Circadian Rhythms of Frequency Domain Measures of Heart Rate Variability in Healthy Subjects and Patients With Coronary Artery Disease

Effects of Arousal and Upright Posture

Heikki V. Huikuri, MD; Matti J. Niemelä, MD; Sirkku Ojala, MD; Asko Rantalä, MD; Markku J. Ikkäimo, MD; K.E. Juhani Airaksinen, MD

**Background** Altered neural regulation of the cardiovascular system may be an important factor for various manifestations of ischemic heart disease. This research was designed to compare the circadian rhythm of cardiac neural regulation and autonomic responses to arousal and upright posture between patients with uncomplicated coronary artery disease (CAD) and age-matched subjects with no evidence of heart disease.

**Methods and Results** Twenty-four-hour heart rate variability (HRV) in the frequency domain was analyzed in 20 male patients (mean age, 52±7 years) with angiographic evidence of CAD without prior myocardial infarction and in 20 healthy men (mean age, 51±8 years) with no clinical, echocardiographic, or exercise ECG evidence of heart disease. None of the 24-hour average frequency-domain components of HRV differed significantly between the two groups. Healthy subjects had a significant circadian rhythm of normalized units of high-frequency (HF) power of HRV with higher values during sleep. Normalized units of low-frequency (LF) power and the LF/HF ratio also showed a significant circadian rhythm in healthy subjects, with higher values during the daytime. No significant circadian rhythms in any of the normalized spectral components of HRV were observed in patients with CAD, and the night-day difference in LF/HF ratio was smaller in the patients with CAD than in the healthy subjects (0.5±1.4 versus 1.8±0.7, P<.001). Awakening when in the supine position resulted in a significant increase in the LF/HF ratio (P<.01) in the healthy subjects, but no significant changes in HRV were observed after awakening in patients with CAD. Assumption of upright position resulted in a comparable decrease in the components of HRV between the groups.

**Conclusions** The circadian rhythm of cardiac neural regulation is altered in patients with uncomplicated CAD. Reduced autonomic responses to sleep-wake rhythm suggest that the modulation of cardiac autonomic function by stimuli from the central nervous system is impaired in CAD. (*Circulation.* 1994;90:121-126.)

**Key Words** • circadian rhythm • ischemia

Clinical and experimental studies provide strong evidence of the importance of cardial autonomic regulation for the pathophysiology of various manifestations of ischemic heart disease.1-3 Autonomic nervous control of the cardiovascular system has a distinct circadian rhythm.4-8 and this may be an important mechanism underlying the diurnal distribution of cardiac events such as myocardial ischemia, myocardial infarction, and cardiac death.9

Analysis of heart rate variability (HRV) in the frequency domain is a novel method of studying cardiovascular neural regulation.10,11 Decrease of HRV is frequently associated with coronary artery disease (CAD).3,5,12-15 and the degree of this impairment is reported to be a predictor of mortality in such patients.16,17 Little information is available, however, on possible alterations in the diurnal rhythm of cardiac autonomic regulation in patients with uncomplicated CAD. This research was designed to compare the circadian rhythms of different frequency-domain measures of HRV between patients with CAD but without any prior myocardial infarction and age-matched subjects with no evidence of heart disease. Temporal responses of HRV to awakening and upright posture were also compared to estimate the relative significance of different exogenous stimuli with respect to diurnal fluctuations in cardiac autonomic function.

**Methods**

**Patient Series**

The series consisted of 20 male patients (mean age, 52±8 years) with a history of stable angina pectoris without prior myocardial infarction who had been referred for angiographic examination. Ten patients had New York Heart Association class III angina pectoris, and 10 had class II. Five patients had three-vessel CAD, 8 had two-vessel CAD, and 7 had one-vessel disease by coronary angiography. The mean ejection fraction was 71±7%, and none of the patients had significant left ventricular wall motion abnormalities. β-Blocking therapy had been withdrawn at least 8 days earlier in all the patients. Eighteen patients were taking long-acting nitrates, 2 patients diltiazem, and 2 nifedipine during the investigation. No other medication was allowed except for sublingual nitroglycerin. No diabetic patients were included.
A similar number of age-matched healthy male subjects (mean age, 51.7 ± 7 years) were selected from among individuals who were participating in a larger trial comparing the characteristics of hypertensive and normotensive subjects, the latter group having been randomly selected from the general population of Oulu on the basis of their social security numbers. They all had a complete physical examination and medical history that revealed no cardiovascular disease or medication. They also had normal blood pressure; 12-lead ECG; M-mode, two-dimensional, and Doppler echocardiography; and 2-hour glucose tolerance test, and none had evidence of ischemic ST segment depression in exercise ECG performed in the form of a symptom-limited bicycle exercise test.

Procedures

All the subjects underwent 24-hour ambulatory ECG during normal daily activities with their normal sleep-wake rhythm but were asked to remain supine in bed for at least 30 minutes after awakening. The two-channel recordings were analyzed with a Delmar Avionics scanner. Left-sided catheterization was performed on the patients with angiography by Judkins’ technique within 2 months of the ambulatory ECG recordings. Left ventricular cineangiograms were performed in a 45° right anterior oblique position, and the ejection fraction was calculated by a single-plane area-length method. Coronary artery stenoses with >50% luminal narrowing were considered significant.

Exercise ECG was performed in the form of a symptom-limited bicycle exercise test, the work load being increased progressively by 15 W/min. A >0.1-mV ST segment depression at 0.08 seconds after the J-point was taken as a sign of ischemia.

A Hewlett-Packard 77020A ultrasound color Doppler system was used for M-mode, two-dimensional, and Doppler echocardiographic recordings using standard techniques, and these were analyzed by a method described previously.19,20

Analysis of HRV

The ECG data were sampled digitally and transferred from the Delmar Avionics scanner to a microcomputer for HRV analysis. A linear detrend was applied to the RR interval data segments of 512 samples to make them more stationary. This was implemented by first fitting a straight line to each segment by the standard least-squares method and then subtracting it from the sample value. The RR interval series was passed through a filter that eliminates unwanted premature beats and noise and fills the resulting gaps with an average value computed in the immediate neighborhood. An RR interval is interpreted as a premature beat if it deviates from the previous qualified interval value by more than a given tolerance level (eg, 30%), which is a programmable parameter dependent on the prematurity index of ectopic beats for each patient. The details of this filtering technique have been described previously.21,22 Only segments with >90% qualified beats were included in the analysis.

An autoregressive model was used to estimate the power spectrum densities of the RR interval variability.23,24 Size 10 was used for the order of the model in analysis of the RR data. The computer program automatically calculates the autoregressive coefficients to define the power spectrum density. Power spectra were quantified by measurement of the area in three frequency bands: total power <0.4 Hz, high-frequency (HF) power from 0.15 to 0.40 Hz, and low-frequency (LF) power from 0.04 to 0.15 Hz. The various spectral components were calculated (1) as absolute units (equal to area under the curves for the spectral densities) and (2) as normalized units by dividing the power of the LF and HF components by the total power, from which the <0.04 Hz had been subtracted, and multiplying by 100. The ratios between the LF and HF spectra in fractional units were also analyzed. The SD of successive RR intervals was also calculated. The average 1-hour and 24-hour RR intervals, SD of RR intervals, and the power spectrum components of HRV were calculated from segments of 512 RR, and the average 30-minute values were calculated both before and after awakening in the supine position and in the upright position immediately after getting up. The mean values for the sleeping hours (midnight to 6 AM), awake hours (9 AM to 9 PM), and total 24-hour period were calculated for each subject.

Statistics

Because of the skewness of the data, logarithmic transformation was performed on the absolute units of the spectral components of HRV, and the resulting logarithmic values and normalized units were compared between the groups by a standard t test. Night-day differences and changes in the data before and after awakening and before and after getting up were compared by a paired t test. P < .05 was considered significant.

The chronobiological analysis of the mean hourly values of parameters of HRV was made by single cosiner method, in which a least-squares procedure is used and a cosine function is fitted to the data. Three parameters characterize this model: mesor, a rhythm-determined average; amplitude, one-half difference between the highest and lowest values in a rhythm defined by the cosine function; and acrophase, the lag from a reference time point (midnight) and time of the highest value in the cosine model fitted to the data. The 95% confidence interval of the mesor and amplitude of parameters of HRV were calculated for both groups.

Results

Circadian Rhythm of HRV

None of the 24-hour average measures of HRV differed significantly between subjects with and without CAD, although there was a trend toward higher values in the healthy subjects (Table 1). The daytime values for all the spectral components were similar between the groups, but the HF component during the sleeping hours was significantly lower in the CAD patients.

Figs 1 and 2 present the 24-hour circadian rhythms of the HF and LF spectral components of HRV analyzed in normalized units. The HF spectral component had a significant circadian rhythm in healthy subjects, with higher values during sleep. The circadian rhythm of the HF component was blunted in the patients with CAD compared with healthy subjects, so the magnitude of its night-day difference was smaller (P < .01) (Fig 1). Normalized units of LF power of HRV also showed a significant circadian rhythm in healthy subjects, with higher values during the daytime, but no significant circadian fluctuations in LF components were observed in the patients with CAD (Fig 2). The healthy subjects also had a significant night-day difference in the LF/HF ratio, which was not observed in the CAD patients (Fig 3, Table 1).

Single cosiner analysis of the circadian rhythms of the components of HRV revealed that healthy subjects had a significant circadian rhythm of the normalized units of HF spectral component (amplitude 12, 95% confidence intervals from 6 to 18) and LF spectral component (amplitude 25, 95% confidence intervals from 10 to 40) and of the LF/HF ratio (amplitude 1.2, 95% confidence intervals from 0.9 to 1.5). None of the spectral measures of HRV showed significant circadian rhythms in patients with CAD.
TABLE 1. Frequency Domain Measures of Heart Rate Variability

<table>
<thead>
<tr>
<th></th>
<th>CAD− (n=20)</th>
<th>CAD+ (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR interval, ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-Hour</td>
<td>856±94</td>
<td>876±114</td>
</tr>
<tr>
<td>Night (midnight to 6 AM)</td>
<td>990±110</td>
<td>963±89</td>
</tr>
<tr>
<td>Night-day difference</td>
<td>227±82*</td>
<td>151±75†</td>
</tr>
<tr>
<td>SD of RR intervals, ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-Hour</td>
<td>76±15</td>
<td>75±17</td>
</tr>
<tr>
<td>Night</td>
<td>91±26</td>
<td>82±22</td>
</tr>
<tr>
<td>Night-day difference</td>
<td>25±22*</td>
<td>13±19†</td>
</tr>
<tr>
<td>Total power, ms²×10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-Hour</td>
<td>445±184</td>
<td>427±253</td>
</tr>
<tr>
<td>Night</td>
<td>631±366</td>
<td>512±297</td>
</tr>
<tr>
<td>Day</td>
<td>348±60</td>
<td>363±263</td>
</tr>
<tr>
<td>Night-day difference</td>
<td>289±325†</td>
<td>154±240‡</td>
</tr>
<tr>
<td>HF power</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-Hour, ms²×10</td>
<td>44±35</td>
<td>36±25</td>
</tr>
<tr>
<td>Normalized units</td>
<td>30±24</td>
<td>30±20</td>
</tr>
<tr>
<td>Night (midnight to 6 AM), ms²×10</td>
<td>82±70</td>
<td>48±33§</td>
</tr>
<tr>
<td>Normalized units</td>
<td>37±24</td>
<td>31±23</td>
</tr>
<tr>
<td>Day (9 AM to 9 PM), ms²×10</td>
<td>25±23</td>
<td>28±23</td>
</tr>
<tr>
<td>Normalized units</td>
<td>21±19</td>
<td>26±23</td>
</tr>
<tr>
<td>Night-day difference, ms²×10</td>
<td>56±56‡</td>
<td>20±27§</td>
</tr>
<tr>
<td>Normalized units</td>
<td>16±14‡</td>
<td>5±12§</td>
</tr>
<tr>
<td>LF power</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-Hour, ms²×10</td>
<td>91±55</td>
<td>77±54</td>
</tr>
<tr>
<td>Normalized units</td>
<td>67±50</td>
<td>69±51</td>
</tr>
<tr>
<td>Night, ms²×10</td>
<td>135±100</td>
<td>93±74</td>
</tr>
<tr>
<td>Normalized units</td>
<td>61±50</td>
<td>66±50</td>
</tr>
<tr>
<td>Day, ms²×10</td>
<td>69±51</td>
<td>67±51</td>
</tr>
<tr>
<td>Normalized units</td>
<td>70±48</td>
<td>69±65</td>
</tr>
<tr>
<td>Night-day difference, ms²×10</td>
<td>66±75‡</td>
<td>26±55§</td>
</tr>
<tr>
<td>Normalized units</td>
<td>11±26‡</td>
<td>2±28§</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-Hour</td>
<td>3.1±1.1</td>
<td>2.8±1.4</td>
</tr>
<tr>
<td>Night</td>
<td>2.2±1.1</td>
<td>2.6±1.6</td>
</tr>
<tr>
<td>Day</td>
<td>3.9±1.7</td>
<td>3.0±1.6</td>
</tr>
<tr>
<td>Night-day difference</td>
<td>−1.8±0.7§</td>
<td>−0.5±1.4‡</td>
</tr>
</tbody>
</table>

*P<.001; †P<.01; ‡P<.05 between night and day. §P<.01; ¶P<.001 between patients with and without coronary artery disease.

Effects of Awakening and Upright Posture

In healthy subjects, awakening resulted in a significant shortening of the average RR interval, a decrease in the HF spectral component, and an increase in the LF component analyzed in normalized units (Table 2). Awakening did not cause any significant changes in the heart rate or in any of the spectral components of HRV in the patients with CAD. Likewise, the LF/HF ratio increased after awakening in the healthy subjects but not in the patients.

Habitual physical activity in the upright position resulted in a significant decrease in the average RR intervals and HF spectral components in both groups (Table 2), with a significant increase in the LF/HF ratio.

Effects of Severity of Ischemic Heart Disease

Angiographic severity, ie, one-vessel, two-vessel, or three-vessel CAD or functional class of the patients had no significant influences on the 24-hour average values of different components of HRV or their circadian rhythm, nor did measures of HRV or their circadian rhythm differ significantly between the 12 CAD patients with severe ischemia during exercise test (>2-mm ST segment depression) and those 8 patients with <2-mm ST segment depression.

Fig 1. Graph showing circadian rhythm of high-frequency (HF) component of heart rate variability analyzed in normalized units in healthy subjects (CAD−) and patients with coronary artery disease (CAD+).

Fig 2. Graph showing circadian rhythm of low-frequency (LF) component of heart rate variability analyzed in normalized units in healthy subjects (CAD−) and patients with coronary artery disease (CAD+).
Discussion

Methodological Considerations

There have been some differences in the methodologies used previously for calculating the frequency-domain components of HRV. Some authors report the area under the frequency-domain bands in absolute units,\(^{11,15,25}\) others report the components in normalized units by dividing the frequency bands by the difference between the total power and the DC component,\(^ {10,26,27}\) and some consider the effects of the variation in the RR interval on each component and divide the square roots of the components by the mean RR interval.\(^ {4,5}\) In general, absolute units of spectral components of HRV can give important prognostic information in postinfarction patients,\(^ {25}\) but normalized units may give more relevant physiological information on temporal reciprocal changes in cardiac autonomic control.\(^ {10,25,27}\) Therefore, both the absolute units and normalized units were used in this study.

There is general agreement that the power of HF oscillations, which are related to respiration, provides a marker of efferent vagal input to the heart.\(^ {10,11,28}\) LF power calculated in normalized units reflects most closely fluctuations in sympathetic tone,\(^ {10,16,27}\) and LF/HF ratio is a marker of sympathovagal balance.\(^ {10,27}\) Total variance of RR intervals is closely related to absolute units of different power spectral components and reflects probably the level of overall autonomic and perhaps renin-angiotensin control of heart rate.\(^ {28}\)

Differences in Neural Regulation Between Patients With Coronary Artery Disease and Healthy Subjects

HF and LF components of HRV had significant circadian rhythms in the healthy subjects. Earlier studies on normal subjects, hypertensive patients, and post-infarction patients have demonstrated similar circadian fluctuations of the spectral components of HRV.\(^ {29-31}\) There were quantitative differences in the amplitude of the circadian rhythms of the power spectral components between the patients with CAD and the healthy subjects. The amplitude of circadian fluctuation in the HF spectral component was blunted in the patients with CAD, suggesting that the vagal responses to endogenous or exogenous stimuli are reduced in CAD. The healthy subjects also showed a circadian rhythm in the LF/HF ratio, which reflects a balance between the sympathetic and vagal tone, but this rhythm was blunted in the patients with CAD. The circadian rhythm of normalized power of the LF component was also blunted in the patients with CAD, suggesting that the diurnal fluctuation of both vagal and sympathetic activity is altered in CAD. Previous studies have demonstrated similar reductions in the amplitudes of circadian rhythm of LF power and LF/HF ratio in hypertensive and diabetic patients compared with healthy subjects.\(^ {32,33}\) Lombardi et al.\(^ {19}\) also reported similar results in post-myocardial infarction patients. However, the LF component and LF/HF ratio were greater in patients after recent myocardial infarction than in control subjects, suggesting a shift toward sympathetic excitatory and a reduced vagal tone. It can therefore be hypothesized that in patients with CAD but without recent myocardial infarction, the alteration of neural regulatory mechanisms is reflected mainly by a diminished fluctuation of spectral indexes of neural modulation.

Previous research has demonstrated that the HRV is reduced in patients with ischemic heart disease,\(^ {4,5,12-16}\) although the series concerned have included mixed groups of patients with and without myocardial infarction who have not necessarily been matched in terms of the age with the control subjects. Chronic myocardial infarction causes destruction of the ventricular neural receptors, which results in altered autonomic regulation,\(^ {34}\) and aging has significant effects on vagal heart rate control.\(^ {31,35}\) In addition, previous recordings have been performed on hospitalized patients, which may also alter the magnitude of the normal diurnal rhythm of autonomic tone.

There may be several reasons for the impaired fluctuation in cardiac autonomic tone observed in CAD.

| TABLE 2. Effects of Awakening and Upright Posture on Heart Rate Variability |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| CAD— (n=20)                     | Asleep          | After Awakening | Upright         | CAD+ (n=20)     | Asleep          | After Awakening | Upright         |
| RR interval, ms                 | 996±100         | 942±101‡        | 787±105*        | 999±146         | 976±154         | 797±105*        |
| HF power, NU                    | 39±35           | 28±26§          | 23±18           | 35±60           | 30±71           | 25±66           |
| LF power, NU                    | 59±41           | 71±51§          | 76±48           | 63±60           | 68±71           | 74±66           |
| LF/HF ratio                     | 2.1±1.5         | 2.8±1.5§        | 4.0±2.5‡        | 2.2±1.5         | 2.6±1.6         | 3.3±1.9‡        |

CAD indicates coronary artery disease; HF, high frequency; NU, normalized units; and LF, low frequency.

\(^*P<.01; \, ‡P<.05\) between supine and upright posture. \(^*P<.01\) and \(^§P<.05\) between asleep and awake.
Physical inactivity may reduce the amplitude of diurnal autonomic regulation, but the present differences were evident only during sleep and therefore cannot be explained by physical inactivity. Conversely, impaired vagal cardiac regulation may be a result of long-term restriction of physical activity. Recent results suggest that HRV is reduced in sedentary subjects relative to physically active ones. Cardiac medication may also alter cardiac autonomic control. Block-4}ing therapy had been discontinued in the present patients with CAD, and only 4 patients were receiving calcium antagonist therapy, which in any case has not been observed to influence vagal tone. The effects of nitrates have not been extensively studied in this respect, but there is no evidence of any connection with cardiac autonomic tone. Thus, it is apparent that the impaired autonomic responses to sleep-wake rhythm are typically related to CAD itself.

**Effects of Awakening and Upright Posture**

Awakening without changes in body posture resulted in significant reduction in the HF oscillations in healthy subjects but not in the CAD patients. The LF/HF ratio also increased in the healthy subjects after awakening, but not in the CAD patients. Similar changes in sympathovagal balance after awakening have also been demonstrated in hypertensive patients without CAD. On the other hand, upright position resulted in similar changes in cardiac autonomic control in subjects with and without CAD, suggesting that autonomic responses to changes in central stimuli are impaired but responses to changes in body posture and habitual physical exercise are normal in patients with CAD. Concurrently with these findings, the autonomic responses to passive tilt have been shown to be normal in patients with a remote myocardial infarction, whereas these responses are blunted in the early postinfarction phase.

**Implications**

These results show that subtle changes in the frequency-domain measures of HRV, especially in the HF component, can be best detected from recordings performed during the sleeping hours. The autonomic responses to the sleep-wake rhythm appeared to be impaired in the patients with CAD, suggesting altered autonomic regulation of the cardiovascular system in response to changes in central stimuli. Defective reflex autonomic responses to awakening in the patients with CAD do not exclude larger neurohumoral responses, i.e., increases in circulating catecholamine levels, which may not have significant influences on measures of HRV but may trigger the temporal onset of cardiovascular events in the morning hours.

**Acknowledgments**

This study was supported by grants from the Finnish Foundation for Cardiovascular Research and the Medical Council of the Academy of Finland, Helsinki. The authors appreciate the technical expertise of Päivi Karjalainen, RN.

**References**

25. Bigger JT, Fleiss J, Steinman RC, Rolnitzky LM, Kleger RE, Rottman JN. Frequency domain measures of heart period vari-
Circadian rhythms of frequency domain measures of heart rate variability in healthy subjects and patients with coronary artery disease. Effects of arousal and upright posture.

_Circulation_. 1994;90:121-126
doi: 10.1161/01.CIR.90.1.121

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 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/90/1/121

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/