Morning Attenuation of Endothelium-Dependent, Flow-Mediated Dilation in Healthy Young Men: Possible Connection to Morning Peak of Cardiac Events?

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Summary

Background and hypothesis: Brachial artery flow-mediated dilation (FMD), a noninvasive, widely used clinical index of endothelial function and magnitude of FMD, has been reported to be closely related to many coronary risk factors and coronary atherosclerosis. However, there has been no study that examines the diurnal change of FMD. We designed this study to reveal the diurnal variation of FMD in healthy volunteers.

Methods: We examined FMD in response to reactive hyperemia by high resolution ultrasound in 13 healthy young men (age 25–32) at four different times over the course of a day.

Results: Mean measures of brachial artery FMD was 4.0% at 8:00, 5.3% at 12:00, 9.7% at 17:00, and 6.9% at 21:00 hours. Flow-mediated dilation at 8:00 and at 12:00 hours was significantly lower than that at 17:00 ($p < 0.05$).

Conclusions: These results show that endothelial function has diurnal variation and is significantly attenuated in the morning. Morning attenuation of endothelial function should be recognized in clinical research and may play an important role in the circadian variation of the occurrence of acute cardiovascular events.

Key words: endothelial function, diurnal variation, brachial artery

Introduction

The incidence of cardiovascular diseases has been reported to exhibit a circadian variation.¹ Cardiovascular diseases, such as myocardial infarction, sudden cardiac death, symptomatic and asymptomatic myocardial ischemia, and stroke, have all been shown to occur more frequently in the morning than at other times of day. Although these diurnal distributions of cardiovascular events can be explained by changes in platelet aggregation,¹ fibrinolytic activity,² sympathetic activity,³ and plasma catecholamines,⁴ endothelial function possibly also contributes to these events. The endothelium plays an important role in cardiovascular diseases^{5–7} by regulating vascular tone, platelet aggregation, leukocyte adhesion and smooth muscle proliferation.

Vasodilatory responses to a shear stress in conduit arteries are reported to be a clinically useful index of endothelial function.^{8,9} Since brachial artery flow-mediated dilation (FMD) in response to reactive hyperemia in human subjects is easily assessable and noninvasive, it has been extensively examined in various clinical settings.^{10–13} Therefore, the present study was designed to determine whether or not a pattern of regular diurnal variations in FMD endothelial function does indeed exist.

Methods

Study Population

After obtaining informed consent, 13 healthy adult males aged 25 to 32 years [28 ± 2 years, mean $\pm$ standard deviation (SD)] were studied. All were normotensive, nondiabetic, non-smokers, nonhypercholesterolemic, and none had a family

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history of premature vascular disease. We also confirmed that all subjects were not taking any medications, including over the counter vitamins or antioxidant supplements.

Brachial Endothelial Function Study

Brachial endothelial function study was performed according to the previously reported methods.¹⁴ All studies were performed in a temperature-controlled room (25°C) with the subject in the resting, supine, and also fasting state. The electrocardiogram (ECG) was continuously monitored. Blood pressure and heart rate were recorded from the left arm every 3 min with an automatic sphygmomanometer (BP-203, Nihon Korin, Tokyo, Japan). The subject's right arm (the dominant arm) was comfortably immobilized in the extended position to allow consistent access to the brachial artery for imaging. The responses of brachial artery vasodilation were obtained by a previously validated technique.^{15, 16} Brachial artery diameter and flow velocity were imaged using a 7.5 MHz linear array transducer ultrasound system (SONOS 1500, Hewlett Packard Company, Andover, Mass.). For each subject, optimal brachial artery images were obtained between 2 and 10 cm above the antecubital fossa. This location was marked, and all subsequent images were obtained at the same location.

First, baseline two-dimensional (2-D) images were obtained. Pulsed-Doppler blood flow velocity was then determined. Brachial arterial flow velocity was obtained using a Doppler signal at a 70° angle to the vessel, with the range gate (1.5 mm) in the center of the artery. After baseline measurements of brachial artery diameter and flow velocity were recorded, a small-width blood pressure cuff (SC-10, Hokanson, Seattle, Wash.) was inflated on the most proximal portion of the forearm to occlusive pressure (200 mmHg) for 5 min in order to induce hyperemia. Throughout the procedure, the ultrasound transducer position was carefully maintained. The cuff was then rapidly deflated. Immediately after deflation, pulsed-Doppler signals were recorded for 15 s. Two-dimensional images of the brachial artery were obtained for 60–120 s after cuff deflation. All images were recorded on super VHS videotape for later analysis. After a 20 min rest period (to allow arterial diameter to return to baseline size), 2-D images of the brachial artery were again obtained at baseline and for 5 min after sublingual administration of nitroglycerin (0.3 mg).

Flow Measurements and Brachial Vasodilator Response

Measurements were taken according to previously reported methods.¹⁴ Brachial artery blood flow at rest and during reactive hyperemia was determined by previously described methods.^{15, 16} Flow volume was calculated by multiplying the velocity-time integral of the Doppler flow signal (corrected for angle) by heart rate and the vessel cross-sectional area (πr^2), using public domain software (SONOS 1500, Hewlett Packard Company). The flow velocity used in this calculation was taken from the center of the brachial artery. Relative increase of blood flow at reactive hyperemia was calculated as the maximum flow recorded in the first 15 s after cuff deflation divid-

ed by the flow at baseline scan, since blood flow increased maximally immediately after cuff deflation.¹⁴

By playback of a videocassette recorder (HR-X3, Victor, Tokyo, Japan), a 10–20 mm segment of the brachial artery was identified for analysis using the anatomic landmarks in each subject. To select images reproducibly at the same point in the cardiac cycle, images at peak systole (maximum dilation, close to the end of the T wave on the ECG) were identified and the diameter of brachial artery was digitized. A quantitative coronary angiography analysis computer (Cardio 500, Kontron Elektronik Corp., Boston, Mass.) containing a digitizing board was used for these measures. For each condition (baseline, reactive hyperemia at 60 s after cuff deflation, repeat baseline, and nitroglycerin at 5 min after administration), three separate images from three different cardiac cycles were digitized. The average segment diameter of these three images was determined. All of these measures were performed in a blinded manner and performed by the same investigator to avoid possible interobserver variability.¹⁴ The percent diameter changes from baseline in response to hyperemia and nitroglycerin were calculated. The intraobserver variabilities (coefficient of variation) for repeated measures of diameter at baseline, reactive hyperemia, and nitroglycerin administration were <3%, as previously reported.¹⁴

Measurement of Basal and Postischemic Forearm Vascular Resistance

Since heart rate and blood pressure were stable during each intervention except for nitroglycerin administration, the averages of heart rate and systolic and diastolic blood pressure during each intervention before the nitroglycerin phase were used for calculation of forearm vascular resistance. Forearm vascular resistance was calculated as the mean blood arterial pressure (mmHg) divided by brachial arterial blood flow (ml/min). These values were converted to common units ($\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5}$) by multiplying them by a constant of 8×10^4 . Mean blood pressure was estimated as 1/3 (systolic blood pressure minus diastolic blood pressure) plus diastolic blood pressure.

Study Protocol

We repeated the same measuring procedures in each subject at four times (8:00, 12:00, 17:00, and 21:00 hours) over the course of a day when all subjects had fasted throughout the entire study. After each of the four measures had been taken, all subjects were allowed to have meals; however, heavy meals including a high-fat diet were prohibited during this study. We provided a traditional Japanese style meal. Also, subjects were instructed not to exercise during the course of the study. All subjects were measured within a 24 h period, but four subjects started at 8:00, three subjects at 17:00, and the remainder at 21:00 hours to avoid a possible time bias. Throughout the study, even early in the morning or late at night, normal laboratory means remained in the same range. The time of measurements was established at these periods in accordance with Quyyumi *et al.*¹⁷

TABLE I Baseline characteristics of the 13 normal healthy adult males used in the present study. Values represent means $\pm$ SD

Characteristic	Value
Age (years)	28 $\pm$ 2
Total cholesterol (mg/dl)	158 $\pm$ 25
Triglycerides (mg/dl)	69 $\pm$ 39
HDL cholesterol (mg/dl)	62 $\pm$ 12
Fasting blood glucose (mg/dl)	90 $\pm$ 5

Abbreviations: SD = standard deviation, HDL high-density lipoprotein.

Statistical Analysis

All data are presented as means $\pm$ SD. The diurnal variation of reactive hyperemia, flow-mediated vasodilation, nitroglycerin-mediated dilation, and other hemodynamic data were examined with repeat-measure analysis of variance (ANOVA). Subsequent alpha values were corrected by the Scheffe method. Statistical significance was accepted if the null hypothesis was rejected at the $p < 0.05$ level.

Results

Selected baseline characteristics of the study population ($n=13$) are shown in Table I. These characteristics are all normal for young adult males in Japan. Heart rates and vessel diameter of the brachial artery were relatively stable during the day, but values of mean blood pressure were significantly ($p < 0.05$) lower at both 8:00 and 12:00 than at either 17:00 or 21:00 hours (Table II).

Baseline brachial artery blood flow and reactive hyperemic flow induced by 5 min occlusion of the forearm vessel were not significantly different throughout the day (Table III). Similarly, basal and postischemic forearm vascular resistance did not differ significantly throughout the day (Table III). However, mean brachial artery FMD response was lowest at 8:00 and highest at 17:00 hours; the values at both 8:00 and 12:00 hours were significantly ($p < 0.05$) lower than at

TABLE II Diurnal changes of blood pressure, heart rate, and the vessel diameter of the brachial artery. Values represent means $\pm$ standard deviation

Time	8:00	12:00	17:00	21:00
Heart rate (beats/min)	59 $\pm$ 12	59 $\pm$ 11	60 $\pm$ 12	63 $\pm$ 14
Mean blood pressure (mmHg)	80 $\pm$ 9 ^{a, b}	81 $\pm$ 7 ^{a, b}	85 $\pm$ 9	86 $\pm$ 9
Vessel diameter (mm)	4.3 $\pm$ 0.5	4.4 $\pm$ 0.5	4.2 $\pm$ 0.5	4.4 $\pm$ 0.5

^a $p < 0.05$ vs. values at 17:00.

^b $p < 0.05$ vs. values at 21:00, by ANOVA and Scheffe.

17:00 hours (Table III). When individual subject responses were examined, FMD was lowest at 8:00 in eight subjects, at 12:00 in three subjects, and in one subject each at 17:00 and 21:00 hours, respectively (Fig. 1). More important, two subjects each at 8:00 and 12:00 hours showed the vasoconstrictive response reflected by the small degree but negative values of FMD, whereas no constrictive response was observed at 17:00 and 21:00 hours. The values of nitroglycerin-induced and endothelium-independent vasodilation were similar throughout the day.

Discussion

The present study demonstrates that FMD of the brachial artery might have a diurnal variation. Since FMD at 17:00 did not significantly differ from that of 21:00 hours, it is not definitely demonstrated that a regular pattern of diurnal variation in FMD does exist. However, FMD is significantly attenuated in the morning to noon (at 8:00 or 12:00) compared with that in the evening (at 17:00). Particularly, a total of four subjects presented vasoconstriction in the morning as shown in Figure 1. These variations of FMD were probably not due to differences in the stimulus for dilation (the degree of reactive hyperemia) at different times, since the shear stress demonstrated by reactive hyperemic flow was not attenuated significantly in the morning and at noon (Table III). The ability of the artery to di-

TABLE III Diurnal changes of brachial blood flow, reactive hyperemic blood flow, endothelial-dependent or -independent vasodilatation, and postischemic forearm vascular resistance. Values represent means $\pm$ standard deviation

Time	8:00	12:00	17:00	21:00
Baseline blood flow (ml/min)	188 $\pm$ 142	176 $\pm$ 99	158 $\pm$ 78	235 $\pm$ 134
Reactive hyperemic blood flow (ml/min)	703 $\pm$ 154	758 $\pm$ 326	896 $\pm$ 316	924 $\pm$ 301
Relative increase of blood flow at reactive hyperemia (X Rest)	5.1 $\pm$ 2.3	4.8 $\pm$ 1.9	6.8 $\pm$ 3.6	4.8 $\pm$ 2.2
Flow-mediated dilation (%)	4.0 $\pm$ 5.3 ^a	5.3 $\pm$ 5.4 ^a	9.7 $\pm$ 7.3	6.9 $\pm$ 5.4
NTG-induced dilation (%)	14.0 $\pm$ 5.7	13.9 $\pm$ 5.9	15.4 $\pm$ 6.2	16.2 $\pm$ 6.3
Basal forearm vascular resistance (dynes·s·cm ⁻⁵)	49340 $\pm$ 29810	44980 $\pm$ 17900	51790 $\pm$ 22100	41000 $\pm$ 24520
Postischemic forearm vascular resistance (dynes·s·cm ⁻⁵)	9429 $\pm$ 2452	10093 $\pm$ 4145	8352 $\pm$ 2667	8084 $\pm$ 2368

^a $p < 0.05$ vs. values at 17:00 hours, by ANOVA and Scheffe. NTG = nitroglycerin.

FIG. 1 Diurnal changes of flow-mediated dilation (FMD) (endothelium-dependent vasodilation) in each subject. Mean values of FMD were lowest at 8:00 and highest at 17:00 hours. Flow-mediated dilation was lowest at 8:00 in eight subjects, at 12:00 in three subjects, and in only one subject at 17:00 and 21:00 hours, respectively. Two subjects each at 8:00 and 12:00 showed the vasoconstrictive response reflected by negative values of FMD. $\dagger$ = mean $\pm$ standard deviation. * $p < 0.05$ vs. 08:00, 12:00 hours.

late by an exogenous source of nitric oxide (nitroglycerin) was also not changed.

The endothelium plays a central role in controlling the biology of the vessel wall. Coronary endothelial function, as well as systemic vascular endothelial function, is an early marker of coronary atherosclerosis.^{6, 16, 18} Examination of brachial endothelial function has been widely used in clinical studies.¹⁰⁻¹³ However, a search of the literature failed to uncover any reports describing a diurnal variation of brachial endothelial function. Measures of brachial artery vasodilation responses to reactive hyperemia differ among previous reports,⁸⁻¹² but the time of day when brachial endothelial function studies were carried out was not always clearly spelled out in these papers. Although it is not clear that these differences are caused by diurnal variations, this possibility should be taken into account.

Basal¹⁹ and postischemic¹⁷ forearm vascular resistance were reported to be higher in the morning than in the afternoon. However, a diurnal variation in forearm vascular resistance was not evident in the present study. The difference in results might be attributable to differences in methods of measuring flow volume; blood flow was measured by plethysmography in the earlier studies and by Doppler flow technique in the present study. The Doppler flow technique is admittedly less precise than plethysmography and therefore represents a limitation in the present study.

Since peripheral endothelial function is reported to reflect coronary endothelial function,^{16, 18} it is reasonable to expect that morning attenuation of FMD of the brachial artery is accompanied by morning attenuation of coronary endothelial function. Concerning this point, decreased morning nitric oxide activity in the coronary circulation may create a potential hazard of coronary thrombosis and vasospasm, which cause

acute coronary events. Our observation of morning attenuation of endothelial function is similar to the morning augmentation of acute coronary events documented by many studies.^{1, 3, 17}

The underlying mechanism of physiologic morning attenuation observed in FMD is unclear. Endothelium produces nitric oxide and prostaglandin I₂ in response to hyperemic blood flow (increased shear stress).²⁰ It is suspected that either nitric oxide production is attenuated or nitric oxide degradation is augmented in the morning. The changes of L-arginine availability or nitric oxide synthase activity might contribute to this mechanism. Further, nitric oxide inactivation might be enhanced in the morning. We demonstrated that FMD is attenuated in the morning in young healthy men, but diurnal variation of the other endothelial functional indices, for example, receptor-dependent endothelial vasodilation, was not examined. These indices need to be examined to elucidate the mechanisms further. Since a prolonged fasting condition can change sympathetic or the other regulatory mechanism of vasculotone, the attenuation of FMD early in the morning (08:00) could possibly be affected by the prolonged fasting condition. Also, we studied only normal subjects, which is another limitation of the present study. The study should be replicated in patients with coronary artery disease. For these reasons, data might not be available for patients with coronary artery disease or subjects on medications such as beta-adrenergic blockers which are known to affect the circadian variation of vasculotone. This is another important limitation of the study.

We evaluate the brachial artery diameter at the end of the T wave, corresponding to peak systolic expansion, as previously described by Uehata *et al.*;¹⁴ and other reports also used the end of T wave as measurement point.^{7, 13, 16} On the other hand, Celermajer *et al.* originally reported that they evaluate brachial artery diameters at R wave.⁸ Many other studies used the R wave as a measurement point. Anderson *et al.* reported a close relation between coronary artery endothelium-dependent vasomotor responses to acetylcholine and flow-mediated vasodilation in the brachial artery; in their study they used the end of the T wave as measurement point.¹⁶ As we intended to demonstrate that the diurnal variation of brachial artery FMD which may have a relation with coronary artery endothelial function, we used the same method as with Anderson *et al.*

Although it has not been demonstrated that vessel compliance affects endothelial function, we have to consider the effect of vessel compliance on the FMD when we assess vessel diameters at the end of the T wave. In fact, carotid artery vessel compliance varied significantly in the Atherosclerosis Risk in Communities Study as did the status of glucose tolerance.²¹ Therefore, we have to pay careful attention to the result of FMD when we evaluate the heterogeneous population by this method. In our study, we examined young volunteers who can be considered to be a homogeneous population, and therefore it seems reasonable to assume that vessel compliance was not different in our population and that vessel compliance effect on FMD might be negligible.

Since endothelial function reflected by FMD in the brachial artery is significantly attenuated in the morning, time of day

must be considered when this technique is applied in human research. Second, there is some possibility that the morning attenuation of endothelial function might play a significant role in the circadian variation of the occurrence of acute cardiovascular events. However, as mentioned in the study limitation, it would be too premature to associate the preliminary findings with the circadian occurrence of cardiac events, so that the findings should be confirmed in patients with coronary artery disease.

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