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1 **Acute impact of different exercise modalities on arterial and**
2 **platelet function**

3
4 Andrew Haynes¹
5 Matthew D Linden²
6 Elisa Robey¹
7 Gerald F Watts^{3,4}
8 P Hugh R Barrett⁵
9 Louise H Naylor¹
10 Daniel J Green^{1,6,7}

11
12 ¹School of Human Sciences (Exercise and Sport Sciences),
13 The University of Western Australia, Crawley, Western Australia

14
15 ²School of Pathology and Laboratory Medicine,
16 The University of Western Australia, Crawley, Western Australia

17
18 ³School of Medicine, Faculty of Health and Medical Sciences,
19 The University of Western Australia, Crawley, Western Australia

20
21 ⁴Department of Cardiology, Royal Perth Hospital, Western Australia

22
23 ⁵School of Biomedical Sciences, Faculty of Health and Medical Sciences,
24 The University of Western Australia, Crawley, Western Australia

25
26 ⁶Research Institute for Sport and Exercise Science, Liverpool John Moores University,
27 Liverpool, United Kingdom

28
29 ⁷Principal Research Fellow, National Health and Medical Research Council, Australia

30
31 **Short title:** Exercise, arterial and platelet function

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38 **Author for Correspondence**

39 Winthrop Professor Daniel J Green
40 35 Stirling Hwy, School of Sports Science, Exercise and Health
41 The University of Western Australia, Crawley, Western Australia, 6009
42 Phone: +61 (8) 6488 2378, Fax: +61 (8) 6488 1039
43 Email: danny.green@uwa.edu.au
44

48 **Abstract**

49 **Purpose:** Acute coronary syndromes and ischemic stroke are associated with arterial events
50 involving platelets, the endothelium and atherosclerosis. Whilst regular physical activity is
51 associated with lower risk of cardiovascular events and mortality, risk is transiently increased
52 during and immediately following participation in an acute bout of exercise. No previous study
53 has investigated the acute impact of exercise on platelet activation and arterial function in the
54 same participants; it is also unknown if responses are dependent on exercise modality. We
55 hypothesised that commonly adopted, yet physiologically distinct, modalities of exercise
56 (“aerobic” versus “resistance”) have differing effects on in vivo platelet activation and conduit
57 artery diameter. **Methods:** Eight apparently healthy middle-aged (53.5 ± 1.6 yrs) male subjects
58 took part in four, 30 min experimental interventions (aerobic AE, resistance RE, combined
59 aerobic/resistance exercise CARE or no-exercise), in random order. Blood samples were
60 collected and the measurement of brachial artery diameter by ultrasound was performed before,
61 immediately after, and one hour after each intervention. Platelet activation was determined by
62 the positive binding of antibodies to surface receptors exposed on activated platelets (anti-
63 CD62P and PAC-1). **Results:** Brachial artery diameter increased immediately following all
64 three exercise modalities ($P < 0.001$), and remained above pre-exercise levels 1hr post-RE and
65 -CARE. No changes were observed in markers of in vivo platelet activation with any
66 experimental protocol. **Conclusion:** These data suggest that post-exercise enhancement in
67 arterial function may mitigate the acute impact of exercise on platelet activation.

68 **Keywords:** ENDOTHELIUM; THROMBOSIS; ACUTE CORONARY SYNDROME;
69 AEROBIC; RESISTANCE; CIRCUIT

70 **Introduction**

71 Acute coronary syndromes and ischemic stroke represent critical events in the chronology of
72 atherosclerotic cardiovascular disease (CVD), often occurring suddenly in previously
73 asymptomatic individuals (1, 2). Arterial thrombosis contributes to sudden ischemia and
74 mortality in acute coronary syndromes, occurring secondary to disruption of atherosclerotic
75 plaque which exposes pro-thrombotic platelet agonist(s) to flowing blood (1, 3).

76

77 Regular physical activity is associated with reduced all-cause and CVD related mortality (4),
78 and is widely recommended in population health guidelines (5, 6). Despite favourable long-
79 term adaptations to exercise, the risk of an adverse CVD related event is transiently increased
80 during and soon after an individual takes part in a single exercise bout (7, 8). The potential
81 mechanisms responsible for this acute increase in cardiovascular risk are poorly understood.
82 However, sedentary individuals may be most susceptible, with there being an inverse
83 relationship between the risk of exercise-induced myocardial infarction and frequency of
84 regular physical activity (7).

85

86 The balance between the production of vasoactive substances that have positive roles within
87 the blood and vascular wall (e.g., nitric oxide NO and prostacyclin PGI₂) (9), and those
88 associated with negative impacts on risk (reactive oxygen species, oxidative stress,
89 inflammation and thromboxane) (3, 10), is of importance to the initiation and progression of
90 CVD in its latent phase, which can span several decades (11, 12). In addition to their well
91 described role in vasodilation, NO and PGI₂ possess anti-thrombotic properties, as both inhibit
92 platelet adhesion, activation and aggregation (9, 13, 14). Endothelial function, reflective of NO
93 and PGI₂ bioavailability, is enhanced following exercise training (15, 16), but some evidence
94 suggests that a transient decrease occurs following a single bout of exercise (17, 18). Acute

95 exercise may also increase platelet activation (19, 20) and reactivity (21, 22). However, no
96 previous study, to our knowledge, has investigated the responses of both artery function and
97 platelet activation, in the same subjects, before and after participation in a bout of exercise.
98 Furthermore, it is not known whether forms of exercise that involve distinct cardiovascular and
99 skeletal muscle loading (e.g., aerobic versus resistance exercise), induce different effects on
100 vascular and/or platelet function. Such observations may provide insight relating to the
101 potential mechanisms underlying the elevated atherothrombotic risk associated with acute
102 bouts of exercise.

103

104 Therefore, the aim of the present study was to measure both arterial function and platelet
105 activation, in vivo, before and after participation in aerobic exercise (AE), resistance exercise
106 (RE) and combined aerobic and resistance exercise (CARE), in a group of sedentary male
107 participants. We hypothesised that distinct modalities of exercise have different effects on
108 arterial function and platelet activation.

109

110

111 **Methods**

112 **Participants**

113 Apparently healthy male participants aged 40-65 years were screened. Exclusion criteria were:
114 participation in exercise exceeding 60mins/wk, regular use of any medications, physical
115 injuries that would hinder participation in exercise and/or previous CVD related events.
116 Participants underwent a resting electrocardiogram (ECG) and had a fasting venous blood test.
117 Abnormal resting ECG and/or urea and electrolytes, fasting glucose and fasting lipids
118 suggestive of kidney disease, hyperglycaemia or hypercholesterolemia were excluded.

119 Participants meeting these criteria underwent an exercise stress test with ECG monitoring, and
120 individuals showing evidence of exercise induced cardiac ischemia were excluded.

121

122 A sample size calculation was conducted for repeated measures using G* Power version 3.1
123 software (23), indicating that with 80% power and an α of 0.05, 6 participants would be
124 sufficient to detect a 2.5% change in brachial artery diameter and 8 participants would be
125 required to detect a 1.6% change in platelet activation.

126

127 Ethical approval

128 All procedures adhered to the Declaration of Helsinki and were approved by the Human
129 Research Ethics Committee of The University of Western Australia. All participants provided
130 written, informed consent prior to any procedures being undertaken.

131

132 Preliminary sessions

133 Participants attended a familiarisation session, during which they were introduced to the
134 equipment, exercises and protocols to be included in subsequent sessions. A further two visits
135 included a graded maximal exercise test on a cycle ergometer, and repetition maximum (RM)
136 strength tests for the six resistance exercises listed below. As recommended in the well-
137 established exercise prescription guidelines of the American College of Sports Medicine (5),
138 percentages of maximum were used to regulate and standardise the intensity of exercise used
139 in the subsequent exercise sessions.

140

141 Experimental Sessions

142 In a repeated-measures crossover design, all participants completed four experimental sessions,
143 in random order, each separated by at least 7 days. These included 30 minutes of AE, RE,

144 CARE or no-exercise (NE). A standardised stretching routine was included in the warm-up of
145 all exercise sessions, composed of a combination of static and dynamic stretches targeting all
146 major muscle groups.

147

148 In preparation for each session, participants were asked to abstain from caffeine consumption
149 for 12 hours and alcohol for 24 hours, prior to arriving at the laboratory at 8am (24, 25). All
150 participants consumed the same carbohydrate based breakfast including toast or cereal,
151 avoiding fruit, vegetables and meat products. A standardised questionnaire was completed
152 upon arrival to each session prior to any measurements being taken, to confirm adherence to
153 the protocol and ensure participants had not used anti-inflammatory, aspirin-containing or other
154 medications that affect platelet or leukocyte function for 10 days prior.

155

156 Participants then began a 20 minute period of quiet rest in a semi-recumbent position, prior to
157 the first blood collection and vascular assessment. This was followed by one of the exercise
158 sessions or the NE condition, the order of which was randomised for each individual. Following
159 each exercise session, participants resumed a semi-recumbent position and remained there for
160 one hour.

161

162 No exercise protocol

163 Participants lay on a bed in a semi-recumbent position for the duration of the NE session, which
164 lasted approximately 2.5 hours.

165

166 Aerobic exercise protocol

167 Participants completed a four minute warm-up on a rowing ergometer achieving 40% heart rate
168 reserve (HRR) by the end of the fourth minute. The main exercise component included 13

169 minutes on a cycle ergometer (Circle Fitness, P&F Brothers Ind., Corp. Taiwan) at 65%
170 Watts(W)max, followed by 13 minutes on a Concept 2 PM3 rowing ergometer (Concept 2 Inc.,
171 Morrisville, VT). Confirmation of steady-state was achieved by continuous monitoring of heart
172 rate and intensity on the rowing ergometer was matched to the heart rate during cycling.
173 Rowing ergometry was intentionally included to make up half of the AE session, to ensure all
174 exercise sessions included a significant upper body contribution.

175

176 Resistance exercise protocol

177 Three sets of each resistance exercise were completed. A 120 second time-period was allocated
178 for each set, made up of 40 seconds working and 80 seconds recovery, to ensure total exercise
179 time was closely matched between all three exercise sessions. Firstly, participants completed
180 one set of each exercise with a resistance of 40% 1RM, followed by two consecutive sets of
181 each exercise at 65% 1RM. Exercise stations included (i) sitting cable chest press, (ii) leg press,
182 (iii) lateral pulldown, (iv) cable shoulder press, (v) sitting machine hamstring flexion and (vi)
183 cable bicep curl with a rope attachment. Repetitions were continued for the entire 40 second
184 working period or until muscular failure, whichever came first.

185

186 Combined aerobic and resistance exercise protocol

187 The CARE training session included all the exercises that made up the AE and RE sessions,
188 but exercises were set at lower intensities and included shorter recovery periods between
189 stations. Participants completed three 10 minute circuits; the first circuit at an intensity of 40%
190 maximum (i.e., Wmax for AE, 1RM for RE), the following two circuits at 55% maximum.
191 Each exercise circuit consisted of 6 RE and 2 AE, performed in the order: 3 RE (sitting cable
192 chest press, leg press, lateral pulldown), 1 AE (bicycle ergometer), 3 RE (cable shoulder press,
193 sitting machine hamstring flexion and cable bicep curl with a rope attachment), 1 AE (rowing),

194 with two minutes recovery between each circuit. Resistance exercises were allocated 60
195 seconds (40 seconds working and 20 seconds transition time) and aerobic exercises were
196 allocated 120 seconds (100 seconds working and 20 seconds transition time).

197

198 Brachial artery diameter

199 The measurement of brachial artery diameter (BAD) was performed on the left arm of each
200 participant, in a quiet, temperature-controlled room in accordance with recent guidelines (24).
201 Measurements were performed on three occasions during each session: pre-exercise,
202 immediately post-exercise and 1hr post exercise, or at identical time-intervals during the NE
203 session. In brief, to examine BAD, the non-dominant arm was extended and positioned at an
204 angle of $\sim 80^\circ$ from the torso. A 10-MHz multi-frequency linear array probe, attached to a high-
205 resolution ultrasound machine (T3200; Terason, Burlington, MA, USA) was used to image the
206 brachial artery in the distal 1/3rd of the upper arm. When an optimal image was obtained, the
207 probe was held stable and the ultrasound parameters were set to optimize the longitudinal, B-
208 mode images of lumen–arterial wall interface. A 1 minute recording of brachial artery diameter
209 was collected (Camtasia Studio 8, TechSmith, Okemos, MI). Post-test analysis of brachial
210 artery diameter was performed using custom-designed edge-detection and wall-tracking
211 software, which is largely independent of investigator bias (26). We have shown that the
212 reproducibility of diameter measurements using this semi-automated software is significantly
213 better than manual methods, reduces observer error significantly, and possesses an intra-
214 observer CV of 6.7% (26).

215

216 Blood collection

217 Venous blood was collected by separate venepunctures at three time-points during each
218 session, as described above. Tourniquet pressure was removed prior to blood collection to

219 prevent blood stasis affecting the sample (27). The first 2mL of blood was collected into a non-
220 additive discard tube, followed by a 4mL 3.2% sodium citrate tube (Vacurette by Greiner bio-
221 one).

222

223 Measurement of Platelet Activation

224 Within ten minutes of collection, whole blood from the sodium citrate tube was diluted 1:5
225 with HEPES saline buffer and incubated for exactly 15 minutes in a cocktail of three
226 fluorescent conjugated antibodies diluted with HEPES saline (28). These included: anti-CD42b
227 PE-Cy5 (platelet identifier), PAC-1 fluorescein (FITC) and anti-CD62P phycoerythrin (PE), or
228 isotype control IgG1κ PE (all BD Pharmingen, San Diego, CA). Positive binding of the PAC-
229 1 antibody to platelets indicate activation of the glycoprotein IIb/IIIa (fibrinogen) receptor, and
230 binding of anti-CD62P indicates platelet granule exocytosis has occurred resulting in exposure
231 of P-selectin on the platelet surface. Two gating and quality controls were included for each
232 blood sample: isotype control and positive control 250 μM TRAP [SFLLRN, Sigma-Aldrich,
233 MO]). Single stained anti-mouse IgG κλ compensation beads (BD Biosciences) were utilised
234 to resolve spectral overlap between the three fluorophores.

235

236 Statistics

237 Statistical analyses were performed using SPSS v23 (IBM, Armonk, NY) software. Two-way
238 repeated measures analysis of variance (ANOVA) tests were conducted to test for differences
239 between modalities, across time and interaction of modality x time effects. If significance was
240 found, multiple repeated measures ANOVA tests were conducted to determine where
241 differences occurred within-modality over time and for corresponding time-points between
242 modalities, with post-hoc Least Significant Difference test. Statistical significance was defined
243 at $P < 0.05$.

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Results

Eight men (53.5 ± 1.6 yrs) completed the study. The characteristics of the participants were detailed elsewhere (29): height 173 ± 1.9 cm, body mass 86.2 ± 4.1 kg, fasting cholesterol 6.0 ± 0.3 mmol/L, fasting glucose 5.2 ± 0.1 mmol/L. No adverse events were reported during any of the experimental sessions. The total volume of work (load kg x reps) performed as a result of the resistance exercises in the RE ($10,576.7 \pm 2,195.5$ kg) and CARE ($10,582.3 \pm 2,125.4$ kg) sessions were not statistically different ($P = 0.984$), as an average of 13 repetitions were performed in the RE session (65% 1RM) and 16 repetitions in the CARE session (55% 1RM).

Brachial artery diameter

Statistical testing of BAD (see Figure 1) measured before, immediately after and 1 hour following the four experimental protocols, indicated there were significant main effects for modality ($P = 0.009$), time ($P = <0.001$) and modality*time interaction ($P = 0.018$). Overall, the BAD measured in all of the exercise sessions were significantly different to the NE session: NE vs RE ($P = 0.007$), NE vs CARE ($P = 0.022$), NE vs AE ($P = 0.041$), but not between the three exercise protocols (all $P > 0.05$). Post-hoc tests at corresponding time-points revealed that no significant differences were present between the four experimental sessions at the pre time-point ($P = 0.773$). However, significant differences were found between NE and all three exercise modalities (all $P = < 0.002$) at the immediately post-exercise time-point. No differences in BAD were found between any of the three exercise modalities at the immediately post-exercise time-point (all $P > 0.05$). At 1hr-post, significant differences in BAD were found between NE vs RE ($P = 0.008$) and NE vs CARE ($P = 0.006$), but not between NE vs AE ($P = 0.325$).

269

270 Within session changes in BAD indicated an increase from pre to post RE ($P = <0.001$), which
271 was still elevated 1hr post-exercise compared to pre-RE ($P = 0.013$), see Figure 1 Panel A. The
272 decrease in BAD from immediately post to 1hr post RE ($P = 0.052$) was borderline significant.
273 With CARE, BAD increased significantly from pre to immediately post-exercise ($P <0.001$)
274 and was still elevated 1hr post- compared to pre-CARE ($P = 0.010$) (see Figure 1 Panel B).
275 The decrease from immediately post to 1hr following CARE was not significant ($P = 0.159$).
276 During the AE session, the increase in BAD from pre to post was significant ($P = 0.001$), but
277 was not different to pre-AE at one hour post ($P = 0.868$) (see Figure 1 Panel C). The decrease
278 from immediately post-AE to one hour post was significant ($P = 0.040$). Over the 3 time-points,
279 no significant ($P = 0.413$) changes in BAD were found during the NE session (see Figure 1
280 Panel D). The significant interaction between modality and time indicates that participation in
281 exercise was required to induce an increase in BAD, which was not observed with NE.

282

283 Platelet PAC-1 and anti-CD62P binding

284 No significant differences for main effects were found for PAC-1 binding: modality ($P=0.159$),
285 time ($P=0.754$) or interaction ($P=0.261$) (see Figure 2 Panel A), or for anti-CD62P binding:
286 modality ($P=0.452$), time ($P=0.222$) or interaction ($P=0.642$) (see Figure 2 Panel B). The
287 positive control included in each experiment caused maximal platelet activation, confirming
288 the validity of the assay (see Figure 2).

289

290

291 **Discussion**

292 This is the first study to measure arterial function and platelet activation, simultaneously,
293 before, immediately after, and one-hour following participation in discrete bouts of distinct but
294 commonly prescribed modalities of exercise. This was conducted in a single group of sedentary
295 middle-aged male participants. We found that the diameter of the brachial artery increased
296 immediately following all three exercise modalities, compared with pre-exercise resting data
297 and a control (no-exercise) condition. These functional changes in the brachial artery were
298 maintained one hour post-exercise following the two protocols that included a resistance
299 component (i.e., RE and CARE). We did not observe changes in indices of platelet activation
300 in vivo with any of the exercise protocols, despite our recent evidence that acute exercise
301 amplified agonist-mediated platelet activation in these subjects (29). Taken together, these
302 findings suggest that acute bouts of exercise, particularly those incorporating some resistance
303 component, induce changes in arterial function favouring vasodilation that are likely mediated
304 by activation of endothelial cells. These endothelium-derived vasodilators may mitigate pro-
305 thrombotic impacts of acute exercise (29, 30).

306

307 There are reports that exercise can cause platelets to become activated following participation
308 in exercise (31-34), which could infer increased risk for spontaneous thrombosis with exercise.
309 Other studies have not found any increases in activated platelets in vivo (22, 35), but have
310 reported increased platelet reactivity to agonists post-exercise (22, 30). In our recent
311 publication (29), based on this experiment, acute exercise caused a significant increase in the
312 sensitivity of platelets to activation when incubated with platelet agonists (i.e., increased
313 platelet reactivity). Despite these findings, the exercise included in this study did not increase
314 platelet activation per se. There are several possible explanations for these findings. Firstly, it
315 is possible that exercise does not exacerbate platelet activation unless there is also the presence

316 of agonists such as thrombin, adenosine diphosphate or arachidonic acid (28, 29), in which case
317 exercise amplifies the pro-thrombotic impacts of these agents (29, 30). This explanation has
318 some relevance in terms of the exercise paradox, in that it infers that exercise is not pro-
319 thrombotic unless there is some underlying endothelial dysfunction or damage that elicits the
320 release of platelet agonists. Hence, exercise may not have the ability to increase the levels of
321 activated platelets in the circulation. But, individuals with occult plaque that may be prone to
322 micro-tears or rupture (which facilitates agonist-induced platelet activation) may be at
323 increased risk if these events occur during or soon after an exercise bout.

324

325 A second possible explanation for the platelet findings we observed is that exercise increases
326 the production of substances such as NO and PGI₂ from the endothelium. It is accepted that
327 exercise induces arterial vasodilation by virtue of shear stress impacts on the endothelium (36)
328 and that endothelium-derived vasodilators also inhibit platelet activation (9). The impact of
329 each exercise bout on artery diameter in the present study reinforces the notion that acute
330 exercise induces endothelium-mediated vasodilation in vivo. It is conceivable that exercise and
331 shear-mediated production of substances such as NO and PGI₂ play a homeostatic role in
332 preventing any increase in platelet activation. This study therefore provides novel information
333 relating to the acute impact that participation in distinct modes of exercise have on both
334 vascular function and platelet activation, and potentially to the mechanisms responsible for
335 exercise-associated thrombotic events.

336

337 Brachial artery dilation remained elevated 1 hour post-RE and -CARE, whereas AE did not
338 induce sustained vasodilation in the majority of participants. However, we did observe some
339 individual differences 1hr following all of the exercise sessions. We expect that brachial artery
340 diameter, in the process of returning to its basal state, may have been differentially affected by

341 oxidative stress, inflammation, sympathetic activation and other factors that can influence
342 arterial tone. There may also be some impact of differences in habitual physical activity levels
343 between participants, although a criterion for inclusion was less than 60 mins/wk in all subjects;
344 this study was not designed or powered to measure these mechanistic outcomes. The
345 explanation for the differences between modalities are unlikely to be related to the “intensity”
346 of exercise, as RE bouts induced a lower cardiovascular demand to the AE bout (29). There
347 may be an impact of the intermittent nature of RE and CARE, compared to sustained steady-
348 state AE, and the inclusion of alternating upper and lower body exercises interspersed with
349 recovery periods. Importantly, it was recently highlighted that few studies have measured shear
350 and flow patterns during exercise, due to technical limitations with blood flow pattern
351 assessment in vascular territories perfusing active areas (37). However, it is well established
352 that distinct modes of exercise induce different shear stress patterns (17, 36) and that these
353 patterns have implications for vascular responses post-exercise (37, 38). Therefore,
354 characterising differences in blood flow and shear stress during different forms of exercise
355 should be the focus of future studies that involve post-exercise assessment of vascular function.

356

357 This study has direct ecological relevance, as current exercise prescription guidelines were
358 used to design the exercise bouts (5), which emulated typical gym session(s) for these
359 modalities. A potential limitation to this study is that only eight participants were included.
360 However, this is compensated by the within-subjects repeated measures study design and the
361 high levels of significance for the vascular findings (i.e., consistency in the responses across
362 subjects). As this study included healthy middle-aged men, we cannot infer that these findings
363 apply to women, specific racial groups, across all ages or to patients with metabolic disorders
364 and/or CVD. Future research could also include a more comprehensive battery of functional
365 tests of arterial / vascular function, and seek to discover potential mechanisms responsible for

366 our findings. Such investigation could include the measurement of shear stress during exercise,
367 catecholamine responses and blood-borne biomarker levels following exercise.

368

369 In summary, we observed that participation in commonly prescribed modalities of exercise
370 induced conduit artery dilation in vivo. This is indicative of increased production of vasoactive
371 compounds that have both vasodilatory and anti-thrombotic effects (9). These impacts on
372 vascular diameter were maintained 1hr following exercise, particularly following exercise
373 involving a resistance component. The absence of change in platelet activation in vivo may
374 indicate that shear-mediated activation of the endothelium provides a homeostatic
375 compensatory mechanism that prevents platelet activation in vivo, counteracting any increase
376 in platelet sensitivity post-exercise. The data presented in this manuscript related to the impacts
377 of exercise on in vivo artery diameter and platelet activation, do not suggest that thrombotic
378 risk was elevated during or soon after these sessions. However, in the presence of a
379 precipitating stimulus such as atherosclerotic plaque rupture or damage which is known to
380 trigger acute coronary syndromes (1), we have shown that exercise may amplify arterial
381 thrombosis (29). Our study therefore has implications for understanding the “exercise paradox”
382 (39) in humans.

383

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389

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397

398

399 **Conflicts of Interest**

400 The authors disclose no conflicts of interest. The results of the study are presented clearly,
401 honestly, and without fabrication, falsification, or inappropriate data manipulation. The
402 results of the present study do not constitute endorsement by the American College of Sports
403 Medicine.

404

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501

502 **Figure Legends**

503

504 **Figure 1**

505 Brachial artery diameter BAD in mm measured by high resolution duplex ultrasound.
506 Assessments were taken pre, post and 1hr post: resistance exercise RE (panel A), combined
507 aerobic and resistance exercise CARE (panel B) aerobic exercise AE (panel C) and no exercise
508 NE (panel D). Individual values are represented by dotted lines (n=8), solid line is mean.
509 Significant within session differences from the “pre” time-point at $P \leq 0.001$ ***, $P \leq 0.010$
510 ** and $P \leq 0.050$ * probability level. † indicates significant within session difference from the
511 “post” time-point $P \leq 0.05$.

512

513 **Figure 2**

514 Percent PAC-1 (panel A) and anti-CD62P (panel B) binding to platelets, from blood tests
515 collected pre, post and 1hr post participation in four experimental sessions: resistance exercise
516 RE, combined aerobic and resistance exercise CARE, aerobic exercise AE and no exercise NE.
517 Confirmation of assay validity is indicated by the positive control Pos Ctrl which caused
518 maximal platelet activation. Data is presented as mean \pm standard error, n=8.

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