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]

[1]*School of Sports Science, Exercise and Health*
University of Western Australia, Crawley, Western Australia

[2]*Research Institute for Sport and Exercise Science*
Liverpool John Moores University, Liverpool, United Kingdom

[3]*Department 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

Abstract

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

Introduction

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

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

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

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

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

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

Methods

Participants

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

Experimental Design

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

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

Experimental Measurements

Assessment of brachial artery endothelial function

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

Brachial artery diameter and blood flow analysis

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

Statistics

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

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

Results

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

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

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

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

Discussion

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

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

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

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

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

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

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

Acknowledgements

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

Funding

Professor Green's research is supported by the National Health and Medical Research Council (NHMRC): grant ID 1045204

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Disclosures

The authors declare that they have no conflict of interest.

References

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

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

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

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

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

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

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

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

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

acute exercise Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme 36:137-144 doi:10.1139/H10-097

Goto C et al. (2003) Effect of different intensities of exercise on endothelium-dependent vasodilation in humans. Role of endothelium-dependent nitric oxide and oxidative stress Circulation 108:530-535

Green DJ (2009) Exercise training as vascular medicine: direct impacts on the vasculature in humans Exerc Sport Sci Rev 37:196-202

Green DJ, Bilsborough W, Naylor LH, Reed C, Wright J, O'Driscoll G, Walsh JH (2005) Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide J Physiol 562:617-628

Green DJ, Cheetham C, Reed C, Dembo L, O'Driscoll G (2002) Assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during lower limb exercise J Appl Physiol 93:361-368

Green DJ, Dawson EA, Groenewoud HM, Jones H, Thijssen DH (2014) Is flow-mediated dilation nitric oxide mediated?: A meta-analysis Hypertension 63:376-382

Green DJ, O'Driscoll G, Joyner MJ, Cable NT (2008) Exercise and cardiovascular risk reduction: time to update the rationale for exercise? J Appl Physiol 105:766-768

Hambrecht R et al. (2003) Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase Circulation 107:3152-3158

Harvey PJ, Picton PE, Su WS, Morris BL, Notarius CF, Floras JS (2005) Exercise as an alternative to oral estrogen for amelioration of endothelial dysfunction in postmenopausal women Am Heart J 149:291-297

Himburg HA, Dowd SE, Friedman MH (2007) Frequency-dependent response of the vascular 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

Table 1. Comparisons between baseline FMD tests and pre-exercise resting shear rate and 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
Baseline FMD Test				
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**

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

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*	*Intenstiy*	*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} ($10^3 \cdot s^{-1}$)	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.