Acute impact of conventional and eccentric cycling on platelet and vascular function in patients with chronic heart failure

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Running Head: Cycling, platelets and endothelial function in heart failure

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Abstract

Evidence-based guidelines recommend exercise therapy for patients with chronic heart failure (CHF). Such patients have increased atherothrombotic risk. Exercise can transiently increase platelet activation and reactivity and decrease vascular function in healthy participants, although data in CHF is scant. Eccentric (ECC) cycling is a novel exercise modality which may be particularly suited to patients with CHF, but the acute impacts of ECC on platelet and vascular function are currently unknown. Our null hypothesis was that ECC and concentric (CON) cycling, performed at matched external workloads, would not induce changes in platelet or vascular function in patients with CHF. Eleven patients with heart failure with reduced ejection fraction (HFrEF) took part in discrete bouts of ECC and CON cycling. Before and immediately after exercise, vascular function was assessed by measuring diameter and flow mediated dilation (FMD) of the brachial artery. Platelet function was measured by the flow cytometric determination of glycoprotein IIb/IIIa activation and granule exocytosis in the presence and absence of platelet agonists. ECC increased baseline artery diameter (pre: 4.0±0.8mm vs post: 4.2±0.7mm, P=0.04) and decreased FMD%. When changes in baseline artery diameter were accounted for the decrease in FMD post-ECC was no longer significant. No changes were apparent after CON. Neither ECC nor CON resulted in changes to any platelet function measures (all P>0.05). These results suggest both ECC and CON cycling at a moderate intensity and short duration can be performed by patients with HFrEF, without detrimental impacts on vascular or platelet function.

New and Noteworthy

This is the first evidence to indicate that eccentric cycling can be performed relatively safely by patients with chronic heart failure, as it did not result in impaired vascular or platelet function compared to conventional cycling. This is important, as acute exercise can transiently increase atherothrombotic risk and eccentric cycling is a novel exercise modality that may be particularly suited to patients with chronic heart failure.

Key words

Eccentric exercise, platelets, vascular function, chronic heart failure

Introduction

Chronic heart failure (CHF) occurs in approximately 10% of individuals aged over 65 years and is expected to rise significantly over the next decade (27). Chronic heart failure is characterized by abnormalities in cardiac structure and/or function, resulting in the inability of the heart to deliver sufficient blood and therefore oxygen to meet the metabolic demands of the body. Individuals with CHF experience impaired physical function (18) and have a greater risk of sudden thrombotic related events compared to healthy individuals (25). Such events include acute coronary syndromes and stroke, which occur in association with compromised vascular function and platelet mediated thrombosis (10, 22). Indeed, impaired vascular function (9, 26), increased platelet activation (39), and a hypercoagulable state (13) have been documented in patients with CHF.

Exercise training is recommended as part of the management of CHF, to alleviate decline in health and physical function and to maintain quality of life (8, 32, 40). Whilst exercise is generally safe and regular exercise training decreases long term risk of cardiovascular events, acute coronary risk is increased during and immediately after participation in a bout of exercise (35). This may relate, in part, to the impact of some forms of exercise on vascular and/or platelet function. Some studies that have tested vascular function before and after acute exercise in healthy participants have revealed transient decreases after exercise (3, 7). The majority of such studies have been performed in healthy volunteers and involved conventional forms of aerobic exercise, with assessments of the brachial artery providing a surrogate for systemic vascular function. Platelet activation and reactivity to agonist exposure have also been reported to be elevated immediately following both moderate and high

intensity exercise in healthy participants (15, 19, 36). Currently there is little evidence regarding the impacts of distinct types of exercise on platelet or vascular function in CHF.

It has been demonstrated in healthy participants, that eccentric (ECC) cycling can be carried out requiring less oxygen uptake compared to conventional concentric (CON) cycling (30). Recently, we provided evidence to suggest that ECC cycling may be a novel and beneficial exercise modality for patients with CHF, as matched exercise workloads can be performed at a lower metabolic demand than CON cycling (4). Few studies have addressed the acute impact of ECC exercise on either platelet or vascular function (31, 33). These studies, performed in separate groups of apparently heathy participants, have reported that ECC based resistance exercise did not increase platelet activation post-exercise (31), but did reduce flow mediated dilation (FMD) 1 hour post-exercise (33). To our knowledge, no previous study has investigated the acute impact of CON or ECC cycling on either platelets or vascular function in patients with CHF. The aim of this study was to therefore compare the impact of short bouts of ECC and CON cycling, matched for external workload, on platelets and vascular function in patients with CHF. Our null hypothesis was that both modalities would have no effects on either platelet or vascular function.

Materials and Methods

A comprehensive account of the recruitment and exercise protocols used in the present study can be found in our recently published paper, which focused on metabolic and hemodynamic outcomes (4). Briefly, patients with reduced left ventricular systolic function (ejection fraction <45%), New York Heart Association class I to III were recruited from the Advanced

Heart Failure and Cardiac Transplantation Unit at Fiona Stanley Hospital, Perth, Western Australia. Ethics approval for the study was provided by the Metro South Health Human Research Ethics Committee (HREC 14-160) and the Human Research Ethics Committee at The University of Western Australia. Exclusion criteria included: resting hypertension (>165/95 mmHg), severe obstructive aortic stenosis, severe rhythm disorders that would exclude safe participation in exercise, severe pulmonary hypertension (systolic >70 mmHg), venous thromboembolic history within the past three months, musculoskeletal comorbidity limiting functional capacity beyond the effect of CHF. Patients continued their routine medical therapy throughout the study period.

A power calculation was conducted *a priori* using (G* Power 3.1.9.2 Software) using data from platelet function assays conducted in our lab, indicating that based on power of 80% and a standard deviation of 5%, 10 participants would be sufficient to detect a change of 5% at a significance level of $P = <0.05$ (11). This was supported by a previously published study (31).

Maximal Exercise Test

In an initial session, participants performed a maximal graded exercise test on a recumbent bicycle ergometer (Corival, Lode BV, Groningen, Netherlands), with power output increasing 20 watts (W) every 3 minutes until volitional exhaustion. The maximal power output (W) achieved during this test was used to prescribe the exercise intensity of subsequent sessions.

General Protocol

To ensure no recent changes were made in relation to participants symptoms, medications, alcohol use and physical activity habits, participants were asked a series of questions relating to this on arrival to the laboratory of each session. The participant sat on the recumbent cycling ergometer that was to be used on that particular day (i.e., ECC or CON) and rested for 10 minutes, after which a venous blood sample was collected. Following another 5 minutes of seated rest, baseline brachial artery diameter and an FMD test were performed on the left arm. The participant then began the exercise protocol (see protocol below). Immediately following the brief cool-down aspect of cycling, a blood sample was taken from the right arm, and vascular tests were performed simultaneously on the left arm. Both the CON and ECC bicycle ergometers were recumbent based apparatus, ensuring body positions were identical for both modalities. Whilst the time of day at which the laboratory visits were conducted varied between participants, it was maintained at the same time within participants.

Eccentric Cycling (ECC)

Seven days following the maximal bicycle ergometer test, participants underwent the ECC protocol. This was performed on a recumbent ergometer (Eccentric Trainer, Metitur, Ltd, Jyväskylä, Finland) with a 1.5 kW motor that powered the cranks in reverse. Participants then performed 11 minutes of continuous ECC cycling, maintaining a cadence of 40 rpm throughout. This was composed of a 3 minute warm-up aiming to achieve 30% Wmax, 5 minutes at 70% Wmax and 3 minutes of active recovery with no resistance. As external workload during ECC cycling is difficult to maintain constant, the watts performed was documented every 10 seconds, and this was used to match the intensity for CON cycling.

Concentric Cycling (CON)

After a further seven days, participants underwent the CON protocol, which was performed at the same time of day as the ECC protocol. CON cycling was performed on the same recumbent bicycle as the maximal exercise test. The total exercise duration, warm-up, main component, active recovery and cadence were identical to that described above for ECC. However, the intensity (watts) of CON was changed manually by a researcher every 30 seconds to match the intensity performed during ECC for each individual subject.

Blood Samples

A venous blood sample was collected from the antecubital fossa with no stasis using a 21G winged needle set (Greiner bio-one, Kremsmuenster, Austria). The first 2 mL was collected into a non-additive discard tube, followed by a 4 mL 3.2% sodium citrate tube (Vacuette by Greiner bio-one, Kremsmuenster, Austria).

Platelet Function Tests

Platelet function was measured by flow cytometric determination of glycoprotein IIb/IIIa activation (measured by PAC-1 binding) and granule exocytosis (measured by surface CD62P expression), in the presence and absence of platelet agonists according to recent recommendations (23). 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. These included: CD42b PE-Cy5 (platelet

identifier), PAC-1 fluorescein (FITC) and anti-CD62P phycoerythrin (PE), or isotype control IgG1K PE (all BD Pharmingen, San Diego, CA). Seven reaction tubes (1.5 mL Protein LoBind, Eppendorf, Germany) were used for platelet immunophenotyping which included: isotype control, positive control (250 µM thrombin receptor activating peptide-6, TRAP [SFLLRN, Sigma-Aldrich, MO]), no agonist, TRAP 2 µM, adenosine diphosphate (ADP) 1.5 µM (Chrono-Log Corp., PA), arachidonic acid AA 10 µg/mL (Sodium arachidonate, Bio/Data Corp., PA) and collagen 1.5 µg/mL (Chrono-Log Corp., PA). Samples were incubated at room temperature with the exception of tubes containing AA and collagen, which were incubated at 37°C using a dry block heater (Ratek DBH20D, Victoria, Australia). Following 15 minutes of incubation, samples were fixed with stabilizing fixative (Becton Dickinson), stored at 4°C and were analyzed within 24 hours by flow cytometry (BD FACSCanto II) at a low flow rate. For each reaction tube, 10,000 platelet positive events were counted and single stained compensation beads were utilized to account for spectral overlap between the three fluorophores (BD Biosciences). ADP at the concentration used (1.5 µM) caused maximal PAC-1 binding in all participants, so was not included in statistical analysis.

Vascular function tests

The vascular assessments were conducted in a quiet, temperature-controlled room in accordance to recent guidelines (34). In brief, to examine baseline brachial artery diameter and FMD, the non-dominant arm was extended and positioned at an angle of ~80° from the torso. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA, USA) was positioned on the forearm, immediately distal to the olecranon process to provide a forearm ischemia stimulus. 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. Continuous Doppler velocity assessments were also obtained using the ultrasound, and were collected using the lowest possible insonation angle (always <60°). Following a 1 minute baseline recording of brachial artery diameter and velocity (Camtasia Studio 8, TechSmith, Okemos, MI), the forearm cuff was inflated (220 mmHg) for 5 min. Diameter and flow recordings resumed 30 seconds prior to cuff deflation and continued for 3 minutes thereafter. 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 (38). Brachial artery FMD is presented as relative (%) rise from the preceding baseline diameter. 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% (37).

Statistics

Statistical analyses were performed using SPSS 22 (IBM, Armonk, NY) software. For data meeting the assumptions of parametric statistical tests, paired *t*-tests were conducted to determine if significant changes occurred within each session over time. For data failing the assumptions of parametric tests, Wilcoxon signed rank tests were conducted. Subsequently, for results revealing a significant change in FMD% post-exercise, a linear mixed model analysis was conducted with logarithmically transformed artery diameter. This procedure accounts for changes in baseline diameter and is appropriate under such circumstances (1).

Results

Eleven participants (9 male) (mean ± SD) age: 52.0 ± 9.3 yrs, height 178.5 ± 9.3 cm, body mass 91.6 ± 19.6 kg, $\dot{V}O_{2\ peak}$ 19.9 ± 4.0 ml.kg.min^{-1} completed the study. The medication use of participants is presented in Table 1. Due to complications with the vascular data files of one participant, ten participants were included in the analysis of peripheral vascular function. Most of these participants were the same as those included in our recent manuscript related to oxygen consumption and hemodynamic variables (4). Briefly, this paper revealed that ECC cycling can be performed at matched external workloads, but lower $\dot{V}O_2$, minute ventilation and respiratory exchange ratio compared to CON cycling.

Vascular function

ECC cycling resulted in a significant (P = 0.04) increase in baseline artery diameter from pre- (4.0 ± 0.8 mm) to post-exercise (4.2 ± 0.7 mm) (see Figure 1). No change (P = 0.43) was observed in baseline artery diameter after CON (pre 4.0 ± 0.7 mm vs post 4.0 ± 0.7 mm). No significant difference (P = 0.18) in peak artery diameter was observed between pre- (4.4 ± 0.8 mm) and post-exercise (4.5 ± 0.7 mm) for ECC, as well as CON (P = 0.53, pre 4.3 ± 0.7 mm vs post 4.4 ± 0.7 mm).

ECC cycling resulted in a significant (P = 0.05) decrease in FMD% from pre- (9.0 ± 2.9 %) to post-exercise (6.0 ± 4.0 %) when changes in baseline diameter (ie changes in the baseline pre to post ECC bout) were not accounted for (Figure 2A). CON cycling did not result in any change (P = 0.94) in FMD% (pre: 8.8 ± 2.8 % vs post: 8.8 ± 3.9 %). When the FMD response

was corrected to account for changes in baseline diameter as a result of the exercise bout (Figure 1), the change in FMD post-ECC was no longer significant (P = 0.26), as shown in Figure 2 (panel B). This suggests the decrease in FMD following ECC was due, at least in part, to the increase in baseline artery diameter following ECC. No significant change was found for time to peak brachial artery diameter for ECC (pre: 68.9 ± 34.0 sec vs post: 77.5 ± 23.3 sec, P = 0.55) or CON (pre: 68.4 ± 26.1 sec vs post: 66.6 ± 27.9 sec, P = 0.64).

Platelet Function

No significant differences (all P = >0.05) were found in either PAC-1 (see Table 2) or anti-CD62P binding (see Table 3) in the absence or presence of canonical platelet agonists following CON or ECC cycling.

Discussion

Acute bouts of exercise involve a transient elevation in the risk of an acute cardiovascular event (35). This may be associated with evidence suggesting that some forms of acute exercise can reduce indices of vascular function (7) and increase platelet activation and sensitivity to agonists (15, 19). This is the first study, to our knowledge, to investigate the acute effect of discrete bouts of CON and ECC cycling, matched for duration and external workload, on platelet and brachial artery vascular function in patients with HFrEF. We assessed the impacts of ECC exercise because it may be particularly relevant in HFrEF, since it requires less oxygen uptake to sustain matched workloads of exercise (30). We found that ECC cycling significantly increased conduit artery diameter, with no such change observed following CON cycling. The decrease in brachial FMD observed following ECC may, at least

partly, have been caused by this significant increase in baseline artery diameter post-exercise, as FMD corrected for changes in baseline diameter was not significantly altered by exercise. This does not exclude the possibility that the changes in FMD% were attributable to the impact of ECC on vasodilator function, but it is appropriate to consider baseline diameter effects on the interpretation of FMD% responses (1).

The vasodilator impact of ECC on baseline arterial diameter occurred despite the workload being matched to the CON condition, with the ECC session performed with ~13% lower $\dot{V}O_2$ requirement (4). Participation in short bouts of CON and ECC cycling at a moderate intensity did not result in any significant change in platelet activation, as measured by PAC-1 or anti-CD62P binding, both of which are sensitive and specific markers of platelet function associated with acute coronary risk (24). These findings suggest that short bouts of moderate intensity ECC or CON cycling have no significant detrimental impacts on vascular or platelet function in patients with HFrEF.

Eccentric exercise is an appealing modality of exercise for patients with impaired cardiac and hemodynamic function, and we have recently demonstrated that ECC cycling is associated with a lower oxygen demand than conventional CON cycling in patients with HFrEF (4). Exercise prescription in heart failure is often challenging, given the extreme deconditioning that characterizes the disease. A form of exercise, such as ECC, which allows greater intensities of exercise to be undertaken at a lower relative systemic burden, should theoretically enhance the benefits of training. However, the acute effects of ECC cycling on peripheral vascular and platelet function, both of which may have implications relating to acute atherothrombotic risk, have not previously been explored in HFrEF. Indeed, acute ECC

exercise data in patients with CHF are sparse, but one study suggests that eccentric resistance exercise decreased brachial FMD post-exercise, even after adjustment for baseline diameter changes (33). This contrasts somewhat with our findings which suggest an increase in arterial function post-ECC, characterized by baseline vasodilation which impacted upon the FMD result. Decreases in FMD need to be considered with caution in cases where significant changes in the baseline diameter have occurred, as we have previously explained (1). It has also been demonstrated that post-exercise changes in FMD are dependent on exercise intensity, with higher intensities conferring greater reduction (3), and we cannot rule out the possibility that exercise performed at a different intensity or for longer duration, may have resulted in a different outcome.

The primary difference in brachial artery response between the cycling modalities was an increase in resting vessel diameter following ECC. No such change was evident following CON. The underlying mechanisms behind this are unknown, but may be linked to differences in hemodynamics, neural and hormonal responses between these contrasting exercise modalities (2). We recently reported that heart rate, mean arterial pressure and rate pressure product are similar between the ECC and CON cycling (4), implying that differences in hemodynamics are not likely to account for the vasodilator effect of ECC. It is well established that hemodynamic effects such as those associated with increased shear stress, transmural wall pressure and heart rate can directly modify artery function (14). Whilst the mechanisms responsible for the dilator effect of ECC are not currently known, our findings suggest that moderate intensity, short duration ECC exercise does not adversely impact on vascular function in HFrEF.

There were no significant changes in circulating activated platelets, or platelet reactivity to physiologically relevant agonists, following either exercise protocol. A previous study in healthy, untrained participants observed that the acute effect of exercise on platelets is intensity dependent (16), and it is possible that the intensity and/or duration of exercise used in the present study were insufficient to induce significant changes in platelet function. This may also explain why our findings contrast with a previous study that reported increased platelet activation following a maximal CON cycling exercise test in CHF (5). ECC exercise is not commonly prescribed to patients with CHF and the acute impacts of ECC on platelets have not previously been reported in such participants. Whilst the exercise protocols included in the present study were somewhat conservative, in part to reduce the risk of skeletal muscle damage and soreness (20, 29), our findings suggest that ECC cycling can be conducted acutely in patients with HFrEF, without the risk of inducing significant platelet activation. We cannot comment on the possible detrimental effects of ECC performed at higher intensities than those used in the present experiment.

Participants were undergoing treatment for HFrEF throughout the study period, and were instructed to maintain their normal regimen of medication, so not to impact upon their therapy. As such, ~63% of participants were prescribed some form of anti-platelet/coagulation medication, and it is possible this may have masked any effect of exercise on platelets in the present study. However, there is evidence to suggest this may not be the case, as aspirin and warfarin use have previously shown to be incapable of inhibiting the effects of maximal exercise on platelets and coagulation markers (6, 17, 21). Another important limitation of this study was that the ECC session had to precede the CON session in all cases, so that we could closely and accurately match the exercise intensities. Because these sessions were not randomized, we cannot exclude the possibility of an order effect, but

the sessions were separated by a minimum of 7 days in an attempt to avoid this problem. Finally, it is germane to emphasize that our study of the acute effect of exercise cannot not be directly extrapolated to a chronic adaptation. Although, logically, repetition of acute responses should lead to adaptation, the nature and direction of such training-induced adaptation may differ from changes seen in response to acute bouts of exercise. This concept been captured in the term "hormesis" (12, 28), taken in this context to indicate that repetitive episodic exposure to stimuli that challenge and compromise function, may lead to upregulation and enhancement in chronic responses. In the present study, we did not observe large responses, either positive or negative, in terms of platelet function, but that does not necessarily mean that training studies will not reveal adaptation.

In summary, we observed a relative vasodilator impact of ECC cycling, but not after CON cycling in patients with HFrEF, however platelet function was unaffected after both exercises. Given that both platelet and vascular function are involved in acute coronary syndromes, our findings provide novel data relating to the impact of ECC cycling in patients with HFrEF, and do not suggest that ECC cycling has greater acute impacts on patients with HFrEF than conventional cycling, when matched for external workload and duration. While the acute effects on vascular function of the brachial artery and platelet activation of ECC exercise do not differ from concentric cycling, future studies will be required before recommendations can emerge regarding the adoption of ECC cycling in routine HFrEF training programs.

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Disclosures

None.

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Figure Caption

Figure 1. Changes in brachial artery diameter before and immediately after concentric (CON) and eccentric (ECC) cycling (A), delta change in artery diameter from pre- to post-exercise time-points (B), individual response changes in baseline diameter during CON (C) and ECC cycling (D). N=10. Data in Panels A and B are mean ± SE, * indicates significant difference from pre-exercise ($P = 0.04$).

Figure 2. Change in flow mediated dilation (FMD%) from pre- to post-concentric (CON) and eccentric (ECC) cycling when not adjusted for baseline diameter changes (A), and when adjusted for baseline diameter change (B). Individual responses in FMD (unadjusted for baseline diameter) pre and post CON (C) and ECC cycling (D). N=10. Data in Panels A and B are mean ± SE, * indicates significant difference from pre-exercise ($P = 0.05$).

Table 1. Medication use of participants

Medication	**N (%)**
Anti-platelet (total)	7 (63.6)
Aspirin	3 (27.3)
Warfarin	4 (36.4)
Rivaroxaban	2 (18.2)
Prasugrel	1 (9.1)
ACE Inhibitors (total)	9 (81.8)
Ramipril	8 (72.7)
Perindopril	1 (9.1)
β-Blockers (Bisoprolol)	9 (81.8)
Statins (Atorvastatin)	7 (63.6)
Anti-arrhythmic (Amiodarone)	4 (36.4)
Aldosterone receptor antagonist	4 (36.4)
Angiotensin II receptor antagonist	1 (9.1)

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Table 6.2 Platelet PAC-1 binding before and after concentric and eccentric cycling

Variable	**% PAC-1 binding**		
	Pre	**Post**	**Statistics**
No Agonist			
Concentric	6.8 ± 5.2	5.6 ± 2.2	*P* = 0.859
Eccentric	4.7 ± 0.8	5.2 ± 0.9	*P* = 0.213
TRAP 2 µM			
Concentric	25.1 ± 3.6	22.8 ± 1.8	*P* = 0.450
Eccentric	23.7 ± 3.6	23.3 ± 3.5	*P* = 0.594
AA 10 µg/ml			
Concentric	29.0 ± 3.3	25.1 ± 3.1	*P* = 0.104
Eccentric	24.8 ± 3.0	22.3 ± 2.8	*P* = 0.284
Collagen 1.5 µg/ml			
Concentric	14.7 ± 2.0	11.4 ± 1.2	*P* = 0.091
Eccentric	11.7 ± 1.5	9.9 ± 1.6	*P* = 0.178

Thrombin Receptor Activating Peptide-6 TRAP, *Arachidonic Acid* AA

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Table 6.3 Platelet anti-CD62P binding with concentric and eccentric cycling

Variable	% anti-CD62P binding		
	Pre	**Post**	**Statistics**
No Agonist			
Concentric	2.0 ± 0.9	2.6 ± 1.0	*P* = 0.450
Eccentric	1.8 ± 1.3	2.0 ± 1.4	*P* = 0.169
TRAP 2 µM			
Concentric	5.5 ± 3.5	5.9 ± 3.8	*P* = 0.374
Eccentric	5.3 ± 4.5	6.3 ± 5.1	*P* = 0.213
ADP 1.5 µM			
Concentric	69.1 ± 25.0	68.9 ± 27.9	*P* = 0.722
Eccentric	69.4 ± 26.3	71.8 ± 26.0	*P* = 0.450
AA 10 µg/ml			
Concentric	12.2 ± 6.1	13.7 ± 8.1	*P* = 0.398
Eccentric	13.2 ± 6.6	13.2 ± 6.1	*P* = 0.981
Collagen 1.5 µg/ml			
Concentric	5.1 ± 4.4	6.1 ± 6.6	*P* = 0.213
Eccentric	5.3 ± 4.8	5.1 ± 4.9	*P* = 0.712

Thrombin Receptor Activating Peptide-6 TRAP, *Adenosine diphosphate* ADP, *Arachidonic Acid* AA