Beneficial impacts of regular exercise on platelet function in sedentary older adults: Evidence from a randomized 6-month walking trial

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Abstract

Platelet activation, including the formation of monocyte platelet aggregates (MPAs), contributes to atherosclerosis, thrombus formation and acute coronary syndromes. Regular participation in exercise can lower cardiovascular risk, but little is known regarding the impact of exercise training on platelet function. We investigated the effect of 6 months of walking exercise on platelet function in sedentary older adults without significant cardiovascular disease. Twenty-seven participants were randomly allocated to 6 months of either: no-exercise (n=13) or 3 x 50 mins/wk of supervised centre-based walking (n=14). Circulating and agonist induced MPAs were assessed using flow cytometry before (month 0 *0M*) and after (month 6 *6M*) the intervention. Circulating MPAs increased from 0M (3.7 ± 1.0%) to 6M (4.7 ± 1.6%) in the no-exercise group ($P = 0.009$), whereas a non-significant decrease was observed in the walking group (0M 4.3 ± 1.7% vs 6M 3.7 ± 1.2, $P = 0.052$). The change in MPAs between groups was significant ($P = 0.001$). There were no differences between groups in platelet responses to agonists across the interventions (all $P > 0.05$). Collectively, these data suggest that the absence of regular exercise may increase MPAs, which are cellular mediators involved in atherosclerosis, whilst regular walking inhibits such increases. The thrombotic function of platelets appear to be relatively unaltered by exercise training. This study provides novel data related to the cardio-protective effects associated with participation in exercise.

New and noteworthy

Monocyte-platelet aggregates contribute to atherosclerosis and exercise can lower cardiovascular risk. This is the first study to discover that a lack of regular physical activity is associated with increased monocyte-platelet aggregates over a six-month intervention period. In contrast, walking exercise inhibits increased monocyte-platelet aggregates in the circulation. This study highlights a novel pathway by which regular participation in exercise exerts its cardio-protective effects.

Key words: platelets, cardiovascular disease, exercise

Glossary:

AA	Arachidonic acid
CVD	Cardiovascular disease
MPAs	Monocyte-platelet aggregates
NE	No-exercise
PA	Physical activity
TRAP	Thrombin receptor activating peptide
W	Walking

Introduction

Cardiovascular disease (CVD) is highly prevalent in Westernized countries; with a recent report indicating that ~30% of deaths in the United States are attributed to CVD (31). The underlying cause attributing to the vast majority of age and lifestyle associated CVD is atherosclerosis (10, 22). Whist platelets have a well-documented role in the acute thrombotic events that occur in the coronary and cerebral arteries in the latter stages of CVD (1, 21), more recent evidence suggests that platelets also have an important role in the initiation and progression of atherosclerosis (39). When platelets undergo activation, α-granule exocytosis results in the expression of P-selectin (CD62P) on the platelet surface, facilitating the interaction between platelets and monocytes (29). The consequent formation of monocyte-platelet aggregates (MPAs), dependent on platelet activation (35), results in the release of pro-inflammatory mediators (36) that promote the adhesion of monocytes to the endothelial cell surface, an atherogenic pathway for cell infiltration into the sub-endothelial space (40). Therefore, MPA assessment may provide an early marker of asymptomatic CVD progression.

Previous studies on the impact of exercise training on platelet function are scant. There is some evidence to suggest adaptation favouring increased fibrinolytic capacity (17), decreased soluble markers of platelet activation (2) and a reduction in agonist-induced platelet aggregation (7) following participation in exercise programs. However, only three studies, to our knowledge, have utilised flow cytometry to assess platelet function in response to exercise training interventions (20, 37, 41), of which one included MPAs (37). This of some importance, as the formation of MPAs are thought to contribute to atherogenesis (25) and the measurement of MPAs by

flow cytometry is a sensitive method of assessing platelet activation *in vivo* (29). No change in circulating MPAs was found following 6 months of either no-exercise or exercise training in patients with peripheral artery disease (37). The remaining two studies only included platelet reactivity to stimulation *in vitro*, indicating the results may be of more relevance to the hemostatic functions of platelets, as opposed to being suggestive of a pro-atherogenic milieu *in vivo*. These studies found that platelet reactivity to high shear stress was increased in a control group, with no change following 8 weeks of exercise training in healthy young men (41); and that platelet sensitivity to ADP was decreased following exercise training in patients with coronary artery disease (20). However, it is possible that the young age of participants (41) and the use of anti-platelet medications (20, 37) may have influenced their observations. To our knowledge, no previous study has investigated the impact of exercise training in older sedentary individuals without diagnosed CVD, or included both circulating and agonist induced MPAs.

Therefore, the aim of the present study was to determine if a 6 month centre-based and supervised walking exercise intervention in apparently healthy older adults, would induce changes in circulating MPAs and platelet reactivity. We assessed responses to a range of agonists (adenosine diphosphate *ADP*, thrombin receptor activating peptide *TRAP*, and arachidonic acid *AA*) which were selected based on their known physiological relevance and because they address distinct activation pathways. Our null hypothesis was that no changes in platelet function would be observed following the exercise intervention or following an identical period of no-exercise.

Materials and Methods

This study was a supplementary experiment to a larger randomised trial, registered as ACTRN12614000017628. The outcome measures included in this study were complimentary to the main purpose of the registered trial, and our focus here is on the land-based exercise and control groups, enabling valid comparison to previous experiments involving weight-bearing exercise effects on platelet function. The study was approved by the University of Western Australia Human Research Ethics Committee, procedures were in accordance with the Declaration of Helsinki and all participants provided written informed consent. Male and post-menopausal female participants were recruited from the general community-dwelling population in the Perth metropolitan area, Western Australia, using multiple recruitment strategies including advertisement in local newspapers, radio stations and posters. Apparently healthy individuals aged 55 years and over were encouraged to contact the research team, resulting in initial phone screening procedures which included questionnaires to determine suitability to attend a formal screening visit. Initial exclusion criteria included serious illness such as cancer, cognitive impairment or dementia, current or past history of ischemic heart disease, angina, stroke, persistent arrhythmias, diabetes mellitus, airway disease, epilepsy, severe mental illness, regular use of anti-platelet medications, engaging in more than 1 hour of physical activity per week, current or recent smokers (within 12 months), pre- or peri-menopausal females and alcohol consumption >28 standard drinks/wk.

Individuals satisfying the initial criteria were invited to the laboratory to attend a screening session, during which a number of measures were collected including:

height, body mass, resting ECG and fasting blood tests (glucose, lipid profile, full blood count, urea and electrolytes) to determine suitability for inclusion. Participants exhibiting abnormal cardiac rhythms, blood test results suggestive of chronic kidney disease, diabetes, or total cholesterol >7mmol/L were excluded. Included participants were then invited to perform an exercise stress test (modified chronotropic protocol) with respiratory gas analysis and ECG monitoring, and those with evidence of exertion-induced myocardial ischemia or significant arrhythmias were excluded from further participation. The exercise test was repeated at the end of the intervention period.

Experimental procedures

Participants satisfying the inclusion criteria were subsequently randomly assigned to one of two groups: no-exercise (NE) or walking (W) group. The intervention period for all participants was 24 weeks (6 months) in duration.

No-exercise (control) group

Participants randomly assigned to the NE group were instructed not to change their current lifestyle or physical activity patterns for the duration of the study period. These participants also attended a monthly seminar as a group, which included topics unrelated to physical activity, lifestyle and health, such as computer literacy and first aid skills. Informal social interaction was a component of each session.

Exercise group

Participants randomly assigned to W were asked to attend the university campus three times per week to take part in walking exercise as a group. Exercise intensity

was individualised for each participant based on heart rate (% heart rate reserve *HRR*) and the duration of exercise was identical for all participants. At week 1, exercise duration commenced at 15 minutes per bout and was progressively increased to reach 50 minutes per bout by the beginning of week 13. The 50 minute duration of each session was then maintained for the remainder of the intervention period (i.e., 11 weeks). Exercise intensity was initially set at 40-45% HRR and progressively increased to 60-65% HRR by the beginning of week 13. This resulted in participants achieving a total of 150 mins/wk of moderate intensity exercise, which is in accordance with physical activity guidelines (14). Continuous walking was conducted outside in the natural environment. All participants wore a heart rate monitor (Polar RS300X, Polar Electro Oy, Finland) for the duration of each session, and were monitored by an exercise physiologist to ensure target heart rates were achieved and maintained.

Blood collection

Resting blood samples were collected under identical conditions before (month 0 *0M*) and following completion of the 6 month (6M) intervention period. The blood tests at 6M were conducted between 2 - 14 days following the final walking session. Participants arrived at the laboratory in the morning between 7:00 - 9:30am following an overnight fast, having abstained from the consumption of caffeine and alcohol for 12 and 24 hours, respectively, and not taken part in physical exercise for 24 hours. Adherence to the protocol was confirmed by questionnaire on arrival. Prior to attending the laboratory for data collection, participants were instructed to be clear of symptoms for 7 days if they had recently suffered with acute conditions including respiratory tract infection, cold and flu. Participants taking prescription medications

were instructed to maintain their usual routine of administration. However, the use of non-prescribed medications such as anti-inflammatory, anti-histamine, antibiotic, aspirin, cold and flu medications were ceased for at least 7 days prior to blood collection. Participants lay supine in a cool temperature controlled room for 15 minutes, after which a venous blood sample was collected for the assessment of platelet function. The first 2ml of blood was collected into a no-additive discard tube, followed by a 3.5ml 3.2% sodium citrate tube (both Vacuette by Greiner bio-one, Kremsmünster, AT).

Monocyte-platelet aggregates

Within 10 minutes of collection, blood was passed from the 3.2% sodium citrate tube and processed for the assessment MPAs in 7 x 1.5ml Eppendorf Protein Lobind tubes (Eppendorf AG, Hamburg, DE). Each reaction tube contained saturating concentrations of two antibodies diluted in HEPES saline (pH 7.3): anti-CD14 (monocyte identifier) conjugated to the fluorophore Brilliant Violet (BV) 421 (Clone M5E2, BioLegend, CA, cat #301830) and anti-CD42b (platelet identifier) conjugated to allophycocyanin (APC) (Clone HIP1, BioLegend, CA, cat #303912), with one tube containing IgG1K isotype control (BioLegend, CA, cat #400122). For each blood collection there were two gating and quality controls, an isotype control and a positive control containing 250 µM of TRAP (SFLLRN, Sigma-Aldrich, MO, cat #T1573-5mg) to cause activation of all platelets. One reaction tube contained no agonist (i.e., HEPES saline) to be representative of the levels of MPAs in circulation when the blood sample was collected. Other reaction tubes contained sub-maximal concentrations of one of three agonists: 1.5 µm ADP (Chrono-Log Corp., PA, cat #P/N 384), 5 µM TRAP or 10 µg/ml AA (Sodium arachidonate, Bio/Data Corp., PA,

cat #BDC101297). Absence of spectral overlap was confirmed by single-colour comp bead controls (Becton Dickinson *BD* Biosciences, CA).

Samples were incubated at room temperature, with the exception of both AA, which must be incubated at 37°C to function effectively (23). This was achieved using a dry block heater (Ratek DBH20D, Victoria, AU). Following exactly 15 minutes incubation, all samples were fixed and red cells lysed with 800 µL of BD FACSLyse solution (BD Biosciences, CA, cat #349202) diluted with ultrapure water to manufacturer specifications. After a further 10 minutes at room temperature to allow complete lysis, samples were stored at 4°C in the dark and analyzed by flow cytometry (BD FACSCanto™ II, BD Biosciences) within 24 hours. All samples were run at a low flow rate for 10 minutes per tube, to avoid coincident events (18). The assessment of MPAs is reproducible and correlates well with markers of platelet activation (5). Data output from flow cytometry was analyzed using FlowJo v.X software (FlowJo LLC., CA). First, a gating strategy was devised to eliminate leukocyte-doublet events (26). Monocytes were then identified by characteristic laser scatter and differential expression of CD14. Monocyte-platelet aggregates were identified by CD42b expression on CD14 positive monocyte events, and data are expressed as a percentage of the total monocyte population. MPA gates were determined by isotype control.

Statistics

Participants were de-identified using individual coding, and group allocation was not known to the individual that analysed the samples. Data was tested for changes in MPAs over time and time*group interaction effects using 2 x 2 mixed design ANOVA

tests. Where necessary, post-hoc tests were conducted to test for within group (paired *t*-tests) and between group (independent *t*-tests) differences in MPAs. Independent *t*-tests were used to test for differences in baseline characteristics between groups. A sample size calculation was conducted using G*Power 3.1.9.2 software using previous MPA data collected in our laboratory, indicating that with the primary objective of observing a significant within-subject change in MPAs (effect size of 1% with no agonist and 10% with agonists), with a significance level of 5% and a power of 80%, a minimum of 10 participants would be required.

Results

A total of 27 participants (22 female, 5 male), age 60.3 ± 6.0 yrs (mean ± SD) completed the study (see Table 1 for detailed characteristics). Thirteen participants were entered into the NE group (n = 2 male, 11 female) and fourteen to the W group (n = 3 male, 11 female). Blood pressure medication was taken by NE = 0, W = 2; cholesterol medication by NE = 1, W = 2; anti-depressant medications by NE = 1, W = 0, and all medications were taken regularly during and for at least 6 months prior to admission into the study. No participants were taking anti-platelet medications. No significant changes in total body mass or body mass index were found in either group from 0M to 6M (all $P > 0.05$, see Table 2). An unintentional occurrence was that the majority of participants randomly enrolled into the study were female. Therefore, a sub-group analysis on MPAs was conducted including female participants only. No participants dropped out of the study. Of the 72 possible attendances across the 6 month intervention period in the walking group, 61.6 ± 12.6 sessions (85.6 ± 17.5 %) were attended.

Peak Exercise Test

Technical issues (n=2) and failure to attend a 6M follow-up exercise test (n=1), resulted in exercise test results for 3 participants (NE 1 male, W 1 male, 1 female) being excluded from analysis. The exercise test results presented are therefore derived from 12 participants in each group. Exercise performance in terms of time to exhaustion increased significantly from 0M to 6M in the W group (P = 0.005), but no change was found with NE (P = 0.979) (Table 2). No significant changes were observed in $\dot{V}O_{2peak}$, either in absolute ($L.min^{-1}$) or relative ($ml.kg.min^{-1}$) terms (all P > 0.05).

Monocyte-platelet aggregates

There were no significant differences in MPAs (no agonist) for main time effects (P = 0.404), but group*time interaction effects were significant (P = 0.001). Post-hoc testing for within-group changes indicated that MPAs increased significantly from 0M to 6M with NE (P =0.009), see Figure 1. In contrast, no change in MPAs was observed from 0M to 6M in the W group (P = 0.052). The change in MPAs from 0M to 6M between NE and W was significant (P = 0.001, Figure 1C).

In blood samples incubated with ADP, there were significant time effects for MPAs (P = 0.002), but the group*time interaction was not significant (P = 0.346, Figure 2 Panel A). Post-hoc tests indicated that platelet sensitivity to ADP increased from 0M and 6M with NE (P = 0.015), but no change was observed in the W group (P = 0.068). There were no differences in ADP sensitivity between groups at either the 0M (P = 0.835) or 6M (P = 0.511) time-points. No significant differences in MPAs

were found for time or interaction effects respectively, when samples were incubated with either TRAP (P = 0.226, P = 0.839) or AA (P = 0.992, P = 0.374, see Figure 2)

Female participant sub-group analysis

With no agonist, MPAs were unchanged for main time effects (P = 0.420), but there was a significant time*group interaction (P = 0.007, Table 3). Post-hoc tests indicated that a significant increase in MPAs occurred from 0M to 6M in the NE group (P = 0.030), whilst there was no change with W (P = 0.118). Data in male participants were directionally similar (data not shown).

For samples incubated with ADP, there was a significant time effect (P = <0.001), but the modality*time interaction effects were not significant (P = 0.330). Post-hoc tests indicated that MPAs increased significantly from 0M to 6M in both the NE (P = 0.003) and W group (P = 0.023). No significant differences were found when samples were incubated with either TRAP (P = 0.160, P = 0.449) or AA (P = 0.150, P = 0.356) for either time or time*group interaction effects respectively (see Table 3).

Discussion

Through complex interactions with cell messengers and pro-inflammatory mediators, platelet activation is linked to the early stages of CVD, contributing to the low-grade inflammation associated with atherosclerosis, monocyte adhesion to the endothelial cell surface and transmigration of monocytes into the sub-intimal space (13, 15, 28, 40, 42). The adoption of walking by sedentary individuals can enhance functional capacity and induce favourable changes to traditional CVD risk factors (32, 33). In

this study, we sought to determine if the presence of *in vivo* MPAs and platelet reactivity to agonists would be modified by 6 months of walking exercise in previously sedentary older adults. We found that MPAs increased significantly in the control group, whereas the adoption of walking inhibited such increases from occurring. There were no differences between these groups in agonist sensitivity, suggesting that the pro-thrombotic functions of platelets were relatively unaffected by the exercise undertaken. Our findings therefore highlight a novel mechanism by which regular walking exerts cardio-protective effects through inhibiting elevation of MPAs and supports the current hypotheses related to the relevance of MPAs and platelet activation *in vivo* to CVD (25).

To our knowledge, only three studies have previously assessed platelet function before and after a period of exercise training using flow cytometry (20, 37, 41). Only one of these studies measured MPAs, which included only circulating levels (37), whereas the remaining two studies simply looked at platelet reactivity to stimuli *in vitro* (20, 41). No changes in MPAs were found following 6 months of either best medical treatment (BMT) or BMT plus exercise (50 mins intermittent walking 2 x wk) in patients with peripheral artery disease. However, it is possible that the use of medications as part of BMT, which included anti-platelet therapy (37), may have inhibited any increases in MPAs from occurring and/or potentially masked any inhibiting effect of exercise. Furthermore, the BMT only group were given written encouragement to take part in unsupervised exercise, the adoption of which was not reported. In healthy men aged ~24 years, 8 weeks of cycle ergometer training resulted in no change in platelet CD62P expression at rest when blood samples were exposed to different shear stress patterns *in vitro*, whereas an increase was

observed in sedentary controls (41). This is in accordance with our findings. It is possible to speculate that the combination of increased platelet activation in the basal state (i.e., MPAs), alongside increased sensitivity to activation by ADP, could be deleterious under conditions of high shear stress such as that present in stiff and/or stenosed arteries. However, the *in vitro* outcome measure included in the study by Wang and colleagues (41) may have less relevance to atherogenesis than platelet activation *in vivo,* whilst also being influenced by the young age of the participants relative to those included in the present study. Although only a small increase in MPAs was observed in the NE group (mean ~1%), this occurred over a 6 month intervention period and differences may become more pronounced over years or decades. Such increases may be associated with aging (16) and/or detrimental changes to the internal vascular environment, including increased oxidative stress (11) and decreased endogenous NO production (38), both of which may promote platelet activation (30) and increase MPA levels in the circulation.

Platelet sensitivity to ADP, although directionally similar in both groups, increased significantly in the NE group only, with no differences between the groups. However, when only female participants were included, both groups exhibited elevated reactivity to ADP. This is in contrast with a previous study including patients with coronary artery disease, in which all but one were taking daily aspirin and did not include a no-exercise control group (20). They found that high (40-60 mins of walking 5-7 days/wk) but not moderate (25-40 mins 3 days/wk) levels of PA conducted for 4 months, decreased ADP induced platelet CD62P expression (20). Although there are differences in the health status and medication use between our studies, the findings of Keating and colleagues (20) may suggest that exceeding current PA guidelines

(i.e., 3 x 50 mins/wk) could induce reductions in agonist induced platelet activation. However, more research is required to support this in apparently healthy individuals. We did not find any differences over time or between groups with any other agonist (i.e., TRAP or AA). In general, these data suggest that walking does not alter the propensity for agonist-induced platelet activation in a manner that differs significantly to remaining inactive (i.e., NE). That is, hemostatic responses to vascular injury are likely to be similar between groups, despite the apparent beneficial effects of walking on preventing increased MPAs in the circulation.

Time to exhaustion during the exercise test increased by an average of almost two minutes in the W group, despite no apparent change in peak oxygen consumption. It is possible that improvements in time to exhaustion reflect increased confidence during treadmill exercise and/or improved walking economy as a result of neuro-muscular adaptations (9). Indeed, a previous study found that 7 weeks of walking exercise in older adults improved exercise test performance, increased preferred walking speed and improved gait performance (speed and energy cost) of walking, with no improvement in peak oxygen uptake (27). Regardless, these findings highlight the importance of regular physical activity at maintaining or improving functional capacity and preventing age-related decline in aerobic capacity in physically inactive individuals.

The exercise programs implemented in the current study were closely supervised and individually tailored by an experienced Exercise Physiologist. They were designed to meet the PA recommendations according to evidence based guidelines (3, 4, 14). It is possible that a different exercise regimen (i.e., greater frequency,

duration, intensity) than that implemented in the current study, may have had a different/greater effect on MPAs or agonist-induced platelet activation than what we observed (e.g., a reduction in circulating MPAs). Indeed, studies with a variety of other outcome measures have indicated that the benefits gained from exercise training may be dose-dependent (8, 19, 34). Future research may investigate the impact of more vigorous exercise training on these outcome measures. Whilst most previous studies have involved relative brief intervention periods (6-12 weeks), our study benefited from a longer duration which enabled observation in the no-exercise control group of changes in MPAs, which are associated with atherosclerotic progression. All participants taking prescribed medications were stable (>6 months) prior to admission to the study, so that any impact of adopting these medication(s) should have occurred prior to commencing the study, and change during the study should reflect adaptation associated with the intervention. Whilst some anti-depressant medications can impact platelet function, the one participant taking such medication was in the no-exercise control group and exhibited a 1.1% *decrease* in MPAs. Removing this subject from the analysis would therefore serve to exaggerate the findings we observed in the control group. The majority of participants included in this study were female, and future research should investigate whether our findings are maintained in a larger male cohort with similar characteristics.

Taken together, the findings of the present study indicate that, if previously sedentary older adults take up regular walking at a volume that meets current PA guidelines (14), it may prevent increases in MPAs that would otherwise occur over time. This may have significant implications for vascular health, as the presence of MPAs is associated with pro-inflammatory and atherogenic adaptation (6, 12, 28,

42), with consequences for coronary artery disease and future cardiac events (24). This study also suggests that MPAs may be an early and sensitive index of atherosclerotic progression, as we observed changes over a relatively short time period in the sedentary group. The lack of difference between groups in terms of platelet responses to agonist exposure indicates that exercise training, at least moderate intensity walking 3 x 50 mins, was relatively ineffective at modifying the hemostatic function of platelets. Previous studies in this area are few and have primarily included only *in vitro* methodologies and clinical patients taking anti-platelet medications (20, 37). For these reasons the current investigation specifically included older aged individuals, not taking regular anti-platelet medications, over a relatively long intervention period. Our results, derived primarily from a female cohort, are therefore particularly novel and relevant. Collectively, these findings suggest that exercise training may prevent increases in the pro-inflammatory and atherogenic functions of platelets *in vivo*, which possibly contributes to the reduced athero-thrombotic risk observed in those who are physically active. However, in the event of physiological agonist exposure typical of that which occurs following plaque disruption initiating acute coronary events (10), exercise training may not alter the thrombogenic response or alter susceptibility to the consequences of agonist-induced platelet activation. The present investigation has highlighted a novel mechanistic pathway, which supports the importance of being physically active across the lifespan.

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Disclosures

None.

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Figure Legends

Figure 1

Circulating levels of monocyte-platelet aggregates (MPAs) collected before (month 0 *0M*) and after (month 6 *6M*) an intervention consisting of either no-exercise *NE* (panel A) or walking exercise *W* (panel B). Dotted lines are individual responses and solid line are mean. The delta from 0M and 6M is presented (Panel C) as mean ± SE. Statistics are paired (panel A) and independent (panel B) student's *t*-tests, * denotes significance at $P<0.01$.

Figure 2

Monocyte-platelet aggregates (MPAs) in samples incubated with *ADP* (Panel A), thrombin receptor activating peptide *TRAP* (Panel B) and arachidonic acid *AA* (Panel C), measured before (month 0 *0M*) and after (month 6 *6M*) an intervention consisting of either no-exercise *NE* or walking exercise *W*. Statistics are paired student's *t*-test * denotes significance at $P<0.05$. Data are mean ± SE.

664 **Tables**

Table 1 Baseline characteristics of participants

Variable		All participants	Differences between groups (*P* =)
Age			
	All	60.3 ± 6.0	
	NE	60.6 ± 6.5	0.795
	W	60.0 ± 5.7	
Body mass (kg)			
	All	73.3 ± 13.3	
	NE	70.6 ± 15.0	0.328
	W	75.8 ± 11.5	
Resting heart rate (beats.min^{-1})			
	All	62 ± 6.5	
	NE	62 ± 6.9	0.882
	W	62 ± 6.2	
Resting blood pressure (mmHg)			
Systolic			
	All	118 ± 10.7	
	NE	117 ± 11.2	0.652
	W	119 ± 10.6	
Diastolic			
	All	70 ± 7.3	
	NE	68 ± 6.3	0.249
	W	71 ± 8.0	
Mean arterial pressure			
	All	89 ± 7.9	
	NE	87 ± 7.5	0.318
	W	91 ± 8.3	
Cholesterol			
	All	5.4 ± 0.8	
	NE	5.3 ± 0.8	0.694
	W	5.5 ± 0.8	
Triglycerides			
	All	1.0 ± 0.4	
	NE	1.0 ± 0.4	0.493
	W	1.1 ± 0.4	
LDL			
	All	3.4 ± 0.7	
	NE	3.5 ± 0.8	0.780
	W	3.4 ± 0.7	
HDL			
	All	1.5 ± 0.4	
	NE	1.4 ± 0.3	0.375
	W	1.6 ± 0.4	
Glucose			
	All	5.1 ± 0.5	
	NE	5.0 ± 0.4	0.289
	W	5.2 ± 0.5	

All n=27, no-exercise *NE* n=13 (n=11 female), walking *W* n=14 (n=11 female). All blood tests results are mmol/L. Statistics are independent *t*-tests between groups.

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Table 2 Body mass and measures of aerobic fitness collected before and after a 6 month walking intervention

Variable	Baseline (0M)	Post-intervention (6M)	Time *P* =	Group*time interaction *P* =
Total body mass				
NE	70.6 ± 15.0	71.3 ± 14.4	0.395	0.458
W	75.8 ± 11.5	75.8 ± 11.7		
Body mass index				
NE	26.1 ± 4.0	26.4 ± 4.0	0.375	0.325
W	27.3 ± 3.2	27.3 ± 3.1		
Time to exhaustion				
NE	17.39 ± 1.93	17.39 ± 2.01	0.006	0.006
W	16.84 ± 3.65	18.50 ± 2.93*		
$\dot{V}O_{2\ peak}$				
NE	28.67 ± 5.48	27.18 ± 3.45	0.497	0.117
W	28.14 ± 9.27	28.74 ± 7.85		
$\dot{V}O_{2\ peak}$				
NE	1.98 ± 0.52	1.90 ± 0.40	0.531	0.179
W	2.19 ± 0.85	2.22 ± 0.78		

Data collected from participants randomly allocated to No Exercise *NE* and Walking *W* before *0M* and after *6M* the intervention. Body mass (kg) and body mass index (kg/m^2) includes n=13 in NE and n=14 in W, exercise test data: time to exhaustion (mins), $\dot{V}O_{2\ peak}$ ($ml.kg.min^{-1}$) and $\dot{V}O_{2\ peak}$ ($L.min^{-1}$) includes n=12 in both NE and W groups. Time and group*time interaction results are derived from 2 x 2 mixed design ANOVA tests, * indicates significant within group change from 0M to 6M (*P* = <0.01) from post-hoc paired student's *t*-tests. Data are presented as mean ± SD.

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Table 3 Monocyte-platelet aggregates (%) in female participants

No agonist	**0M**	**6M**
NE	3.8 ± 0.3	4.7 ± 0.5*
W	4.3 ± 0.6	3.8 ± 0.4
ADP		
NE	40.2 ± 4.6	59.6 ± 6.4†
W	46.9 ± 4.9	59.6 ± 5.7*
TRAP		
NE	70.5 ± 7.4	77.9 ± 9.0
W	69.4 ± 8.9	71.7 ± 9.2
AA		
NE	13.1 ± 2.9	25.5 ± 8.5
W	20.2 ± 7.2	23.0 ± 9.7

Samples collected before (0M) and after (6M) a 6 month intervention consisting of either no-exercise (NE) or walking (W). Blood samples were incubated with no agonist, *ADP,* thrombin receptor activating peptide *TRAP* and arachidonic acid *AA*. Statistical significance at $P = < 0.05$* and $P = < 0.01$† derived from post-hoc paired student's *t*-tests. Data are mean ± SE.

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674 **Figure 1**

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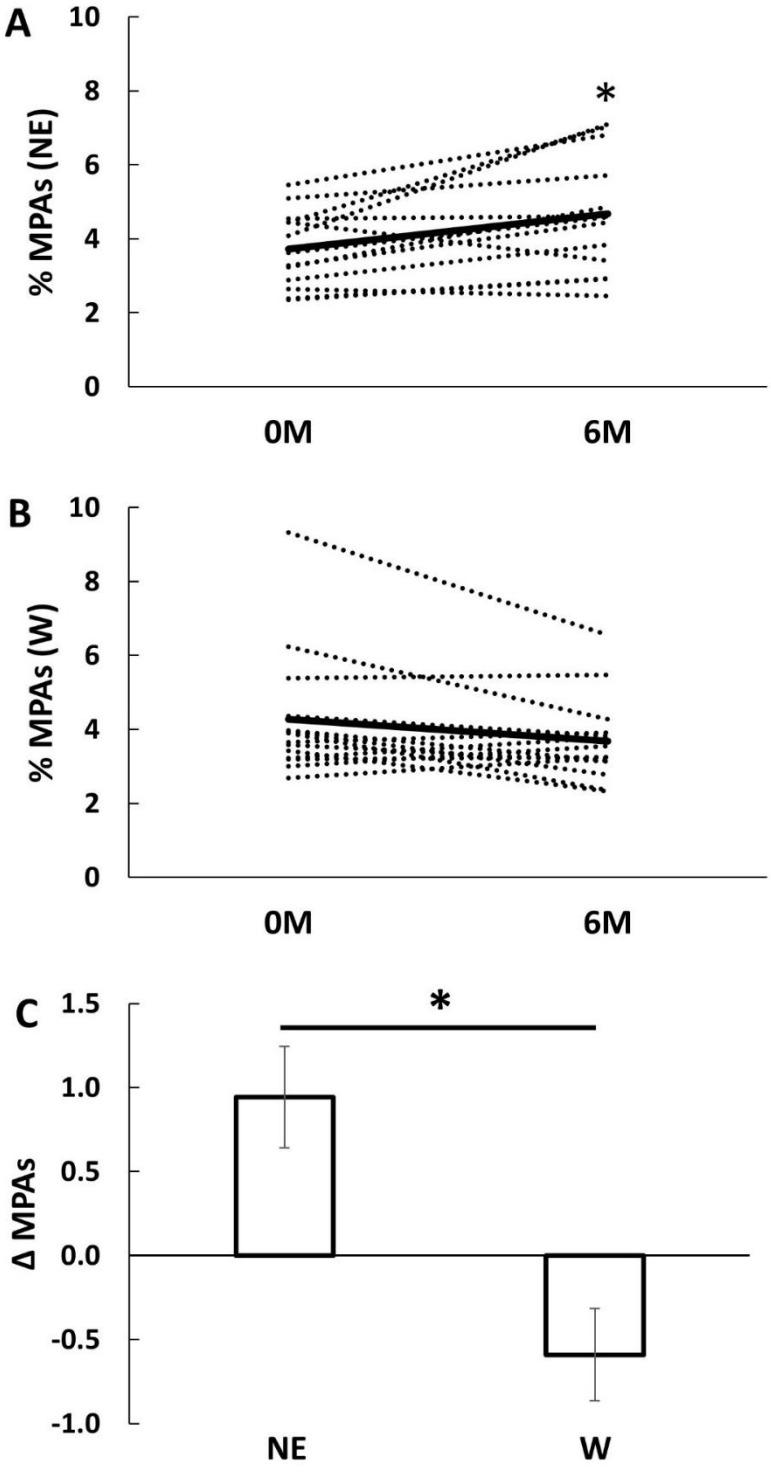
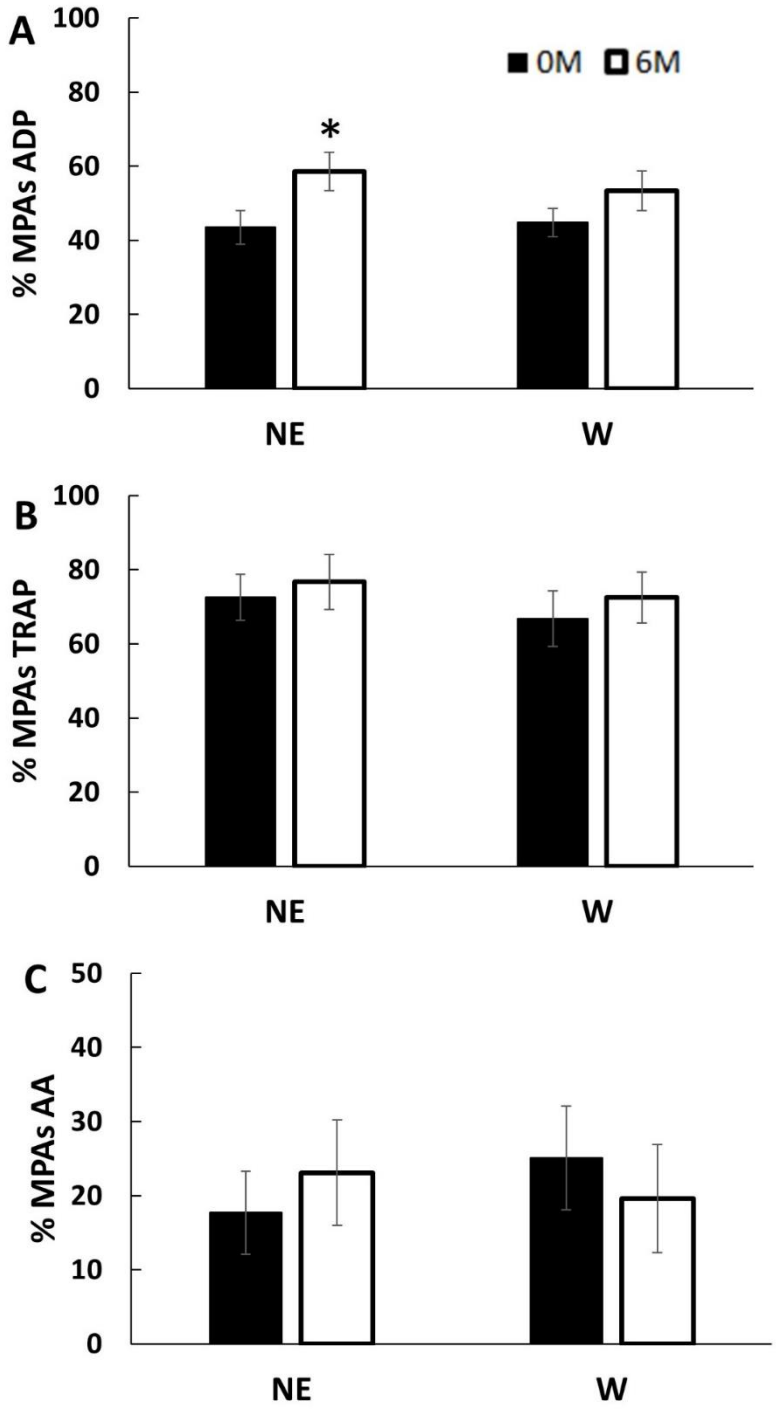


676 **Figure 2**



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