Early Morning Attenuation of Endothelial Function in Healthy Humans

Maria E. Otto, MD, PhD; Anna Svatikova, BA; Rodrigo Bellio de Mattos Barretto, MD; Simone Santos, MD; Michal Hoffmann, MD; Bijoy Khandheria, MD; Virend Somers, MD, PhD

Background—Cardiovascular events such as myocardial infarction, sudden death, and stroke have a peak incidence in the early hours after waking. The mechanisms involved in this circadian variation are not clear. Endothelial dysfunction is associated with increased risk for cardiovascular events. We tested the hypothesis that endothelial function is reduced in the early morning, around the time of waking, compared with measurements obtained both before sleep and later in the day in healthy humans.

Methods and Results—We studied 30 subjects (19 men, 11 women; mean age, 41.6 years). All participants underwent complete overnight polysomnography to exclude obstructive sleep apnea or other sleep disorders. Brachial artery flow–mediated endothelium-dependent vasodilation (FMD) and endothelium–independent dilation (non-FMD) were measured on 3 different occasions: before subjects went to sleep (9 PM), the next morning immediately after waking (6 AM), and during the late morning 5 hours after waking (11 AM). All subjects had normal sleep with good sleep efficiency of 84±2%. Compared with before sleep, FMD decreased markedly in the early morning after waking and recovered by late morning (9 PM, 7.5±1%; 6 AM, 4.4±0.7%; 11 AM, 7.7±1%; P=0.02). Non-FMD was similar for the 3 periods of observation (9 PM, 17.3±1.6%; 6 AM, 17.2±1.3%; 11 AM, 18.5±1.7%).

Conclusions—FMD is blunted in the early morning in healthy subjects. Decreased endothelial function in the early morning may have implications for our understanding of the morning peak in cardiac and vascular events. (Circulation. 2004;109:2507-2510.)

Key Words: brachial artery ■ endothelium ■ circadian rhythm ■ sleep

Cardiovascular events such as myocardial infarction, sudden death, and stroke have a peak incidence in the early hours after waking.1–5 The mechanisms involved in the morning increase in cardiovascular events are not clear. Blood pressure6,7 and forearm vascular resistance8 have been shown to be increased in the morning. Rapid eye movement–related increased sympathetic activation may also be especially evident in the early morning before waking.10,11 Acute increases in sympathetic activity may induce endothelial dysfunction.12

Impaired endothelial function is associated with increased risk for cardiovascular events.13–17 Furthermore, acute early morning changes in endothelial function may contribute to the circadian pattern of cardiac and vascular vulnerability.

Whether there is indeed a morning decrease in endothelial function in healthy subjects is controversial. Shaw et al18 reported increased flow-mediated vasodilation in resistance vessels in normal subjects in the morning. Kawano et al19 detected no differences in conduit vessels of healthy subjects in the morning compared with late afternoon, and Elherik et al20 observed a morning decrease in flow-mediated vasodilation as measured by laser Doppler in forearm skin.

High-resolution ultrasound of brachial artery flow–mediated dilation, well established as a noninvasive technique to evaluate endothelial function,21–23 is significantly associated with Framingham risk factor score23 and provides an index of future cardiovascular risk.15–17 We tested the hypothesis that endothelial function is altered in the early morning around the time of waking compared with measurements obtained either before sleep or later in the day.

Methods

The study was approved by the Institutional Review Board of the Mayo Clinic. All subjects signed an informed written consent to participate. We studied 30 healthy normal volunteers (19 men, 11 women, mean age, 41.6 years; mean body mass index, 28.4 kg/m²) recruited from the general population. All subjects had no history of cardiovascular or other disease and were not taking any medication. None of the subjects had a history of smoking, except for 1 woman who had quit smoking 1 month before the study.

Vascular Studies

All subjects were asked to abstain from the use of alcohol and caffeine for 24 hours before the study. Studies were performed in patients in the supine position in a quiet, temperature-controlled (24°C to 27°C) room at the General Clinical Research Center. Subjects underwent complete overnight polysomnography to ensure
adequate sleep quality and to exclude obstructive sleep apnea or other sleep pathology. Subjects were awakened at 6 AM.

Endothelial function was evaluated by ultrasound measures of flow-mediated endothelium-dependent vasodilation (FMD) and endothelium-independent vasodilation (NFMD) of the brachial artery. FMD and NFMD were measured on 3 occasions: in the evening (9 PM) after 4 hours of fasting, in the early morning (6 AM) after waking and before breakfast, and in the late morning 4 hours after breakfast (11 AM). Blood samples for norepinephrine concentration were also taken on the 3 occasions and analyzed by high-performance liquid chromatography.

The right brachial artery images were acquired above the antecubital fossa in the longitudinal plane of the artery with an Acuson ultrasound machine (Acuson Computed Sonography) with a 6-MHz linear transducer. When the first baseline images were obtained, the skin was marked so that the artery could be scanned at the same place for all 3 observations. A continuous ECG was recorded for timing diastole. Subjects rested undisturbed in the supine position for 10 minutes before each observation period.

Reactive hyperemia (FMD) was induced by inflation of a blood pressure cuff around the forearm to 200 mm Hg for 5 minutes and then released. The diameter of the brachial artery was assessed 60 to 90 seconds after deflation of the cuff. Thereafter, a 10-minute period was allowed for recovery of the vessel, after which a second baseline image of the brachial artery was obtained. A sublingual dose of nitroglycerin tablet (0.4 mg) was administered, and the brachial artery dilation response (NFMD) was assessed by imaging the artery continuously for 4 minutes. The images for measurement of diameter were obtained between the third and fourth minutes of recording. All measurements, acquired by 3 experienced investigators, were stored digitally and recorded on VHS tape.

Diameter Measurements
Brachial artery diameter was measured at the onset of the R wave, and an average of 5 measurements from 3 cardiac cycles was used. Measurements were made manually offline on a workstation by 2 blinded investigators. The intraobserver reproducibility was 95%, and the interobserver reproducibility was 93%.

Statistical Analysis
The percent changes in FMD and NFMD were compared for the 3 different time points. Values are expressed as mean±SE. Baseline measurements obtained during the 3 different observations were compared by use of ANOVA. The percent variations of diameter among the 3 observations were compared by use of MANOVA. For nonparametric analysis, Wilcoxon’s rank test was used. Data were analyzed with JMP software (Statistical Discovery Software from SAS). A value of P<0.05 was considered significant.

Results
Subject characteristics are shown in Table 1. All subjects were free of obstructive sleep apnea and had good sleep efficiency of 84±2%. Vascular, hemodynamic, and humoral measurements obtained at the 3 different time points are shown in Table 2.

FMD Findings
FMD at 6 AM was markedly decreased compared with the measurements at 9 PM and 11 AM (9 PM, 7.5±1%; 6 AM, 4.4±0.7%; 11 AM, 7.7±1%; P=0.02) (the Figure).

The trend toward decreased endothelial function was present in the morning in both men and women. In women, brachial artery dilation was 7.4±0.9% at 9 PM, decreasing to 5.0±1.3% at 6 AM (P=0.07) and recovering to 8.1±1.6% at 11 AM (P=0.11). The findings were similar in men, with

### TABLE 1. Clinical Characteristics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Subjects (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, M/F, n</td>
<td>19/11</td>
</tr>
<tr>
<td>Age, y</td>
<td>41.6±2.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.4±0.9</td>
</tr>
<tr>
<td>BMI events/h</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL*</td>
<td>177.4±9.1</td>
</tr>
<tr>
<td>HDL, mg/dL*</td>
<td>39.3±2.2</td>
</tr>
<tr>
<td>Triglycerides, mg/dL*</td>
<td>155±33.6</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL*</td>
<td>94±1.3</td>
</tr>
</tbody>
</table>

*Measured in 25 patients. BMI indicates body mass index; AHI, apnea-hypopnea index.

### TABLE 2. Vascular, Hemodynamic, and Humoral Changes Through the Night and Morning in All Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>9 PM</th>
<th>6 AM</th>
<th>11 AM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP, mm Hg</td>
<td>121±2.2</td>
<td>119±2.3</td>
<td>119±2.1</td>
<td>0.49</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>75±1.4</td>
<td>75±2.1</td>
<td>75±1.9</td>
<td>0.21</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>64±1.8</td>
<td>63±2.0</td>
<td>63±2.5</td>
<td>0.95</td>
</tr>
<tr>
<td>Norepinephrine, pg/mL</td>
<td>241±29</td>
<td>213±25</td>
<td>246±25</td>
<td>0.62</td>
</tr>
</tbody>
</table>

DBA indicates diameter of brachial artery; SBP, systolic blood pressure; DBP, diastolic blood pressure; and HR, heart rate.

Percent changes of brachial artery FMD and NFMD in 30 healthy control subjects. FMD: P=0.02 for repeated measures by MANOVA, P=0.006 between 9 PM and 6 AM, and P=0.007 between 11 AM and 6 AM. NFMD: P=0.61 for repeated measures by MANOVA. Data are mean±SEM.
brachial artery dilation decreasing from 7.5±1.4% at 9 PM to 4.0±0.8% at 6 AM (P=0.03) and improving to 7.4±1.2 at 11 AM (P=0.01). There were no differences in changes from 9 PM to 6 AM and to 11 AM between men and women.

**NFMD Findings**
Percent changes in brachial artery diameter after nitroglycerin (NFMD) were similar at the 3 observations (9 PM, 17.3±1.6%; 6 AM, 17.2±1.3%; 11 AM, 18.5±1.7%; P=0.61) (the Figure). Findings were very similar in both women (9 PM, 21.4±2.4%; 6 AM, 19.0±2.5%; 11 AM, 21.2±2.5%; P=0.6) and men (9 PM, 15.5±1.8%; 6 AM, 15.6±1.6%; 11 AM, 16.8±2.2%; P=0.7).

**Discussion**
The novel and important finding of the present study is the blunted brachial artery endothelial-dependent vasodilation but preservation of endothelial-independent vasodilation in the early morning. The magnitude of attenuation in endothelial function in the morning in healthy normal subjects is very similar to the attenuation of brachial artery reactivity noted in smokers and in diabetics compared with healthy control subjects. These findings may have implications for our understanding of the increased incidence of cardiac and vascular events in the morning hours.

The peak incidence in cardiac events is noted to occur between 6 AM and 11 AM in many studies. Integrity of endothelial function is important to multiple homeostatic mechanisms that influence cardiovascular risk. These include control of vascular tone, platelet aggregation, and fibrinolysis. Blunted endothelial function in the early morning hours may predispose to enhanced vasoconstriction and intravascular thrombosis, especially in the setting of increased sympathetic activation and heightened α-receptor sensitivity in the early morning.

Any interrelationship between endothelial function and cardiac event peak would be affected by a number of other considerations, including the time of awakening. Another important consideration is that the pathological processes linking decreased endothelial function to a cardiovascular event are not instantaneous but probably are gradual and complex, perhaps extending over several hours and eventually leading to overt ischemia. Thus, the presence of decreased endothelial function at 6 AM may be linked with the beginning of the cardiovascular event surge. Furthermore, the progressive nature of the development of a coronary thrombosis, which may persist for hours before progressing to infarction, speaks to the likelihood that reduced endothelial function could contribute to the initiation of a process that may present several hours later in the morning.

Our data contrast with earlier work showing either no change or improved endothelial function in the morning. However, our study is consistent with data showing attenuated skin vasodilation in the early morning hours. Nonetheless, changes in skin vascular physiology cannot be easily extrapolated to an understanding of the systemic circulation. Indeed, in many conditions, the responses of the skin vasculature are directly opposite those noted in the muscle and other vascular beds. Thus, the physiology and pathology of skin blood vessels differ strikingly from other vascular beds, and the implications of changes in the regulation of skin blood vessels for understanding cardiovascular pathophysiology may be limited. Furthermore, although there is compelling evidence linking brachial artery endothelial dysfunction to disease states and to poorer cardiovascular prognosis, little is known about the interaction between skin vasodilation and either cardiovascular disease or prognosis.

One strength of the present study is the polysomnographic confirmation of nighttime sleep quality and the absence of sleep apnea or other sleep disorders. No prior studies have included polysomnographic evaluation. Occult sleep apnea may influence measures of vascular and endothelial function, particularly in the early morning after a night of sleep apnea independently of any true physiological fluctuation in vasoregulation. Another strength of this study is that all subjects were healthy and on no medications. Thus, we excluded the possibility that overnight abstinence from vasodilating medication could have elicited an artifactual impairment of morning measures of endothelial function. Additionally, our subjects remained in the laboratory throughout the course of all 3 measurements, limiting the effects of external variables on these measures, allowing endothelial function to be measured consistently around the time of waking from sleep, and allowing confirmation of good sleep quality. Inadequate sleep may also have consequences for circulatory control that, in the absence of measurements of sleep quality, could be misinterpreted as changes secondary to morning fluctuations in cardiovascular physiology. Finally, the use of nitroglycerin-induced dilation as an internal control makes more robust our findings of a selective impairment of FMD but not NFMD.

A limitation of our study is that we cannot identify the specific mechanisms responsible for the morning-related endothelial impairment. Potential candidates may include increases in sympathetic activation and heightened adrenergic receptor sensitivity. Insofar as plasma norepinephrine levels are able to detect changes in sympathetic activity, and in agreement with data from Schoff et al., we did not observe any increase in norepinephrine, blood pressure, or heart rate in the morning. A further limitation relates to data from Hjalmarson et al., who observed a lower second peak in the incidence of myocardial infarction in patients with congestive heart failure, those with non-Q-wave infarction, smokers, and diabetics. Our study was not designed to address this secondary peak, and we cannot draw inferences regarding the potential mechanism involved.

In conclusion, we have noted a significant reduction in morning levels of endothelium-mediated vasodilation in healthy subjects. This morning decrease in flow-mediated vasodilation needs to be recognized in clinical studies of endothelial function so that comparisons of measurements are performed at approximately the same time of day. The morning-related attenuation of endothelial function may also have implications for our understanding of the morning peak in cardiac and vascular events.
Acknowledgments
M.E.O., A.S., and V.S. are supported by NIH grants HL-65176, HL-65176, HL-70602, HL-73211–01, and M01-RR00585. M.E.O. was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação E. J. Zerbini (InCor), São Paulo Brazil. We appreciate the expert secretarial assistance of Debra Pfeifer in preparing this manuscript.

References
35. Early effects of tissue-type plasminogen activator added to conventional therapy on the culprit coronary lesion in patients presenting with ischemic cardiac pain at rest: results of the Thrombolysis in Myocardial Ischemia (TIMI IIIa) Trial. Circulation. 1993;87:38–52.
Early Morning Attenuation of Endothelial Function in Healthy Humans
Maria E. Otto, Anna Svatikova, Rodrigo Bellio de Mattos Barretto, Simone Santos, Michal Hoffmann, Bijoy Khandheria and Virend Somers

_Circulation._ 2004;109:2507-2510; originally published online May 10, 2004;
doi: 10.1161/01.CIR.0000128207.26863.C4
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/21/2507

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/