Sympathetic stimulations by exercise-stress testing and by dobutamine infusion induce similar changes in heart rate variability in patients with chronic heart failure

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1. Heart rate variability can be used to evaluate autonomic balance, but it is unclear how inotropic therapy may affect the findings. The aim of the study was to assess whether heart rate variability can differentiate between sympathetic stimulation induced by inotrope infusion or by physical exercise.

2. Ten patients with chronic heart failure (64.3 ± 5.4 years of age) underwent four dobutamine infusions (8-min steps of 5 μg min⁻¹ kg⁻¹) and four supine bicycle exercise tests (5-min steps of 25 W). Plasma noradrenaline was evaluated, as well as the SD of R-R intervals, together with low-frequency (0.03–0.14 Hz) and high-frequency (0.15–0.4 Hz) components of heart rate variability using autoregressive spectral analysis.

3. Exercise and inotrope infusion produced similar changes in heart rate variability. An exercise load of 50 W and a dobutamine infusion of 15 μg min⁻¹ kg⁻¹ gave the following results respectively: heart rate, 120.3 ± 3.0 beats/min versus 110.2 ± 3.0 beats/min; SD, 16.0 ± 1.1 ms versus 16.3 ± 2.5 ms; low-frequency component, 4.3 ± 0.3 ln-ms² versus 4.4 ± 0.3 ln-ms² and high-frequency component, 2.6 ± 0.3 ln-ms² versus 2.2 ± 0.3 ln-ms². All comparisons were non-significant. The variables of heart rate variability showed high reproducibility in the same subject during different conditions. Noradrenaline was elevated by exercise from 326.0 ± 35.2 pg/ml to 860.1 ± 180.4 pg/ml (P < 0.05), but was unchanged by dobutamine infusion.

4. Heart rate variability changes cannot differentiate between dobutamine infusions and physical exercise, indicating that we should be cautious in evaluating patients undergoing inotropic therapy. The degree of receptor stimulations, rather than the level of sympathetic drive, would appear to determine the changes in heart rate variability.

Key words: chronic heart failure, dobutamine, exercise, heart rate variability, inotrope stimulation, reproducibility.

Abbreviations: HF, high-frequency; HRV, heart rate variability; LF, low-frequency; nu, normalized units.

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INTRODUCTION

No single adequate measure of cardiovascular autonomic tone exists. In addition to a battery of cardiovascular tests, comprising the Valsalva manoeuvre, deep breathing, sustained handgrip, cold-face stimulus and orthostatic tests, which are widely accepted for routine diagnostic use in the assessment of autonomic neuropathy [1], in recent years, heart rate variability (HRV) has been proposed as a test of autonomic cardiovascular function. The R–R interval signal contains information which may be expressed in both the time and the frequency domain. Both methods are currently used to evaluate HRV and both have been proposed as measures of autonomic cardiovascular control.

The clinical importance of HRV was first established by Hon and Lee in 1965 [2]. Since this report, HRV analysis has been developed and used as a prognostic and non-invasive technique to study many diseases, including coronary artery disease [3–6], congestive heart failure [7–9] and autonomic neuropathy associated with diabetes mellitus [10, 11]. It has also been used to monitor graft rejection in cardiac transplant patients [12]. HRV has been proposed as a reliable quantitative estimate of the autonomic control of the cardiovascular system and the balance between its parasympathetic and sympathetic components [13, 14].

Positive inotropic support is often used in patients in intensive therapy units. This may affect the HRV findings, confounding the analysis of autonomic cardiovascular control by this technique. In this study, we evaluated the ability of HRV parameters to differentiate between sympathetic stimulation induced by physical exercise and adrenergic receptor stimulation induced by an exogenous catecholaminergic agent. Physical exercise at a high
workload results in a generalized sympathetic activation of the peripheral organs by the central nervous system [15, 16] Dobutamine is a synthetic, inotropic, racemic mixture of D- and L-dobutamine, which acts as a potent agonist of the $\beta_1$- and $\alpha$-receptors, and also has a weak $\beta_2$-action [17]. In addition, the reproducibility of the HRV response to these interventions was assessed. Plasma noradrenaline was measured as an independent index of sympathetic activity [18].

**METHODS**

**Patients**

Ten patients with chronic heart failure in stable sinus rhythm (eight secondary to ischaemic heart disease and two with dilated cardiomyopathy) gave informed consent for this trial, which was approved by the local ethics committee. At the time of the study, none had symptomatic angina, ECG evidence of ischaemia limiting exercise or ventricular arrhythmias on 24 h ECG monitoring. Patients in unstable conditions, or with diabetes mellitus or neuropathy, were excluded. Pharmacological treatment was stable for 3 months before and during the study. Table 1 shows the characteristics of the patients.

**Study design**

We used a cross-over design to compare the changes in heart rate, HRV and plasma noradrenaline during exercise and inotrope infusion. Before the test, patients underwent a 2–4 week familiarization and baseline evaluation phase, during which reproducible exercise and autonomic tests were obtained. Subsequently, the patients underwent four supine exercises and four dobutamine infusions on a different day (2–30 days apart), at the same time of the day (09:00 hours), in a laboratory kept at 22–24°C.

On the day of the test, the patients were fasting for at least 3 h, took no tea, coffee or cigarettes and delayed taking their daily medications until after the test. First they were requested to lie down supine on a bed for 30 min, while all measurement methods were set up and a small cannula (Venflon; Viggo-Spectramed, Stockholm, Sweden) was placed in an antecubital vein and maintained patent with a slow infusion of isotonic saline. A respiratory signal from an impedance device was superimposed on lead II of the ECG and was displayed on a monitor (Minimon 7136; Kontron Instruments Ltd, U.K.). The blood pressure was measured manually by the Korotkoff method with a Riva-Rocci sphygmomanometer (phase V). At the end of the 30-min rest, baseline assessments were performed. During the last 5 min of the resting period, a blood sample was taken for measurement of plasma noradrenaline, and heart rate, blood pressure (average of three measurements) and respiratory signals were recorded.

**Sympathetic stimulation by exercise-stress testing**

On the days of exercise-stress testing, the patients were asked to pedal a supine bicycle ergometer (Elema-Schonander AM 368, Stockholm, Sweden) at the rate of 50 times/min until exhaustion. The tests were performed in 5-min stages, with 25 W increments to the limit of tolerance, while continuous records of heart rate and respiration were obtained throughout the exercises and during the first 5 min of recovery. Blood pressure was measured at the end of each stage and at 5 min of recovery: A venous blood sample for measurement of plasma noradrenaline was taken at peak exercise.

**Sympathetic stimulation by dobutamine**

On the days of dobutamine infusion, while the patients were maintaining a comfortable supine position, a dobutamine infusion was initiated by increasing infusion rates (8-min steps of 5 $\mu$g min$^{-1} \cdot$ kg$^{-1}$) through a pump syringe until 80% maximal heart rate was achieved, which was then maintained for a total infusion duration of 30 min. Heart rate and respiratory signals were recorded continuously during the infusion period and during the first 5 min of recovery. Blood pressure was measured at the end of each stage and after 5 min of recovery. A venous blood sample for measurement of plasma noradrenaline was taken during the last minute of infusion.

**HRV**

Heart period (derived from the ECG) and the respiratory signal were digitized on-line by a 12-bit analog-to-digital converter (NB-MIO-16 board; National Instruments, Austin, TX, U.S.A.) at a sampling rate of 500 samples/s. The converter was connected to a Macintosh IIcx computer (Apple Inc., Coupertino, CA, U.S.A.) equipped with a 5-megabyte RAM memory and a 60-megabyte hard disk. A 'C' language program identified all the QRS
Heart rate variability

**Fig. 1.** Power spectral analysis of R-R interval and respiration variability in a representative patient in the supine position. The time series of the signals (a) and the respective autoregressive power spectra (b) are shown. On the R-R interval spectrum, it is possible to identify the HF (corresponding with the respiratory signal) and the LF component.

complexes in each sequence, located the peak of the R-wave and identified the R–R intervals. Steady-state sections of 256 beats of data were selected by visual examination and analysed. Relatively short data records were chosen, since the physiological changes that occurred during exercise and inotrope stimulation were inherently non-stationary on a longer time-scale.

Power spectral analysis of the R–R interval and respiratory signals was performed by an autoregressive model [19, 20]. It is well recognized that breathing parameters (both tidal volume and respiratory frequency) strongly influence the fluctuations of R–R interval, and the HRV estimation should always be associated with the evaluation of the respiratory signal [21, 22]. Model coefficients were evaluated according to a modification of the Burg algorithm [23]. Model order was assessed by Akaike criteria [24]. The range of the model order used for the autoregressive analysis was 9–13; in most cases, a model order of 11 was adequate.

Spectral components were obtained by a decomposition method, which was also used to measure the area below each spectral peak [20, 25, 26]. R–R interval variability was evaluated by SD. The absolute power of the harmonic components in the regions between 0.03 and 0.14 Hz [low-frequency (LF) component], and between 0.15 and 0.40 Hz [high-frequency (HF) component], was calculated. The LF/HF ratio, largely used to assess the sympatho-vagal balance [14], was also computed.

The frequency distributions of the R–R interval powers were plotted and assessed for skewness. As the absolute values derived from the spectral analysis were not normally distributed but markedly skewed, a logarithmic transformation was applied (ln-ms<sup>2</sup>) [6, 9, 12, 20]. The relative predominance of each frequency component after exercise or dobutamine infusion (when the heart rate was increased and the HRV was reduced) was computed by the relative power of each component as a percentage of the total variability [normalized units (nu)] [14]. An example of power spectral analysis is shown in Fig. 1.

It has been shown that power spectral analysis of HRV fails to identify LF components where there is a predominant respiratory-related component (HF) or reduced variability due to physiological (severe physical exercise) [20] or pathological (severe chronic heart failure) conditions [14]. Therefore, in the present analysis, we excluded the tracings which showed no LF components during the tests (30.5% of the recordings). In order to obtain a better understanding of the complex nature of the mechanisms responsible for the generation of the spectral components, we considered separately the tracings which showed no LF components during sympathetic stimulations.

**Reproducibility of HRV variables**

The reproducibility of the HRV variables was assessed by calculating the distribution of the differences between the readings obtained at the different phases of the study, as recommended by Bland and Altman [27]. This was done in absolute units and as percentage differences.

**Noradrenaline determinations**

A 10ml sample was obtained and immediately centrifuged at 1000g for 10min. The plasma was aspirated immediately, frozen and stored at −40°C. The noradrenaline concentrations were measured by
Dobutamine dose infused was 20pg\textperiodcentered min\textsuperscript{-1} while in the remaining two patients it was 20pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}. Results are expressed as means \pm SEM (40 recordings for each test day). There were no significant differences in the baseline values of any variable between the two tests. SD, time domain index of HRV; LF and HF, powers in the low- and high-frequency bands, respectively, in absolute values (ln-ms\textsuperscript{2}) and in nu.

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<tr>
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<th>Dobutamine infusion</th>
<th>Exercise</th>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113.7 \pm 4.5</td>
<td>113.8 \pm 4.5</td>
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<td>Diastolic blood pressure (mmHg)</td>
<td>7.2 \pm 1.2</td>
<td>7.45 \pm 1.7</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>75.4 \pm 2.5</td>
<td>78.3 \pm 2.7</td>
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<tr>
<td>HRV</td>
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<tr>
<td>SD (ms)</td>
<td>28.2 \pm 2.3</td>
<td>28.9 \pm 2.4</td>
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<tr>
<td>LF components ln-ms\textsuperscript{2}</td>
<td>5.5 \pm 0.6</td>
<td>5.5 \pm 0.2</td>
</tr>
<tr>
<td>nu</td>
<td>56.9 \pm 3.0</td>
<td>55.7 \pm 2.6</td>
</tr>
<tr>
<td>HF components ln-ms\textsuperscript{2}</td>
<td>4.5 \pm 0.2</td>
<td>4.5 \pm 0.2</td>
</tr>
<tr>
<td>nu</td>
<td>36.2 \pm 12</td>
<td>34.0 \pm 2.7</td>
</tr>
<tr>
<td>LF/HF ratio</td>
<td>2.8 \pm 0.5</td>
<td>2.9 \pm 0.6</td>
</tr>
<tr>
<td>Noradrenaline (pg/ml)</td>
<td>405.1 \pm 47.1</td>
<td>326.0 \pm 35.2</td>
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HPLC [28]. The mean inter-assay and intra-assay coefficients of variation for these determinations were 9.6\% and 8.7\% respectively. The normal range of plasma noradrenaline in our laboratory was 120–300 pg/ml (in non-hospital-based subjects).

**Statistical analysis**

For each variable, comparisons with respective baseline values, and between the endogenous and exogenous sympathetic stimulations at matched heart rate, were made by analysis of variance (corrected for multiple comparisons by Scheffe’s procedure). Results are expressed as means \pm SEM.

**RESULTS**

No significant differences were observed in the baseline values before the exercises and inotrope stimulations (Table 2). No major adverse side-effects were observed during the exercise tests or during the inotrope infusions, and all the patients completed the study. In eight patients, the maximal dobutamine dose infused was 20pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}, while in the remaining two patients it was 25pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}. During the higher doses of inotrope infusions (above 15pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}), all patients felt palpitations but none complained of nausea or vomiting. No significant ventricular arrhythmias, hypertension or hypotension occurred during or after the infusions. For four patients, the maximal exercise load reached was 50 W, with 75 W being achieved by the remaining six patients. During the supine exercise tests, the patients did not develop any significant ventricular arrhythmias. The major limiting symptoms were breathlessness and/or leg fatigue.

**Heart rate and blood pressure**

During exercise, heart rate increased by 18.76\% at 25 W, by 42.02\% at 50 W and by 55.34\% at 75 W. During inotrope infusion, heart rate increased by 0.87\% at 5pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}, 17.48\% at 10pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}, by 34.88\% at 15pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1} and by 48.03\% at 20pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}. At 5 min of recovery, the heart rate was still increased both after exercise (by 17.07\%) and after dobutamine infusion (by 26.37\%) (Fig. 2). We compared the changes in HRV during exercise and dobutamine infusion at 25 W and 10pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}, at 50 W and 15pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}, at 75 W and 20pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}, and during the respective recovery periods. Systolic blood pressure progressively increased during exercise, whereas during inotrope infusion it was significantly elevated only at higher doses of dobutamine. At 50 and 75 W, systolic pressure was significantly higher than at 15 and 20pg\textperiodcentered min\textsuperscript{-1} kg\textsuperscript{-1}, respectively (Fig. 2). No changes were detected in diastolic pressure.
components were excluded from the analysis. Therefore, during dobutamine infusions, 28 recordings at 20 pg min$^{-1}$ were considered, and during exercise-stress testing and dobutamine infusion. At 75 W were considered.

In the frequency domain, HF and LF components, both in absolute (Fig. 3) and relative values (nu), showed similar patterns during exercise and dobutamine infusion. At 75 W, HF decreased by 74.9% (8.5 ± 1.1 nu, $P<0.05$ compared with baseline). LF was reduced by 20.0% at 75 W (3.0 ± 0.3 ms versus 3.2 ± 0.3 ms, $P<0.05$), on the infusion days, 3.2 ± 0.3 ms (P not significant). Also, the LF component increased by 51.5% at 75 W (84.4 ± 3.4 nu, $P<0.05$ compared with baseline), and by 42.2% at 20 μg min$^{-1}$ kg$^{-1}$ (81.0 ± 1.7 nu, $P<0.05$ compared with baseline). Also, during the recovery periods after the two different forms of sympathetic stimulation, similarities in the HRV variables were maintained (Figs. 3 and 4).

The LF/HF ratio significantly increased during the two sympathetic stimulations [7.6 ± 3.9 at 15 μg min$^{-1}$ kg$^{-1}$ and 6.0 ± 1.7 at 50 W, 12.0 ± 2.0 at 20 μg min$^{-1}$ kg$^{-1}$ and 15.9 ± 5.7 at 75 W ($P<0.05$ compared with respective baseline values)]. The power spectra during exercise and dobutamine infusion in a representative patient are shown in Fig. 5.

At comparable heart rates, the respiratory frequency was more elevated during supine exercise in comparison with dobutamine infusion, although not significantly (at 50 W, 0.27 ± 0.03 Hz versus 0.24 ± 0.02 Hz, $P$ not significant).

Patients without LF fluctuations during sympathetic stimulations

Four patients showed this pattern of HRV, which was consistent during the recordings at the same level of sympathetic stimulation. This subgroup of patients showed no significant differences in peak oxygen uptake (12.6 ± 1.8 ml min$^{-1}$ kg$^{-1}$ versus 12.2 ± 0.7 ml min$^{-1}$ kg$^{-1}$), left ventricular ejection fraction (22.2 ± 4.8% versus 24.0 ± 4.4%), or New York Heart Association class compared with the other patients in our study population ($P$ not significant for all comparisons). However, they presented lower resting SDs [on the exercise days, 23.3 ± 3.2 ms versus 34.1 ± 3.2 ms ($P<0.005$), on the infusion days, 15.8 ± 3.0 ms versus 32.3 ± 2.3 ms ($P<0.005$)], and lower LF components [on the exercise days, 4.2 ± 0.3 In-ms$^{-2}$ versus 5.0 ± 0.2 In-ms$^{-2}$ ($P<0.05$), on the infusion days, 3.2 ± 0.3 In-ms$^{-2}$ versus 5.0 ± 0.1 In-ms$^{-2}$ ($P<0.05$)]. Also, the LF components were reduced in absolute terms [on the exercise days, 4.9 ± 0.3 In-ms$^{-2}$ versus 5.7 ± 0.2 In-ms$^{-2}$ ($P<0.05$), on the infusion days, 4.1 ± 0.4 In-ms$^{-2}$ versus 5.5 ± 0.2 In-ms$^{-2}$ ($P<0.05$)], but proportional LF (nu) increased [on the exercise days, 60.8 ± 3.0 nu versus 53.8 ± 5.3 nu ($P$ not significant), on the infusion days, 64.6 ± 5.7 nu versus 53.9 ± 3.5 nu ($P<0.05$)].

Reproducibility of HRV variables

Table 3 presents the distribution of the differences between the readings (in absolute units and as percentage difference) at rest before, during and after the stimulations. The LF index showed good reproducibility (varying from 0.1 ± 0.4 In-ms$^{-2}$ to 0.2 ± 0.7 In-ms$^{-2}$). SDs and the HF components were less reproducible at rest and at the higher levels of sympathetic stimulation (dobutamine infusion and exercise). Overall, the time domain measures of HRV were slightly less reproducible than the fre-

HRV in the time and frequency domain

As specified above, the recordings without LF components were excluded from the analysis. Therefore, during dobutamine infusions, 28 recordings at 10 μg min$^{-1}$ kg$^{-1}$ and 15 μg min$^{-1}$ kg$^{-1}$, and 24 at 20 μg min$^{-1}$ kg$^{-1}$ were considered, and during exercise, 24 recordings at 25 W, 24 at 50 W and 16 at 75 W were considered.

In the time domain analysis, the SDs of R–R intervals showed very similar changes during the exercise-stress testing and dobutamine infusion. At 75 W, the SD decreased by 47.7% (15.1 ± 1.0 ms, $P<0.05$ compared with baseline), and by 52.1% (13.5 ± 2.6 ms, $P<0.05$ compared with baseline) at 20 μg min$^{-1}$ kg$^{-1}$ (Fig. 3). There were no significant differences between the two tests. In the frequency domain, HF and LF components, both in absolute (In-ms$^{-2}$) and relative values (nu), showed similar patterns during exercise and dobutamine infusion (Fig. 4). At 75 W, HF decreased by 57.7% (1.9 ± 0.3 In-ms$^{-2}$, $P<0.005$ compared with baseline), and at 20 μg min$^{-1}$ kg$^{-1}$, by 62.2% (1.7 ± 0.6 In-ms$^{-2}$, $P<0.005$ compared with baseline). In relative terms (nu), at 75 W, HF decreased by 74.9% (8.5 ± 1.1 nu, $P<0.005$ compared with baseline), and by 67.3% at 2 μg min$^{-1}$ kg$^{-1}$ (11.1 ± 1.7 nu, $P<0.05$ compared with baseline). LF was reduced by 20.0% at 75 W (3.0 ± 0.3 ms versus 3.2 ± 0.3 ms, $P<0.05$), on the infusion days, 3.2 ± 0.3 ms (P not significant). Also, the LF component increased by 51.5% at 75 W (84.4 ± 3.4 nu, $P<0.05$ compared with baseline), and by 42.2% at 20 μg min$^{-1}$ kg$^{-1}$ (81.0 ± 1.7 nu, $P<0.05$ compared with baseline). Also, during the recovery periods after the two different forms of sympathetic stimulation, similarities in the HRV variables were maintained (Figs. 3 and 4).

The LF/HF ratio significantly increased during the two sympathetic stimulations [7.6 ± 3.9 at 15 μg min$^{-1}$ kg$^{-1}$ and 6.0 ± 1.7 at 50 W, 12.0 ± 2.0 at 20 μg min$^{-1}$ kg$^{-1}$ and 15.9 ± 5.7 at 75 W ($P<0.05$ compared with respective baseline values)]. The power spectra during exercise and dobutamine infusion in a representative patient are shown in Fig. 5.

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Fig. 4. Changes in LF (a) and HF (b) components of HRV in absolute values (ln-ms²) and mnu. For an explanation see the legend to Fig. 2.

Fig. 5. Power spectra of HRV during exercise (a) and dobutamine infusion (b) in a representative patient. Different sympathetic stimulations induced similar changes in HRV.
frequency domains. Similar results were observed during inotrope infusion and during exercise.

Noradrenaline determinations

During exercise, a significant increase in plasma noradrenaline was observed (from 326.0 ± 35.2 pg/ml at rest to 860.1 ± 180.4 pg/ml at peak exercise, P < 0.05). No significant changes were observed during inotrope infusion (from 405.1 ± 47.1 pg/ml to 421.6 ± 26.1 pg/ml; Fig. 3). Therefore, at peak exercise, plasma noradrenaline was significantly higher than during dobutamine therapy (P < 0.05).

DISCUSSION

HRV is a relatively new technique for estimating autonomic cardiac modulation. It uses non-invasive recordings, and is said to provide a quantitative evaluation of the sympatho-vagal interaction during changes in cardiovascular function. Important pathophysiological conditions, such as arterial hypertension, myocardial ischaemia and heart failure, in which autonomic dysfunction may play a promoting and an aggravating role, can be studied with important practical implications.

Present study findings

First we evaluated the reproducibility of power spectral measures of HRV during different conditions. The reproducibility of HRV variables obtained from short-term sampling periods in static conditions has been confirmed [29]. The results of the present study extend these findings to dynamic conditions, because reasonably reproducible data of HRV were observed during physical exercise and inotrope stimulations. This stability may have relevance in the evaluation of patients in different clinical settings.

In fact, patients admitted to intensive therapy units as a consequence of acute illness, often in unstable conditions, frequently require inotropic support. This therapy may therefore affect the HRV results concerning the autonomic control of these patients. We studied how HRV measures are influenced by the administration of a sympathomimetic drug (dobutamine) compared with the relative results of endogenous sympathetic stimulation (exercise). As expected [20], physical exercise induced an increase in the sympathetic indices and reductions in the R-R interval SD and HF component, both in absolute and relative values, while the LF component was reduced in absolute terms but increased in relative terms. LF was reduced in absolute terms (ln-ms²), partly as a consequence of the fall in HRV and partly because of tachycardia. After normalization for the overall HRV, proportional LF (nu) increased because the reduction in HRV involved mainly the HF component. Venous noradrenaline was also increased, supporting a generalized sympathetic activation. Thereafter, we evaluated the effect of the infusion of a sympathetic stimulator (dobutamine), which induced virtually identical modifications in the spectral analysis of HRV to those seen during moderate exercise. Figures 3, 4 and 5 show the HRV changes during and after exercise at 25 W, 50 W and 75 W, and during and after dobutamine infusions of 10 μg min⁻¹ kg⁻¹, 15 μg min⁻¹ kg⁻¹ and 20 μg min⁻¹ kg⁻¹, respectively, verified by similar heart rate values obtained in both conditions. In these figures, the similarity in HRV patterns induced by the two different types of sympathetic stimulation is evident.

The absence of any increase in venous noradrenaline suggested that there was no activation of endogenous sympathetic drive during dobutamine infusion. Colucci et al. [30] similarly observed that intracoronary infusion of dobutamine in patients

### Table 3. Reproducibility of the measured HRV variables during the different study conditions, in absolute units and as percentage difference (means ± SD). Definitions are as given in Table 2.

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<th>Dobutamine infusion</th>
<th>Supine exercise (W)</th>
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<td></td>
<td>At rest 10 15 20 Recovery</td>
<td>At rest 25 50 75 Recovery</td>
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<td></td>
<td>SD</td>
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**Table 3. Reproducibility of the measured HRV variables during the different study conditions, in absolute units and as percentage difference (means ± SD). Definitions are as given in Table 2.**

<table>
<thead>
<tr>
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<th>Dobutamine infusion</th>
<th>Supine exercise (W)</th>
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<tr>
<td></td>
<td>At rest 10 15 20 Recovery</td>
<td>At rest 25 50 75 Recovery</td>
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<tr>
<td></td>
<td>SD</td>
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with chronic heart failure induced withdrawal of the endogenous sympathetic drive (estimated by noradrenaline), and that the decrease in plasma noradrenaline was dose-related. Therefore, HRV seems unable to differentiate between stimulation of the $\beta_1$- and $\alpha$-receptors induced by endogenous or exogenous stimulation.

**Possible mechanisms**

One possible explanation of the observed findings may be that the changes in autonomic tone assessed by HRV are related to the degree of peripheral receptor stimulation, rather than to the extent of the central sympathetic drive. In fact, central drive was not activated during exogenous sympathetic receptor stimulation, as confirmed by the unchanged concentration of plasma noradrenaline during dobutamine infusion.

Another possible mechanism is the reduction of baroreflex sensitivity present during exercise [31] and during catecholamine infusion [32-34]. The baroreflex-dependent fluctuation in heart rate would likewise be expected to be reduced during the two tests. It is therefore possible that the observed changes in HRV may be related to a reduction in both the vagal and sympathetic limbs of the baroreflex arc. It has been suggested that both LF and HF components of the power spectrum originate from the actions of the arterial baroreflex [34, 35]. In particular, the HF component has been described as a baroreceptor-dependent oscillation in heart rate mediated by the vagus in response to respiratory fluctuations in blood pressure, whereas the LF is thought to originate as a result of resonance induced by the interaction of the fast vagal efferent response with a baroreflex stimulus, with the delay caused by the slower sympathetic response. The link between LF components and baroreflex function was clearly shown in animal models by Cerutti et al. [36], and by Di Rienzo et al. [37] The De Boer model has also been confirmed in our laboratory in normal human subjects [38, 39].

In this study, both HF and LF components were reduced in absolute terms, in agreement with the theory that considers both components of the HRV spectrum analysis to be generated by the baroreflex.

We also observed different patterns in blood pressure between the two different sympathetic stimulation tests: systolic pressure increased immediately at the beginning of exercise, but only at the higher doses of dobutamine infusion. This rise would have induced a greater stimulus to the baroreceptors when the gain of the baroreceptors was simultaneously depressed [40]. We do not know how quickly catecholamines in humans depress the baroreflex. As the HRV indices were identical in all aspects, it seems unlikely that the differences in blood pressure per se might have played any significant role in the observed results, thus implying that both LF and HF components are not entirely or exclusively dependent on baroreceptor function. This of course has to be tested under more stable (stationary) conditions.

The rise in respiratory frequency observed during exercise may have selectively affected the power of the respiratory-related HF component of HRV [21, 22], which instead showed similar changes during the exercise-stress testing and inotrope stimulations. Therefore, either the changes in the respiration during supine exercise were too small to affect the HRV pattern and/or the respiratory stimulation was counterbalanced by a more marked baroreceptor inhibition during exercise.

Similar changes in HRV spectral analyses have been experimentally induced by a vasodilator drug (nitroglycerin) [41]. It is possible that the autonomic changes reported here may be reflex responses to a generalized vasodilatation produced by exercise (due to vasodilatation of the exercising limb) and by dobutamine (via $\beta_2$-receptor stimulation).

**Patients without LF fluctuations during sympathetic stimulations**

These presented lower SDs and HF components and higher LF components compared with the other patients at rest, but no significant differences were observed in peak oxygen uptake, left ventricular ejection fraction or New York Heart Association class. In fact, it is well known that the decrease in the components of HRV is not simply related to left ventricular dysfunction [7]. Diminished HRV and respiratory-related components of HRV are associated with lower vagal tone, while high LF components are attributed to an enhanced sympathetic tone. In chronic heart failure, impairment of control sensory mechanisms, such as cardiopulmonary and arterial baroreflexes, are important pathogenetic mechanisms underlying the parasympathetic withdrawal. At the same time, discharges from arterial chemoreceptors and skeletal muscle metaboreceptors (afferents sensitive to the muscle work) are the major inputs to sympatho-excitation [42, 43]. However, more advanced stages of heart failure [44], like other situations of severe sympathetic stimulation [14, 20], are associated with impairment of the HRV indexes, low values of HRV and an absence of LF power. It could therefore be hypothesized that the subgroup of patients showing no LF fluctuations during sympathetic stimulations may include patients with a more advanced stage of heart failure and autonomic impairment, evident by spectral analysis of HRV.

**Limitations**

This study was only carried out in patients with heart failure. It would have been of interest to obtain data from normal subjects as well, to check the validity of extrapolating the conclusions of the present study to subjects with normal cardiovascu-
lar control. Ethical considerations did not allow a control group to receive dobutamine infusion for no clinical purpose, therefore we could not address this question specifically in normal subjects. However, our findings are similar to those reported by Binkley et al. [45], who observed an increased LF component in normal subjects after isoprenaline infusion. Breuer et al. [46], comparing the exercise-induced changes in HRV with the changes induced by intravenous infusion of atropine and catecholamines in healthy subjects, reported results consistent with our findings. In fact, after catecholamine infusion, increases in heart rate and systolic blood pressure were associated with falls in HRV, with a concomitant reduction in the absolute values of the LF and HF components. The hypothesis of a negative-feedback effect of increased plasma catecholamines on the sympathetic control of heart rate, proposed by Breuer et al. [46], is not confirmed by our findings, because we observed that noradrenaline levels were elevated after exercise but were unchanged by dobutamine. Also, a more recent study [47] has confirmed the similarity of HRV indices induced by exercise and isoproterenol infusion (isoprenaline).

In our study, dobutamine was infused and endogenous noradrenaline were measured. In order to evaluate the effects of sympathetic stimulation on HRV, it would have been advantageous to use the same exogenous agent as the endogenous catecholamines produced in exercise. However, noradrenaline infusions in patients with heart failure carry well-recognized risks of inducing arrhythmias.

In this study, the data were compared during different levels of sympathetic stimulation after matching for similar heart rates. Matching for mean heart rate may have made it difficult to detect a statistically significant difference in HRV, given the relatively small number of patients studied. However, the changes in spectral analysis data and catecholamine levels were consistent in all the patients.

Implications

Even taking into account these limitations, the results of this study may have important implications for clinical practice. They strongly suggest that it is necessary to be more critical in the use of ‘simple’ indices of autonomic function, and that, in particular, LF and HF powers cannot be considered as specific markers of autonomic activity in all instances: in fact, they may be powerfully influenced by other confounding factors such as the degree of receptor stimulation due to different and changing physiological or therapeutic interventions (drugs, exercise, clinical status, stress). Therefore, the ability of spectral analysis to provide a specific estimate of autonomic modulation is an issue which should be dealt with more cautiously. This is particularly important as clinicians are now encouraged to look at HRV indices to compare different patients or the same patient in unstable conditions, on different therapies and at different times (i.e. after acute myocardial infarction, unstable chronic heart failure or different therapeutic interventions), and to use these parameters to predict prognosis.

ACKNOWLEDGMENTS

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