Single and 7-day handgrip and squat exercise prevents endothelial ischaemia-reperfusion injury in individuals with cardiovascular disease risk factors

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Background: Whole-body exercise provides protection against endothelial ischaemia-reperfusion (IR) injury. In this crossover study, we examined the effects of 1) single bout of local exercise (handgrip, squats) on endothelial responses to IR, and 2) if 7 days of daily local exercise bolsters these effects in individuals with cardiovascular disease (CVD) risk factors.

Methods: Fifteen participants (9 women, 58±5 years, ≥2 CVD risk factors) attended the laboratory for 6 visits. Subsequent to familiarization (visit 1), on visit 2 (control) brachial artery flow-mediated dilation (FMD) was measured before and after IR (15-minutes upper-arm ischemia, 15-minutes reperfusion). One week later, participants were randomized to 4x5-min unilateral handgrip (50% maximal voluntary contraction, 25 rpm) or squat exercises (15 rpm), followed by IR plus FMD measurements. Subsequently, home-based exercise was performed (six days), followed by another visit to the laboratory for the IR protocol plus FMD measurements (18-24 h after the last exercise bout). Following a two-week washout period, procedures were repeated with the alternative exercise mode.

Results: For a single exercise bout, we found a significant IR injury*exercise mode interaction (P<0.01), but no main effect of injury (P=0.08) or condition (P=0.61). A lower post-IR FMD was evident after control (pre-IR: 4.3±2.1% to post-IR: 2.9±1.9%, P<0.01), but not after handgrip (pre-IR: 3.8±1.6% to post-IR: 3.4±1.5%, P=0.31) or squats (pre-IR: 3.9±1.8% to post-IR: 4.0±1.9%, P=0.74). After 7 days of daily exercise, we found no change in FMD post-IR following handgrip (pre-IR: 4.3±1.9% to post-IR: 4.7±3.2%) or squats (pre-IR: 3.7±2.1% to post-IR: 4.7±3.0%, P>0.05).

Conclusions: Single bouts of dynamic, local exercise (handgrip, squats) provides remote protection against endothelial IR-induced injury in individuals with CVD risk factors, with one-week daily, home-based exercise preserving these effects for up to 24h following the last exercise bout.

New & Noteworthy: We show that single bouts of dynamic handgrip and squat exercise provide remote protection against endothelial IR-induced injury in individuals with CVD risk factors, with one-week daily, home-based exercise preserving these effects for up to 24 hours following the last exercise bout.

Introduction

Regular exercise training protects against cardiovascular disease (CVD)-related morbidity and mortality [1, 2]. These benefits cannot be fully explained by improvements in traditional CVD risk factors but may also relate to structural and functional vascular adaptations [3, 4]. Interestingly, single or short-term exercise provides immediate protection against ischaemia-reperfusion (IR) injury [5, 6]. This seems relevant as IR is central in mediating injury following cardiac surgery, such as bypass surgery, but also after myocardial infarction [7, 8]. Studies in animals reveal that a single bout of exercise is associated with a significantly smaller infarct size compared to non-exercising animals [5, 6, 9-11]. Subsequent studies in animals demonstrate this protection can persist for several days [12] and can be bolstered with repeated exercise bouts [13], although presence of CVD or risk factors attenuate these effects [14, 15].

Translation of this work to humans reinforces that single bouts of whole-body exercise can provide immediate cardiac [16] and vascular protection [17]. However, these previous studies on the effects of exercise on IR injury focused on healthy individuals. This is important to consider since populations with increased cardiovascular risk or established CVD show attenuated efficacy of remote ischaemic preconditioning (RIPC) [14, 15, 18]. Interestingly, these attenuated responses to RIPC in individuals with CVD or risk factors, appear to be mitigated in habitual endurance trained middle-aged to older individuals [19, 20] and following a 12-week cycling exercise training in heart failure patients [21]. Whilst this highlights the potency of exercise for immediate benefits, for example pertaining to cardiac surgery, whole-body exercise is associated with practical limitations in relation to cardiac surgery due to accessibility and population constraints. For this reason, we have recently explored the effects of handgrip exercise, and reported remote protection against endothelial IR injury in young, healthy participants following an acute bout of exercise [22]. This raises the question whether protective effects of local exercise modes, such as handgrip, are also present in individuals with elevated CVD risk, and whether a longer duration of exercise and/or a greater exercise stimulus (whole-body squat exercise) may be needed to achieve such protection.

Another practical aspect to consider in translating short-term, exercise-induced protection in the setting of cardiac surgery, is that the protective effects from acute exercise ('first window') often disappear 1-2 h following exercise [11, 23]. This makes the timing challenging when applied prior to elective cardiac surgery. Supported by previous work in animals [12, 24, 25], short-term daily exercise (1-week) may lead to preserving the effects of the final exercise bout for up to 24h or longer, making short-term exercise feasible in the context of elective surgery.

To facilitate translation of the potential benefits of short-term exercise-induced protection in humans, our first objective was to evaluate the effects of a single bout of local dynamic exercise (small and large muscle mass) on endothelial protection against IR injury in individuals with CVD risk factors. Secondly, we examined whether 7 days of daily (handgrip or squat) exercise leads to protection against IR injury that occurs between 18-24h following the last exercise bout. We hypothesised that both modes of exercise, handgrip and squat exercise, will prevent IR injury in individuals with CVD risk factors, whilst these effects are bolstered when local daily exercise is performed for 7 days. These objectives will provide important insight for translating (single and/or short-term) smaller muscle mass exercise types to the clinical arena in contexts where IR injury is present.

Methods

Participants

Of the 20 participants we recruited that were over the age of 50 without established CVD from the Liverpool, Merseyside greater community, 17 participants met the study criteria, and 15 participants (58±5 years) completed all parts of the study at the cardiovascular laboratory at Liverpool John Moores University. Nine of these participants were women (57±5 years) and were 6±3 years postmenopausal. Participants were included based on having ≥2 of the following CVD risk factors: sedentary (<3 hours of structured exercise per week), (pre)hypertension (systolic >120 mmHg and/or diastolic >80 mmHg) or diagnosed hypertension controlled with medication, elevated cholesterol (total >5.0 mmol/L, triglycerides >2.3 mmol/L, or LDL >3 mmol/L) or diagnosed hypercholesterolaemia controlled with medication, body mass index >30 kg/m^2 or waist circumference ≥94 cm for males and ≥80 cm for females. Exclusion criteria were smoking, pregnancy, or the diagnosis of diabetes mellitus, peripheral vascular disease, angina pectoris, previous myocardial infarction, stroke or thrombosis, or any leg or arm injury which could prevent application of the IR injury protocol or exercise. All participants provided written informed consent before taking part in the study. The study was approved by the Liverpool John Moores University Research Ethics Committee and adhered to the standards set forth in the *Declartion of Helsinski*.

Experimental Design

We adopted a crossover design, that involved a total of 6 visits: the first being a screening and familiarization visit, followed by the control experimental visit. During the control day, we examined the impact of IR on brachial artery endothelial function (using the flow-mediated dilation (FMD) technique). Subsequently, participants reported to our laboratory one week later and were randomized using open-source online software (randomization.com) in a counter-balanced manner to handgrip or squat exercise (8/15 participants performed squats first). Participants arrived at the laboratory at the same time of day for each visit (between 7-10 am) and refrained from food and caffeine for 12 hours and alcohol and vigorous physical activity for 24 hours, as these factors can influence vascular outcomes (Thijssen et al 2019b). During all experimental visits, resting blood pressure was measured after 10 minutes of rest and was followed by vascular assessments performed in the right arm before and after ischaemia (15-minutes of upper arm occlusion) and reperfusion (15-min following release of the occlusion cuff); the IR injury protocol [26]. To minimize the possibility of an increase in resting brachial artery diameter following injury, which would influence comparisons of FMD[27], we shorted the ischemic period to 15 minutes instead of the more frequently applied 20-minute ischemic period[18]. Importantly, we previously demonstrated that using the current model led to a significant decline in FMD without resulting in changes to the resting brachial artery diameter in young individuals[22]. To evaluate the immediate effects of exercise, participants performed the exercise intervention immediately following the baseline assessment of brachial artery FMD. After exercise, participants proceeded with the IR injury protocol. Following the visit to the laboratory where an acute bout of exercise was performed, participants then performed 6 consecutive days of the same exercise protocol at home, supported using online supervision and guidance from video clips. Participants recorded completion of the exercise in a log each day. Participants returned to the laboratory 18-24 hours after completing the last exercise session to examine the effects of 7 days of exercise on responses to IR by repeating the protocol outlined above. Upon completion of the

7-day exercise program, a washout period of two weeks was undertaken before examining the acute and 7-day effects of the other mode of local exercise (Figure 1).

Participant screening

Participants received a finger-stick capillary blood collection kit (MonitorMyHealth, NHS, UK) either at home or in the cardiovascular laboratory at Liverpool John Moores University, which was used to determine blood cholesterol levels (random, unfasted samples). During the first experimental visit, height, weight, and waist circumference were assessed. Blood pressure and heart rate were measured after 10 minutes of seated rest (Dinamap Carescape V100, GE Medical Systems Ltd, US). Furthermore, participants were asked to complete two questionnaires on physical activity: the International Physical Activity Questionnaire Short Form (IPAQ-SF) and the Physical Activity Readiness Questionnaire (PAR-Q). The IPAQ-SF provided information on physical activity levels in the past 7 days[28] and was used in our criteria for evaluating cardiovascular risk factors. The PAR-Q helped in confirming safety and preparedness among participants in performing exercises in the study.

Vascular assessments

Brachial artery endothelial function was assessed using the FMD technique described in the most recent published guidelines [29]. This measure is correlated with coronary artery function [30] and several studies have demonstrated the prognostic value of brachial artery FMD for future cardiovascular events [31, 32]. Participants rested in a supine position and the right arm was extended and positioned at an angle of 80-90° abduction from the torso, depending on comfort. A rapid inflating cuff (D.E. Hokanson, Bellevue, WA) was placed around the forearm immediately distal to the olecranon to provide the ischaemic stimulus. A high-resolution ultrasound machine (T3300; Terason, Burlington, MA) with a 15-MHz multifrequency linear array probe was used to image the brachial artery in the distal third of the upper arm. One minute of baseline recording was performed before the cuff was inflated to 220 mmHg for 5 minutes. The brachial artery recording was restarted again at 30 seconds before cuff deflation and continued for 3 minutes after deflation. All FMD measurements were taken by two experienced sonographers, with no interchange within participants to reduce variation. Sonographer 1 had a coefficient of variation in FMD of 18% and a coefficient of variation of 2% for baseline artery diameter. Sonographer 2 had a coefficient of variation in FMD of 16% and a coefficient of variation of 2% for baseline artery diameter.

These values are in line with recommended guidelines for FMD in consecutive scans (Thijssen et al 2019b).

Analysis of brachial artery diameter, blood velocity, and shear rate were performed using automatic edge-detection and wall-tracking software (FMD Studio system, Cardiovascular Suite, Quipu, Pisa, Italy) [33, 34]. Baseline data were calculated across the 1-minute preceding cuff inflation. After cuff deflation, peak diameter was automatically detected by the software system and is reported as an average of 4 seconds. FMD was calculated as ((peak diameter – baseline diameter)/ baseline diameter) × 100%. Blood flow was calculated at 30 Hz by multiplying the cross-sectional area of the artery with resting blood velocity. Shear rate was calculated as 4 × mean blood velocity/ diameter. Shear rate area under the curve (AUC) was defined as the area under the curve from the start of cuff release to the time of peak diameter.

Exercise protocols

Handgrip Exercise. At the start of the handgrip visit, forearm maximal voluntary contraction (MVC) of the left arm was assessed using a dynamometric handheld force transducer. Participants performed 3 short maximal contractions, of which the maximum-recorded value (kg) was reported as MVC. The handgrip exercise protocol consisted of 4 periods of rhythmic (25 reps/min, guided by a metronome) handgrip contractions at 50% MVC for 5 minutes on a dynamometric handgrip device. The exercise bouts were separated by 5-minute periods of rest and all exercise sessions were performed in the left arm to evaluate the remote effect of exercise as the IR injury protocol was performed on the right arm and endothelial function was examined in the right brachial artery. This was to ensure consistency in our set-up and measuring the right brachial artery was to improve quality of scanning and, hence, reduce variation. Moreover, research with lower limb[17, 21] and upper limb[22] exercise confirms the remote effect of (handgrip) exercise is independent of which muscles are active. Training was also performed with the left arm, identical to the laboratory protocol, to ensure we evaluate the remote effects of handgrip exercise and not a local effect[35]Participants were given a handgrip to take home for home-based exercise, and were instructed to perform the same resistance applied in the laboratory (50% MVC), as confirmed by daily virtual check-ins. We asked participants to achieve at least an 8/10 on their rating of perceived exertion at the end of each interval.

Squat Exercise. In line with the handgrip exercise protocol, participants performed 4 periods of 5 minutes of rhythmic squatting that was guided by a metronome. Participants performed

15 squats per minute, with the squats being performed without additional weights. For safety reasons and in line with practical feasibility, participants were instructed to use a chair to perform sit-to-stand procedures to perform squats. After each 5-minute exercise bout there was a 5-minute rest period. Home-based exercise training was performed in the same manner as during the laboratory visit.

Statistical Analysis

To answer the first objective of the present study, i.e., to evaluate the effects of a single session of small (handgrip exercise) and larger (squat) muscle mass dynamic exercise on responses to IR, we performed a repeated measures, within-subjects general linear model, with condition (3 levels: control, handgrip, squat) and injury (2 levels: pre-IR, post-IR). Subsequently, to address our second objective of the present study, i.e., to compare the effects of a single versus 1-week daily exercise on responses toIR, and if exercise mode plays a modulatory role, we employed a 3-factor repeated measures general linear model with exercise duration (2 levels: single, short-term), exercise mode (2 levels: handgrip, squat) and injury (2 levels: pre-IR, post-IR) on FMD outcomes. Within this model, we also explored the impact of one week of daily exercise on resting vascular function prior to the IR protocol. These analyses were repeated after allometric scaling of FMD responses to adjust for the influence of baseline diameter changes across trials [36]. Statistically significant interactions were followed up with the Bonferroni post-hoc comparison approach to correct for multiple comparisons. Analysis was conducted using Statistical Package for Social Sciences (Version 26: SPSS Inc., Chicago, IL). Statistical significance was delimited at $p<0.05$ and data are presented in the text as mean ± standard deviation.

Results

Participant characteristics

All participants exhibited at least 2 or more cardiovascular risk factors. Out of the 15 participants, one was prescribed cholesterol-lowering medication (statin) and 2 were prescribed calcium channel blockers for hypertension at the time of the study and were using these medications for 3 months or longer. Of the 9 women, 3 were taking menopausal hormone therapy for at least one year. Using the QRISK3 assessment tool [37], the average 10-year risk for CVD was 6.5 ±1.5%.

Single handgrip and squat exercise versus endothelial IR

Results of the repeated measures general linear model revealed a mode*injury-interaction effect (Figure 2). In the control condition, FMD declined from pre- to post-IR (pre-IR: 4.3±2.1% to post-IR: 2.9±1.9%, $p<0.01$), whilst this decline was absent after the handgrip (pre-IR: 3.8±1.6% to post-IR: 3.4±1.5%, $p=0.31$) and squats (pre-IR: 3.9±1.8% to post-IR: 4.0±1.9%, $p=0.74$; Figure 2). Baseline FMD was similar across all three conditions prior to IR ($p>0.05$, Table 2). Analyses conducted with allometric scaled FMD reinforced our initial observations (Table 2). There was a main effect of injury on time to peak ($p=0.04$) and shear AUC to peak diameter ($p<0.01$), indicating an overall decline in time to peak and shear AUC from pre- to post-IR that was not significantly different between conditions (Table 2).

Single + short-term (7 days), daily handgrip and squat exercise versus endothelial IR

Upon reviewing daily logs for recording home-based exercise, 13 out of 15 participants complied with performing all daily sessions, whilst 2 participants did not complete 1-2 sessions of the 6 home-based squat program due to reported muscle soreness. The general linear model analyses conducted indicated no significant change in FMD following IR ('injury'), an effect that was not different between the single bout of exercise *versus* short-term effects (7-days) for handgrip (pre-IR: 4.3±1.9% to post-IR: 4.7±3.2%) and squat exercise (pre-IR: 3.7±2.1% to post-IR: 4.7±3.0%) (all $p>0.05$; Table 3). There were no differences in baseline FMD and brachial artery diameter after 1-week handgrip or squat exercise (all $p>0.05$; Table 2). Resting, seated blood pressure was not different following both handgrip (pre: 126±14/77±8 mmHg, post: 125±17/77±8 mmHg, $p>0.05$) and squat exercise training (pre: 124±14/77±6 mmHg, post: 124±14/75±8 mmHg, $p>0.05$).

Discussion

The aim of this study was to evaluate the effect of a single bout of local exercise (handgrip and squats) on endothelial responses to IR injury in individuals with CVD risk factors, and subsequently, test whether 1 week of daily exercise affords remote protection against IR injury. We present the following findings. First, we found that a single session of local, dynamic exercise (4 bouts, 5-minutes/bout), either performed as handgrip or squat exercise, effectively prevents IR-induced endothelial injury of the (remote) brachial artery in individuals with CVD risk factors. Second, we demonstrated the ability for remote protection against endothelial IR to remain present for at least 18-24h following 1-week of daily exercise, independent of the exercise mode. Collectively, our findings show that even local modes of exercise can provide immediate, remote protection against endothelial IR injury in individuals with CVD risk factors; an effect that seems largely independent of the volume of exercising muscle.

Acute exercise and protection against IR injury

The finding that a single session of handgrip or squat exercise is effective in preventing IR-induced endothelial injury is especially relevant for clinical populations who may be limited in performing more strenuous, whole-body exercises involving greater muscle mass. Especially handgrip exercise, when performed in an episodic manner, represents a feasible approach and more accessible option than other types of whole-body exercises (cycling, running). We show that exercise performed in the lower limbs or unilaterally in the upper limb exerts protection in the contralateral arm exposed to IR injury, suggesting the presence of a systemic protective effect that is consistent with previous work in healthy individuals demonstrating that one bout of interval cycling exercise prevents upper arm-induced endothelial injury [17]. Our observations are also in line with more recent data in healthy individuals, who exhibit attenuated endothelial IR injury after performing handgrip exercise in the contralateral arm [22]. Unlike the attenuated effects of classic ischaemic preconditioning (cuff-induced) in populations that are older [18] and/or increased CVD risk [14, 15], we show that the preconditioning effect from dynamic handgrip or squat exercise remains intact in individuals with CVD risk factors.

The immediate protection following one session of exercise in preventing endothelial IR injury may relate to several protective pathways that are upregulated through the contracting muscle, as well as the intermittent nature of the exercise protocol itself. Previously we have shown that handgrip exercise elicits a comparable tissue deoxygenation and reperfusion profile to the traditional remote ischaemic preconditioning (RIPC) protocol, which involves brief periods of ischaemia prior to IR [22]. While differences seem present in prostacyclin formation at the microvascular level following handgrip and ischemic preconditioning protocols [38], recent proteomic analyses in older individuals with small vessel disease in the cerebral arteries suggest the presence of shared anti-inflammatory pathways triggered following both stimuli [39]. Specifically, this overlap with acute handgrip and RIPC intervention existed in reductions in Flt3L and FGF-21, pro-inflammatory markers that are both implicated in IR injury [40, 41], and these levels remained depressed after 4 days of repeated handgrip and IPC exposure[39]. The temporal pattern of tissue ischemia may also be responsible for these protective effects, as corroborated by improved resistance to injury with acute interval exercise, but not continuous exercise in healthy individuals [17]. Apart from the downstream ischaemic pattern achieved with intermittent exercise, humoral factors (e.g. adenosine, bradykinin, opioids) that seem to rely on opioid receptor activation[42] and circulating molecules released by the contracting muscle itself such as cytokines (e.g. IL-6, TNFα) or myokines (e.g. myonectin), may play a role in providing remote cardiac and vascular protection [6]. While efficacy of RIPC attenuates with aging, these latter processes, involving factors released by contracting muscle, may help to explain how exercise appears to restore preconditioning protection in aged rat hearts [43 44].

As squat exercises involves activation of a larger muscle mass compared to handgrip exercises, we expected squats would lead to the release of an increased number of circulating molecules [45], which in turn would result in greater protection against IR injury. Overall, we show that handgrip exercise provides equivalent protection from vascular injury to squat exercise, suggesting that sufficient immediate protection can be achieved with even small muscle mass contractions. It could also be possible that the relative contributions of protective pathways activated following each exercise stimulus differs, however, we can only speculate based on the observational nature of our study. Unfortunately, our study was not powered to assess the potential impact of sex on our outcomes. An underpowered analysis suggests that acute handgrip exercise is less effective in women in preserving endothelial function following IR injury than in men (women: pre-IR: 3.9±1.2 % to post-IR:3.0±1.6 %; men: pre-IR: 3.7±2.1 % to post-IR: 4.0±1.1 %), however this was not significant (p=0.17) and such differences were not observed for squats and/or following 7-days exercise. Although speculative, potential sex differences may relate to distinct role of functional sympatholysis in relation to preconditioning between men and women.[46]. Further research is needed to interrogate potential sex differences in exercise-induced protection against IR injury.

Short-term exercise and protection against IR injury

Successfully applying exercise preconditioning to patients awaiting surgery rests on maintaining a preconditioned state until the time of intervention. Although we demonstrate that a single session of exercise can prevent endothelial injury 1 hour before IR, previous work demonstrates this effects wanes 1-2 h following exercise [11]. Timing of the preconditioning stimulus is a frequently raised concern regarding the poor clinical translation, mainly relating to the short-lived effects of preconditioning [47]. In clinical trials, RIPC administered after induction of anaesthesia before surgery failed to show cardioprotection [48], but when RIPC was performed in the ambulance during hospital transport (~2 hours before primary percutaneous intervention) patients showed greater myocardial salvage [49] and improved long-term clinical outcomes [50] than those who received standard care. Even though handgrip exercise is a readily accessible mode of exercise, feasibility immediately prior to surgery may present challenges. Alternatively, enlarging the 'operating window' of the effects of preconditioning would be more beneficial. Interestingly, we show that protection against IR injury is preserved at least 18-24 hours following the last session of a 1-week daily exercise regimen. Recent analyses from a large standalone cardiac centre in the UK reports the median time from referral to operation for non-elective coronary artery bypass graft (CABG) is 7-8 days [51]. The short-term exercise program used in the current study is consistent with this wait period and may therefore be feasible and suited to implement, as demonstrated in a feasibility study in patients scheduled for cardiac surgery[52]. In the current study, we show that handgrip exercises can be completed at home with high compliance and limited supervision. Although we cannot simply translate our observations to those with established CVD, vascular protection afforded from 12 weeks of endurance exercise in patients with heart failure [21] suggests that prolonged effects of preconditioning can indeed be achieved in CVD populations.

The preserved protection conferred with 1-week of daily handgrip or squat exercise raises questions on the mechanisms that underlie these observations. One potential explanation may relate to vascular adaptations [4, 53, 54]. However, we found no change in resting endothelial function and brachial artery diameter, suggesting that alternative pathways were involved. An alternative explanation relates to a biphasic pattern of cardioprotection, which is typically observed following ischaemic preconditioning stimuli. The early phase (within minutes to hours) offers a strong protection, while the second phase provides a delayed (12 hours to days) mild protection against IR injury [11, 12, 23]. Since we assessed responses to IR injury between 18-24 hours following the last exercise bout, the protection observed may relate to the second window of protection from the last exercise session or, alternatively, may reflect continuous protection that is achieved from consecutive exercise bouts [13, 55]. To interrogate this further, exploring whether protection is maintained up to 24 hours after a single bout of exercise in humans is required.

Methodological considerations. Some limitations exist in the present study. We applied a frequently used model of IR in the upper limb, which may not translate to injury occurring in the myocardium during surgical intervention or myocardial infarct. Nonetheless, previous work shows this model indeed produces transient impairments in endothelial function [26], and significantly decreases plasma nitrite and nitrate concentrations, indicating reduced nitric oxide bioavailability following injury [56]. Varying protocols of IR involving longer durations of ischemia and/or reperfusion have been adopted in previous work[18, 57], which makes direct study comparisons with the current IR protocol challenging. The crossover design of our study meant that all participants performed both exercises for 1-week, which may have led to carry over effects from potential sustained protection from the first week of exercises completed. We attempted to minimize this by implementing a 2-week washout period between exercise modes, as well as by counterbalancing the intervention. Importantly, we did not find statistical differences in FMD measures at baseline or in response to IR between the two exercise modes ($p>0.05$). We did not assess endothelial responses to IR the day following the acute session of exercise, which could provide insight into whether a second window of protection presents in this population after the first bout of exercise and if this differs across exercise modes. We recognize that with the omission of this testing day it becomes difficult to disentangle whether the protection observed after 7 days of exercise was a result of continuous protection from repeated bouts of exercise and/or was attributed to the second window of protection emerging 24 hours after the last exercise bout. Another limitation to consider is that we did not include a testing arm to evaluate responses to IR 7 days following the control visit where during that time no exercise intervention would be prescribed. While we did not include this to minimize participant burden, such testing would have provided a control comparison for the short-term exercise conditions.

In conclusion, we show that a single session of handgrip or squat exercise effectively prevents IR-induced endothelial injury in individuals with CVD risk factors. Endothelial protection against IR injury remains present for at least 18-24 hours following a week of daily exercise, independent of the exercise mode. Taken together, our study suggests that even local modes of exercise can provide immediate, remote protection against endothelial IR injury in individuals at increased risk for CVD. This carries important clinical relevance for patients awaiting surgical intervention who may benefit from such protection and represents an important next step for future investigation.

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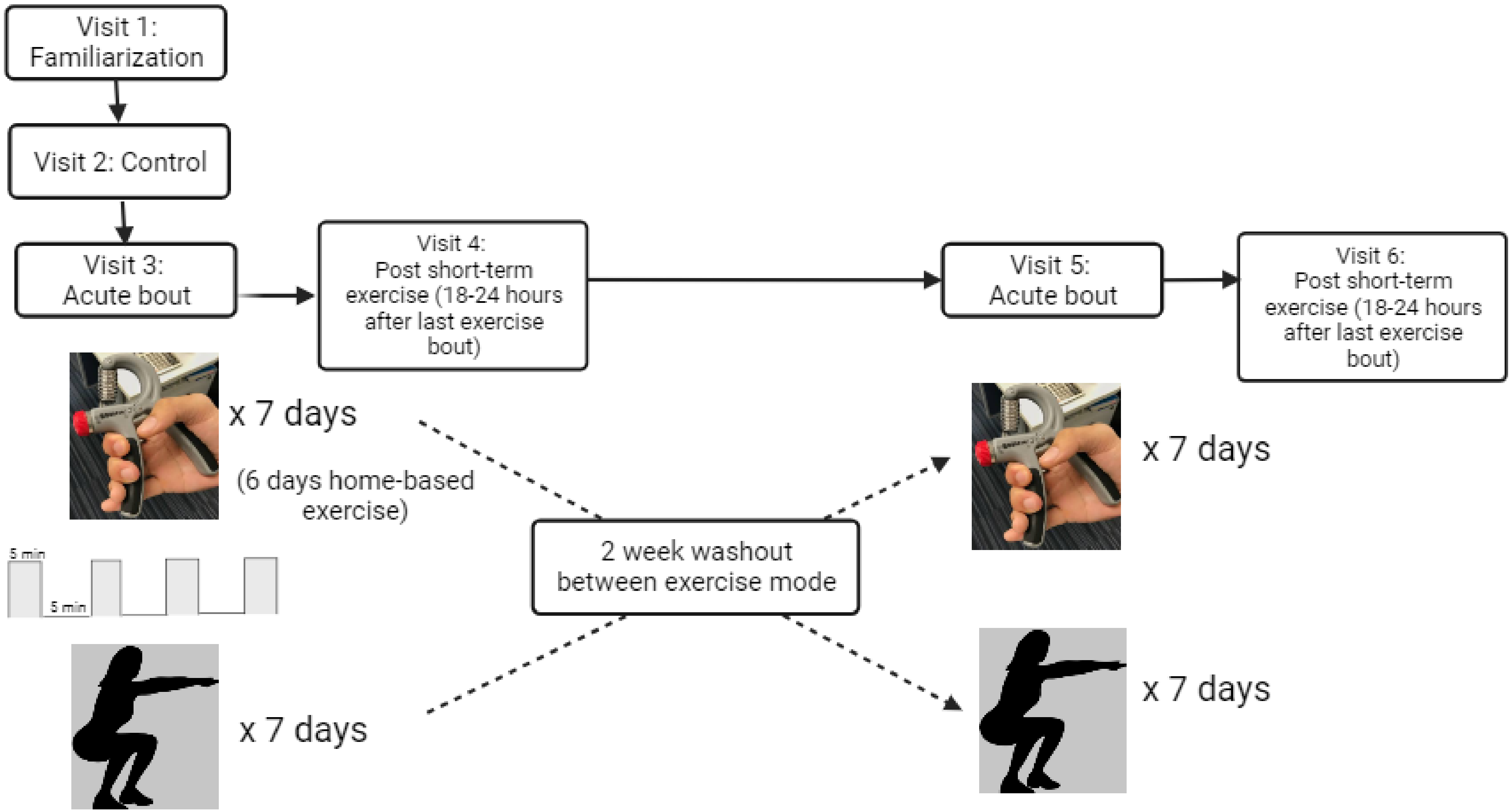
Figure Captions:

Figure 1. Schematic of study design displaying laboratory visits 1-6.

Figure 2. Comparison of A) control, a single bout of dynamic handgrip exercise, and squat exercise on flow-mediated dilation (FMD) at baseline and after ischaemia-reperfusion (IR) injury (Post IR), and comparison of a single session to B) 1-week of daily handgrip and squat exercise on FMD at baseline and post-IR in individuals with elevated CVD risk (n=15, 9 women). A 2-way repeated measures (RM) ANOVA to evaluate the acute effect of exercise revealed a significant interaction effect *Denotes statistical significance of Bonferroni corrected pairwise comparisons to interrogate the exercise mode*time interaction, $p<0.05$. A 3-way RM ANOVA to compare the acute and short-term effect of exercise and whether exercise mode moderated this revealed no statistically significant interaction or main effects, all $p>0.05$.


Visit 1: Familiarization
Visit 2: Control
Visit 3: Acute bout
Visit 4: Post short-term exercise (18-24 hours after last exercise bout)
Visit 5: Acute bout
Visit 6: Post short-term exercise (18-24 hours after last exercise bout)
x 7 days
(6 days home-based exercise)
5 min
5 min
x 7 days
2 week washout between exercise mode
x 7 days
x 7 days

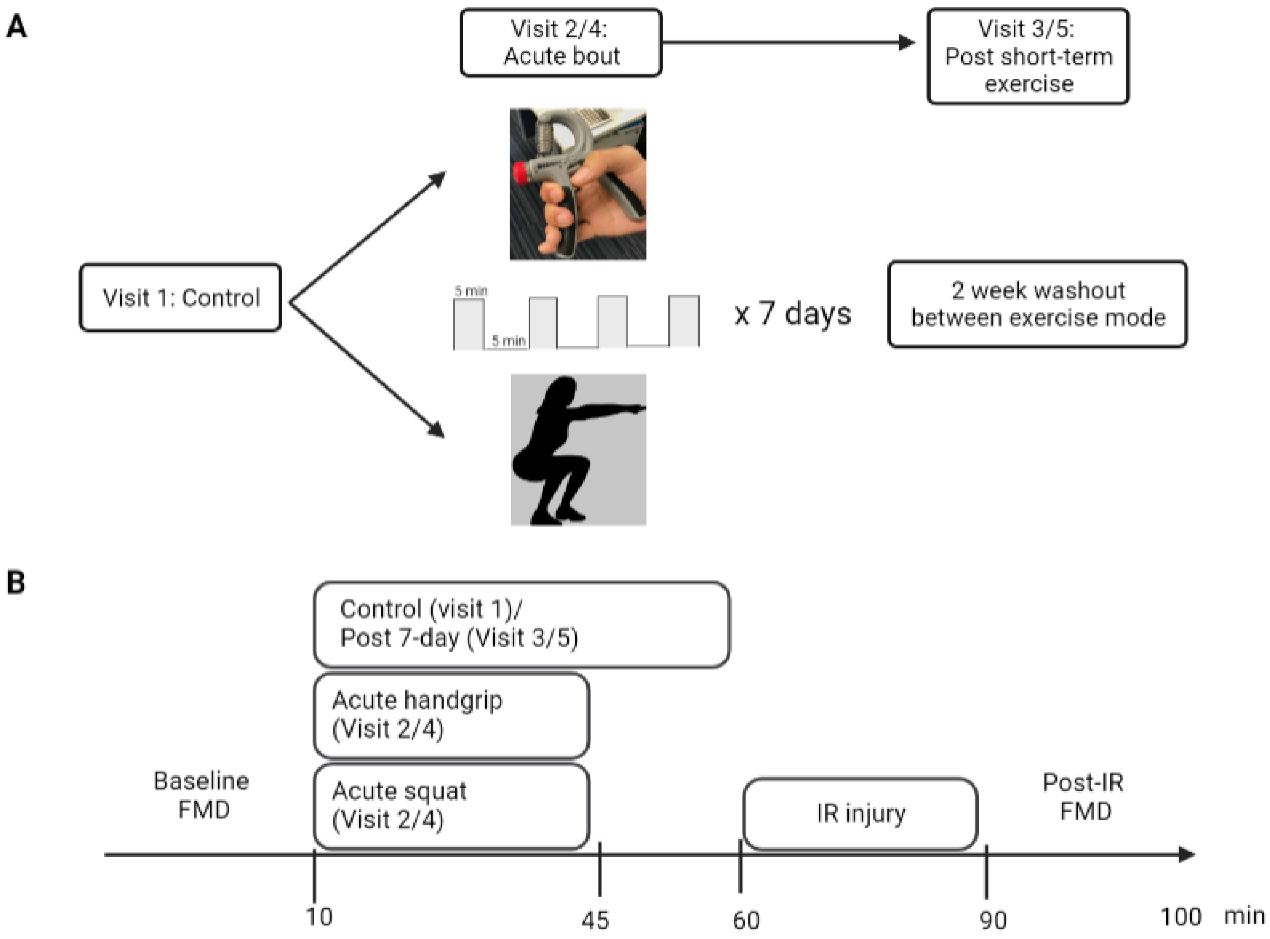


Figure 1. Schematic of A) study design and B) timeline for experimental protocol. *FMD, flow-mediated dilation, IR, ischaemia-reperfusion injury*

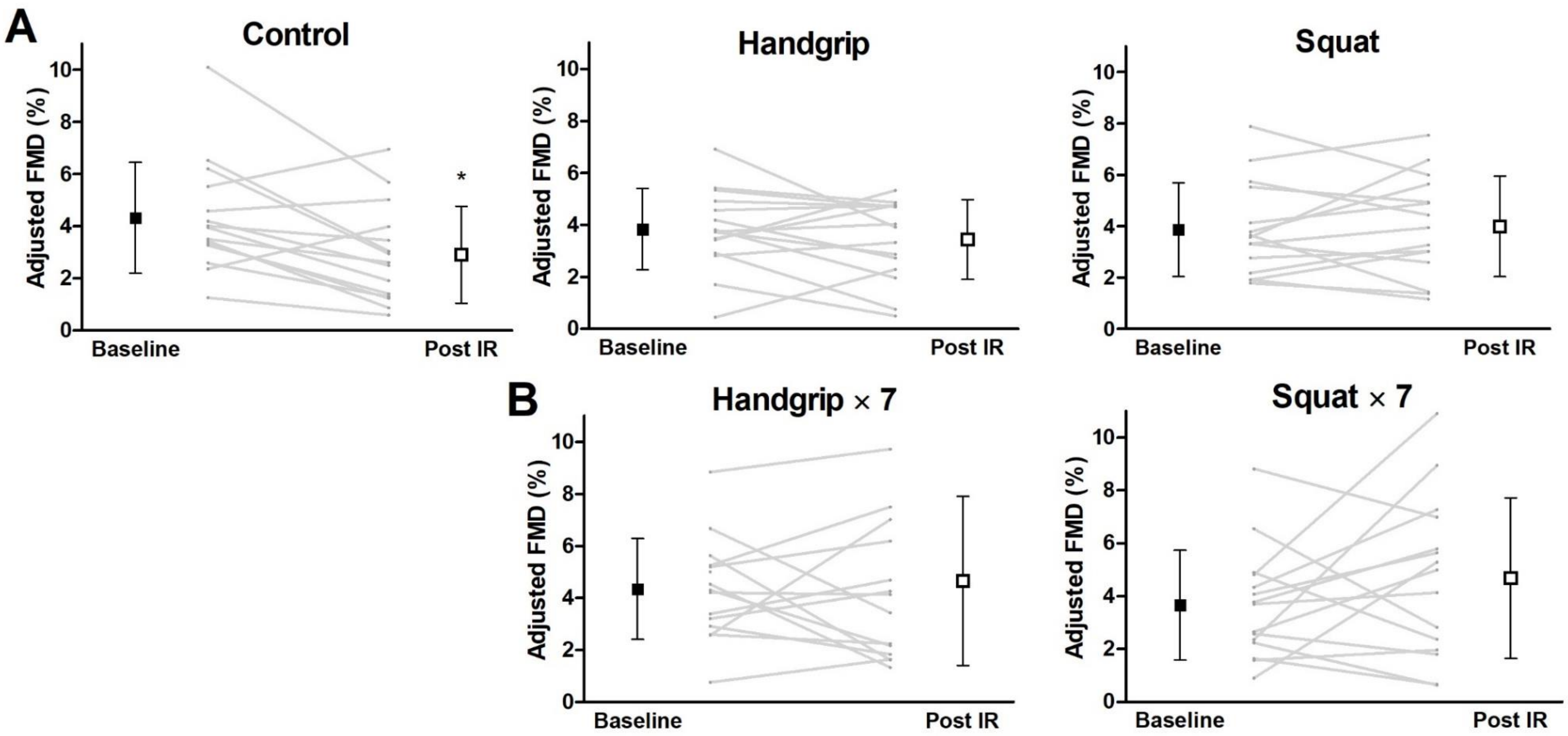


Figure 2. Comparison of **A)** control, a single bout of dynamic handgrip exercise, and squat exercise on flow-mediated dilation (FMD) at baseline and after ischaemia-reperfusion (IR) injury (Post IR), and comparison of a single session to **B)** 1-week of daily handgrip and squat exercise on FMD at baseline and post-IR in individuals with elevated CVD risk (n=15, 9 women). *Denotes statistical significance of Bonferroni corrected pairwise comparisons to interrogate the exercise mode*time interaction, $p<0.05$

Table 1. Participant characteristics

	n=15	Participants with risk factor, n
Sex, men/women	6/9	
Age, years	58±5	
Weight, kg	76.5±14.4	
Height, cm	167±7	
BMI, kg/m^2	27.4±4.0	4
Waist circumference, cm	93±14	8
Resting systolic BP, mmHg	121±13	8
Resting diastolic BP, mmHg	75±7	1
Resting HR, beats/minute	67±8	
Total cholesterol, mmol/l	6.0±1.1	11
Triglyceride, mmol/l	1.8±0.8	3
HDL, mmol/l	1.7±0.3	
LDL, mmol/l	3.4±0.9	9
Physical activity, MET×min/week	2175±1628	10
CVD risk factors, n		
2	5	
3	4	
4	3	
5	3	

Values are mean±SD. BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; HR, heart rate; LDL, low-density lipoprotein. MET, metabolic equivalent of task; CVD, cardiovascular disease.

Table 2. Brachial artery flow mediated dilation (FMD) measured at baseline and following ischaemia-reperfusion (Post-IR) after control (rest), acute, dynamic handgrip, and squat exercises in individuals with CVD risk factors (n=15). Repeated measures general linear models were performed to compare the change in FMD from baseline to Post-IR ('Injury') between control, handgrip, and squat exercise ('Mode'). *Values are means±SD. FMD, flow mediated dilation, AUC, area under curve.*

Single Exercise Bout	Control		Handgrip		Squat		*General Linear Model, P values*		
	Baseline	**Post-IR**	**Baseline**	**Post-IR**	**Baseline**	**Post-IR**	**Mode**	**Injury**	**Mode*Injury**
Resting Diameter (cm)	0.39±0.07	0.40±0.08	0.40±0.08	0.40±0.09	0.40±0.08	0.41±0.09	0.08	0.37	0.35
Peak Diameter (cm)	0.40±0.07	0.41±0.09	0.41±0.08	0.41±0.09	0.42±0.08	0.42±0.09	0.07	0.64	0.59
FMD%	4.3±2.1	2.9±1.9	3.8±1.6	3.4±1.5	3.9±1.8	4.0±1.9	0.61	**0.08**	**<0.01**
Allometric Scaled FMD%	4.3±2.0	2.9±1.8	3.8±1.5	3.3±1.7	3.9±1.7	3.6±2.0	0.87	**0.03**	**0.04**
Time to Peak (sec)	53±21	46±16	62±24	51±15	55±17	52±20	0.24	**0.04**	0.56
Shear AUC (10^3)	13.0±5.6	8.1±4.5	14.2±7.1	9.1±5.5	13.6±7.2	11.0±5.0	0.39	**<0.01**	0.29

Table 3. Brachial artery flow mediated dilation (FMD) measured at baseline and following ischaemia-reperfusion (post-IR) following 1 session, and 7 days of daily dynamic handgrip and squat exercises. A repeated measures general linear model was performed to compare the effects of a single bout of exercise to 1-week of daily exercise (duration) on IR injury (injury) and interrogate whether mode of exercise (mode) modifies these responses. *Values are means± SD. FMD, flow mediated dilation, AUC, area under curve.*

	Single Bout		1-week		3-way general linear model, P values			
Handgrip	Baseline	Post-IR	Baseline	Post-IR	Duration*Mode *Injury	Duration* Injury	Mode* Injury	Injury
Resting Diameter (cm)	0.40±0.08	0.40±0.09	0.39±0.07	0.39±0.09	0.94	0.25	0.27	0.99
Peak Diameter (cm)	0.41±0.08	0.41±0.09	0.41±0.07	0.41±0.08	0.74	0.49	0.12	0.97
FMD%	3.8±1.6	3.4±1.5	4.3±1.9	4.7±3.2	0.79	0.27	0.13	0.55
Allometric Scaled FMD%	3.8±1.5	3.3±1.7	4.3±1.9	4.5±3.1	0.49	0.17	0.09	0.74

Time to Peak (sec)	62±24	51±15	55±18	43±16	0.55	0.85	**0.02**	0.06
Shear AUC (10^3)	14.2±7.1	9.1±5.5	13.4±7.9	9.4±3.9	0.85	0.62	0.19	**<0.01**
Squat								
	Baseline	**Post-IR**	**Baseline**	**Post-IR**				
Resting Diameter (cm)	0.40±0.08	0.41±0.09	0.40±0.07	0.40±0.09				
Peak Diameter (cm)	0.42±0.08	0.42±0.09	0.41±0.07	0.41±0.09				
FMD%	3.9±1.8	4.0±1.9	3.7±2.1	4.6±3.0				
Allometric Scaled FMD%	3.9±1.7	3.6±2.0	3.6±2.1	4.6±2.9				
Time to Peak (sec)	55±17	52±20	55±16	56±19				
Shear AUC (10^3)	13.6±7.2	11.0±5.0	13.0±6.7	11.1±7.1				