ANG}$, along with the
223 $CD4^+CD31^+$ $T_{ANG}$ and $CD8^+CD31^+$ $T_{ANG}$ subsets, were considerably higher ($p<0.05$) post-
224 exercise following SPRINT (figure 2). Smaller increases ($p<0.05$) were evident post-exercise
225 in $CD3^+CD31^+$ $T_{ANG}$ and the $CD8^+CD31^+$ $T_{ANG}$ subset following CONTEX (figure 2). The
226 percentage ingress and egress data (figure 3) shows a greater ($p<0.05$) ingress and subsequent
227 egress of $CD3^+CD31^+$ $T_{ANG}$, $CD4^+CD31^+$ $T_{ANG}$ and $CD8^+CD31^+$ $T_{ANG}$ in SPRINT compared
228 to CONTEX. There was a greater ($p<0.05$) ingress of $CD8^+$ $T_{ANG}$ compared to $CD4^+$ $T_{ANG}$
229 (figure 3). There was also a greater ingress of $CD31^-$ compared to $CD31^+$ cells in the $CD3^+$,
230 $CD4^+$ and $CD8^+$ pools (data not shown). There was no trial x timepoint interaction for CXCR4
231 expression on any T cell subset. There were significant ($p<0.05$) main effects for timepoint
232 however for CXCR4 expression (MFI ratio) on $CD3^+CD31^+$, $CD4^+CD31^+$ and $CD8^+CD31^+$
233 cells with the MFI ratio increased at 24 h post-exercise compared to pre-exercise (figure 4).

234 There was a trial by timepoint interaction ($p<0.05$) for circulating $Tie2^+$Mon1, $Tie2^+$Mon2, and
235 $Tie2^+$Mon3. When compared to pre-exercise values, these subsets of angiogenic monocytes
236 were considerably higher ($p<0.05$) post-exercise following SPRINT, with no changes evident
237 following CONTEX (figure 2). The ingress data and egress data (figure 3) shows a greater
238 ($p<0.05$) ingress and subsequent egress of $Tie2^+$Mon2, and $Tie2^+$Mon3 cells in SPRINT
239 compared to CONTEX. There was a greater ($p<0.05$) ingress and subsequent egress of
240 $Tie2^+$Mon2 and $Tie2^+$Mon3 cells compared to $Tie2^+$Mon1 cells following SPRINT (figure 3).
241 Tie2 positivity was greater ($p<0.05$) on Mon2 and Mon3 compared to Mon 1 (data not shown).

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

247 There was no trial x timepoint interaction for serum cortisol but a main effect for timepoint
248 ($p<0.05$) was observed. Timepoint (trial averaged) cortisol concentrations were 39.8 ± 2.9
249 $ng{\cdot}mL^{-1}$, 41.5 ± 5.0 $ng{\cdot}mL^{-1}$, 29.3 ± 3.0 $ng{\cdot}mL^{-1}$ and 32.6 ± 2.9 $ng{\cdot}mL^{-1}$ at pre-exercise, post-
250 exercise 2 h and 24 h post-exercise respectively with values lower ($p<0.05$) at 2 h and 24 h
251 post-exercise compared to pre-exercise.

252

253 **Discussion**

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

260

261 To date, only a limited number of studies (Lansford et al. 2016; Ross et al. 2018a; Ross et al.
262 2016; Ross et al. 2018b; Shill et al. 2016) examining the effects of acute exercise on circulating
263 $T_{ANG}$ have been undertaken. The results of the present study are similar to those observed
264 following a 10 km treadmill time-trial (Ross et al. 2016), where increases in circulating

265 $CD3^+CD31^+$, $CD8^+CD31^+$ and $CD4^+CD31^+$ cells were reported immediately post-exercise
266 with values back to pre-exercise levels at 1 h post-exercise. Two other studies (Ross et al.
267 2018a; Ross et al. 2018b) have reported that the response of these $T_{ANG}$ cells to continuous
268 exercise is age-dependent, with an impaired ingress and egress of these cells in older
269 participants. The 45 min duration of exercise in CONTEX is similar to that in the 10 km time-
270 trial. The percentage changes following the time trial (+100%) was greater however than that
271 observed following CONTEX (+14%). The relative exercise intensity during the self-paced
272 time-trial is likely to have been higher than during CONTEX, particularly in the last minutes
273 where a 1 km to completion notice was given to participants. "Sprint finishes" were not
274 permitted in our study. Protocol differences may also be relevant however as the 10 km trial
275 analysis was based on PBMC. Direct comparisons with the other two works (Lansford et al.
276 2016; Shill et al. 2016) are more difficult as these reported increased CAC levels after exercise
277 as percentage CAC changes within PBMC, compared to our absolute cell count changes. Of
278 relevance to the present study however were the changes observed in surface marker and gene
279 expression (Lansford et al. 2016), underlining the need to track both quantitative and qualitative
280 cell changes in the post-exercise period.

281 Tie2 expression was higher on Mon2 and Mon3 than on Mon1, consistent with a greater pro-
282 angiogenic role for these subsets (Shantsila et al. 2011). There were changes of 58% - 159%
283 evident following SPRINT in all TEM subsets. The increases in $Tie2^+$Mon2 and $Tie2^+$Mon3
284 subsets were particularly large, the largest observed of any circulating angiogenic subset. The
285 only comparable angiogenic monocyte ($CD14^{++}CD16^+CCR2^+$) study also reported increases
286 following a maximal exercise test (Van Craenenbroeck et al. 2014). Unlike $T_{ANG}$, circulating
287 Mon2, Mon3 and all TEM subsets were unchanged post-exercise in CONTEX. The
288 $Tie2^+$Mon2 and $Tie2^+$Mon3 ingress data following SPRINT would indicate that these subsets
289 are particularly responsive to acute exercise. The absence of any exercise effect on these TEM

290 subsets following CONTEX, deserves attention from a methodological perspective. The
291 enumeration of low frequency events using flow cytometry is challenging. Unlike previous
292 studies which analysed PBMC (Ross et al. 2016), our analysis was undertaken on fresh whole
293 blood. Whereas this may decrease the signal to noise ratio in certain assays, whole blood has
294 been recommended for monocyte assays (Weber et al. 2016) as monocytes are sensitive to
295 sample processing. The use of additional angiogenic markers, such as CCR2 (Weber et al.
296 2016), can improve subset delineation and biological relevance. As technological advances in
297 cytometry allow for the simultaneous detection of a greater number of markers, there is a trade-
298 off however between the desire to enumerate novel CAC subsets of interest and the need for
299 reproducible data.

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

309 The increase in CAC was not uniform across all T lymphocytes and monocyte subsets; the
310 increase in $CD31^{-}$ T lymphocytes was greater than that of $CD31^{+}$ T lymphocytes, the increase
311 in CD8 $T_{ANG}$ was greater than that of CD4 $T_{ANG}$ and the increases in $Tie2^{+}$Mon2 and
312 $Tie2^{+}$Mon3 were greater than that of $Tie2^{+}$Mon1. The T lymphocyte ingress data observed,
313 with greater increases in the $CD8^{+}$ and $CD31^{-}$ subsets, mirror those of Ross et al. (Ross et al.
314 2016) following the 10 km treadmill time-trial, also in recreationally active men. The

315 preferential mobilisation of exercise responsive lymphocyte and monocyte subsets appears to
316 be dependent on β2 adrenergic signalling (Graff et al. 2018), with preferential mobilisation of
317 cytotoxic $CD8^+$ over $CD4^+$ T lymphocytes and pro-inflammatory $CD16^+$ over $CD14^+$
318 monocytes (Dimitrov et al. 2010). Less clear are the factors explaining the preferential
319 mobilisation of the non-angiogenic $CD31^-$ cells but this may relate to T cell differentiation
320 status (Ross et al. 2018a; Simpson et al. 2007). Regardless of these preferential release
321 patterns, it should be noted that all angiogenic subsets were increased post-exercise in SPRINT
322 by between 38% and 145%.

323 Sprint interval exercise has greater effects than continuous moderate intensity exercise on a
324 number of vascular outcomes including flow-mediated dilatation (FMD) (Sawyer et al. 2016),
325 vascular eNOS content (Cocks et al. 2013) and muscle capillarization (Jensen et al. 2004).
326 Sprint exercise may also have a greater effect on $VO_2max$ (Milanovic et al. 2015). In an
327 eloquent study, Tsai and colleagues (2016) demonstrated concomitant post-training increases
328 in circulating angiogenic cells, vastus lateralis perfusion and the preservation of vascular
329 endothelial integrity, that were greater following 6 weeks of high intensity interval training
330 than following moderate intensity continuous training. Exercise, in part via adrenergic
331 mechanisms, serves to ensure a re-distribution of T lymphocytes from the spleen through the
332 circulation to target organs including the lungs, bone marrow and Peyer's patch (Kruger et al.
333 2008). Just as this redistribution of T cells to target organs is likely to play a role in immune
334 surveillance, the regular redistribution of angiogenic cells from marginal pools with high
335 intensity exercise training may be an important stimulus for angiogenesis and vascular
336 development. The greater post-exercise increases in CAC following SPRINT may explain, at
337 least in part, the enhanced vascular adaptations observed in other studies. Although back to
338 pre-exercise levels at the 2 h timepoint, some of the liberated cells are likely to be redistributed
339 through the circulation where they home to ischemic tissue via CXCR4, Tie2 and VEGFR2,

340 adhere to an activated endothelium via CD31 and stimulate vascular growth via multiple
341 secreted angiogenic factors. The factors known to be secreted by stimulated $CD31^+$ T cells
342 include VEGF, IL-8, G-CSF, IL-17 and MMP-9 (Hur et al. 2007; Kushner et al. 2010a; Weil
343 et al. 2011). An important issue not addressed in the present study is the fate of the mobilised
344 angiogenic T cells and monocytes as they egress from the circulation following SPRINT.
345 Direct evidence of their accumulation in exercised muscle following sprint and/or continuous
346 exercise should be a consideration in future CAC research studies.

347 Exercise increased the expression of the chemokine receptor CXCR4 on $CD3^+$ $T_{ANG}$, $CD4^+$
348 $T_{ANG}$ and $CD8^+$ $T_{ANG}$ at 24 h post exercise without any differences between trials. The
349 CXCR4/SDF-1α axis is essential for T lymphocyte and EPC migration along an SDF-1α
350 gradient to ischaemic tissue (Mao et al. 2014) where they stimulate endothelial cell
351 proliferation and vascular repair in a paracrine fashion. A strength of this study is that
352 participants were followed for 24 h post-exercise, necessary to reveal this increase in CXCR4
353 expression. As circulating $CD3^+CD31^+$ cells were back to pre-exercise levels by 24 h, the
354 change in MFI is less likely to be due to preferential mobilisation of $CXCR4^+$ cells and more
355 likely to reflect increased protein expression. SDF-1α is also increased following continuous
356 moderate to vigorous intensity exercise (Chang et al. 2015). The stimulus for the increases in
357 CXCR4 expression is unclear. Cortisol has been shown to increase CXCR4 and CCR2
358 expression on T cells and monocytes respectively *in vitro* (Okutsu et al. 2005; Okutsu et al.
359 2008) but the cortisol data in this study do not support such a role, where values were reduced
360 at the 2 h and 24 h timepoint. The present results do justify short-term training studies
361 involving multiple exercise bouts utilising both exercise modes to identify late changes in
362 CXCR4 expression on $T_{ANG}$, beyond the 24 h timepoint. The results also justify T cell
363 functional studies to determine if changes in CXCR4 expression impact on $T_{ANG}$ migration and
364 angiogenic function.

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

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

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386

387 **Author Contribution Statement**

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

391

392 **References**

393 Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B,
394 Schatteman G, Isner JM (1997) Isolation of putative progenitor endothelial cells for
395 angiogenesis Science 275:964-967

396 Caligiuri G, Rossignol P, Julia P, Groyer E, Mouradian D, Urbain D, Misra N, Ollivier V,
397 Sapoval M, Boutouyrie P, Kaveri SV, Nicoletti A, Lafont A (2006) Reduced
398 immunoregulatory CD31+ T cells in patients with atherosclerotic abdominal aortic aneurysm
399 Arterioscler Thromb Vasc Biol 26:618-623 doi:10.1161/01.ATV.0000200380.73876.d9

400 Capoccia BJ, Shepherd RM, Link DC (2006) G-CSF and AMD3100 mobilize monocytes into
401 the blood that stimulate angiogenesis in vivo through a paracrine mechanism Blood
402 108:2438-2445 doi:10.1182/blood-2006-04-013755

403 Chang E, Paterno J, Duscher D, Maan ZN, Chen JS, Januszyk M, Rodrigues M, Rennert RC,
404 Bishop S, Whitmore AJ, Whittam AJ, Longaker MT, Gurtner GC (2015) Exercise induces
405 stromal cell-derived factor-1alpha-mediated release of endothelial progenitor cells with
406 increased vasculogenic function Plast Reconstr Surg 135:340e-350e
407 doi:10.1097/PRS.0000000000000917

408 Cocks M, Shaw CS, Shepherd SO, Fisher JP, Ranasinghe AM, Barker TA, Tipton KD,
409 Wagenmakers AJ (2013) Sprint interval and endurance training are equally effective in
410 increasing muscle microvascular density and eNOS content in sedentary males J Physiol
411 591:641-656 doi:10.1113/jphysiol.2012.239566

412 De Biase C, De Rosa R, Luciano R, De Luca S, Capuano E, Trimarco B, Galasso G (2013)
413 Effects of physical activity on endothelial progenitor cells (EPCs) Front Physiol 4:414
414 doi:10.3389/fphys.2013.00414

415 De Palma M, Murdoch C, Venneri MA, Naldini L, Lewis CE (2007) Tie2-expressing
416 monocytes: regulation of tumor angiogenesis and therapeutic implications Trends Immunol
417 28:519-524 doi:10.1016/j.it.2007.09.004

418 Dimitrov S, Lange T, Born J (2010) Selective mobilization of cytotoxic leukocytes by
419 epinephrine J Immunol 184:503-511 doi:10.4049/jimmunol.0902189

420 Dopheide JF, Geissler P, Rubrech J, Trumpp A, Zeller GC, Daiber A, Munzel T, Radsak MP,
421 Espinola-Klein C (2016) Influence of exercise training on proangiogenic TIE-2 monocytes
422 and circulating angiogenic cells in patients with peripheral arterial disease Clin Res Cardiol
423 105:666-676 doi:10.1007/s00392-016-0966-0

424 Gibala MJ, Little JP, Macdonald MJ, Hawley JA (2012) Physiological adaptations to low-
425 volume, high-intensity interval training in health and disease J Physiol 590:1077-1084
426 doi:10.1113/jphysiol.2011.224725

427 Graff RM, Kunz HE, Agha NH, Baker FL, Laughlin M, Bigley AB, Markofski MM, LaVoy
428 EC, Katsanis E, Bond RA, Bollard CM, Simpson RJ (2018) beta2-Adrenergic receptor
429 signaling mediates the preferential mobilization of differentiated subsets of CD8+ T-cells,
430 NK-cells and non-classical monocytes in response to acute exercise in humans Brain Behav
431 Immun doi:10.1016/j.bbi.2018.08.017

432 Hur J, Yang HM, Yoon CH, Lee CS, Park KW, Kim JH, Kim TY, Kim JY, Kang HJ, Chae
433 IH, Oh BH, Park YB, Kim HS (2007) Identification of a novel role of T cells in postnatal
434 vasculogenesis: characterization of endothelial progenitor cell colonies Circulation 116:1671-
435 1682 doi:10.1161/CIRCULATIONAHA.107.694778

436 Jaipersad AS, Shantsila A, Lip GY, Shantsila E (2014) Expression of monocyte subsets and
437 angiogenic markers in relation to carotid plaque neovascularization in patients with pre-
438 existing coronary artery disease and carotid stenosis Ann Med 46:530-538
439 doi:10.3109/07853890.2014.931101

440 Jensen L, Bangsbo J, Hellsten Y (2004) Effect of high intensity training on capillarization
441 and presence of angiogenic factors in human skeletal muscle J Physiol 557:571-582
442 doi:10.1113/jphysiol.2003.057711

443 Kruger K, Alack K, Ringseis R, Mink L, Pfeifer E, Schinle M, Gindler K, Kimmelmann L,
444 Walscheid R, Muders K, Frech T, Eder K, Mooren FC (2016) Apoptosis of T-Cell Subsets
445 after Acute High-Intensity Interval Exercise Med Sci Sports Exerc 48:2021-2029
446 doi:10.1249/MSS.0000000000000979

447 Kruger K, Lechtermann A, Fobker M, Volker K, Mooren FC (2008) Exercise-induced
448 redistribution of T lymphocytes is regulated by adrenergic mechanisms Brain Behav Immun
449 22:324-338 doi:10.1016/j.bbi.2007.08.008

450 Kushner EJ, MacEneaney OJ, Morgan RG, Van Engelenburg AM, Van Guilder GP, DeSouza
451 CA (2010a) CD31+ T cells represent a functionally distinct vascular T cell phenotype Blood
452 Cells Mol Dis 44:74-78 doi:10.1016/j.bcmd.2009.10.009

453 Kushner EJ, Weil BR, MacEneaney OJ, Morgan RG, Mestek ML, Van Guilder GP, Diehl KJ,
454 Stauffer BL, DeSouza CA (2010b) Human aging and CD31+ T-cell number, migration,
455 apoptotic susceptibility, and telomere length J Appl Physiol (1985) 109:1756-1761
456 doi:10.1152/japplphysiol.00601.2010

457 Lansford KA, Shill DD, Dicks AB, Marshburn MP, Southern WM, Jenkins NT (2016) Effect
458 of acute exercise on circulating angiogenic cell and microparticle populations Exp Physiol
459 101:155-167 doi:10.1113/EP085505

460 Mao L, Huang M, Chen SC, Li YN, Xia YP, He QW, Wang MD, Huang Y, Zheng L, Hu B
461 (2014) Endogenous endothelial progenitor cells participate in neovascularization via
462 CXCR4/SDF-1 axis and improve outcome after stroke CNS Neurosci Ther 20:460-468
463 doi:10.1111/cns.12238

464 Milanovic Z, Sporis G, Weston M (2015) Effectiveness of High-Intensity Interval Training
465 (HIT) and Continuous Endurance Training for VO2max Improvements: A Systematic
466 Review and Meta-Analysis of Controlled Trials Sports Med 45:1469-1481
467 doi:10.1007/s40279-015-0365-0

468 Murias JM, Kowalchuk JM, Ritchie D, Hepple RT, Doherty TJ, Paterson DH (2011)
469 Adaptations in capillarization and citrate synthase activity in response to endurance training
470 in older and young men J Gerontol A Biol Sci Med Sci 66:957-964
471 doi:10.1093/gerona/glr096

472 Okutsu M, Ishii K, Niu KJ, Nagatomi R (2005) Cortisol-induced CXCR4 augmentation
473 mobilizes T lymphocytes after acute physical stress Am J Physiol Regul Integr Comp Physiol
474 288:R591-599 doi:10.1152/ajpregu.00438.2004

475 Okutsu M, Suzuki K, Ishijima T, Peake J, Higuchi M (2008) The effects of acute exercise-
476 induced cortisol on CCR2 expression on human monocytes Brain Behav Immun 22:1066-
477 1071 doi:10.1016/j.bbi.2008.03.006

478 Patel AS, Smith A, Nucera S, Biziato D, Saha P, Attia RQ et al. (2013) TIE2-expressing
479 monocytes/macrophages regulate revascularization of the ischemic limb EMBO Mol Med
480 5:858-869 doi:10.1002/emmm.201302752

481 Ross M, Ingram L, Taylor G, Malone E, Simpson RJ, West D, Florida-James G (2018a)
482 Older men display elevated levels of senescence-associated exercise-responsive CD28(null)
483 angiogenic T cells compared with younger men Physiol Rep 6:e13697
484 doi:10.14814/phy2.13697

485 Ross M, Tormey P, Ingram L, Simpson R, Malone E, Florida-James G (2016) A 10 km time
486 trial running bout acutely increases the number of angiogenic T cells in the peripheral blood
487 compartment of healthy males Exp Physiol 101:1253-1264 doi:10.1113/EP085771

488 Ross MD, Malone EM, Simpson R, Cranston I, Ingram L, Wright GP, Chambers G, Florida-
489 James G (2018b) Lower resting and exercise-induced circulating angiogenic progenitors and
490 angiogenic T cells in older men Am J Physiol Heart Circ Physiol 314:H392-H402
491 doi:10.1152/ajpheart.00592.2017

492 Ross MD, Wekesa AL, Phelan JP, Harrison M (2014) Resistance exercise increases
493 endothelial progenitor cells and angiogenic factors Med Sci Sports Exerc 46:16-23
494 doi:10.1249/MSS.0b013e3182a142da

495 Rouhl RP, Mertens AE, van Oostenbrugge RJ, Damoiseaux JG, Debrus-Palmans LL,
496 Henskens LH, Kroon AA, de Leeuw PW, Lodder J, Tervaert JW (2012) Angiogenic T-cells
497 and putative endothelial progenitor cells in hypertension-related cerebral small vessel disease
498 Stroke 43:256-258 doi:10.1161/STROKEAHA.111.632208

499 Sawyer BJ, Tucker WJ, Bhammar DM, Ryder JR, Sweazea KL, Gaesser GA (2016) Effects
500 of high-intensity interval training and moderate-intensity continuous training on endothelial
501 function and cardiometabolic risk markers in obese adults J Appl Physiol (1985) 121:279-288
502 doi:10.1152/japplphysiol.00024.2016

503 Shantsila E, Wrigley B, Tapp L, Apostolakis S, Montoro-Garcia S, Drayson MT, Lip GY
504 (2011) Immunophenotypic characterization of human monocyte subsets: possible
505 implications for cardiovascular disease pathophysiology J Thromb Haemost 9:1056-1066
506 doi:10.1111/j.1538-7836.2011.04244.x

507 Shill DD, Marshburn MP, Hempel HK, Lansford KA, Jenkins NT (2016) Heterogeneous
508 Circulating Angiogenic Cell Responses to Acute Maximal Exercise Med Sci Sports Exerc
509 48:2536-2543 doi:10.1249/MSS.0000000000001029

510 Simpson RJ, Florida-James GD, Cosgrove C, Whyte GP, Macrae S, Pircher H, Guy K (2007)
511 High-intensity exercise elicits the mobilization of senescent T lymphocytes into the
512 peripheral blood compartment in human subjects J Appl Physiol (1985) 103:396-401
513 doi:10.1152/japplphysiol.00007.2007

514 Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I (1996) The ISHAGE
515 guidelines for CD34+ cell determination by flow cytometry. International Society of
516 Hematotherapy and Graft Engineering J Hematother 5:213-226 doi:10.1089/scd.1.1996.5.213

517 Van Craenenbroeck AH, Van Ackeren K, Hoymans VY, Roeykens J, Verpooten GA, Vrints
518 CJ, Couttenye MM, Van Craenenbroeck EM (2014) Acute exercise-induced response of
519 monocyte subtypes in chronic heart and renal failure Mediators Inflamm 2014:216534
520 doi:10.1155/2014/216534

521 Weber C, Shantsila E, Hristov M, Caligiuri G, Guzik T, Heine GH et al. (2016) Role and
522 analysis of monocyte subsets in cardiovascular disease. Joint consensus document of the
523 European Society of Cardiology (ESC) Working Groups "Atherosclerosis & Vascular
524 Biology" and "Thrombosis" Thromb Haemost 116:626-637 doi:10.1160/TH16-02-0091

525 Weil BR, Kushner EJ, Diehl KJ, Greiner JJ, Stauffer BL, Desouza CA (2011) CD31+ T cells,
526 endothelial function and cardiovascular risk Heart Lung Circ 20:659-662
527 doi:10.1016/j.hlc.2011.06.003

528 Witkowski S, Jenkins NT, Hagberg JM (2011) Enhancing treatment for cardiovascular
529 disease: exercise and circulating angiogenic cells Exerc Sport Sci Rev 39:93-101
530 doi:10.1097/JES.0b013e31820a595e

531 Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prchal JT,
532 Ingram DA (2007) Redefining endothelial progenitor cells via clonal analysis and
533 hematopoietic stem/progenitor cell principals Blood 109:1801-1809 doi:10.1182/blood-2006-
534 08-043471

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536 **Table 1** - Influence of continuous endurance exercise (CONTEX) and sprint interval exercise
537 (SPRINT) on circulating mononuclear cells (n=12).

| **Cell subset** | **Trial** | **Pre-Ex** | **Post-Ex** | **2 h post** | **24 h post** | **Trial x timepoint interaction** |
|---|---|---|---|---|---|---|
| Leukocytes (cells x $10^6 \cdot mL^{-1}$) | 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 x $10^6 \cdot mL^{-1}$) | 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 | |
| $CD3^+$ ($cells \cdot \mu L^{-1}$) | CONTEX | 1132 ± 91 | 1285 ± 83 * | 1138 ± 91 | 1115 ± 114 | <0.001 |
| | SPRINT | 1092 ± 94 | 1831 ± 163 * | 1040 ± 143 | 1094 ± 114 | |
| $CD3^+CD4^+$ ($cells \cdot \mu L^{-1}$) | CONTEX | 698 ± 67 | 765 ± 68 [a] | 716 ± 66 | 686 ± 82 | 0.001 |
| | SPRINT | 675 ± 54 | 944 ± 83 * | 653 ± 86 | 651 ± 0.68 | |
| $CD3^+CD8^+$ ($cells \cdot \mu L^{-1}$) | CONTEX | 354 ± 41 | 416 ± 38 * | 344 ± 40 | 329 ± 45 | <0.001 |
| | SPRINT | 334 ± 37 | 677 ± 77* | 311 ± 48 | 324 ± 32 | |
| Mon1 ($cells \cdot \mu L^{-1}$) | CONTEX | 488 ± 65 | 599 ± 62 * | 638 ± 59 * | 487 ± 33 | 0.013 |
| | SPRINT | 531 ± 71 | 833 ± 110 * | 676 ± 97 * | 512 ± 42 | |
| Mon2 ($cells \cdot \mu L^{-1}$) | 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 ($cells \cdot \mu L^{-1}$) | 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 ($cells \cdot mL^{-1}$) | 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 ($cells \cdot mL^{-1}$) ǂ | CONTEX | 245 ± 55 | 331 ± 83 | 267 ± 65 | 231 ± 34 | 0.88 |
| | SPRINT | 193 ± 37 | 260 ± 35 | 193 ± 36 | 114 ± 23 | |

538

539 *Values shown are mean ± SEM.* Mon1, Mon2 and Mon3 correspond to the classical ($CD14^{++}CD16^-$),
540 intermediate ($CD14^{++}CD16^+$) and non-classical ($CD14^+CD16^{++}$) monocyte subsets respectively.
541 Analysis based on two-way (trial x timepoint) repeated measures Analysis of Variance with pairwise
542 comparisons following significant interaction or main effect. * $p<0.05$ compared to pre-exercise in
543 same trial. [a] $p=0.06$ compared to pre-exercise. ǂ significant main effect for timepoint, Pre-Ex vs Post-
544 Ex, $p<0.05$.

545

546

547 **Figure legends**

548 **Figure 1** – Representative profile of the flow cytometric gating strategy for angiogenic T cell
549 (A – C) and Tie2-expressing monocyte (D – H) analysis.

550 A- Initial T cell gate (A) on CD3 vs SS. B- CD3 vs CD31 gated on (A) from previous plot A.
551 C- Overlay histogram of CXCR4 (solid line) and isotype control sample (dotted line) events,
552 both gated on gated (B) from previous plot B. CD4 and CD8 subset analysis followed the
553 approach in A – C with initial gating on CD3 vs SS in (A). D- Initial monocyte gate on CD14
554 vs SS with initial gate (A) encompassing all CD14 positive events and a portion of lymphocyte
555 events. E- CD14 vs CD16 events gated on (A) from previous plot D with exclusion of CD16
556 positive and negative lymphocytes outside of gate (B). F- CD14 vs CD16 monocytes with
557 Mon1, Mon2 and Mon3 subsets in the lower right, top right and top left quadrants respectively.
558 Additional gate (C) on Mon3 for subsequent analysis. G and H- Tie 2 positive Mon3 events
559 (upper portion of plot H), with threshold determined using isotype control sample (plot G),
560 both gated on (C) from plot F. Determination of Tie2 positive Mon1 and Mon2 events followed
561 the approach in D – H with movement of gate (C) to the appropriate quadrant in plot F.

562

563 **Figure 2** – Circulating angiogenic T cell (A – C) and Tie2-expressing monocyte (D – F)
564 subsets at pre-, post-, 2 h and 24 h post-exercise following continuous exercise (CONTEX)
565 and sprint interval exercise (SPRINT) (n=12).

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

570 timepoint interaction, $p<0.05$ in A, C, D, E and F, $p=0.51$ in B. * $p<0.05$ compared to pre-
571 exercise in SPRINT. ǂ $p<0.05$ compared to pre-exercise in CONTEX

572

573 **Figure 3** - Percentage ingress (A) and egress (B) of angiogenic T lymphocyte ($T_{ANG}$) and
574 Tie2-expressing monocyte subsets following continuous exercise (CONTEX) and sprint
575 interval exercise (SPRINT) (n=12).

576 *Values shown are mean ± SEM.* Mon1, Mon2 and Mon3 correspond to the classical
577 ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocyte
578 subsets respectively. [a] $p<0.05$ compared to $CD4^{+}$ $T_{ANG}$ SPRINT trial change, paired t-test. [b]
579 $p<0.05$ compared to $Tie2^{+}$Mon1 SPRINT trial changes, one-way repeated measures Analysis
580 of Variance with post-hoc pairwise comparisons. * $p<0.05$ compared to corresponding
581 CONTEX change, paired t-tests.

582

583 **Figure 4** – CXCR4 mean fluorescence intensity (MFI) ratio on (A) $CD3^{+}CD31^{+}$ (B) $CD4^{+}CD31^{+}$
584 and (C) $CD8^{+}CD31^{+}$ angiogenic T cells at pre-, post-, 2 h and 24 h post-exercise following
585 continuous exercise (CONTEX) and sprint interval exercise (SPRINT) (n=12).

586

587 *Values shown are mean ± SEM.* Analysis based on two-way (trial x timepoint) repeated
588 measures Analysis of Variance. * $p<0.05$ compared to Pre-Ex, main effects for timepoint
589 pairwise comparison, no significant interaction.

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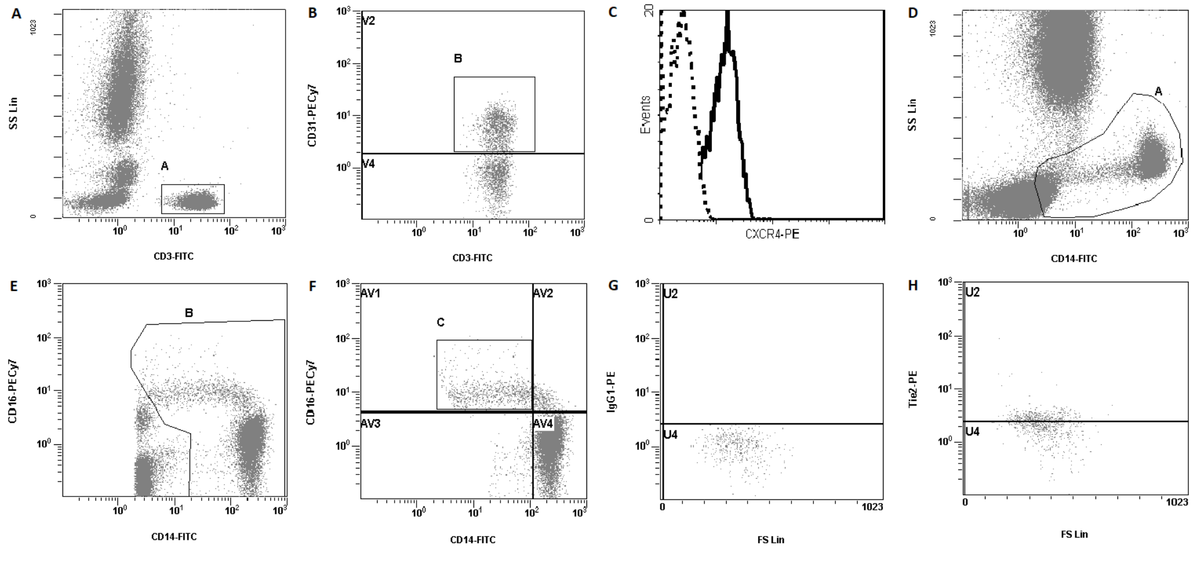


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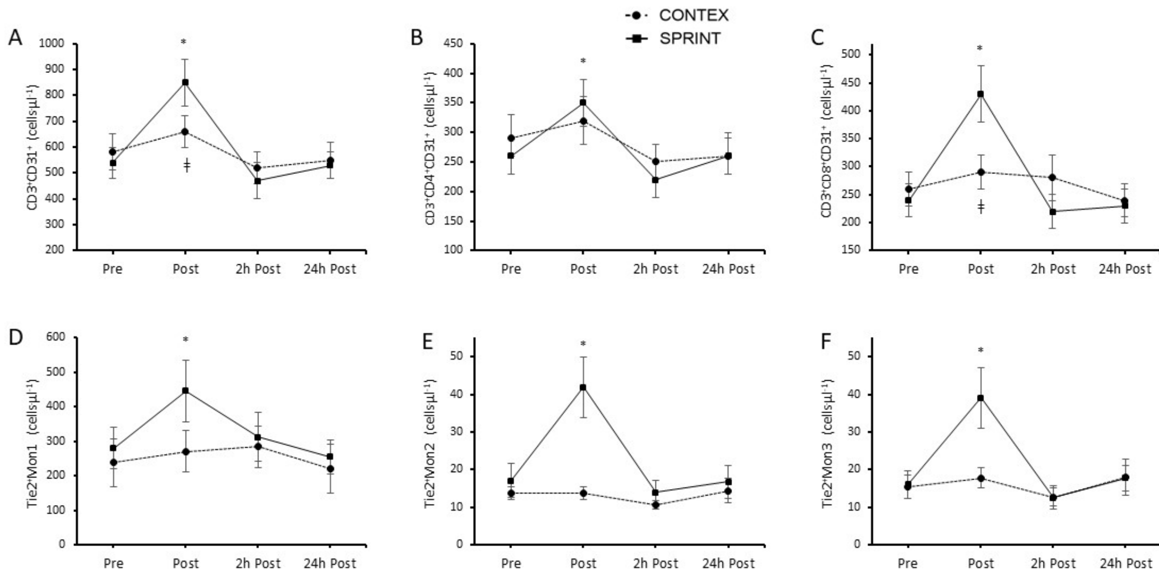


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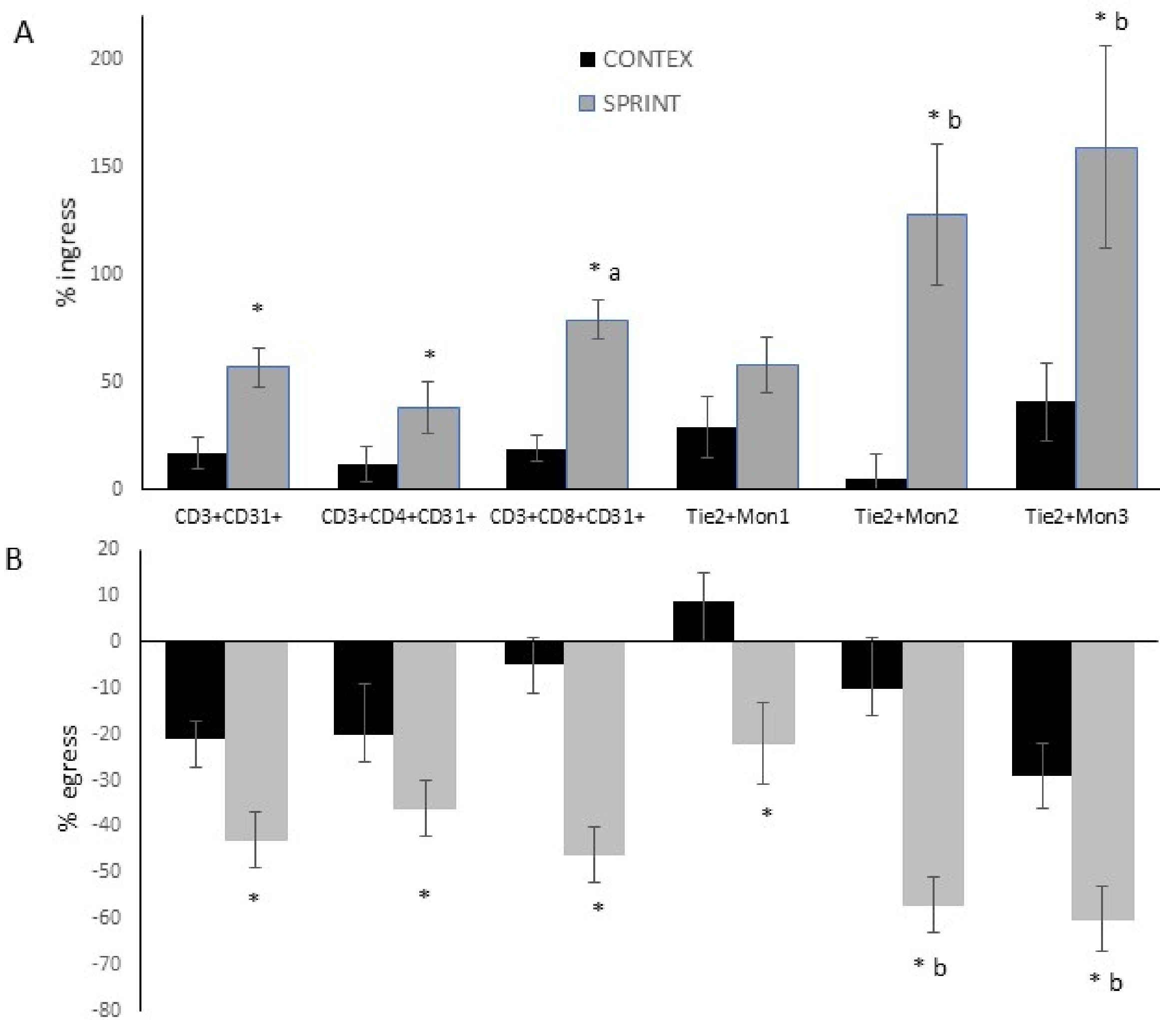


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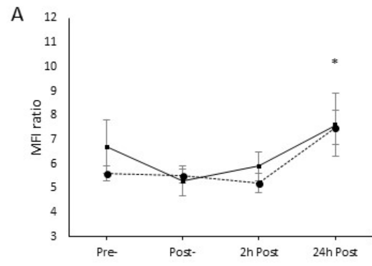

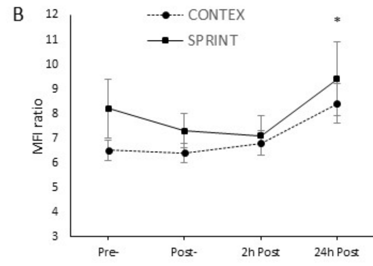

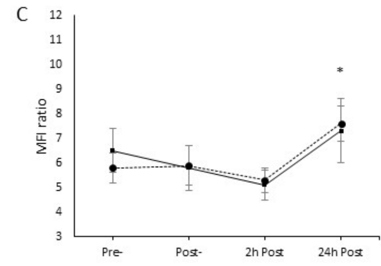


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