Acute impact of different exercise modalities on arterial and platelet function

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Short title: Exercise, arterial and platelet function

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Abstract

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

Keywords: ENDOTHELIUM; THROMBOSIS; ACUTE CORONARY SYNDROME; AEROBIC; RESISTANCE; CIRCUIT

Introduction

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

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

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

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

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

Methods

Participants

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

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

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

Ethical approval

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

Preliminary sessions

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

Experimental Sessions

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

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

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

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

No exercise protocol

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

Aerobic exercise protocol

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

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

Resistance exercise protocol

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

Combined aerobic and resistance exercise protocol

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

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

Brachial artery diameter

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

Blood collection

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

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

Measurement of Platelet Activation

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

Statistics

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

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 ± 2,195.5 kg) and CARE (10,582.3 ± 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).

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

Platelet PAC-1 and anti-CD62P binding

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

Discussion

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

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

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

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

Acknowledgements

The authors acknowledge the facilities, and the scientific and technical assistance of the Australian Microscopy & Microanalysis Research Facility at the Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, a facility funded by the University, State and Commonwealth Governments.

Sources of Funding

This work was supported by funding from the National Heart Foundation of Australia G12P6417.

Professor Green is a National Health and Medical Research Council Principal Research Fellow (APP1080914).

Associate Professor Linden is an International Society for Advancement of Cytometry (ISAC) Marylou Ingram Scholar.

Conflicts of Interest

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

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Figure Legends

Figure 1

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

Figure 2

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