TRANSLATIONAL PHYSIOLOGY

Saturation of high-frequency oscillations of R-R intervals in healthy subjects and patients after acute myocardial infarction during ambulatory conditions

Antti M. Kiviniemi,1,2 Arto J. Hautala,1,2 Tapio Seppänen,3 Timo H. Mäkikallio,2 Heikki V. Huikuri,2 and Mikko P. Tulppo1,2

1Merikoski Rehabilitation and Research Center, 2Division of Cardiology, Department of Medicine; and 3Department of Electrical and Information Engineering, University of Oulu, FIN-90101 Oulu, Finland

Submitted 10 May 2004; accepted in final form 6 July 2004

Kiviniemi, Antti M., Arto J. Hautala, Tapio Seppänen, Timo H. Mäkikallio, Heikki V. Huikuri, and Mikko P. Tulppo. Saturation of high-frequency oscillations of R-R intervals in healthy subjects and patients after acute myocardial infarction during ambulatory conditions. Am J Physiol Heart Circ Physiol 287: H1921–H1927, 2004.—This study was designed to assess the relationship between R-R interval length and heart rate (HR) variability in healthy subjects and patients after an acute myocardial infarction (AMI). Twenty-four-hour ambulatory ECG recordings were obtained for 76 healthy subjects and 82 post-AMI patients. The high-frequency (HF, 0.15–0.4 Hz) spectral power of R-R intervals was analyzed in 5-min sequences over 24 h and plotted as a function of the corresponding mean R-R interval length. Quadratic regression model was used to study the relationship between R-R interval length and HF power. If a distinct deflection point (R0) occurred in the quadratic regression (r > 0.50) model before maximum R-R interval, indicating the plateau of HF power, the relationship between R-R interval and HF power was defined as saturated. Otherwise, the relationship was defined as linear (r > 0.50) or low correlated (r > 0.50). The relationship was saturated in 35, linear in 38, and low correlated in 3 healthy subjects. In post-AMI patients, the relationship was saturated in 9 subjects, linear in 44 subjects, and low correlated in 29 patients. The HF power analyzed from the 24-h period did not differ between the saturated and linear groups, but when analyzed from the linear portion only, HF spectral power was smaller in the linear than the saturated group both among groups, but when analyzed from the linear portion only, HF spectral power was smaller in the linear than the saturated group both among healthy subjects (P < 0.05) and post-AMI patients (P < 0.05). Saturation of the HF oscillations of R-R intervals is a common phenomenon in healthy subjects and also present in post-AMI patients during ambulatory conditions. This saturation effect may bias the quantification of cardiac vagal function when HR variability is analyzed from Holter recordings.

Analysis of heart rate (HR) variability from 24-h ambulatory recordings is a widely used noninvasive tool in the assessment of autonomic regulation in various physiological and clinical settings (13). The ability of HR variability, particularly the measurement of high-frequency (HF, 0.15–0.4 Hz) oscillations of HR, to quantify vagal outflow to the sinus node has been documented in several previous studies (2, 11, 22). However, pharmacological modulation of autonomic regulation has revealed a dissociation between vagal activity and HR variability, particularly at low HR levels (5, 8, 28). In these experimental settings, the relationship between the magnitude of HR variability and R-R interval length has been suggested to be quadratic rather than linear (9). It has also been proposed that HR variability is saturated and may not reveal the changes in vagal activity at HR levels <50 beats/min (7, 9). Despite these experimental findings obtained in laboratory conditions, it is unknown whether similar saturation occurs during free-running ambulatory conditions.

This study was designed to assess the relationship between R-R interval length and HR variability in healthy subjects and patients after an acute myocardial infarction (AMI). We hypothesized that saturation of HR variability is detectable at low HR levels during ambulatory conditions.

METHODS

Subjects. Healthy adults (n = 83) were recruited by advertising in a newspaper. All subjects were nonsmokers and without any medication or cardiovascular disorders (Table 1). Subjects with higher body mass index [BMI = weight (kg)/height^2 (m), kg/m^2] than 30 kg/m^2 were excluded. A consecutive series of patients with a recent AMI (n = 82, Table 2) were drawn from a population described earlier (14). All post-AMI patients were under steady-state β-blocking medication. The protocol was approved by the ethics committee of the University of Oulu, and all subjects gave their written informed consent.

Protocol. Healthy subjects underwent an ambulatory 24-h two-channel Holter recording during a nonexercise day (Oxford Medilog 4500, Oxford Medical). R-R intervals were simultaneously measured by a digital R-R recorder (Polar Electro R-R recorder; Kempele, Finland) at an accuracy of 1 ms (24). In post-AMI patients, ambulatory 24-h R-R intervals were recorded between days 3 and 14 after the AMI (Oxford Medilog 4500, Oxford Medical). The length of recording ranged from 19 to 24 h in post-AMI patients. The Holter recordings were examined by one of the authors (H. V. Huikuri), and seven subjects were excluded from further analysis due to significant changes in P-wave morphology in ECG data. R-R recordings were edited by visual inspection based on Holter recordings. Interpolation degree 0 was used to replace undesirable beats with the average of the preceding R-R intervals for more accurate HR variability analysis, as previously described by Salo et al. (25). An average of 2 ± 2% of all R-R intervals were replaced in healthy subjects and 4 ± 4% in post-AMI patients.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Table 1. Characteristics of healthy subjects divided into saturated, linear, and low-correlated groups based on the relationship between R-R interval length and HF power

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Saturated</th>
<th>Linear</th>
<th>( r &lt; 0.50 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>76</td>
<td>35</td>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td>Male</td>
<td>54</td>
<td>24</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
<td>11</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Age, yr</td>
<td>23±4</td>
<td>23±4</td>
<td>23±4</td>
<td>23±6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177±9</td>
<td>176±9</td>
<td>178±9</td>
<td>179±14</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72±12</td>
<td>70±13</td>
<td>73±10</td>
<td>76±25</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23±3</td>
<td>23±3</td>
<td>23±2</td>
<td>23±4</td>
</tr>
<tr>
<td>BP sys, mmHg</td>
<td>127±15</td>
<td>124±15</td>
<td>128±14</td>
<td>142±20</td>
</tr>
<tr>
<td>BP dia, mmHg</td>
<td>78±9</td>
<td>76±8</td>
<td>79±9</td>
<td>88±16</td>
</tr>
<tr>
<td>BRS, ms/mmHg</td>
<td>11.7±4.4</td>
<td>11.9±4.5</td>
<td>11.7±4.5</td>
<td>9.0±3.2</td>
</tr>
<tr>
<td>VO(_2) max, ml/kg·min(^{-1})</td>
<td>48±9</td>
<td>50±9</td>
<td>47±8</td>
<td>41±3</td>
</tr>
<tr>
<td>HR max, beats/min</td>
<td>191±9</td>
<td>192±9</td>
<td>191±9</td>
<td>189±13</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n \), number of subjects. HF, high frequency; BMI, body mass index; BP sys, systolic blood pressure; BP dia, diastolic blood pressure; BRS, baroreflex sensitivity; VO\(_2\) max, maximal O\(_2\) consumption; HR max, maximal heart rate.

Table 2. Characteristics of post-AMI patients divided into saturated, linear, and low-correlated groups based on the relationship between R-R interval length and HF power

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Saturated</th>
<th>Linear</th>
<th>( r &lt; 0.30 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>82</td>
<td>9</td>
<td>44</td>
<td>29</td>
</tr>
<tr>
<td>Male</td>
<td>61</td>
<td>7</td>
<td>32</td>
<td>22</td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>2</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Age, yr</td>
<td>59±11</td>
<td>54±11</td>
<td>60±11</td>
<td>60±10</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171±8</td>
<td>172±9</td>
<td>170±8</td>
<td>171±9</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>80±13</td>
<td>86±6</td>
<td>78±14</td>
<td>81±13</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27±4</td>
<td>30±4</td>
<td>27±4</td>
<td>27±4</td>
</tr>
<tr>
<td>BP sys, mmHg</td>
<td>127±19</td>
<td>116±15</td>
<td>130±18</td>
<td>126±20</td>
</tr>
<tr>
<td>BP dia, mmHg</td>
<td>82±12</td>
<td>78±15</td>
<td>83±11</td>
<td>82±13</td>
</tr>
<tr>
<td>BRS, ms/mmHg</td>
<td>9.9±7.5</td>
<td>15.4±8.5</td>
<td>10.7±8.2</td>
<td>6.8±4.2*</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>46±2</td>
<td>49±6</td>
<td>47±8</td>
<td>44±8</td>
</tr>
<tr>
<td>Max exercise, watts</td>
<td>110±39</td>
<td>129±29</td>
<td>107±38</td>
<td>110±39</td>
</tr>
<tr>
<td>HR max, beats/min</td>
<td>122±20</td>
<td>122±14</td>
<td>122±22</td>
<td>120±19</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n \), number of subjects. AMI, acute myocardial infarction. * \( P < 0.05 \), low-correlated group vs. both linear and saturated groups.
Fig. 1. High-frequency (HF) power of R-R intervals and R-R interval length analyzed in 5-min sequences during ambulatory recording in healthy subject (A) and post-acute myocardial infarction (AMI) patient (B). Despite a marked increase of R-R interval at night hours, corresponding HF power of R-R intervals shows no increase in both cases indicating saturated HF power.

Fig. 2. An example of saturated (A), linear (B), and low-correlated (C) behavior between R-R interval length and corresponding HF power in healthy subjects analyzed in 5-min intervals over 24-h ambulatory recording (top). Middle, location of the 5-min examples of tachograms (a–c) in the 24-h analysis (top); bottom, corresponding R-R interval spectra.
The correlation between R-R interval length and HF power was

RESULTS

Relationship between HF power and R-R interval length. The correlation between R-R interval length and HF power was 0.81 ± 0.15 in healthy subjects and 0.57 ± 0.24 in post-AMI patients (P < 0.001 between the groups). The relationship between R-R interval length and the corresponding HF power was saturated in 35, linear in 38, and low correlated in 3 healthy subjects. In post-AMI patients, the relationship was saturated in 9, linear in 44, and low correlated in 29 cases.

Figure 3, A and B, displays the three types of regression curves (saturated, linear, and low correlated) of the relationship between R-R interval length and HF spectral power observed in healthy subjects and post-AMI patients, respectively. In the group of healthy subjects with a saturated relationship, the average deflection point R-R₀ was observed at an R-R interval length of 1,238 ± 160 ms (49 ± 6 beats/min, range 1,010–1,788 ms) and in post-AMI patients at 1,119 ± 84 ms (54 ± 4 beats/min, range 1,037–1,245 ms, P < 0.05 between the groups). The relative R-R₀ was 92 ± 5% of the maximal R-R interval length in healthy subjects and 89 ± 7% in post-AMI patients (P = not significant).

Association between the behavior of HF power and other variables. Among healthy subjects, the maximal R-R interval length was significantly longer and the long-term HR variability indexes (VLF and SDNN) were significantly higher in the saturated than the linear group (Table 3). BRS and VO₂max were also lower among those with a low correlation, but the small number of healthy subjects with a low correlation limits statistical comparisons (Table 1). No other differences were observed in the traditional HR variability measures.

Among the post-AMI patients with a low correlation between HF power and the R-R interval, BRS was lower compared with both the linear and the saturated groups, P < 0.05 for both. Exercise capacity also tended to be highest in the saturated group and lowest in the low-correlated group among the post-AMI patients (Table 2). HF, LF, and VLF power were also significantly lower among those with a low correlation compared with those with a saturated relationship (Table 4).

HF index in healthy and post-AMI patients. Among healthy subjects, the HF index was significantly higher in the saturated group compared with the linear group (Table 3). Among post-AMI patients, the HF index was significantly higher in the saturated group than in either the linear or the low-correlated group.
DISCUSSION

The present study shows that the saturation of HF power, expressed as a plateau of the HF power spectral component of HR variability regardless of the lengthening of the R-R interval, is a common phenomenon in healthy subjects and also present in post-AMI patients during “free-running” ambulatory conditions. These findings suggest that the quantification of cardiac vagal function by standard measurement of HR variability indexes from ambulatory ECG recordings may be biased, because the saturation effect underestimates the vagal outflow at low HR levels.

Measurement of cardiac vagal outflow during ambulatory conditions. Graded electrical stimulation of the vagus nerve has revealed a good linear relationship between R-R interval length and the magnitude of vagal activity in animal experiments (20). However, the human regulation pattern of HR involves continuous interaction between the vagal and sympathetic nervous activities. These antagonistic effects on R-R interval length do not ordinarily summate algebraically but rather in a complex manner (18, 23, 30), and the measurement of the average R-R interval length alone may not be the best index of vagal outflow to the sinus node. Therefore, spectral analysis of R-R interval time series was introduced as a way to study in more detail both vagal and sympathetic regulation of HR (2).

HF oscillation of R-R variability is related to respiration as it quantifies the magnitude of respiratory sinus arrhythmia (2). The magnitude of respiratory sinus arrhythmia (16) and HF power of R-R interval variability have been shown to measure vagal outflow to the heart in several studies (2, 11, 22). HF power of R-R intervals, measured from ambulatory ECG recordings, has also been widely used as an index of vagal activity in various clinical settings (13).

Dissociation between the HF power of R-R intervals and R-R interval length was first discovered during pharmacological modulation of autonomic tone (5, 8, 9, 28). The results were observed during graded infusions of phenylephrine or norepinephrine, which result in baroreflex-mediated increases in cardiac vagal activity and lengthen the R-R intervals. Dissociation was observed in the presence (9) or absence of (5, 8, 28) sympathetic blockade. In these experiments, HF power was shown to increase linearly as R-R interval increased up to the HR level ~50 beats/min in healthy subjects, whereafter a plateau or even a decrease of HF power was observed upon a further R-R interval increase (9). In the present study, too, HF power reached a plateau as a function R-R interval length in half of the healthy subjects during ambulatory conditions and also in a smaller proportion of patients with a recent AMI.

There are salient differences between the present study and the previous ones assessing the saturation effect of vagal activity. First, in the earlier studies, the sympathetic branch was blocked by propranolol (9). In the present study, no autonomic blockade was used in healthy subjects. However, all post-AMI patients with a saturation effect were on steady-state β-blocking medication, suggesting that withdrawal of sympathetic activity is not the predominant factor determining the further decrease of HR at the plateau phase of HF oscillations of HR even during ambulatory conditions. Second, enhanced vagal activity was induced via a blood pressure increase in the earlier studies (5, 9, 28), and the dissociation between R-R interval length and HF power was observed at lower HR levels than the baseline HR levels of those subjects. In the present study, the results were obtained during ambulatory 24-h recording of R-R intervals without artificial modulation of the cardiovascular system. The data show that the saturation effect also occurs during free-running conditions without an increase of blood pressure and baroreflex-mediated vagal activation. The majority of the saturation effects was observed during the sleeping hours, when blood pressure is usually low rather than high.

Behavior of HF oscillations of HR in healthy subjects. In a previous study (9), subjects with saturated dynamics between HF power and R-R intervals (n = 24) had significantly lower HR levels during baroreflex-mediated parasympathetic stimulation than subjects with linear relationship (n = 5). Concurrent with the previous findings, the saturated HF oscillation of R-R intervals was also here associated with longer maximum R-R interval length among healthy subjects. In addition to the experimental laboratory settings (9), the present results show some new findings on the relationship between R-R interval length and the HF oscillation of R-R intervals during ambulatory conditions. First, there was wide interindividual variation in the values of the deflection point (range 1,010–1,788 ms) among the subjects with a saturated HF oscillation pattern. In other words, the saturation of the HF oscillation of R-R intervals could occur at a relatively high HR level (~60 beats/min) or at a very low HR level (~40 beats/min). Second, long-term HR variability indexes over 24-h recordings were significantly higher in the saturated group compared with the linear group. Subjects with a saturated relationship also had tendency to higher aerobic capacity than other groups, which is in line with a previous study on the relationship between cardiac vagal activity and physical fitness (29). These findings together indicate higher vagal activity in the saturated group than in the linear group despite the equal HF power analyzed traditionally over 24-h R-R interval recordings.

Behavior of HF oscillations of HR in post-AMI patients. The correlation between R-R interval length and HR variability was remarkably lower in post-AMI patients compared with healthy subjects. A higher proportion of patients had a low correlation between HF power and R-R interval length, suggesting that nonautonomic mechanisms may partly contribute to the HF oscillations of HR in patients with cardiac disease. This could also mean that R-R interval length is less dependent on the vagal modulation of HR in post-AMI patients. Because of the significant difference in age between healthy subjects and post-AMI patients in the present study, it could not be separated whether age or cardiac disease itself contributed to the lower correlation between the HF oscillation of R-R interval and R-R interval length. However, in 10% of post-AMI patients, the relationship between R-R interval and HF power was defined as saturated. This was associated with higher overall HR variability and better baroreflex sensitivity, suggesting that the autonomic function is better preserved in the post-AMI patients with a saturated relationship. Post-AMI patients with a saturated relationship tended to have higher exercise capacity compared with other groups, suggesting that functional capac-
ity partly determines the saturation effect of HF power (29). Thus assessment of the relationship between R-R intervals and HF power may have practical implications even in the assessment of autonomic function from ambulatory ECG recordings among patients with heart disease.

Possible physiological mechanisms underlying saturated HF power dynamics. Goldberger et al. (9) explained their results by the dose response of the heart to the acetylcholine secreted by vagal nerve ending. The dose response to acetylcholine has been considered to be linear until its concentration reaches the level at which a further increase in acetylcholine concentration does not produce a change in the response (6). It has been suggested that rapid discharge of the vagal nerve during expiration may produce acetylcholine secretion intense enough to maintain a major parasympathetic effect even during inspiration, which results in a saturation of respiratory sinus arrhythmia and HF power (9). Another mechanism may involve a loss of phasic respiratory changes in vagal nerve discharges in the presence of pharmacologically increased blood pressure (17). From the present data, the former mechanism seems likely and the latter unlikely because the increased blood pressure does not seem to be a prerequisite for the occurrence of the saturation effect. The present data are also well in line with the human respiratory gate theory, which suggests that, at the very high levels of vagal tone, HF oscillation of R-R intervals does not increase in a linear fashion upon a further increase of vagal activity (4).

New insight into the analysis of vagal function during ambulatory conditions. Possible saturation of HF power should perhaps be taken into account in the analysis of vagal function during ambulatory conditions. It is evident that the saturation effect underestimates the cardiac vagal function in several cases. Therefore, we also analyzed HF power only from the linear portion of the relationship. In this analysis, HF power was significantly higher in the saturated group than in the linear group, which was in line with the better long-term HR variability measures among those with a saturation effect and suggests better vagal activity.

Measurement of the HF power spectral component from 24-h ECG recordings has not provided prognostic information in previous post-AMI studies (3, 14) despite the experimental evidence of the cardioprotective role of vagal activity. This may be partly due to methodological bias in the measurement of 24-h HF power caused both by the saturation effect and by the occurrence of erratic nonautonomic HF oscillations (low-correlated group). In this respect, the analysis of the type of regression curve between R-R interval length and HF power and/or the measurement of HF power only from the linear portion of the regression curve might yield new clinical and prognostic information. This will be assessed in the ongoing further studies.

Limitations. We did not measure ambulatory respiration, blood pressure, or physical activity. It is well known that these factors have marked effects on HR variability (10). It is possible that the saturated and linear groups differ in terms of respiratory pattern, blood pressure fluctuation, or physical activity. Second, it is not possible to measure ambulatory sympathetic activity. Another limitation of the study is the different age of the healthy and post-AMI patients, because age has a significant effect on HR variability and autonomic function (21). Therefore, age may be one of the factors explaining the different patterns of the regression curve among the healthy subjects and the post-AMI patients. Finally, the results of this study apply only to patients with a recent AMI. A different saturation pattern might be observed among those with a remote AMI and recovered HR variability (15).

Implications and conclusions. Because of the relatively common phenomenon of saturation of HF oscillations of HR, measurement of total HF power from 24-h R-R intervals alone may not be an exact measure of cardiac vagal function. The magnitude of HF power of R-R intervals should ideally also be analyzed at different R-R interval lengths to detect the possible deflection point and the saturation effect. Future studies will reveal the practical implications of the assessment of the type of regression curve between R-R interval length and HF power and the measurement of HF power only from the linear portion of the relationship.

ACKNOWLEDGMENTS
The authors appreciate the technical support received from Polar Electro Oy (Kempele, Finland) and from Heart Signal (Oulu, Finland). Special thanks to Suvi Tiianen from the Department of Physical Sciences, University of Oulu (Oulu, Finland), for analysis software programming.

GRANTS
This research was funded by grants from the Medical Council of the Academy of Finland (Helsinki, Finland) and the Ministry of Education (Helsinki, Finland).

REFERENCES