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

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

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

32 **Results:** For a single exercise bout, we found a significant IR injury*exercise mode
33 interaction (P<0.01), but no main effect of injury (P=0.08) or condition (P=0.61). A lower
34 post-IR FMD was evident after control (pre-IR: 4.3±2.1% to post-IR: 2.9±1.9%, P<0.01), but
35 not after handgrip (pre-IR: 3.8±1.6% to post-IR: 3.4±1.5%, P=0.31) or squats (pre-IR:
36 3.9±1.8% to post-IR: 4.0±1.9%, P=0.74). After 7 days of daily exercise, we found no change
37 in FMD post-IR following handgrip (pre-IR: 4.3±1.9% to post-IR: 4.7±3.2%) or squats (pre-
38 IR: 3.7±2.1% to post-IR: 4.7±3.0%, P>0.05).

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

43

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

48 **Introduction**

49 Regular exercise training protects against cardiovascular disease (CVD)-related
50 morbidity and mortality^{1, 2}. These benefits cannot be fully explained by improvements in
51 traditional CVD risk factors but may also relate to structural and functional vascular
52 adaptations^{3, 4}. Interestingly, single or short-term exercise provides immediate protection
53 against ischaemia-reperfusion (IR) injury^{5, 6}. This seems relevant as IR is central in
54 mediating injury following cardiac surgery, such as bypass surgery, but also after myocardial
55 infarction^{7, 8}. Studies in animals reveal that a single bout of exercise is associated with a
56 significantly smaller infarct size compared to non-exercising animals^{5, 6, 9-11}. Subsequent
57 studies in animals demonstrate this protection can persist for several days¹² and can be
58 bolstered with repeated exercise bouts¹³, although presence of CVD or risk factors attenuate
59 these effects^{14, 15}.

60 Translation of this work to humans reinforces that single bouts of whole-body
61 exercise can provide immediate cardiac¹⁶ and vascular protection¹⁷. However, these
62 previous studies on the effects of exercise on IR injury focused on healthy individuals. This is
63 important to consider since populations with increased cardiovascular risk or established
64 CVD show attenuated efficacy of remote ischaemic preconditioning (RIPC)^{14, 15, 18}.
65 Interestingly, these attenuated responses to RIPC in individuals with CVD or risk factors,
66 appear to be mitigated in habitual endurance trained middle-aged to older individuals^{19, 20}
67 and following a 12-week cycling exercise training in heart failure patients²¹. Whilst this
68 highlights the potency of exercise for immediate benefits, for example pertaining to cardiac
69 surgery, whole-body exercise is associated with practical limitations in relation to cardiac
70 surgery due to accessibility and population constraints. For this reason, we have recently
71 explored the effects of handgrip exercise, and reported remote protection against endothelial
72 IR injury in young, healthy participants following an acute bout of exercise²². This raises the
73 question whether protective effects of local exercise modes, such as handgrip, are also
74 present in individuals with elevated CVD risk, and whether a longer duration of exercise
75 and/or a greater exercise stimulus (whole-body squat exercise) may be needed to achieve
76 such protection.

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

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

94

95 **Methods**

96 *Participants*

97 Of the 20 participants we recruited that were over the age of 50 without established CVD
98 from the Liverpool, Merseyside greater community, 17 participants met the study criteria,
99 and 15 participants (58±5 years) completed all parts of the study at the cardiovascular
100 laboratory at Liverpool John Moores University. Nine of these participants were women
101 (57±5 years) and were 6±3 years postmenopausal. Participants were included based on
102 having ≥2 of the following CVD risk factors: sedentary (<3 hours of structured exercise per
103 week), (pre)hypertension (systolic >120 mmHg and/or diastolic >80 mmHg) or diagnosed
104 hypertension controlled with medication, elevated cholesterol (total >5.0 mmol/L,
105 triglycerides >2.3 mmol/L, or LDL >3 mmol/L) or diagnosed hypercholesterolaemia
106 controlled with medication, body mass index >30 kg/m² or waist circumference ≥94 cm for
107 males and ≥80 cm for females. Exclusion criteria were smoking, pregnancy, or the diagnosis
108 of diabetes mellitus, peripheral vascular disease, angina pectoris, previous myocardial

109 infarction, stroke or thrombosis, or any leg or arm injury which could prevent application of
110 the IR injury protocol or exercise. All participants provided written informed consent before
111 taking part in the study. The study was approved by the Liverpool John Moores University
112 Research Ethics Committee and adhered to the standards set forth in the *Declaration of*
113 *Helsinki*.

114 *Experimental Design*

115 We adopted a crossover design, that involved a total of 6 visits: the first being a screening
116 and familiarization visit, followed by the control experimental visit. During the control day,
117 we examined the impact of IR on brachial artery endothelial function (using the flow-
118 mediated dilation (FMD) technique). Subsequently, participants reported to our laboratory
119 one week later and were randomized using open-source online software (randomization.com)
120 in a counter-balanced manner to handgrip or squat exercise (8/15 participants performed
121 squats first). Participants arrived at the laboratory at the same time of day for each visit
122 (between 7-10 am) and refrained from food and caffeine for 12 hours and alcohol and
123 vigorous physical activity for 24 hours, as these factors can influence vascular outcomes
124 (Thijssen et al 2019b). During all experimental visits, resting blood pressure was measured
125 after 10 minutes of rest and was followed by vascular assessments performed in the right arm
126 before and after ischaemia (15-minutes of upper arm occlusion) and reperfusion (15-min
127 following release of the occlusion cuff); the IR injury protocol²⁶. To minimize the possibility
128 of an increase in resting brachial artery diameter following injury, which would influence
129 comparisons of FMD²⁷, we shorted the ischemic period to 15 minutes instead of the more
130 frequently applied 20-minute ischemic period¹⁸. Importantly, we previously demonstrated
131 that using the current model led to a significant decline in FMD without resulting in changes
132 to the resting brachial artery diameter in young individuals²². To evaluate the immediate
133 effects of exercise, participants performed the exercise intervention immediately following
134 the baseline assessment of brachial artery FMD. After exercise, participants proceeded with
135 the IR injury protocol. Following the visit to the laboratory where an acute bout of exercise
136 was performed, participants then performed 6 consecutive days of the same exercise protocol
137 at home, supported using online supervision and guidance from video clips. Participants
138 recorded completion of the exercise in a log each day. Participants returned to the laboratory
139 18-24 hours after completing the last exercise session to examine the effects of 7 days of
140 exercise on responses to IR by repeating the protocol outlined above. Upon completion of the

141 7-day exercise program, a washout period of two weeks was undertaken before examining the
142 acute and 7-day effects of the other mode of local exercise (Figure 1).

143 *Participant screening*

144 Participants received a finger-stick capillary blood collection kit (MonitorMyHealth, NHS,
145 UK) either at home or in the cardiovascular laboratory at Liverpool John Moores University,
146 which was used to determine blood cholesterol levels (random, unfasted samples). During the
147 first experimental visit, height, weight, and waist circumference were assessed. Blood
148 pressure and heart rate were measured after 10 minutes of seated rest (Dinamap Carescape
149 V100, GE Medical Systems Ltd, US). Furthermore, participants were asked to complete two
150 questionnaires on physical activity: the International Physical Activity Questionnaire Short
151 Form (IPAQ-SF) and the Physical Activity Readiness Questionnaire (PAR-Q). The IPAQ-SF
152 provided information on physical activity levels in the past 7 days²⁸ and was used in our
153 criteria for evaluating cardiovascular risk factors. The PAR-Q helped in confirming safety
154 and preparedness among participants in performing exercises in the study.

155 *Vascular assessments*

156 Brachial artery endothelial function was assessed using the FMD technique described in the
157 most recent published guidelines²⁹. This measure is correlated with coronary artery function
158³⁰ and several studies have demonstrated the prognostic value of brachial artery FMD for
159 future cardiovascular events^{31, 32}. Participants rested in a supine position and the right arm
160 was extended and positioned at an angle of 80-90° abduction from the torso, depending on
161 comfort. A rapid inflating cuff (D.E. Hokanson, Bellevue, WA) was placed around the
162 forearm immediately distal to the olecranon to provide the ischaemic stimulus. A high-
163 resolution ultrasound machine (T3300; Terason, Burlington, MA) with a 15-MHz
164 multifrequency linear array probe was used to image the brachial artery in the distal third of
165 the upper arm. One minute of baseline recording was performed before the cuff was inflated
166 to 220 mmHg for 5 minutes. The brachial artery recording was restarted again at 30 seconds
167 before cuff deflation and continued for 3 minutes after deflation. All FMD measurements
168 were taken by two experienced sonographers, with no interchange within participants to
169 reduce variation. Sonographer 1 had a coefficient of variation in FMD of 18% and a
170 coefficient of variation of 2% for baseline artery diameter. Sonographer 2 had a coefficient of
171 variation in FMD of 16% and a coefficient of variation of 2% for baseline artery diameter.

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

174 Analysis of brachial artery diameter, blood velocity, and shear rate were performed using
175 automatic edge-detection and wall-tracking software (FMD Studio system, Cardiovascular
176 Suite, Quipu, Pisa, Italy)^{33, 34}. Baseline data were calculated across the 1-minute preceding
177 cuff inflation. After cuff deflation, peak diameter was automatically detected by the software
178 system and is reported as an average of 4 seconds. FMD was calculated as $((\text{peak diameter} -$
179 $\text{baseline diameter}) / \text{baseline diameter}) \times 100\%$. Blood flow was calculated at 30 Hz by
180 multiplying the cross-sectional area of the artery with resting blood velocity. Shear rate was
181 calculated as $4 \times \text{mean blood velocity} / \text{diameter}$. Shear rate area under the curve (AUC) was
182 defined as the area under the curve from the start of cuff release to the time of peak diameter.

183 *Exercise protocols*

184 *Handgrip Exercise.* At the start of the handgrip visit, forearm maximal voluntary contraction
185 (MVC) of the left arm was assessed using a dynamometric handheld force transducer.
186 Participants performed 3 short maximal contractions, of which the maximum-recorded value
187 (kg) was reported as MVC. The handgrip exercise protocol consisted of 4 periods of rhythmic
188 (25 reps/min, guided by a metronome) handgrip contractions at 50% MVC for 5 minutes on a
189 dynamometric handgrip device. The exercise bouts were separated by 5-minute periods of
190 rest and all exercise sessions were performed in the left arm to evaluate the remote effect of
191 exercise as the IR injury protocol was performed on the right arm and endothelial function
192 was examined in the right brachial artery. This was to ensure consistency in our set-up and
193 measuring the right brachial artery was to improve quality of scanning and, hence, reduce
194 variation. Moreover, research with lower limb^{17, 21} and upper limb²² exercise confirms the
195 remote effect of (handgrip) exercise is independent of which muscles are active. Training
196 was also performed with the left arm, identical to the laboratory protocol, to ensure we
197 evaluate the remote effects of handgrip exercise and not a local effect³⁵ Participants were
198 given a handgrip to take home for home-based exercise, and were instructed to perform the
199 same resistance applied in the laboratory (50% MVC), as confirmed by daily virtual check-
200 ins. We asked participants to achieve at least an 8/10 on their rating of perceived exertion at
201 the end of each interval.

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

204 15 squats per minute, with the squats being performed without additional weights. For safety
205 reasons and in line with practical feasibility, participants were instructed to use a chair to
206 perform sit-to-stand procedures to perform squats. After each 5-minute exercise bout there
207 was a 5-minute rest period. Home-based exercise training was performed in the same manner
208 as during the laboratory visit.

209 **Statistical Analysis**

210 To answer the first objective of the present study, i.e., to evaluate the effects of a single
211 session of small (handgrip exercise) and larger (squat) muscle mass dynamic exercise on
212 responses to IR, we performed a repeated measures, within-subjects general linear model,
213 with condition (3 levels: control, handgrip, squat) and injury (2 levels: pre-IR, post-IR).
214 Subsequently, to address our second objective of the present study, i.e., to compare the
215 effects of a single versus 1-week daily exercise on responses to IR, and if exercise mode plays
216 a modulatory role, we employed a 3-factor repeated measures general linear model with
217 exercise duration (2 levels: single, short-term), exercise mode (2 levels: handgrip, squat) and
218 injury (2 levels: pre-IR, post-IR) on FMD outcomes. Within this model, we also explored the
219 impact of one week of daily exercise on resting vascular function prior to the IR protocol.
220 These analyses were repeated after allometric scaling of FMD responses to adjust for the
221 influence of baseline diameter changes across trials³⁶. Statistically significant interactions
222 were followed up with the Bonferroni post-hoc comparison approach to correct for multiple
223 comparisons. Analysis was conducted using Statistical Package for Social Sciences (Version
224 26: SPSS Inc., Chicago, IL). Statistical significance was delimited at $p < 0.05$ and data are
225 presented in the text as mean \pm standard deviation.

226

227 **Results**

228 *Participant characteristics*

229 All participants exhibited at least 2 or more cardiovascular risk factors. Out of the 15
230 participants, one was prescribed cholesterol-lowering medication (statin) and 2 were
231 prescribed calcium channel blockers for hypertension at the time of the study and were using
232 these medications for 3 months or longer. Of the 9 women, 3 were taking menopausal
233 hormone therapy for at least one year. Using the QRISK3 assessment tool³⁷, the average 10-
234 year risk for CVD was $6.5 \pm 1.5\%$.

235

236 *Single handgrip and squat exercise versus endothelial IR*

237 Results of the repeated measures general linear model revealed a mode*injury-interaction
238 effect (Figure 2). In the control condition, FMD declined from pre- to post-IR (pre-IR:
239 4.3±2.1% to post-IR: 2.9±1.9%, $p<0.01$), whilst this decline was absent after the handgrip
240 (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:
241 4.0±1.9%, $p=0.74$; Figure 2). Baseline FMD was similar across all three conditions prior to
242 IR ($p>0.05$, Table 2). Analyses conducted with allometric scaled FMD reinforced our initial
243 observations (Table 2). There was a main effect of injury on time to peak ($p=0.04$) and shear
244 AUC to peak diameter ($p<0.01$), indicating an overall decline in time to peak and shear AUC
245 from pre- to post-IR that was not significantly different between conditions (Table 2).

246

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

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

259 **Discussion**

260 The aim of this study was to evaluate the effect of a single bout of local exercise
261 (handgrip and squats) on endothelial responses to IR injury in individuals with CVD risk
262 factors, and subsequently, test whether 1 week of daily exercise affords remote protection
263 against IR injury. We present the following findings. First, we found that a single session of
264 local, dynamic exercise (4 bouts, 5-minutes/bout), either performed as handgrip or squat
265 exercise, effectively prevents IR-induced endothelial injury of the (remote) brachial artery in

266 individuals with CVD risk factors. Second, we demonstrated the ability for remote protection
267 against endothelial IR to remain present for at least 18-24h following 1-week of daily
268 exercise, independent of the exercise mode. Collectively, our findings show that even local
269 modes of exercise can provide immediate, remote protection against endothelial IR injury in
270 individuals with CVD risk factors; an effect that seems largely independent of the volume of
271 exercising muscle.

272 *Acute exercise and protection against IR injury*

273 The finding that a single session of handgrip or squat exercise is effective in
274 preventing IR-induced endothelial injury is especially relevant for clinical populations who
275 may be limited in performing more strenuous, whole-body exercises involving greater muscle
276 mass. Especially handgrip exercise, when performed in an episodic manner, represents a
277 feasible approach and more accessible option than other types of whole-body exercises
278 (cycling, running). We show that exercise performed in the lower limbs or unilaterally in the
279 upper limb exerts protection in the contralateral arm exposed to IR injury, suggesting the
280 presence of a systemic protective effect that is consistent with previous work in healthy
281 individuals demonstrating that one bout of interval cycling exercise prevents upper arm-
282 induced endothelial injury¹⁷. Our observations are also in line with more recent data in
283 healthy individuals, who exhibit attenuated endothelial IR injury after performing handgrip
284 exercise in the contralateral arm²². Unlike the attenuated effects of classic ischaemic
285 preconditioning (cuff-induced) in populations that are older¹⁸ and/or increased CVD risk¹⁴,
286¹⁵, we show that the preconditioning effect from dynamic handgrip or squat exercise remains
287 intact in individuals with CVD risk factors.

288 The immediate protection following one session of exercise in preventing endothelial
289 IR injury may relate to several protective pathways that are upregulated through the
290 contracting muscle, as well as the intermittent nature of the exercise protocol itself.
291 Previously we have shown that handgrip exercise elicits a comparable tissue deoxygenation
292 and reperfusion profile to the traditional remote ischaemic preconditioning (RIPC) protocol,
293 which involves brief periods of ischaemia prior to IR²². While differences seem present in
294 prostacyclin formation at the microvascular level following handgrip and ischemic
295 preconditioning protocols³⁸, recent proteomic analyses in older individuals with small vessel
296 disease in the cerebral arteries suggest the presence of shared anti-inflammatory pathways
297 triggered following both stimuli³⁹. Specifically, this overlap with acute handgrip and RIPC

298 intervention existed in reductions in Flt3L and FGF-21, pro-inflammatory markers that are
299 both implicated in IR injury^{40, 41}, and these levels remained depressed after 4 days of
300 repeated handgrip and IPC exposure³⁹. The temporal pattern of tissue ischemia may also be
301 responsible for these protective effects, as corroborated by improved resistance to injury with
302 acute interval exercise, but not continuous exercise in healthy individuals¹⁷. Apart from the
303 downstream ischaemic pattern achieved with intermittent exercise, humoral factors (e.g.
304 adenosine, bradykinin, opioids) that seem to rely on opioid receptor activation⁴² and
305 circulating molecules released by the contracting muscle itself such as cytokines (e.g. IL-6,
306 TNF α) or myokines (e.g. myonectin), may play a role in providing remote cardiac and
307 vascular protection⁶. While efficacy of RIPC attenuates with aging, these latter processes,
308 involving factors released by contracting muscle, may help to explain how exercise appears
309 to restore preconditioning protection in aged rat hearts^{43 44}.

310 As squat exercises involves activation of a larger muscle mass compared to handgrip
311 exercises, we expected squats would lead to the release of an increased number of circulating
312 molecules⁴⁵, which in turn would result in greater protection against IR injury. Overall, we
313 show that handgrip exercise provides equivalent protection from vascular injury to squat
314 exercise, suggesting that sufficient immediate protection can be achieved with even small
315 muscle mass contractions. It could also be possible that the relative contributions of
316 protective pathways activated following each exercise stimulus differs, however, we can only
317 speculate based on the observational nature of our study. Unfortunately, our study was not
318 powered to assess the potential impact of sex on our outcomes. An underpowered analysis
319 suggests that acute handgrip exercise is less effective in women in preserving endothelial
320 function following IR injury than in men (women: pre-IR: 3.9 \pm 1.2 % to post-IR: 3.0 \pm 1.6 %;
321 men: pre-IR: 3.7 \pm 2.1 % to post-IR: 4.0 \pm 1.1 %), however this was not significant (p=0.17)
322 and such differences were not observed for squats and/or following 7-days exercise.
323 Although speculative, potential sex differences may relate to distinct role of functional
324 sympatholysis in relation to preconditioning between men and women.⁴⁶ Further research is
325 needed to interrogate potential sex differences in exercise-induced protection against IR
326 injury.

327 *Short-term exercise and protection against IR injury*

328 Successfully applying exercise preconditioning to patients awaiting surgery rests on
329 maintaining a preconditioned state until the time of intervention. Although we demonstrate

330 that a single session of exercise can prevent endothelial injury 1 hour before IR, previous
331 work demonstrates this effects wanes 1-2 h following exercise ¹¹. Timing of the
332 preconditioning stimulus is a frequently raised concern regarding the poor clinical translation,
333 mainly relating to the short-lived effects of preconditioning ⁴⁷. In clinical trials, RIPC
334 administered after induction of anaesthesia before surgery failed to show cardioprotection ⁴⁸,
335 but when RIPC was performed in the ambulance during hospital transport (~2 hours before
336 primary percutaneous intervention) patients showed greater myocardial salvage ⁴⁹ and
337 improved long-term clinical outcomes ⁵⁰ than those who received standard care. Even though
338 handgrip exercise is a readily accessible mode of exercise, feasibility immediately prior to
339 surgery may present challenges. Alternatively, enlarging the ‘operating window’ of the
340 effects of preconditioning would be more beneficial. Interestingly, we show that protection
341 against IR injury is preserved at least 18-24 hours following the last session of a 1-week daily
342 exercise regimen. Recent analyses from a large standalone cardiac centre in the UK reports
343 the median time from referral to operation for non-elective coronary artery bypass graft
344 (CABG) is 7-8 days ⁵¹. The short-term exercise program used in the current study is
345 consistent with this wait period and may therefore be feasible and suited to implement, as
346 demonstrated in a feasibility study in patients scheduled for cardiac surgery⁵². In the current
347 study, we show that handgrip exercises can be completed at home with high compliance and
348 limited supervision. Although we cannot simply translate our observations to those with
349 established CVD, vascular protection afforded from 12 weeks of endurance exercise in
350 patients with heart failure ²¹ suggests that prolonged effects of preconditioning can indeed be
351 achieved in CVD populations.

352 The preserved protection conferred with 1-week of daily handgrip or squat exercise
353 raises questions on the mechanisms that underlie these observations. One potential
354 explanation may relate to vascular adaptations ^{4, 53, 54}. However, we found no change in
355 resting endothelial function and brachial artery diameter, suggesting that alternative pathways
356 were involved. An alternative explanation relates to a biphasic pattern of cardioprotection,
357 which is typically observed following ischaemic preconditioning stimuli. The early phase
358 (within minutes to hours) offers a strong protection, while the second phase provides a
359 delayed (12 hours to days) mild protection against IR injury ^{11, 12, 23}. Since we assessed
360 responses to IR injury between 18-24 hours following the last exercise bout, the protection
361 observed may relate to the second window of protection from the last exercise session or,
362 alternatively, may reflect continuous protection that is achieved from consecutive exercise

363 bouts^{13, 55}. To interrogate this further, exploring whether protection is maintained up to 24
364 hours after a single bout of exercise in humans is required.

365 *Methodological considerations.* Some limitations exist in the present study. We
366 applied a frequently used model of IR in the upper limb, which may not translate to injury
367 occurring in the myocardium during surgical intervention or myocardial infarct. Nonetheless,
368 previous work shows this model indeed produces transient impairments in endothelial
369 function²⁶, and significantly decreases plasma nitrite and nitrate concentrations, indicating
370 reduced nitric oxide bioavailability following injury⁵⁶. Varying protocols of IR involving
371 longer durations of ischemia and/or reperfusion have been adopted in previous work^{18, 57},
372 which makes direct study comparisons with the current IR protocol challenging. The
373 crossover design of our study meant that all participants performed both exercises for 1-week,
374 which may have led to carry over effects from potential sustained protection from the first
375 week of exercises completed. We attempted to minimize this by implementing a 2-week
376 washout period between exercise modes, as well as by counterbalancing the intervention.
377 Importantly, we did not find statistical differences in FMD measures at baseline or in
378 response to IR between the two exercise modes ($p>0.05$). We did not assess endothelial
379 responses to IR the day following the acute session of exercise, which could provide insight
380 into whether a second window of protection presents in this population after the first bout of
381 exercise and if this differs across exercise modes. We recognize that with the omission of this
382 testing day it becomes difficult to disentangle whether the protection observed after 7 days of
383 exercise was a result of continuous protection from repeated bouts of exercise and/or was
384 attributed to the second window of protection emerging 24 hours after the last exercise bout.
385 Another limitation to consider is that we did not include a testing arm to evaluate responses to
386 IR 7 days following the control visit where during that time no exercise intervention would
387 be prescribed. While we did not include this to minimize participant burden, such testing
388 would have provided a control comparison for the short-term exercise conditions.

389 In conclusion, we show that a single session of handgrip or squat exercise
390 effectively prevents IR-induced endothelial injury in individuals with CVD risk factors.
391 Endothelial protection against IR injury remains present for at least 18-24 hours following a
392 week of daily exercise, independent of the exercise mode. Taken together, our study suggests
393 that even local modes of exercise can provide immediate, remote protection against
394 endothelial IR injury in individuals at increased risk for CVD. This carries important clinical

395 relevance for patients awaiting surgical intervention who may benefit from such protection
396 and represents an important next step for future investigation.

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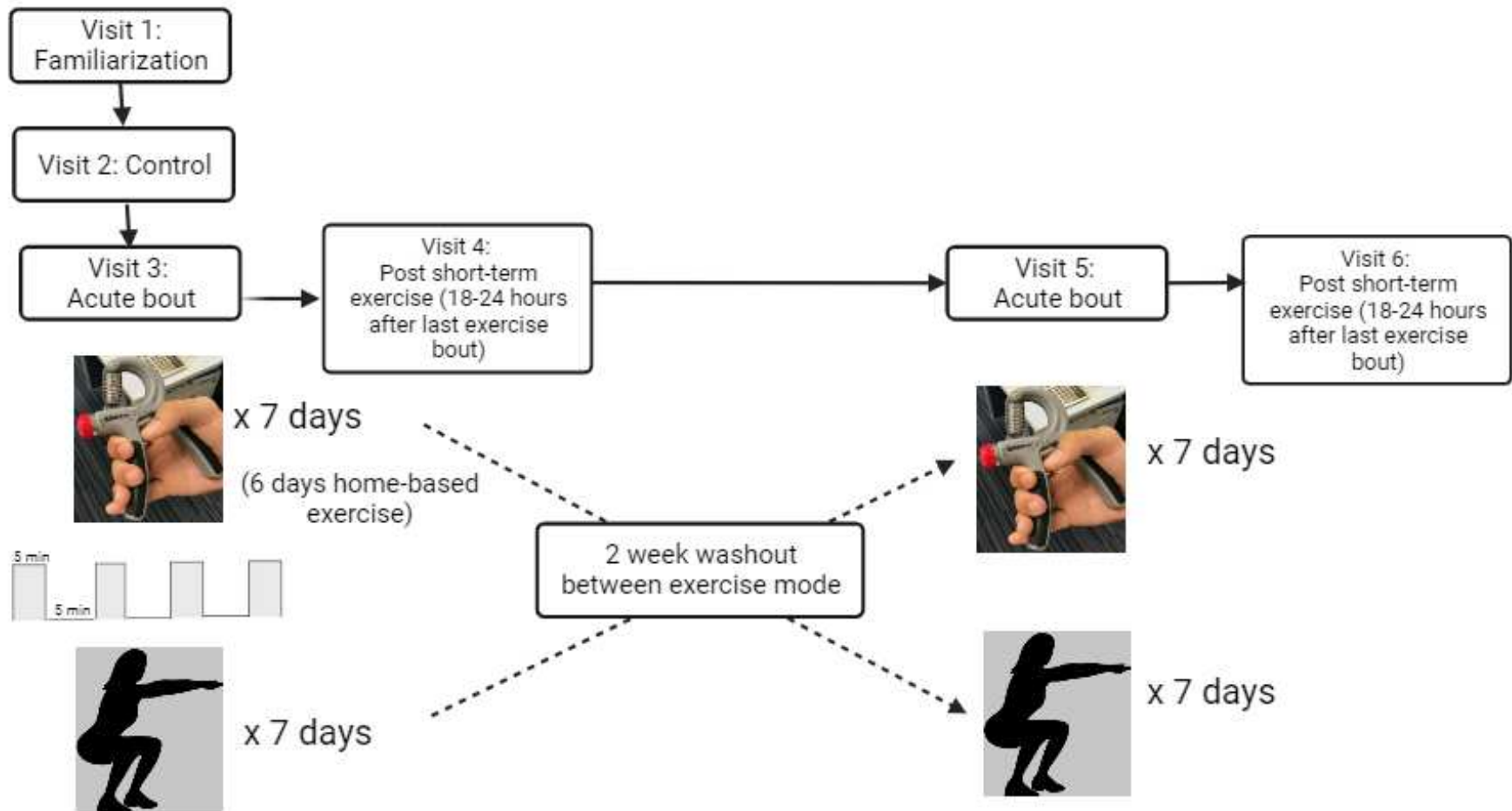
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570

571 **Figure Captions:**

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

573 **Figure 2.** Comparison of A) control, a single bout of dynamic handgrip exercise, and squat
574 exercise on flow-mediated dilation (FMD) at baseline and after ischaemia-reperfusion (IR)
575 injury (Post IR), and comparison of a single session to B) 1-week of daily handgrip and squat
576 exercise on FMD at baseline and post-IR in individuals with elevated CVD risk (n=15, 9
577 women). A 2-way repeated measures (RM) ANOVA to evaluate the acute effect of exercise
578 revealed a significant interaction effect *Denotes statistical significance of Bonferroni
579 corrected pairwise comparisons to interrogate the exercise mode*time interaction, p<0.05. A
580 3-way RM ANOVA to compare the acute and short-term effect of exercise and whether
581 exercise mode moderated this revealed no statistically significant interaction or main effects,
582 all p>0.05.



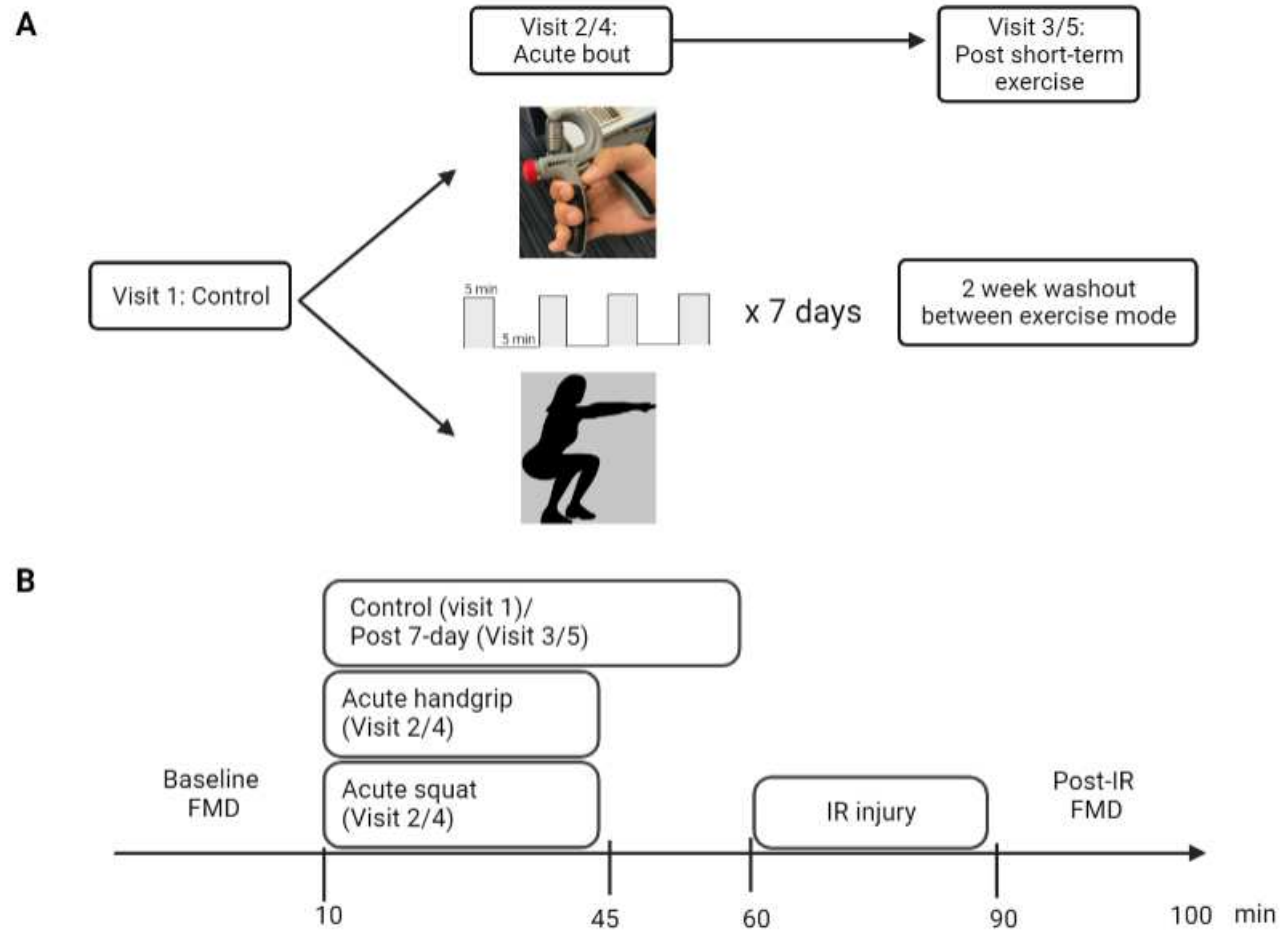


Figure 1. Schematic of A) study design and B) timeline for experimental protocol. *FMD*, *flow-mediated dilation*, *IR*, *ischaemia-reperfusion injury*

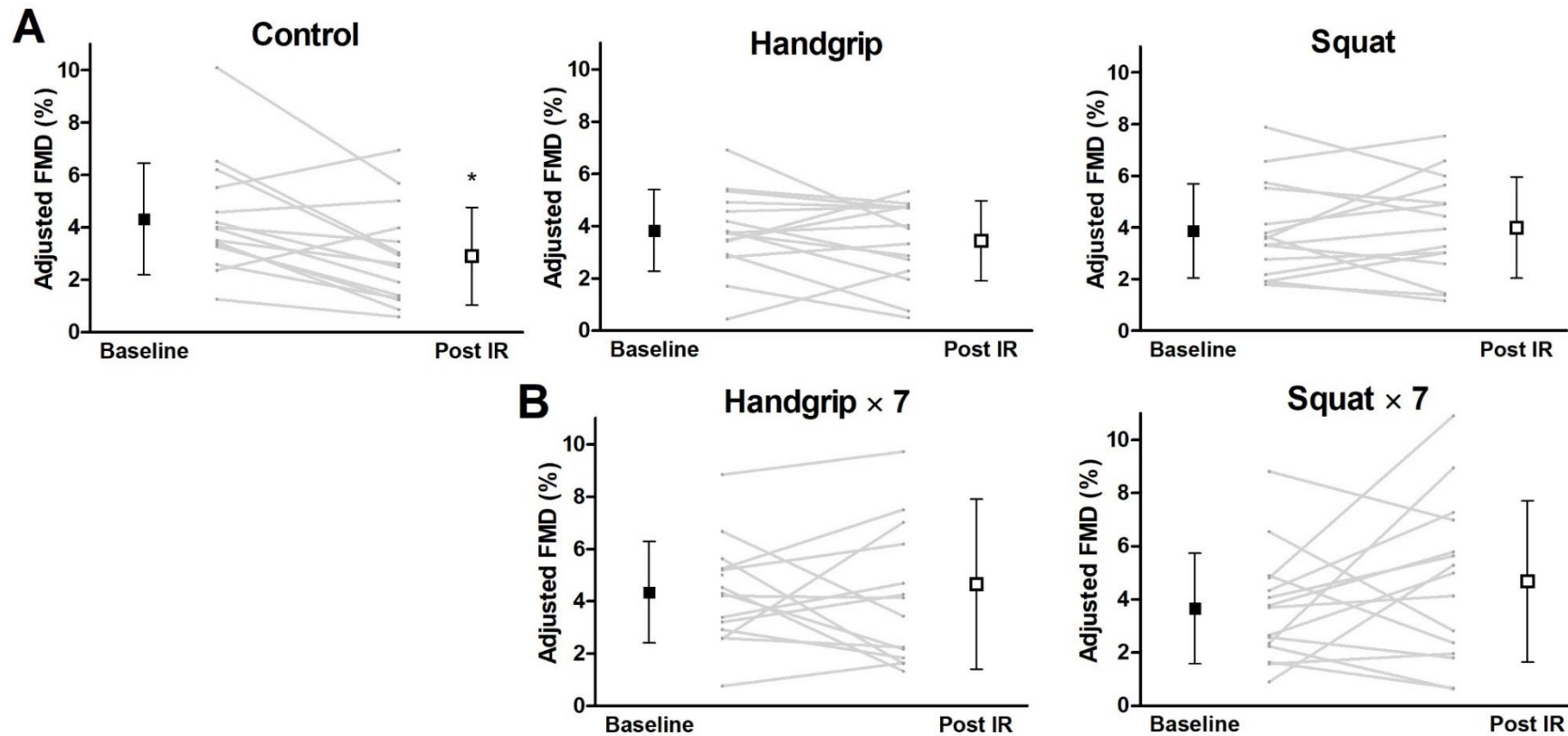


Figure 2. Comparison of **A)** control, a single bout of dynamic handgrip exercise, and squat exercise on flow-mediated dilation (FMD) at baseline and after ischaemia-reperfusion (IR) injury (Post IR), and comparison of a single session to **B)** 1-week of daily handgrip and squat exercise on FMD at 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/m²	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		<i>General Linear Model, P values</i>		
	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 (10³)	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

<i>Handgrip</i>	Single Bout		1-week		3-way general linear model, P values			
	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

these responses. Values are means ± SD. FMD, flow mediated

dilation, AUC, area under curve.

Time to Peak (sec)	62±24	51±15	55±18	43±16	0.55	0.85	0.02	0.06
Shear AUC (10³)	14.2±7.1	9.1±5.5	13.4±7.9	9.4±3.9	0.85	0.62	0.19	<0.01
<i>Squat</i>								
	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 (10³)	13.6±7.2	11.0±5.0	13.0±6.7	11.1±7.1				