# Effects of Exercise Intensity on Flow Mediated Dilation in Healthy Humans

Authors

Affiliations

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## Abstract

Previous studies have demonstrated conflicting results on the effects of acute exercise on FMD. The aim of the study was to examine brachial artery FMD before and after 3 bouts of acute exercise performed at different intensities. 10 healthy males (mean $\pm$ SD age: 22 $\pm$ 1 years) completed 30 min of cycling at 50, 70 and 85% maximal heart rate (HR<sub>max</sub>). Brachial artery FMD and the shear rate area-under-the-curve (cuff deflation to peak dilation; SR<sub>AUC</sub>) were assessed pre- and immediately post-exercise using high-resolution echo-Doppler. A generalized estimating equation (GEE) analysis was used to estimate the effect magnitudes of exercise intensity and

time (pre/post) on FMD, whilst controlling for the influence of baseline diameter and SR<sub>AUC</sub>. Both baseline diameter and SR<sub>AUC</sub> were elevated by exercise. With covariate-control of these variables, the decrease in brachial artery FMD was negligible after exercise at 50% HR<sub>max</sub> ( $6.3 \pm 2.6$ vs.  $5.9 \pm 2.5\%$ ; 95%Cl for difference: -0.59-1.34%) with larger decreases in FMD after exercise at 70% ( $6.1 \pm 1.8$  vs.  $4.7 \pm 1.9\%$ ; 95%Cl for difference: 0.08 - 2.58%) and at 85% HR<sub>max</sub> ( $6.6 \pm 1.6$  vs.  $3.6 \pm 2.2\%$ ; 95%Cl: 0.41 - 5.42%). In conclusion, even after accounting for exercisemediated changes in shear and baseline diameter, our data indicate that a negative relationship exists between exercise intensity and FMD.

## Introduction

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Exercise training reduces primary [4] and secondary cardiovascular events [32], effects which cannot be fully explained by a reduction in traditional risk factors [19]. It has been suggested that exercise has a direct anti-atherogenic impact on the vasculature [17], which may be mediated through a shear-stress induced improvement in flow mediated dilation (FMD, a measure of endothelial-dependent dilation) [9]. Whilst the effects of exercise training on the vasculature are well documented, less is known about acute (short-term) changes in vascular function in response to exercise which may provide the foundation for prolonged adaptations to exercise training.

Studies that have examined the acute effect of exercise on FMD have reported conflicting results with increases [10, 12, 15, 37, 39, 42], decreases [6, 10, 15, 16, 27] or no change [6, 12, 16, 27, 40] in FMD following acute bouts of aerobic and resistance exercise. In our own, and others, experiments, there is a suggestion that the intensity

and/or load of exercise performed may modify the FMD response post exercise [6,15,37]. A proportion of the disparity in previous studies examining the acute effect of exercise on FMD may therefore relate to differences in exercise intensity.

FMD responses are inversely proportional to baseline arterial diameter [34]. Acute exercise impacts upon arterial diameter and may consequently impact upon the magnitude of FMD. particularly following strenuous exercise. For example, the decrease in FMD immediately postexercise in a recent study by Johnson et al. [15] may relate to the increase in baseline arterial diameter preceding the ischemic FMD stimulus. Nonetheless, the impact of different intensities of acute exercise on FMD and the influence of baseline diameter has not previously been addressed. The purpose of this study was to examine brachial artery FMD before and immediately following 30 min of acute cycling exercise at 3 different intensities (50, 70 and 85% maximal heart rate) whilst accounting for changes in shear rate and baseline diameter. We hypothesised that the magnitude of decrease in FMD would be greater with increasing exercise intensity, independent of changes in resting artery diameter.

## Methods

## **Subjects**

10 young male subjects (age: 22±1 years; height: 180±5cm; weight: 79.8±11.1 kg) were recruited. This sample size was similar to those recruited for previous studies on the effects of exercise intensity, 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%. Subjects were healthy; none reported having been diagnosed with cardiovascular disease, diabetes, insulin resistance or cardiovascular risk factors, such as hypercholesterolemia or hypertension. Subjects who smoked or were on medication of any type were excluded. All subjects took part in ≤2h of physical activity per week, as determined via questionnaire. Informed consent was gained from all subjects prior to the experimental procedures. The study procedures were approved by the Liverpool John Moores Ethics Committee, adhered to the Declaration of Helsinki and meets the ethical standards of the International Journal of Sports Medicine [11].

## **Experimental design**

We measured brachial artery diameter and FMD in one arm (randomized between subjects, but once selected all measurements were always performed in the same arm) before and immediately after upright cycling exercise. At least 48 h was taken between subsequent exercise bouts, whilst all tests were performed at the same time of day within subjects and within 14 days to minimize variability of the primary outcome variables. Exercise was performed for 30 min at 50, 70 and 85% of predicted maximal heart rate (HR<sub>max</sub>) (in a randomized order).

## **Experimental procedures**

Subjects were instructed to abstain from strenuous exercise for 24h and from caffeine and alcohol ingestion for 18h before attending the laboratory. They were also asked to fast for 6h prior to each visit. Subjects rested in the supine position for 20 min, followed by assessment of heart rate (HR) and blood pressure (BP) using an automated sphygmomanometer (GE Pro 300V2, Dinamap, Tampa, FL). This was followed by assessment of brachial artery diameter and velocity. Subsequently, participants cycled, without an external cooling fan, for 30 min on an electrically braked ergometer (Monark 874E, Sweden), with resistance adjusted to maintain a stable and individually determined exercise intensity at 50, 70 and 85% of predicted maximal HR (HR<sub>max</sub>) [31]. Heart rates were also monitored throughout exercise and recorded every 5 min and then averaged from 5-30 min. During the exercise bout both arms were rested comfortably on an adjustable table. Duplex ultrasound (T3000; Terason, Burlington, MA) was utilized to simultaneously image brachial artery diameter and velocity in one arm. Recordings were performed at baseline and during the final 15 min of cycling and immediately post-exercise. Subjects returned to the bed immediately following exercise and FMD and blood pressure measurements were repeated (<10 min post-exercise). Previous data from this laboratory has demonstrated that 30 min of a sham condition does not significantly alter FMD on 3 separate

# testing days. The coefficients of variation for these testing days were 6.7%, 10.9% and 9.5% [35].

## Brachial artery flow-mediated dilation

To examine brachial artery FMD, the arm was extended and positioned at an angle of ~80° from the torso. A rapid inflation and deflation pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned on the forearm, immediately distal to the olecranon process to provide a stimulus to forearm ischemia. A 10-MHz multi-frequency linear array probes, attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA), was then used to image the brachial artery in the distal <sup>1</sup>/<sub>3</sub>r<sup>d</sup> of the upper arm. When an optimal image was obtained, the probe was held stable and the ultrasound parameters were set to optimize the longitudinal, B-mode image of the lumen-arterial wall interface. Continuous Doppler velocity assessments were also obtained using the ultrasound and were collected using the lowest possible insonation angle (always <60°). Following baseline assessments, the forearm cuff was inflated (>200 mmHg) for 5 min. Diameter and flow recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter, in accordance with recent technical specifications [3,41].

## Brachial artery diameter and blood flow analysis

Analysis of brachial artery diameter was performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias. Recent papers contain detailed descriptions of our analysis approach [3,41]. From synchronized diameter and velocity data, blood flow (the product of lumen cross-sectional area and Doppler velocity) were calculated at 30Hz. Shear rate (SR, an estimate of shear stress without viscosity) was calculated as 4 times mean blood velocity/vessel diameter [26]. Reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods, reduces observer error significantly, and possesses an intra-observer CV of 6.7% [41].

## **Statistics**

Our primary outcome was FMD. We deemed it important to control for the influence of known moderators of FMD (shear rate and baseline diameter), since it was likely that these moderators would also be influenced by exercise [33]. As discussed in our recent guideline document, shear rate (SR) can impact FMD, but calculating a ratio of FMD/SR violates important statistical fundamentals [1,33]. We therefore analyzed the effects of exercise intensity and time on the change in logarithmically transformed diameter using a Generalized Estimating Equation (GEE), which incorporated baseline diameter and shear rate as covariates. The resulting mean differences between exercise intensities and pre/post were back-transformed to the original units of FMD (%). 2-factor general linear models (GLM) with repeated measures were employed to analyze the effect magnitudes of time and intensity on all other study outcomes. A 1-factor GLM was used to examine changes in shear rate during exercise. Post-hoc analysis was performed using the least significant difference (LSD) method, with no adjustment for multiple comparisons [24]. Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, Illinois) software. All data are reported as mean±SD unless stated otherwise. Exact P-values and 95% confidence intervals for the effect magnitudes are also cited.

intensities (50, 70, 85% HR<sub>max</sub>).

	50%		70%		85%		Table 1         Mean arterial blood
	Pre	Post	Pre	Post	Pre	Post	pressure (MAP) and heart rate (HR) pre and post exercise at all
MAP (mmHg)	91±4	91±3	93±8	92±6	90±6	88±6	exercise intensities (50, 70, and 85% of HR <sub>max</sub> ).
heart rate (beats/min)	67±14	65±11	62±13	75±13*	62±11	90±11*	
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Data are presented as mean ± SD. \* Statistically significant difference from pre-exercise at P<0.05

	Pre	50%	70%	85%	P-value	Table 2         Antegrade and retrograde           brachial artery shear rate at baseline
antegrade shear rate (s-1)	$109 \pm 42$	243±66*	322±63*†	324±40*†	< 0.001	and during cycling exercise (during final 15 min cycling) at all exercise
retrograde shear rate (s-1)	$-6 \pm 4$	$-18\pm24$	-15±14	$-12 \pm 10$	0.47	

P-values refer to one way GLM. Data are presented mean  $\pm$  SD. Post-hoc significantly different from \*pre (P<0.05) or  $\dagger$ 50 %HR<sub>max</sub> (P<0.05). Data in the table are based on n = 7 due to difficulty tracking velocity measures at higher exercise intensities



**Fig. 1** Brachial artery mean shear rate at rest and during a 30-min leg cycling exercise bout at 50, 70 and 85% of the individual maximal heart rate in healthy subjects (n=7). Error bars represent SE. Significantly different at P<0.05 from \*pre-exercise, †significantly different from 50%HR<sub>max</sub> (P<0.05).

## Results

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Participants completed the 30-min cycle exercise bouts at heart rates of  $101\pm6$ ,  $135\pm4$  and  $162\pm4$  bpm, which corresponded closely with 50, 70 and 85% of HR<sub>max</sub> (193±1 bpm). The changes in mean arterial pressure (MAP) after exercise were small in clinical terms ( $\leq 2$  mmHg) and not statistically significant across the 3 different exercise intensities ( $\bigcirc$  Table 1). The magnitude of exercise-mediated tachycardia post-exercise increased as exercise intensity became higher ( $\bigcirc$  Table 1).

Acute effects of cycle exercise on brachial artery shear rate

Shear rate data for 3 subjects, across all intensities, were removed from the analysis due to inadequate brachial artery diameter and velocity recordings during intense exercise (**• Table 2**). Mean and antegrade shear rate during exercise were higher compared to baseline data (**• Fig. 1** and **• Table 2**, respectively). Moreover, mean and antegrade shear rate demonstrated an increase with exercise intensity (one factor GLM; *P*<0.001, **• Fig. 1**, **• Table 2**).

Acute effects of cycle exercise on brachial artery flowmediated dilation

Exercise was associated with an increase in baseline diameter, the magnitude of which depended on exercise intensity. The pre-to-post exercise changes were 4.06±0.43-4.15±0.32 mm, 3.91±0.44-4.18±0.37 mm and 4.06±0.39-4.43±0.43 mm for the 50%, 70% and 85% HR<sub>max</sub> intensities. There was a significant difference in peak diameter pre vs. post exercise at 70 and 85% (P<0.05). Time to reach peak diameter increased after exercise at 50, 70 and 85%HR<sub>max</sub> ( $51\pm22-70\pm23$  s,  $57\pm21-65\pm15$  s and 54±18–78±23 s, respectively, 2 factor GLM main effect for time, P=0.003). The pre-to-post changes in peak diameter were  $4.32 \pm 0.46 - 4.39 \pm 0.36$  mm,  $4.17 \pm 0.45 - 4.37 \pm 0.38$  mm and 4.31±0.42–4.58±0.44 mm. Exercise also induced an increase in the FMD related post cuff deflation shear rate area-under-thecurve, (SR<sub>AUC</sub>; from cuff deflation to peak dilation) immediately after exercise at 50, 70 and 85%HR<sub>max</sub> (21.8±13.2- $26.9 \pm 8.4 \text{ s}^{-1} \times 10^3$ ,  $20.9 \pm 6.0 - 25.4 \pm 8.1 \text{ s}^{-1} \times 10^3$  and  $19.6 \pm 7.0 - 10^3$  $37.7 \pm 12.8 \,\mathrm{s}^{-1} \times 10^3$ , respectively, two factor GLM, interaction; P = 0.04).

Due to exercise-mediated changes in baseline diameter and SR<sub>AUC</sub>, we performed the GEE for FMD with baseline diameter and SR<sub>AUC</sub> entered as covariates. This analysis indicated that the decrease in FMD, after correction for the impacts of baseline diameter and shear rate, was negligible after exercise at 50%HR<sub>max</sub>, whilst larger and significant decreases were apparent after 70%HRmax exercise and after 85%HR<sub>max</sub> (**• Fig. 2**). Under all conditions, FMD returned to baseline values by 1h post-exercise  $(6.4\pm2.9\% \text{ vs. } 6.9\pm1.9\%, 6.7\pm2.1\% \text{ vs. } 5.9\pm2.7\%$  and  $6.1\pm1.2\% \text{ vs. } 6.8\pm2.7\%$ , pre vs. 1h post for 50, 70 and 85%HR<sub>max</sub>, respectively).

## Discussion

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The purpose of the present study was to determine the effect of 30 min leg cycling exercise performed at each of 3 exercise intensities (50, 70 and 85%) on brachial artery FMD immediately after cycle exercise. Our principal finding was that FMD decreased to a greater degree immediately after exercise performed at higher exercise intensities. The decrease in FMD induced by exercise was accompanied, but not fully explained by, increased baseline diameter. Taken together, our data suggest that exercise intensity impacts upon the magnitude of FMD reduction in an intensity-dependent manner.



**Fig. 2** Top Panel: Brachial artery flow-mediated dilation (FMD%, presented as a relative change from baseline diameter) before and immediately after a 30-min leg cycling exercise bout performed at 50, 70 and 85% of the individual maximal heart rate in healthy subjects (n = 10). Data are presented as mean ± SE. Data calculated from the GEE (and therefore corrected for shear rate and baseline diameter) are presented. \*Post hoc statistically significantly different from pre-exercise, † statistically significantly different from 50% HR<sub>max</sub> (P<0.05). Lower Panel: Individual delta changes in FMD% pre to post exercise at all exercise intensities.

There are several previous studies which have reported increases [10, 12, 15, 37, 39, 42], decreases [6, 10, 15, 16, 27] or no change [6, 12, 16, 27, 40] in FMD following acute bouts of exercise. This literature is difficult to interpret due to differences in the exercise intensities and modalities used [6, 15, 39, 40], the timing of FMD measurements after exercise [15,27,37,39,42], comparisons made between subjects or groups and technical differences related to artery diameter measures and FMD techniques such as discrete time points used to determine peak diameter and cuff placement during the FMD test. Each of these factors may independently influence FMD and it is therefore difficult to tease out the role of exercise intensity on acute changes in vascular function. This experiment attempted to minimize several of these sources or error and to systematically manipulate exercise intensity in isolation, whilst accounting for the impacts of exercise on FMD-associated shear and baseline diameter change. We observed an exercise intensity dependent decrease in function, which is consistent with some previous findings [6, 15, 27, 40]. As with our findings, Johnson and colleagues [15] reported a decrease in FMD at higher intensity only immediately post exercise, which returned to normal 1 h post-exercise.

The rationale for assessing the acute impact of exercise on FMD relates to the impact of repeated bouts of exercise on arterial

adaptation. Whilst it is tempting to speculate that a bout of exercise should acutely enhance arterial function if training effects are to induce beneficial adaptations, this may be a simplistic assumption. Decreases in FMD as a result of an acute bout of exercise may not necessarily be associated with down-regulation as an adaptive response [30]. This hypothesis was highlighted by Padilla et al. [22], who also note that there are many examples in integrative human physiology of up-regulation to stimuli which acutely challenge pathways, a notion encapsulated in the concept of "hormesis". Incidentally, we found that FMD had returned to baseline levels when measured 1 h post bout under all conditions.

Some previous data support our suggestion that exercise intensity plays an important role in modulating adaptations in vascular function with exercise [8,9,15]. In a carefully performed study, Goto and colleagues [8] introduced the notion that vascular adaptations to exercise training may be dependent upon repetitive increases in shear stress, offset at higher exercise intensities by the impact of oxidative stress. More specifically, it was suggested that high intensity exercise may impair endothelium-dependent vasodilation due to an increase in reactive oxygen species, resulting in a reduction in NO bioavailability [2]. Our findings provide support for this notion, as exercise at higher intensity was associated with larger acute impacts upon FMD.

It is pertinent that the largest decrease in FMD in the present study was also accompanied by the largest increase in post-exercise baseline diameter. Our observation of an increase in brachial artery diameter in response to lower limb exercise confirms the findings of a recent study which reported systemic post-exercise increases in resting diameter [23]. As previously noted, the potential change in artery diameter and shear rate post-exercise makes the use of FMD immediately post-exercise problematic [21], as baseline diameter is used in this calculation. While expressing the change in diameter as a percentage of resting diameter is relatively simple, this expression clearly does not control fully for the influence of baseline diameter per se, since it is well documented that baseline diameter is inversely related to FMD [34]. We noted that the slope of the relationship between logarithmically-transformed baseline and peak diameters was lower <1. Under such circumstances, differences in baseline diameter within or between subjects should be accounted for with more appropriate allometric models involving covariate control of baseline diameter [34,36]. We therefore undertook analysis of this type to determine whether exercise-induced changes in baseline diameter accounted for the observed effects on FMD. The exercise intensity dependent decrease in FMD was still found following covariate control for both baseline diameter and shear rate, suggesting that exercise intensity has an independent impact on artery function following exercise.

The discussion above also raises the question of whether the decreases in FMD we observed post-exercise at higher intensities in this experiment can be attributed to encroachment on a "ceiling diameter", due to increases in baseline diameters. We do not think this is the case for several reasons. A previous study of acute exercise, albeit at lower intensity, assessed GTN responses in addition to FMD responses post exercise. Whilst the FMD responses post exercise were around 6–10%, the GTN responses after identical exercise bouts were ~15–20% [37]. Secondly, the FMD peak arterial diameters (mm) after exercise at 50%, 70% and 85% were 4.39 ± 0.11, 4.37 ± 0.12 and 4.58 ± 0.14. There was a significant difference in FMD post-exercise between the 50 and

70% bouts, despite the peak diameter following the 70% bout not approaching 4.58mm. We therefore maintain that we have observed *bone fide* impairment in vasodilator function at higher exercise intensities, which is not simply a consequence of exercise-induced increases in baseline diameters.

Shear stress stimulates the endothelium to release several vasodilators, in particular nitric oxide [18,25]. It has therefore been suggested that shear stress mediated NO production is a key stimulus for conduit artery vasodilation during exercise [9,29,37,38]. Our data indicate that, whilst brachial artery diameter increased as a result of exercise, artery function assessed by FMD was diminished at higher levels of exercise (**•** Fig. 1). It is conceivable that large and/or sustained increases in shear that occur during exercise bouts may be associated with attenuated FMD because stimulation of vasodilation post-exercise reveals pathway limitations. For example, NO biosynthesis is dependent upon the continual availability of L-arginine [14] and several co-factors. Whilst arginine stores do not usually limit basal NO production in healthy humans, availability of this substrate may be limiting under some circumstances, particularly where NOS function is augmented in the face of enhanced NO degradation by oxidant species [20]. Provision of exogenous arginine enhances FMD under such conditions. It is therefore possible that high levels of shear during intense exercise reduce the bioavailability of NO and that increases in oxidative stress at higher exercise intensities amplify this [8]. Indeed, a recent study observed increases in an index of oxidative stress at higher exercise intensities, which was also associated with decreased FMD [15]. Future studies could employ nitric oxide donors or anti-oxidant strategies to determine the role of substrate depletion or oxidative stress on post-exercise vascular function.

## Limitations

A potential limitation of this study is that healthy male volunteers were recruited and we cannot extrapolate these findings to subjects with cardiovascular disease, women or older subjects. We did not measure indices of oxidative stress in our study, but previous research has demonstrated that exercise at higher intensities, consistent with those observed in the present experiment, is associated with production and circulation of reactive oxidant species [5, 13]. Studies have also attempted to overcome the production and circulation of reactant oxidant species using antioxidant strategies, with some suggesting that these approaches may modulate NO function [7,28]. A further limitation is that we did not assess the impact of different exercise durations on FMD. Future studies might match total work done to address the relative impacts of duration and intensity on FMD. Interestingly, a recent paper by Johnson and colleagues suggested that FMD exhibits similar changes following energy expenditure equivalent to 30 min at 50% VO<sub>2</sub>peak, regardless of intensity or duration [15]. Finally, it is possible that different exercise intensities induced differences in skin blood flows and this may have contributed to differences in shear rate stimulus to FMD. Nonetheless, our principal finding was that larger shear stimuli were associated with greater decreases in FMD at higher exercise intensities.

In conclusion, the present study demonstrates an 'exercise intensity-dependent' effect of leg exercise on brachial artery FMD. An immediate and substantial reduction in vasodilator function, which was not related to exercise-mediated changes in baseline diameter or shear stress, was observed at higher exercise intensities. This decrease in FMD may form the basis for adaptive vascular responses to episodic exercise stimuli.

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