



LJMU Research Online

Atkinson, CL, Carter, HH, Dawson, EA, Naylor, LH, Thijssen, DHJ and Green, DJ

Impact of handgrip exercise intensity on brachial artery flow-mediated dilation.

<http://researchonline.ljmu.ac.uk/id/eprint/851/>

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Atkinson, CL, Carter, HH, Dawson, EA, Naylor, LH, Thijssen, DHJ and Green, DJ (2015) Impact of handgrip exercise intensity on brachial artery flow-mediated dilation. European Journal of Applied Physiology. ISSN 1439-6327

LJMU has developed [LJMU Research Online](#) for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

<http://researchonline.ljmu.ac.uk/>

IMPACT OF HANDGRIP EXERCISE INTENSITY ON BRACHIAL ARTERY FLOW-MEDIATED DILATION

Atkinson, Ceri L.¹

Carter, Howard H.¹

Dawson, Ellen A.²

Naylor, Louise H.¹

Thijssen, Dick H.J.^{2,3}

Green, Daniel J.^{1,2}

¹School of Sports Science, Exercise and Health

University of Western Australia, Crawley, Western Australia

²Research Institute for Sport and Exercise Science

Liverpool John Moores University, Liverpool, United Kingdom

³Department of Physiology,

Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands

Running Head:

Exercise intensity and endothelial function

Author for correspondence:

Ceri Atkinson

School of Sport Science, Exercise and Health,

The University of Western Australia, Crawley, Western Australia, 6009

Phone: +61 (8) 6488 2378, Fax: +61 (8) 6488 1039

Email: ceri.atkinson@research.uwa.edu.au

1 Abstract

2 **Purpose.** Previous studies that have examined the impact of exercise intensity on conduit
3 artery endothelial function have involved large muscle group exercise which induces local
4 and systemic effects. The aim of this study was to examine flow mediated dilation (FMD)
5 before and after incremental intensities of handgrip exercise (HE), to assess the role of local
6 factors such as blood flow and shear rate on post-exercise brachial artery function. **Methods.**
7 Eleven healthy men attended the laboratory on 3 occasions. Subjects undertook 30 minutes of
8 handgrip exercise at 3 intensities (5, 10 or 15% MVC). Brachial artery FMD, shear and blood
9 flow patterns were examined before, immediately after, and 60 minutes post exercise.
10 **Results.** Handgrip exercise increased mean and antegrade shear rate (SR) and blood flow
11 (BF), and reduced retrograde SR and BF (all $P<0.01$). Exercise intensity was associated with
12 a dose-dependent increase in both mean and antegrade BF and SR (interaction, $P<0.01$).
13 Post-hoc tests revealed that, whilst handgrip exercise did not immediately induce post-
14 exercise changes, FMD was significantly higher 60 minutes post-exercise following the
15 highest exercise intensity (5.9 ± 2.8 to $10.4\pm 5.8\%$, $P=0.01$). **Conclusions.** HE leads to
16 intensity-and time-dependent changes in conduit artery function, possibly mediated by local
17 increases in shear, with improvement in function evident at 1 hour post-exercise when
18 performed at a higher intensity.

19

20 Introduction

21 It is well established that exercise training reduces cardiovascular risk (Green et al. 2008), in
22 part due to the direct effects of exercise on the function and health of the vessel wall (Green
23 2009; Joyner and Green 2009; Laughlin and McAllister 1992). Episodic exercise-induced
24 increases in blood flow, shear stress and perhaps transmural pressure are now recognized as
25 important physiological stimuli for training-based improvements in vascular health (Laughlin
26 et al. 2008; Tinken et al. 2009). Despite the chronic effect of exercise training on the vascular
27 endothelium being well studied, the acute impacts of distinct forms and intensities of exercise
28 are less well described.

29

30 Vascular function is commonly assessed using the flow-mediated dilation (FMD) technique,
31 a partially nitric oxide- and endothelium-dependent (Green et al. 2014) conduit artery
32 response to a brief ischemic stimulus (Corretti et al. 2002; Thijssen et al. 2011) which
33 predicts cardiovascular events (Gocke et al. 2003; Takase et al. 1998). Studies of the FMD
34 response to acute exercise have reported increases (Johnson et al. 2012; Tinken et al. 2009;
35 Tyldum et al. 2009; Zhu et al. 2010), decreases (Dawson et al. 2008; Goel et al. 2007; Jones
36 et al. 2010; Llewellyn et al. 2012; Rognmo et al. 2008), or no change (Dawson et al. 2008;
37 Harvey et al. 2005; Jones et al. 2010). These inconsistencies have been linked to
38 methodological differences between studies, the timing of post-exercise FMD measures and
39 the possibility of intensity and modality-specific exercise effects (Dawson et al. 2013). A
40 recent study conducted by Birk and colleagues (Birk et al. 2013) addressed some of these
41 issues by assessing FMD at multiple time-points following 30 minutes of lower limb cycle
42 exercise of differing intensities. A 'biphasic' response pattern, characterized by an initial
43 post-exercise FMD reduction, followed by a normalization 60 minutes post-exercise, was
44 observed. Furthermore, the reduced FMD following cycle exercise was intensity dependent,

45 with larger reductions observed following higher exercise intensity (Birk et al. 2013). These
46 changes in FMD may be related to the pattern of blood flow and shear stress through the
47 brachial artery during cycling, but endocrine and reflex effects cannot be excluded, given the
48 systemic nature of exercise stimulus.

49

50 The primary aim of the present study was to describe the time-dependent effects of handgrip
51 exercise, performed at different exercise intensities, on FMD immediately post-exercise and
52 60-minutes following exercise cessation. Our rationale for adopting handgrip exercise, which
53 utilizes a small muscle mass, was that it may provide insight into whether localized
54 mechanisms (particularly those related to changes in blood flow and shear stress) contribute
55 to the acute effects of exercise on arterial function. We hypothesized that an intensity-
56 dependent decrease in FMD would occur immediately after exercise, with higher intensities
57 of handgrip exercise leading to a larger post-exercise reduction in FMD, followed by a return
58 of function to baseline levels after 60-minutes of recovery.

59

60 **Keywords:** exercise intensity; endothelial function; shear stress; cardiovascular risk

61

62

63 **Abbreviations:**

64 ANOVA: analysis of variance

65 BF: blood flow

66 DBP: diastolic blood pressure

67 FMD: flow mediated dilation

68 HE: handgrip exercise

69 LMM: linear mixed model

70 LSD: least significant difference

71 MAP: mean arterial pressure

72 MVC: maximal voluntary contractile

73 ROS: reactive oxygen species

74 SBP: systolic blood pressure

75 SR: shear rate

76

77 Methods**78 Participants**

79 Eleven healthy male subjects (age: 27 ± 3 years, height: 1.78 ± 0.08 m weight: 75.2 ± 11.5 kg)
80 volunteered for the study. Women were excluded due to the cyclical effects of estrogen on
81 vascular function (Williams et al. 2001). Based on detailed medical questionnaires, we
82 excluded subjects with pre-existing medical conditions (including cardiovascular diseases)
83 and cardiovascular risk factors such as hypertension, hypercholesterolemia and type 1 or 2
84 diabetes, as well as those taking any medications. The study was approved by the University
85 of Western Australia's institutional ethics committee and adhered to the Declaration of
86 Helsinki. Informed consent was obtained from all participants before undertaking
87 experimental procedures.

88

89 Experimental Design

90 Participants attended all testing sessions in a fasted state (>6 hrs), having avoided strenuous
91 exercise, alcohol and caffeine for 18 hours prior to arrival (Thijssen et al. 2011). Participants
92 undertook 3 separate experimental sessions over two weeks, with a minimum of 48 hours
93 between sessions. Upon arrival at the laboratory, participants were required to complete an
94 assessment of maximal voluntary contractile (MVC) handgrip strength. This involved three
95 maximal contractions, separated by 5 minute rest intervals, using a grip strength
96 dynamometer. An average of the three contractions was used to calculate MVC which was
97 used in subsequent sessions to set exercise intensities. After a 20 minute supine rest period on
98 a bed, subjects performed 30 minutes of unilateral handgrip exercise (HE) at either 5, 10 or
99 15% of maximal voluntary handgrip contraction strength (MVC) at a rate of 30 isotonic
100 contractions a minute, based on similar protocols adopted by our research team (Tinken et al.
101 2009). The order of testing was randomised between subjects. The arm selected to undertake

102 the 30 minute exercise stimulus (preferred vs non-preferred) was also randomised between
103 subjects, although we maintained the same arm within subjects across all three sessions.
104 Brachial arterial endothelial function was assessed prior to, immediately after, and 60-
105 minutes following cessation of handgrip exercise using the flow mediated dilation (FMD)
106 technique (Thijssen et al. 2011). We also continuously recorded brachial artery diameter,
107 blood flow and shear rate throughout each HE session. Finally, blood pressure was
108 continuously monitored throughout the exercise duration (Finapres Medical Systems,
109 Netherlands),

110

111 **Experimental Measurements**

112 *Assessment of brachial artery endothelial function*

113 After a 20-minute rest period, brachial artery endothelial function was assessed using the
114 flow-mediated dilation (FMD) technique in accordance with recently published guidelines
115 (Thijssen et al. 2011). Briefly, the arms were extended to an ~80° angle from the torso. A
116 rapid inflation/deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was placed around
117 the forearm, distal to the olecranon process. Using a 10-MHz multi-frequency linear array
118 probe, attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA),
119 an optimal B-mode image of the brachial artery was acquired. Settings were optimized,
120 recorded and used for subsequent repeat sessions. Following image acquisition, one minute of
121 baseline imaging was performed, after which the forearm cuff was inflated to 220mmHg for
122 5 minutes. Thirty seconds prior to cuff deflation, recordings of diameter and velocity resumed
123 and were continued for 3 minutes following cuff deflation, in accordance with previous
124 studies (Woodman et al. 2001). This procedure was repeated immediately following cessation
125 of the handgrip exercise, and again 60-minutes thereafter.

126

127 *Brachial artery diameter and blood flow analysis*

128 Brachial artery diameter and velocity were analysed using custom-designed edge-detection
129 and wall-tracking software, which is largely independent of investigator bias (Woodman et al.
130 2001). Specific details of analysis techniques are described elsewhere (Thijssen et al. 2009a).
131 Continuous (30Hz) diameter and velocity data were used to calculate blood flow (the product
132 of lumen cross-sectional area and Doppler velocity). Shear rate was also calculated as 4 times
133 mean blood velocity/vessel diameter. Reproducibility of diameter measurements using this
134 semi-automated software is significantly better than manual methods, reduces observer error
135 significantly, and possesses an intra-observer coefficient of variation of 6.7% (Woodman et
136 al. 2001). FMD was calculated in absolute (mm) and relative (%) terms as the increase from
137 the resting baseline diameter, as described in detail in previously (Thijssen et al. 2011). The
138 shear rate stimulus to FMD, SRAUC, was calculated in each individual by plotting SR
139 collected at 30Hz continuously from the time of cuff deflation to the time of peak diameter
140 attainment, and then summing the areas of successive post-occlusion trapezoids (each with a
141 base of 3-sec). This approach is consistent with literature best practice and the body of
142 literature on this topic, largely pioneered by Tschakovsky and Pyke (Thijssen et al. 2011).
143 We also analysed brachial artery diameter and velocity data across 30-second epochs, at 5
144 minute intervals, throughout the 30 minute unilateral handgrip exercise bout. In the present
145 study, all files were coded such that the individual undertaking the automated analysis was
146 nonetheless blinded to each subject's identity and the exercise intensity at which the file in
147 question was collected.

148

149 **Statistics**

150 Statistical analysis was performed using SPSS 21.0 (SPSS, Chicago, IL) software. A two-way
151 repeated measures ANOVA was used to compare changes in FMD%, SR, blood pressure

152 across ‘time’ (pre, post & 60mins post) and whether these changes occurred across differing
153 ‘intensity’ bouts (5, 10 & 15% MVC). Post-hoc analysis was performed using the least
154 significant difference (LSD) method. All data are reported as mean (\pm SD) unless stated
155 otherwise and statistical significance was assumed at $P < 0.05$. Additional statistics were
156 adopted in order to account for influences known to affect FMD responses, principally
157 arterial diameter and SR_{AUC} changes (Atkinson et al. 2013). To do so, we analysed the effects
158 of intensity and time, as well as the interaction effect for FMD, on the change in
159 logarithmically transformed diameter using a Linear Mixed Model (LMM). This model
160 incorporated baseline arterial diameter and shear rate area under the curve as covariates,
161 where these baseline arterial diameter data were specific to each FMD test. This sample size
162 was similar to those reported in previous studies on the effects of exercise (Johnson et al.
163 2012; Padilla et al. 2006; Padilla et al. 2011), and we estimated that a 2 % change in FMD
164 would be detected with 10 participants assuming that the standard deviation of this change is
165 2 % with statistical power of 80 % (Birk et al. 2013).

166

167

168 **Results**

169 There were no differences in resting (pre-exercise) brachial diameter, FMD%, FMDmm,
170 shear rate or SR_{AUC} across the three testing days ($P > 0.05$) (Table 1). Participants completed
171 30 minutes of isotonic HG exercise (30 contractions per minute) at each exercise intensity.
172 Mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP)
173 and heart rate all increased in an exercise intensity “dose-dependent” manner (interaction-
174 effect: all $P < 0.05$), with the largest elevations seen during 30 minutes of HG exercise at 15%
175 MVC (Table 2).

176

177 *Brachial artery blood flow and diameter during handgrip exercise*

178 Unilateral handgrip exercise resulted in an immediate increase in both mean and antegrade
179 blood flow and shear rate, which remained elevated throughout the 30-minute exercise bout
180 (Figure 1a). Higher exercise intensity was associated with a larger increase in both mean and
181 antegrade blood flow and shear rate across the exercise duration (interaction-effect: $P<0.01$).
182 Post-hoc t-tests using an LSD approach revealed differences at all-time points of exercise for
183 antegrade SR at 15% MVC compared to both 10% (all $P<0.01$) and 5% (all $P<0.01$).
184 Retrograde shear rate during each 30 minute bout of handgrip exercise was reduced (time
185 effect: $P<0.01$), although this reduction did not significantly differ between intensities
186 (interaction-effect: $P=0.07$, Figure 1b). Exercise at a low intensity (5% MVC) did not
187 significantly alter brachial artery diameter following exercise ($P=0.64$, Table 3), whereas
188 10% MVC evoked a decrease in diameter 60 minutes post-exercise ($P=0.04$). At 15% MVC,
189 diameter increased immediately following exercise ($P=0.01$), but had returned to baseline
190 values at 60 minutes ($P=0.16$).

191

192 *Brachial artery flow-mediated dilation*

193 Two-way ANOVA revealed a significant time*intensity interaction-effect ($P=0.01$) for
194 FMD% (Table 3). Post-hoc analysis revealed a significant increase in FMD 60-minutes after
195 the 15% MVC handgrip exercise bout ($P<0.01$), whilst no change in FMD was observed
196 during at other time-points or exercise intensities. Similar changes were observed for absolute
197 FMD, with a significant interaction-effect ($P<0.01$) and a significant increase in FMD 60-
198 minutes after 15% MVC handgrip exercise session (Table 3). Given that FMD responses are
199 inversely proportional to baseline arterial diameter (Thijssen et al. 2008), the interaction

200 effect observed for FMD% may have been influenced by acute exercise impacts on baseline
201 arterial diameter (Birk et al. 2013; Dawson et al. 2013; Padilla et al. 2007). In order to
202 account for potential impacts of baseline diameter, we undertook the LMM for FMD with
203 baseline diameter as a covariate. This analysis reinforced our initial findings and revealed a
204 significant interaction effect between time (pre, post, 60-post) and exercise intensity (5, 10
205 & 15% MVC) ($P=0.03$).

206

207 The tabulated FMD% data are summarized in Figure 2, in which change in FMD (expressed
208 as the immediate post-exercise FMD% minus baseline pre-exercise FMD%) was not
209 significantly different between exercise intensities ($P=0.67$, Figure 2A). However, change in
210 FMD, 60 minutes post-exercise vs baseline, showed a dose-response relationship with
211 exercise intensity ($P<0.01$), with a 0.7% decreased FMD at the lowest exercise intensity, and
212 a 4.5% increased FMD following HG exercise at the highest intensity (t-test: $P=0.01$, Figure
213 2B).

214

215 The shear rate stimulus to FMD (FMDSR_{AUC}) was significantly higher immediately post-
216 exercise under each of the three conditions (Table 3 $P=<0.01$), however, it had returned to
217 pre-exercise levels by the time the 60-minutes post-exercise FMD test was performed (Table
218 3). There were no differences in FMD SR_{AUC} between the exercise conditions ($P=0.11$).
219 Statistically accounting for changes in SR_{AUC} in our LMM did not alter the significant
220 interaction effect for FMD 1 hour following 3 different intensities of HG exercise ($P<0.01$).

221

222

223 Discussion

224 The purpose of the present study was to determine the effect of different intensities of
225 handgrip exercise on post-exercise brachial artery endothelium-dependent conduit artery
226 function. Previous research has produced conflicting findings pertaining to conduit artery
227 functional responses to acute exercise, with increases (Johnson et al. 2012; Tinken et al.
228 2009; Tyldum et al. 2009; Zhu et al. 2010), decreases (Dawson et al. 2008; Goel et al. 2007;
229 Jones et al. 2010; Llewellyn et al. 2012; Rognum et al. 2008) and no change (Dawson et al.
230 2008; Harvey et al. 2005; Jones et al. 2010) reported. As recently reviewed, these disparate
231 findings may be related to differences in experimental methodology (Dawson et al. 2013),
232 idiosyncratic effects of distinct exercise modes and intensities (Birk et al. 2013; Dawson et al.
233 2013; Padilla et al. 2007) and/or the timing of post-exercise measurements. Another issue is
234 that previous experiments have involved large muscle group “systemic” exercise bouts,
235 which may induce complex localized, reflex and humoral responses. We adopted handgrip
236 exercise in the present study in an attempt to understand the localized impacts of distinct
237 exercise intensities on post-exercise FMD measures of artery function.

238

239 Our principal finding was that intensity of handgrip exercise modified the post-exercise FMD
240 response, with the highest exercise intensity (15% MVC) demonstrating a large and
241 significant elevation in FMD, one hour post-exercise. The intensity-dependent effect on post-
242 exercise FMD we observed may be related to the impact of exercise-induced shear stress.
243 Shear stress patterns are known to directly induce changes in the FMD/endothelial response
244 to acute exercise (Green et al. 2005; Green et al. 2002; Laughlin et al. 2008; Thijssen et al.
245 2009c; Tinken et al. 2009). Elevated antegrade shear, in the absence of changes in retrograde
246 shear, has been linked to improvement in endothelium-dependent vasodilation (Tinken et al.
247 2009), whilst increases in oscillatory shear and/or retrograde flow may be associated with

248 increased endothelin-1 expression (Chappell et al. 1998; Himburg et al. 2007), the up-
249 regulation of reactive oxygen species (Hwang et al. 2003) and dose-dependent attenuation of
250 endothelial function in humans (Schreuder et al. 2014; Thijssen et al. 2009c). In contrast to
251 some forms of lower limb exercise, handgrip exercise is not associated with increases in
252 retrograde flow and shear during exercise (see Figure 3), due largely to the decrease in distal
253 vascular resistance (Green et al. 2005; Thijssen et al. 2009b). In contrast, there are large
254 increases in retrograde shear during the early phases of leg cycling, which induces an
255 oscillatory pattern of flow and shear in the brachial artery (Green et al. 2005; Green et al.
256 2002). When comparing shear rate patterns in the current study to our previous studies (Birk
257 et al. 2013), clear differences can be observed in both the pattern and magnitude of shear
258 responses to cycle versus handgrip exercise (Figure 3). It is plausible that the increase in
259 antegrade shear observed, particularly at the highest handgrip exercise intensity in the present
260 study, induces a post-exercise improvement in FMD. This would be consistent with what is
261 known regarding the impacts of acute exercise stress on eNOS bioavailability and consequent
262 chronic adaptation and upregulation (Hambrecht et al. 2003). Our data are also suggestive of
263 a possible threshold effect of antegrade shear on artery function. We observed significantly
264 higher antegrade shear at 15% compared to the other exercise intensities and the 15%
265 condition was the only one associated with an elevation in FMD post exercise. Further
266 studies will be required to address the idea of an ‘antegrade shear threshold’ and to examine
267 the impact of shear rate manipulation during handgrip exercise on arterial adaptation in
268 function and structure.

269

270 In the present study, we observed no reduction in FMD in the immediate post-exercise period
271 in response to any intensity of HE. This contrasts with some previous studies examining the
272 acute impacts of larger muscle group exercise on arterial function. Studies that have focused

273 predominantly on leg exercise with multiple post-exercise FMD measures (Birk et al. 2013;
274 Johnson et al. 2012), have typically observed a decrement in function immediately following
275 exercise which appears to be larger at higher intensities. Both localized handgrip exercise *and*
276 large muscle group exercise lead to a (dose-dependent) increase in brachial artery blood flow.
277 Therefore, the distinct immediate post-exercise changes in FMD seen in studies of large
278 muscle group exercise may relate to the impact of such exercise on retrograde shear, the
279 production of reactive oxygen species (ROS) (Goto et al. 2003) or increases in adrenergic
280 tone. However, as we did not assess oxidative stress or adrenergic tone in the present study,
281 we cannot rule out impacts of handgrip exercise on these parameters. Our findings are
282 therefore limited to the observation that handgrip and cycling exercise produce distinct
283 immediate effects on artery function in humans compared to larger muscle group exercise
284 and it seems reasonable to assume that larger muscle group exercise would have greater
285 impact on humoral or neural responses.

286

287 In another previous study performed by our group, we observed increases in FMD after 30
288 mins of handgrip exercise, in keeping with the current results. However, in that experiment,
289 the increase in FMD was observed immediately, rather than one hour after the cessation of
290 the exercise bout. The magnitude of increase in SR during handgrip exercise in the Tinken *et*
291 *al.* study was similar to that observed in response to the lowest exercise intensity (5% MVC)
292 in the current experiment, but FMD% increased significantly in Tinken *et al.*, whereas
293 changes in the current study, though directionally consistent, were more modest. One
294 possible explanation for this disparity relates to a minor difference in the exercise modality:
295 although both experiments involved the same contractile frequency (30 per min), the study of
296 Tinken *et al.* utilised isometric hand-gripping against a fixed handpiece and resistance,
297 whereas in the current study subjects performed isotonic exercise using a custom built device

298 that allowed movement in the handpiece during contraction. These findings therefore raise
299 the intriguing possibility that differences may exist between modalities of exercise, even
300 when identical muscle groups are involved. This finding adds to previous work which
301 suggested that small differences in the rate of handgrip contraction can alter the FMD
302 response (Gonzales et al. 2011). Future studies will be necessary to specifically address this
303 question.

304

305 We utilized handgrip exercise as an experimental tool to isolate the localized effects of
306 exercise and it was not our intention to study whether exercise training of the forearm might
307 translate into systemic health benefits. Nonetheless, our results warrant future studies that are
308 focused on the potential differences in factors that may contribute to the distinct changes in
309 FMD following different modalities of training. Another limitation of our study is that we
310 were unable to assess responses to an exogenous NO donor. Since it is possible that our
311 strenuous exercise in the current experiment may have induced an up-regulation of
312 endothelium-derived vasodilators *or* augmented smooth muscle cell sensitivity to NO, future
313 studies should address the impact of exercise intensity on responses to glyceryl trinitrate.

314

315 In summary, our findings contribute to the existing literature in that we have studied, for the
316 first time, the impact of distinct intensities of localized handgrip exercise on artery function.
317 Impacts of such exercise on arterial function are less likely to have resulted from systemic,
318 reflex or circulating factors than responses associated with lower limb large muscle group
319 exercise (Birk et al. 2013). Further studies for the acute and chronic effects of different forms
320 of exercise will be important to shed light on phenomena which relate initial physiological
321 challenge to chronic adaptation in humans.

322

323 **Acknowledgements**

324 We would like to thank the participants for their time in completing this research study.

325

326 **Funding**

327 Professor Green's research is supported by the National Health and Medical Research

328 Council (NHMRC); grant ID 1045204

329

330 **Ethical Approval**

331 All procedures performed in studies involving human participants were in accordance with

332 the ethical standards of the institutional and/or national research committee and with the 1964

333 Helsinki declaration and its later amendments or comparable ethical standards. Informed

334 consent was obtained from all individual participants included in the study.

335

336 **Disclosures**

337 The authors declare that they have no conflict of interest.

338

339 **References**

340

341 Atkinson G, Batterham AM, Thijssen DH, Green DJ (2013) A new approach to improve the
342 specificity of flow-mediated dilation for indicating endothelial function in
343 cardiovascular research *J Hypertens* 31:287-291

344 Birk GK, Dawson EA, Batterham AM, Atkinson G, Cable T, Thijssen DH, Green DJ (2013)
345 Effects of exercise intensity on flow mediated dilation in healthy humans *Int J Sports*
346 *Med* 34:409-414

347 Chappell DC, Varner SE, Nerem RM, Medford RM, Alexander RW (1998) Oscillatory shear
348 stress stimulates adhesion molecule expression in cultured human endothelium *Circ*
349 *Res* 82:532-539

350 Corretti MC et al. (2002) Guidelines for the ultrasound assessment of flow-mediated
351 vasodilation of the brachial artery *J Am Coll Cardiol* 39:257-265

352 Dawson EA, Green DJ, Cable NT, Thijssen DH (2013) Effects of acute exercise on flow
353 mediated dilatation (FMD) in healthy humans *J Appl Physiol* 115:1589-1598

354 Dawson EA et al. (2008) Changes in vascular and cardiac function after prolonged strenuous
355 exercise in humans *J Appl Physiol* 105:1562-1568

356 Gocke N, Keaney JF, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, Vita JA
357 (2003) Predictive value of non-invasively determined endothelial dysfunction for
358 long-term cardiovascular events in patients with peripheral vascular disease *J Am J*
359 *Cardiol* 41:1769-1775

360 Goel R et al. (2007) Exercise-induced hypertension, endothelial dysfunction, and coronary
361 artery disease in a marathon runner *Am J Cardiol* 99:743-744

362 Gonzales JU, Thompson BC, Thistlethwaite JR, Scheuermann BW (2011) Association
363 between exercise hemodynamics and changes in local vascular function following

- 364 acute exercise *Applied physiology, nutrition, and metabolism = Physiologie*
365 *appliquee, nutrition et metabolisme* 36:137-144 doi:10.1139/H10-097
- 366 Goto C et al. (2003) Effect of different intensities of exercise on endothelium-dependent
367 vasodilation in humans. Role of endothelium-dependent nitric oxide and oxidative
368 stress *Circulation* 108:530-535
- 369 Green DJ (2009) Exercise training as vascular medicine: direct impacts on the vasculature in
370 humans *Exerc Sport Sci Rev* 37:196-202
- 371 Green DJ, Bilsborough W, Naylor LH, Reed C, Wright J, O'Driscoll G, Walsh JH (2005)
372 Comparison of forearm blood flow responses to incremental handgrip and cycle
373 ergometer exercise: relative contribution of nitric oxide *J Physiol* 562:617-628
- 374 Green DJ, Cheetham C, Reed C, Dembo L, O'Driscoll G (2002) Assessment of brachial
375 artery blood flow across the cardiac cycle: retrograde flows during lower limb
376 exercise *J Appl Physiol* 93:361-368
- 377 Green DJ, Dawson EA, Groenewoud HM, Jones H, Thijssen DH (2014) Is flow-mediated
378 dilation nitric oxide mediated?: A meta-analysis *Hypertension* 63:376-382
- 379 Green DJ, O'Driscoll G, Joyner MJ, Cable NT (2008) Exercise and cardiovascular risk
380 reduction: time to update the rationale for exercise? *J Appl Physiol* 105:766-768
- 381 Hambrecht R et al. (2003) Regular physical activity improves endothelial function in patients
382 with coronary artery disease by increasing phosphorylation of endothelial nitric oxide
383 synthase *Circulation* 107:3152-3158
- 384 Harvey PJ, Picton PE, Su WS, Morris BL, Notarius CF, Floras JS (2005) Exercise as an
385 alternative to oral estrogen for amelioration of endothelial dysfunction in
386 postmenopausal women *Am Heart J* 149:291-297
- 387 Himburg HA, Dowd SE, Friedman MH (2007) Frequency-dependent response of the vascular
388 endothelium to pulsatile shear stress *Am J Physiol Heart Circ Physiol* 293:645-653

- 389 Hwang J et al. (2003) Pulsatile versus oscillatory shear stress regulates NADPH oxidase
390 subunit expression: implication for native LDL oxidation. *Circ Res* 93:1225–1232
- 391 Johnson BD, Padilla J, Wallace JP (2012) The exercise dose affects oxidative stress and
392 brachial artery flow-mediated dilation in trained men *Eur J Appl Physiol* 112:33-42
- 393 Jones H, Green DJ, George K, Atkinson G (2010) Intermittent exercise abolishes the diurnal
394 variation in endothelial-dependent flow-mediated dilation in humans *Am J Physiol*
395 *Regul Intergr Comp Physiol* 298:427-432
- 396 Joyner MJ, Green DJ (2009) Exercise protects the cardiovascular system: effects beyond
397 traditional risk factors *J Physiol* 587:5551-5558
- 398 Laughlin MH, McAllister RM (1992) Exercise training-induced coronary vascular adaptation
399 *J Appl Physiol* 73:2209-2225
- 400 Laughlin MH, Newcomer SC, Bender SB (2008) Importance of hemodynamic forces as
401 signals for exercise-induced changes in endothelial cell phenotype *J Appl Physiol*
402 104:588-600
- 403 Llewellyn TL, Chaffin ME, Berg KE, Meendering JR (2012) The relationship between shear
404 rate and flow-mediated dilation is altered by acute exercise *Acta Physiol* 205:394-402
- 405 Padilla J, Harris R, Fly A, Rink L, Wallace J (2006) The effect of acute exercise on
406 endothelial function following a high-fat meal *Eur J Appl Physiol* 98:256-262
- 407 Padilla J, Harris RA, Wallace JP (2007) Can the measurement of brachial artery flow-
408 mediated dilation be applied to the acute exercise model? *Cardiovascular Ultrasound*
409 5:45
- 410 Padilla J, Simmons GH, Vianna LC, Davis MJ, Laughlin MH, Fadel PJ (2011) Brachial
411 artery vasodilatation during prolonged lower limb exercise: role of shear rate *Exp*
412 *Physiol* 96:1019-1027

- 413 Rognmo O et al. (2008) Endothelial function in highly endurance-trained men: effects of
414 acute exercise *J Strength Cond Res* 22:535-542
- 415 Schreuder TH, Green DJ, Hopman MT, Thijssen DH (2014) Acute impact of retrograde shear
416 rate on brachial and superficial femoral artery flow-mediated dilation in humans
417 *Physiological reports* 2:e00193
- 418 Takase B et al. (1998) Endothelium-dependent flow-mediated vasodilation in coronary and
419 brachial arteries in suspected coronary artery disease. *Am J Cardiol* 82:1535-1539
- 420 Thijssen DHJ et al. (2011) Assessment of flow mediated dilation (FMD) in humans: a
421 methodological and physiological guideline *Am J Physiol Heart Circ Physiol* 300:2-
422 12
- 423 Thijssen DHJ et al. (2009a) Does arterial shear explain the magnitude of flow-mediated
424 dilation?: a comparison between young and older humans. *Am J Physiol Heart Circ*
425 *Physiol* 296:57-64
- 426 Thijssen DHJ, Dawson EA, Black MA, Hopman MTE, Cable NT, Green DJ (2009b) Brachial
427 artery blood flow responses to different modalities of lower limb exercise. *Med Sci*
428 *Sports Exerc* 41:1072-1079
- 429 Thijssen DHJ, Dawson EA, Tinken TM, Cable NT, Green DJ (2009c) Retrograde flow and
430 shear rate acutely impair endothelial function in humans *Hypertension* 53:986 - 992
- 431 Thijssen DHJ et al. (2008) The impact of baseline diameter on flow mediated dilation (FMD)
432 differs in young and older humans *Am J Physiol Heart Circ Physiol* 295:1594 - 1598
- 433 Tinken TM et al. (2009) Impact of shear rate modulation on vascular function in humans
434 *Hypertension* 54:278-285
- 435 Tyldum GA, Schjerve IE, Tjonna AE, Kirkeby-Garstad I, Stolen TO, Richardson RS, Wisloff
436 U (2009) Endothelial dysfunction induced by post-prandial lipemia: complete

437 protection afforded by high-intensity aerobic interval exercise J Am Coll Cardiol
438 53:200-206

439 Williams MRI, Westerman RA, Kingwell BA, Paige J, Blombery PA, Sudhir K, Komesaroff
440 PA (2001) Variations in endothelial function and arterial compliance during the
441 menstrual cycle J Clin Endocrinol Metab 86:5389-5395

442 Woodman RJ et al. (2001) Improved analysis of brachial artery ultrasound using a novel
443 edge-detection software system J Appl Physiol 91:929-937

444 Zhu W, Zeng J, Yin J, Zhang F, Wu H, Yan S, Wang S (2010) Both flow-mediated
445 vasodilation procedures and acute exercise improve endothelial function in obese
446 young men Eur J Appl Physiol 108:727-732

447

448

449 **Table 1.** Comparisons between baseline FMD tests and pre-exercise resting shear rate and
 450 blood flow on each of the three testing days, with significance as $P \leq 0.05$.

	5% MVC Handgrip	10% MVC Handgrip	15% MVC Handgrip	P Value
<i>Baseline FMD Test</i>				
Diameter (mm)	3.82±0.42	3.91±0.39	3.89±0.58	0.39
FMD (%)	6.54±2.98	6.41±1.64	5.93±2.77	0.73
FMD (mm)	0.02±0.01	0.02±0.01	0.02±0.01	0.69
Baseline mean SR_{AUC} ($10^3 \cdot s^{-1}$)	17.9±9.0	16.6±7.7	16.7±8.2	0.82

451 Values are mean \pm SD. MVC: Maximal Voluntary contraction, SR_{AUC} : shear rate area under
 452 curve. P value reflects one-way ANOVA

453

Table 2. Hemodynamic parameters (5 min averages) at baseline and 30 minutes of unilateral handgrip exercise at distinct intensities (5, 10 and 15% MVC) with corresponding one-way ANOVA effects for time and two-way ANOVA (Time 2 levels, Intensity 3 levels).

	Handgrip Exercise Time (minutes)		ANOVA		
	0	30	Time	Intensity	Time*Intensity
MAP					
5%	93±9	99±14*			
10%	90±7	100±10*	<0.01	0.02	<0.01
15%	90±5	108±10*			
DBP					
5%	72±15	74±12			
10%	71±5	78±8*	<0.01	0.05	<0.01
15%	70±5	84±8*			
SBP					
5%	130±16	138±17*			
10%	126±9	137±10*	<0.01	0.09	0.02
15%	126±9	144±7*			
PP					
5%	58±12	64±9			
10%	55±6	59±5*	<0.01	0.03	0.97
15%	56±9	61±6*			
HR					
5%	58±6	59±6			
10%	58±5	63±8*	<0.01	<0.01	<0.01
15%	59±6	66±10*			

Data are presented as mean ± SD. *indicates statistically significant from baseline (0 values) by *t*-test at $P \leq 0.05$

Table 3. Diameter, SR_{AUC} and FMD responses pre, post and 60-minutes after cessation of 30 minutes of unilateral handgrip exercise at distinct intensities (5, 10 and 15% MVC).

	5% MVC Handgrip			10% MVC Handgrip			15% MVC Handgrip			2-way ANOVA		
	Pre	Post	Post 60mins	Pre	Post	Post 60mins	Pre	Post	Post 60mins	Intensity	Time	Intensity *Time
Diameter (mm)	3.8±0.4	3.8±0.3	3.9±0.5	3.9±0.4	4.0±0.5	3.8±0.4*	3.9±0.6	4.1±0.5*	3.8±0.4	0.50	<0.01	0.01
FMD (mm)	0.02±0.01	0.03±0.01*	0.02±0.01	0.02±0.01	0.03±0.01	0.03±0.01	0.02±0.01	0.02±0.02	0.04±0.02*	0.64	0.16	0.01
FMD SR _{AUC} (10 ³ ·s ⁻¹)	17.9±9.0	24.2±9.7*	17.2±7.7	16.6±7.7	30.0±14.1*	20.0±11.5	16.7±8.2	32.4±18.5*	17.3±9.0	0.25	<0.01	0.11
FMD (%)	6.5±3.0	7.9±3.2	5.9±3.1	6.4±1.6	6.6±3.1	7.0±4.4	5.9±2.8	6.2±5.5	10.4±5.8*	0.73	0.20	0.01
FMD (%) ADJUSTED	2.8±1.3	3.4±1.3	2.5±1.3	2.7±0.7	2.8±1.3	3.0±1.8	2.5±1.2	2.6±2.3	4.4±2.4*	0.48	0.04	<0.01

Data are presented as mean ± SD. *indicates statistically significant from baseline values at $P \leq 0.05$ by *t*-test. FMD (%) ADJUSTED values indicated FMD when statistically accounting for changes in baseline diameter and SR_{AUC} using a Linear Mixed Model (LMM).

Figure Legends

Fig. 1 a. Brachial artery antegrade shear rate before and during 30 minutes of handgrip exercise at 5% (black bars), 10% (light grey bars) and 15% (dark grey bars) MVC. **b.** Brachial artery retrograde shear rate before and during 30 minutes of handgrip exercise at 5% (black bars), 10% (light grey bars) and 15% (dark grey bars) MVC. Error bars represent SD.

Fig. 2 a. Change in FMD expressed as the immediate post-exercise FMD% minus baseline FMD% at 5% (black bars), 10% (light grey bars) and 15% (dark grey bars) MVC. **b.** Change in FMD expressed as the 60 minutes post-exercise FMD% minus baseline FMD% at 5% (black bars), 10% (light grey bars) and 15% (dark grey bars) MVC. Error bars represent SD. Significance value $P < 0.05$. * indicates statistical significance between highest (15% MVC) and lowest (5% MVC) intensity. † indicates statistical significance between moderate (10% MVC) and highest (15% MVC) intensity

Fig. 3 Brachial artery mean (average of the last 15mins) antegrade shear rate (black bars) during **a.** 30 minutes of handgrip exercise at 5, 10 and 15% MVC, compared to **b.** 30 minutes of cycle exercise at 50, 70 and 85% VO_2 max. Brachial artery mean (average of the last 15mins) retrograde shear (grey bars) during **c.** 30 minutes of handgrip exercise at 5, 10 and 15% MVC, compared to **d.** 30 minutes of cycle exercise at 50, 70 and 85% VO_2 max. Error bars represent SD.