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**Acute Impact of Different Exercise Modalities on Arterial and Platelet Function.**

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### Article

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

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

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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 ( $53.5 \pm 1.6$  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  $\alpha$  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  $P < 0.05$ .

244

245

## 246 **Results**

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

254

### 255 *Brachial artery diameter*

256 Statistical testing of BAD (see Figure 1) measured before, immediately after and 1 hour  
257 following the four experimental protocols, indicated there were significant main effects for  
258 modality ( $P = 0.009$ ), time ( $P = <0.001$ ) and modality\*time interaction ( $P = 0.018$ ). Overall,  
259 the BAD measured in all of the exercise sessions were significantly different to the NE session:  
260 NE vs RE ( $P = 0.007$ ), NE vs CARE ( $P = 0.022$ ), NE vs AE ( $P = 0.041$ ), but not between the  
261 three exercise protocols (all  $P > 0.05$ ). Post-hoc tests at corresponding time-points revealed  
262 that no significant differences were present between the four experimental sessions at the pre  
263 time-point ( $P = 0.773$ ). However, significant differences were found between NE and all three  
264 exercise modalities (all  $P = < 0.002$ ) at the immediately post-exercise time-point. No  
265 differences in BAD were found between any of the three exercise modalities at the immediately  
266 post-exercise time-point (all  $P > 0.05$ ). At 1hr-post, significant differences in BAD were found  
267 between NE vs RE ( $P = 0.008$ ) and NE vs CARE ( $P = 0.006$ ), but not between NE vs AE ( $P =$   
268  $0.325$ ).

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 PGI<sub>2</sub> 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 PGI<sub>2</sub> 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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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  $P=<0.001$  \*\*\*,  $P=<0.010$   
510 \*\* and  $P=<0.050$  \* probability level. † indicates significant within session difference from the  
511 “post” time-point  $P=<0.05$ .

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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