Intermittent exercise abolishes the diurnal variation in endothelial-dependent flow-mediated dilation in humans

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Intermittent exercise abolishes the diurnal variation in endothelial-dependent flow-mediated dilation in humans. *Am J Physiol Regul Integr Comp Physiol* 298: R427–R432, 2010. First published November 18, 2009; doi:10.1152/ajpregu.00442.2009.—It is currently unclear to what extent diurnal variation and exercise effect endothelium-dependent nitric oxide (NO)-mediated vasodilation. Therefore, we measured brachial artery flow-mediated dilation (FMD) in 10 males (mean age = 28 yr, SD = 7), before and after a bout of intermittent cycling at 70% peak oxygen uptake on separate days beginning either at 0800 or 1600. Edge-detection and wall-tracking software was used to measure changes in arterial diameter, while shear rate (SR) was assessed using simultaneously derived blood flow velocity and B-mode diameter data. The FMD data were analyzed before and after normalization for SR with repeated-measures models. Before exercise, mean ± SD FMD was 7 ± 3% in the morning compared with 11 ± 6% in the afternoon (P = 0.01). This diurnal variation persisted after data were normalized for SR, which was found to be unaffected by time of day (P = 0.33). Postexercise SR was higher than at baseline (pre-exercise) (P = 0.01) to a similar extent at both times of day. FMD was unaffected by exercise in the morning (P = 0.96) but decreased by 4 ± 3% following exercise in the afternoon (P = 0.01) so that postexercise measurements did not differ between times of day. These data indicate that endothelium-dependent FMD is lower in the morning, and this finding was not altered by normalization of FMD for diurnal variation in SR. This infers a reduced function of the intrinsic endothelial NO-vasodilator system in the morning. We also report, for the first time, that a bout of intermittent exercise abolishes this diurnal variation in endothelium-dependent FMD.

The flow-mediated dilation (FMD) technique provides information about endothelial function of conduit arteries and, when the appropriate methodology is used, it has been reported that the FMD-response is nitric oxide (NO) dependent (16, 22, 44). FMD has been shown previously to vary with time of day (5, 13, 14, 30, 34, 40), with lower responses reported in the morning, compared with other times of day, in healthy individuals (13, 14, 34) and in patients with variant angina (30).

In experiments involving direct manipulations of shear rate (SR), within-subject changes in SR have been reported to positively correlate to the changes in FMD response (37–39). In the context of the present study, it is important that SR has also been found to be modulated by time of day and by exercise (27). Nevertheless, none of the previous studies of diurnal variation in FMD have taken into account any changes in SR over times of day, to determine whether the magnitude of the proposed FMD stimulus accounts for the observed effects (37). Therefore, it is currently unknown whether the diurnal variation in FMD is due to diurnal differences in intrinsic endothelial NO-vasodilator system function or in SR related to blood flow changes (i.e., differences in the stimulus per se).

Everyday life for most individuals consists of intermittent bouts of physical activity, which vary in intensity and duration. Intermittent exercise also characterizes many leisure and sports activities. Therefore, it is important to examine the effects of such “real-world” physical activity in the studies on diurnal variation to understand the changes in circulatory control with time of day. Interestingly, recent studies on diurnal variation in postexercise circulatory responses have indicated that postexercise blood pressure and brachial SR are higher in the morning than in the afternoon (25, 27). The purpose of this study was 1) to investigate the contribution of diurnal variation in SR to that in endothelial-dependent FMD, and 2) to explore whether diurnal variation in FMD is present following a preceding bout of intermittent exercise. Our primary hypothesis was that FMD will be lower in the morning compared with the afternoon and that this diurnal variation will not be explained by differences in SR.

**METHODS**

*Participants.* Ten normotensive males aged 28 ± 7 years, with mean ± SD body mass of 75.6 ± 6.3 kg, height of 1.79 ± 0.04 m and peak oxygen uptake (VO2 peak) of 45.7 ± 6.7 ml·kg−1·min−1 participated in the study. All participants were nonsmokers, had no history of cardiovascular disease, were not taking any medication, and all reported that they were engaged in regular physical activity >2 h/wk on a self-report questionnaire. The study conformed to the Declaration of Helsinki and was approved by the Institutional Ethics Committee. All participants were informed of the methods before providing written informed consent.

*Research design.* Participants attended the laboratory on four separate occasions, with the first visit for familiarization purposes, the second visit for measurement of peak oxygen uptake, and then two visits for completion of the main experimental trials involving FMD measurements prior to and following an intermittent exercise protocol in the morning (AM) and the afternoon (PM). The two trials were administered in a counterbalanced order and were separated by 7–10 days. The light intensity in the laboratory was controlled at ~200 lux, and temperature was maintained at 21°C. At both times of day, the protocol began after a 12-h abstinence from caffeine, 24-h abstinence from alcohol, and strenuous exercise, and at least a 4-h fast (participants ate a standard carbohydrate breakfast only between 0800 and 0900 on the day of the afternoon test).

*Familiarization.* During their first visit to the laboratory, participants were familiarized with the equipment and exercise protocol, and anthropometric measurements were recorded. Height (m), body mass...
(kg), and resting blood pressure (three serial measurements with a mercury manual sphygmomanometer) were determined.

**Measurement of peak oxygen uptake.** On the second visit to the laboratory, \( \text{VO}_2 \text{peak} \) was determined using a progressive continuous protocol (7). As a standard warm-up, participants performed 10 min of submaximal cycling on an ergometer (Kettler Sport, Worcestershire, UK). Power output was set initially at 50 W and was increased in 25-W increments every 2 min until volitional exhaustion or the point at which the subject could no longer maintain the required pedal cadence (≥60 rev/min). Expired gases were collected using an on-line collection system that sampled every 10 s (Metamax 1; Cortex Biophysic, Leipzig, Germany). Oxygen uptake was then plotted against work rate, and the exercise work rate (i.e., watts) corresponding to 70% \( \text{VO}_2 \text{peak} \) was interpolated using a linear regression equation.

**Experimental protocol.** Participants reported to the laboratory at 0700 ready to begin exercise at 0800 in the morning exercise condition, and at 1500 ready to begin exercise at 1600 in the afternoon exercise condition. Baseline measurement of conduit artery FMD was obtained prior to any exercise. The exercise protocol consisted of three 10-min bouts of semisupine cycling at 70% \( \text{VO}_2 \text{peak} \), with each bout separated by 10-min rest periods (seated on cycle ergometer). The exercise protocol was performed in all subjects, in the morning and afternoon, respectively. Participants remained seated on the ergometer for an initial rest period. The occluding cuff was then inflated to 220 mmHg and SR, and an ANCOVA-based repeated-measures statistical model with \( \text{SR} \) entered as a changing covariate. In light of current debate about the general magnitude of correlation between \( \text{SR} \) and FMD in different research situations (4), we also calculated the within-subjects correlation coefficient between \( \text{SR} \) and FMD using appropriate methods, which partition the within-subject and between-subject influences properly (9). All data were analyzed using the STATISTICA software (Statsoft, Tulsa, OK). Data are presented in the text as means ± SD, and exact \( P \) values are cited (values of \( P \) of “0.000” provided by the statistics package are reported as “<0.0005”).

**RESULTS**

**Brachial artery FMD and SR stimuli.** A statistically significant interaction between time of day and exercise (referring to baseline/preexercise or postexercise) was evident for brachial FMD (\( P = 0.01 \)). Follow-up contrasts indicated that baseline FMD was 7 ± 3% in the morning compared with 11 ± 6% in the afternoon (\( P = 0.01 \), Fig. 1). Nevertheless, the exercise effect depended on the time of day; following exercise in the afternoon, FMD was reduced to 7 ± 3% (\( P = 0.01 \)), but no change in FMD was evident after exercise in the morning (\( P = 0.96 \)).

SR was unaffected by time of day (time of day main effect: \( P = 0.33 \)). SR was higher after exercise compared with baseline values at both times of day (exercise main effect: \( P < 0.005 \)). The nonsignificant interaction between time of day and exercise (\( P = 0.41 \)) indicated that the exercise-mediated increases of 12,011 ± 12,513 and 7,432 ± 7,138 AU in the morning and afternoon, respectively, were not significantly different (Fig. 2). Baseline and peak artery diameters, as well as the time-to-peak artery diameter, were not found to be influenced by time of day, exercise, or the interaction between these two factors (\( P > 0.23 \), Table 1).

![Fig. 1. Brachial artery flow-mediated dilation (FMD) values preexercise and postexercise in the morning and afternoon. Significant interaction between time of day and exercise was evident in FMD (\( P = 0.05 \)). *Significant difference between time of day preexercise. ^Significant difference between preexercise and postexercise FMD in the afternoon.](http://ajpregu.physiology.org/)

**Statistical analysis.** Baseline (preexercise) and postexercise vascular and blood pressure measurements were analyzed using two-factor general linear models with repeated measures. The factors were time of day (which refers to morning or afternoon) and exercise (which refers to the preexercise or postexercise time point). The sphericity assumption in repeated measures is not relevant in this two-level situation. Statistically significant interactions were followed-up with multiple contrasts corrected for type I error rate using the Newman Keuls procedure. According to current opinion on normalization of FMD for differences in SR (20), we normalized FMD for SR using two statistical approaches; the simple ratio of FMD and SR, and an ANCOVA-based repeated-measures statistical model with SR entered as a changing covariate. In light of current debate about the general magnitude of correlation between SR and FMD in different research situations (4), we also calculated the within-subjects correlation coefficient between SR and FMD using appropriate methods, which partition the within-subject and between-subject influences properly (9). All data were analyzed using the STATISTICA software (Statsoft, Tulsa, OK). Data are presented in the text as means ± SD, and exact \( P \) values are cited (values of \( P \) of “0.000” provided by the statistics package are reported as “<0.0005”).
Using appropriate statistical modeling (9), we found a low and nonsignificant within-subject correlation \( (r = -0.02, P = 0.91) \) between the measurements of SR and FMD in our experimental conditions. Regression lines are shown in Fig. 3 for individual subjects, and it can be seen that the slopes are very variable between subjects. We also found low \( (r < 0.3) \) and nonsignificant between-subject correlations between SR and FMD when examined in each of the four experimental conditions (preexercise and postexercise at the two times of day). Although this absence of a correlation between SR and FMD challenges the usefulness of normalization (4), we proceeded to examine the effects of two SR normalization approaches on our FMD data. When FMD was controlled for the effects of SR with the ANCOVA approach, the interaction between time of day and time was still statistically significant \( (P = 0.01) \). The covariate-adjusted mean FMD at preexercise/baseline was lower in the morning compared with the afternoon \( (P = 0.005) \). Exercise mediated a reduction in FMD but only in the afternoon so that postexercise FMD was not different between times of day \( (P = 0.71) \). When the FMD/SR ratio approach to normalization was adopted, similar trends in the nonnormalized and ANCOVA-normalized data were observed. FMD/SR was lower following exercise in the afternoon but not lower after exercise in the morning. Nevertheless, the \( P \) value \( (0.08) \) for the interaction between time of day and exercise was higher for the FMD/SR data than the nonnormalized and ANCOVA-normalized data.

**Blood pressure.** Systolic and diastolic blood pressures were not different during the FMD test (i.e., pre-post occlusion) \( (P > 0.11) \). No interactive effects between time of day and exercise were evident for diastolic blood pressure \( (P = 0.299) \). There was evidence of an interaction between time of day and exercise for systolic blood pressure \( (P = 0.06, \text{Fig. 4}) \). Resting systolic blood pressure was \( 5 \pm 3 \) mmHg greater in the afternoon compared with the morning \( (P = 0.01) \). Systolic blood pressure was reduced following exercise in the morning and afternoon \( (P < 0.01) \). The mean \( \pm \) SD difference was \( 4 \pm 6 \) and \( 10 \pm 6 \) mmHg in the morning and afternoon, respectively.

**DISCUSSION**

In the present study, we employed the FMD technique to examine diurnal variation in endothelial-dependent FMD at rest and following a bout of intermittent exercise. Previous researchers have found that FMD is lower in the morning compared with other times of day \( (13, 30, 34) \), but these studies have not involved measurements of SR in parallel with measurements of FMD. In support of our primary hypothesis, our data indicate that lower FMD is not explained by differences in SR, since SR was not different in the morning and afternoon. This infers that intrinsic differences in the function of the endothelial NO-vasodilator system contribute to diurnal variation in FMD in arteries of similar size and function as epicardial coronary arteries \( (1, 41, 42) \). We also report, for the first time, that a bout of intermittent, submaximal exercise abolishes this diurnal variation in FMD, since FMD measured postexercise was not different between morning and afternoon test times.

**Diurnal variation in FMD.** Our data indicate that it is unlikely that variation in SR \( (37–39) \) is the explanation for the observed diurnal variation in FMD. Therefore, within the bounds of our measurement technique and study population, our data infer that intrinsic differences in the function of the endothelial NO-vasodilator system mediate the diurnal variation. This could be explained by cyclical changes in NO bioavailability or NO

### Table 1. Resting and peak artery diameters and the time-to-peak artery diameter values preexercise and postexercise in the morning and afternoon

<table>
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<tr>
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<th>Morning</th>
<th>Afternoon</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>Baseline artery diameter, mm</td>
<td>4.43±0.37</td>
<td>4.49±0.37</td>
</tr>
<tr>
<td>Peak artery diameter, mm</td>
<td>4.75±0.44</td>
<td>4.79±0.44</td>
</tr>
<tr>
<td>Time to Peak, s</td>
<td>67±28</td>
<td>75±44</td>
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Values are expressed as means \( \pm \) SD. TOD, time of day.
Potential explanations for the removal of diurnal variation in FMD postexercise could relate to diurnal variation in other components contributing to vascular tone postexercise, other than the NO-vasodilator system. Vascular tone represents the competitive balance between local vasodilator function and SNA (27). Similarly, a reduction in FMD as a consequence of renoreceptor-mediated vasoconstriction has been shown to be elevated in the morning at rest, but this has not been demonstrated post exercise, albeit with an indirect measure of SNA (27). Therefore, the lack of diurnal variation in postexercise FMD in the current study does not explain the greater morning SR observed immediately following exercise in our previous study (27). Diurnal variation was not evident in postexercise SR in the current study. Nevertheless, the higher postexercise SR in the morning compared with the afternoon reported in our previous study was apparent 5 min after exercise, but was relatively short-lived, not being evident 20 min after exercise. In the current study, both FMD and SR were measured 20–30 min postexercise. In addition, it is apparent from the results of the current study that the intrinsic diurnal variation in resting FMD can be influenced by extrinsic stimuli, such as exercise, and that the extent of this influence depends on time of day. This could suggest that diurnal variation in FMD, specifically NO-vasodilator system, is susceptible to effects of physical activity, sleep, and posture, rendering it difficult to describe the endogenous mechanism for diurnal variation. Previous researchers have not attempted to investigate the endogenous mechanism for diurnal variation in FMD (13, 30, 34). Such a study is warranted but would require a number of strict control procedures, including careful control of posture and prior sleep.
acute exercise such as that observed in the afternoon in the current study could potentially be explained by elevated oxidative stress (15, 19) or inflammation (2). Indeed, biomarkers of both oxidative stress and inflammation show diurnal variation at rest (29, 33). Peak concentrations of oxidative stress biomarkers are evident in the early evening (29). It might, therefore, be speculated that more oxidative stress in the afternoon impacts FMD. However, the diurnal variation oxidative stress at rest (i.e., higher in the afternoon) does not explain the resting FMD data in the current study, and no research to date has examined diurnal variation in such markers following exercise.

Only when baseline values of FMD were higher in the afternoon, did we observe an exercise-mediated reduction in FMD. A postexercise reduction in FMD is in agreement with some previous research (2, 11, 21) but not all (6, 15, 21). Generally, there is no consensus within the literature as to the direction of change in FMD immediately after exercise, and the discord could possibly be explained by differences in 1) the population studied; 2) the timing of the measurement postexercise, 3) the exercise mode or intensity and 4) the time of day the FMD measurements were taken. Nevertheless, the debate surrounding direction of change in FMD postexercise does not compromise our finding that exercise abolished the observed diurnal variation in FMD 30 min after an intense exercise bout. It is important to note, however, that the findings of this study are currently limited to young healthy individuals performing intermittent exercise and to measurements recorded 30 min postexercise. Indeed, it is possible that the afternoon reduction in FMD was not long lasting. Nonetheless, our data indicate that such a reduction is not evident in the morning. At this time of day, FMD is similar at rest and postexercise. It is clear from these data and previous studies that exercise-mediated cardiovascular adjustments are different in the morning compared with the afternoon.

Methodological limitations. One potential limitation of this study is the lack of assessment of diurnal variation in vascular smooth muscle function (i.e., endothelium-independent vasodilation). Previous researchers have suggested that there is no diurnal variation at all (14, 30, 31), or greater endothelium-independent vasodilation in the morning (5). However, all of the previous studies involving measurements of endothelium-independent vasodilation have utilized a 4-min assessment period, which may not have been long enough to fully observe the changes that occur with sublingual nitroglycerin administration (8). Therefore, further research is warranted to accurately quantify diurnal variation in endothelium-independent vasodilation. Another possible limitation of the present study relates to the lack of control of sleep prior to the two trials. Potentially, diurnal differences in resting FMD could be due to the longer period of inactivity (i.e., during sleep) or nocturnal sleep per se, prior to the morning measurements compared with the typical everyday activities, which participants undertook prior to the afternoon measurements. Although we have shown recently, using a protocol that controlled for the amount of prior sleep, that diurnal variation in blood pressure at rest and following exercise cannot be explained by sleep-related influences (26), FMD was not measured in this study. In addition, a long period of inactivity (3 days) has been shown to negatively affect FMD measurements (17).

Conclusion. The data from this study indicate that the diurnal variation in resting endothelium-dependent FMD is not explained by changes in SR. This suggests that intrinsic endothelial function mediates diurnal variation in FMD in conduit arteries. Diurnal variation in FMD was not evident following a bout of intermittent exercise, which suggests that diurnal variation in FMD is influenced by the rest-activity cycle over 24 h. To unravel the relative endogenous and exogenous influences on diurnal variation in FMD, it is clear from our data that the effects of physical activity should be taken into account.

Perspectives and Significance

A reduction in FMD in the morning has previously been implicated as a potential mechanism, which contributes to the morning peak in cardiovascular events (34). We present data that support some previous findings of diurnal variation in FMD and add the observation that this variation in FMD as a function of time of day is intrinsically mediated and not related to diurnal change in SR. Although our findings are specific to healthy normotensive males with no evidence of cardiovascular disease, lower FMD in the morning has been shown previously in individuals with cardiovascular disease at rest (30). Therefore, it can be reasonably assumed that a similar diurnal pattern in FMD will be evident in individuals with cardiovascular disease. Future research studies should focus upon determining the relative contribution of NO to the diurnal variation in endothelial function together with competing vasoconstrictor control mechanisms, ideally via pharmacological blockade.

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


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