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# Power Spectral Analysis of Heart Rate and Arterial Pressure Variabilities as a Marker of Sympatho-Vagal Interaction in Man and Conscious Dog

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In 57 normal subjects (age 20–60 years), we analyzed the spontaneous beat-to-beat oscillation in R–R interval during control recumbent position, 90° upright tilt, controlled respiration ( $n = 16$ ) and acute ( $n = 10$ ) and chronic ( $n = 12$ )  $\beta$ -adrenergic receptor blockade. Automatic computer analysis provided the autoregressive power spectral density, as well as the number and relative power of the individual components. The power spectral density of R–R interval variability contained two major components in power, a high frequency at  $\sim 0.25$  Hz and a low frequency at  $\sim 0.1$  Hz, with a normalized low frequency:high frequency ratio of  $3.6 \pm 0.7$ . With tilt, the low-frequency component became largely predominant ( $90 \pm 1\%$ ) with a low frequency:high frequency ratio of  $21 \pm 4$ . Acute  $\beta$ -adrenergic receptor blockade (0.2 mg/kg IV propranolol) increased variance at rest and markedly blunted the increase in low frequency and low frequency:high frequency ratio induced by tilt. Chronic  $\beta$ -adrenergic receptor blockade (0.6 mg/kg p.o. propranolol, t.i.d.), in addition, reduced low frequency and increased high frequency at rest, while limiting the low frequency:high frequency ratio increase produced by tilt. Controlled respiration produced at rest a marked increase in the high-frequency component, with a reduction of the low-frequency component and of the low frequency:high frequency ratio ( $0.7 \pm 0.1$ ); during tilt, the increase in the low frequency:high frequency ratio ( $8.3 \pm 1.6$ ) was significantly smaller. In seven additional subjects in whom direct high-fidelity arterial pressure was recorded, simultaneous R–R interval and arterial pressure variabilities were examined at rest and during tilt. Also, the power spectral density of arterial pressure variability contained two major components, with a relative low frequency:high frequency ratio at rest of  $2.8 \pm 0.7$ , which became  $17 \pm 5$  with tilt. These power spectral density components were numerically similar to those observed in R–R variability. Thus, invasive and noninvasive studies provided similar results. More direct information on the role of cardiac sympathetic nerves on R–R and arterial pressure variabilities was derived from a group of experiments in conscious dogs before and after bilateral stellectomy. Under control conditions, high frequency was predominant and low frequency was very small or absent, owing to a predominant vagal tone. During a 9% decrease in arterial pressure obtained with IV nitroglycerin, there was a marked increase in low frequency, as a result of reflex sympathetic activation. Bilateral stellectomy prevented this low-frequency increase in R–R but not in arterial pressure autospectra, indicating that sympathetic nerves to the heart are instrumental in the genesis of low-frequency oscillations in R–R interval. (*Circulation Research* 1986;59:178–193)

**T**HE whole history of brain electrophysiology has proved that rhythms can often be markers of normal functional states such as wakefulness or sleep, or of abnormal states such as epilepsy. In the present context, the general hypothesis is that rhythmical beat-to-beat oscillations of one cardiovascular controlled variable might provide some criteria to interpret the complex interplay among the neural regulatory outflows. On the other hand, the existence, under stable conditions, of rhythmic fluctuations in car-

diovascular variables such as heart rate or arterial pressure has been recognized for a long time.<sup>1–7</sup> In recent years, the possibility of quantifying these oscillations by using computer techniques, particularly on the variability of electrocardiographic R–R interval, has aroused a growing interest.<sup>8–15</sup>

Indeed, as instantaneous heart rate depends on the interaction between sympathetic and parasympathetic efferent activities and pacemaker properties, it has been suggested that this analysis could lead to a noninvasive assessment of the “tonic” autonomic regulation of heart frequency.<sup>12</sup> The nonrandom components of R–R variability usually are assessed with spectral techniques based on the fast Fourier transform, in spite of the consideration that the heart rate variability signal is not strictly periodical, as requested by the deterministic nature of the algorithm.<sup>16</sup>

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In conscious dogs and in man, a high-frequency component ( $\sim 0.25$  Hz) has been consistently found in the power spectrum and has been interpreted as a quantitative assessment of respiratory arrhythmia.<sup>15</sup> One or two low-frequency components have also been described, respectively, about 0.1 Hz and 0.03 Hz. The former of these, the so-called 10-second period rhythm, has been considered particularly interesting as it has the same frequency of the better known Mayer's waves.<sup>17</sup>

As to the neural mechanisms underlying these fluctuations, vagal efferent activity has been interpreted as being primarily responsible for the high-frequency component of heart rate variability on the basis of experiments on vagotomized decerebrated cats,<sup>10</sup> conscious dogs,<sup>12</sup> and man with muscarinic receptor blockade.<sup>15</sup>

Both vagal and sympathetic outflows were considered to determine the low-frequency components.<sup>12,15</sup>

The aim of this study on normal human subjects and conscious dogs was to assess the relative role of vagal and sympathetic activities in determining the variability in heart rate and arterial pressure at rest and during induced changes of autonomic regulation. A totally automatic computation of the autospectra and of their individual components was implemented, using an autoregressive modeling algorithm, in order to take advantage of its inherent better definition, compared to the more current spectral techniques.<sup>18,19</sup>

### Subjects and Methods

Fifty-seven subjects without any clinically evident disease (20–60 years old) were used for this study. They were healthy volunteers, randomly selected from hospital staff, medical students, and their relatives. Seven additional subjects consulted our hypertension clinic and were eventually found to be normotensive in the hospital environment. These subjects underwent 24-hour continuous monitoring of electrocardiogram (ECG) and high-fidelity intraarterial systemic pressure recording<sup>20</sup> as part of their clinical workup for suspected hypertension.

All subjects included in this study had no signs of organ or systemic disease. Subjects smoking more than five cigarettes a day were excluded from the study. No one was taking any medication, and each subject was instructed to avoid beverages containing alcohol or caffeine after 10 pm of the day preceding the study and to come to the laboratory after a light breakfast. Studies were performed between 10:30 AM and 12:30 PM. All subjects were carefully instructed about the study, and all gave their informed consent.

### Tilting

Subjects were placed on an electrically driven tilt table (provided with a mattress and a footrest) and connected to a conventional electrocardiographic amplifier (lead II, Cardioline) and FM tape recorder (Racal). After 15–30 minutes, allowed for stabilization, the ECG was continuously recorded for 30 minutes at rest, followed by 20 minutes after the subjects had

been passively moved to an upright 90° position by electrically rotating the table. During tilting, the subjects rested on their feet, although they had been loosely strapped to the table to avoid accidental falls.

The arterial blood pressure was recorded at the beginning and at the end of "rest" and "tilt" periods using a conventional sphygmomanometer.

Reproducibility of the results was assessed by repeating the study in 10 subjects after a period of 1 week to 12 months. Additionally, in 4 subjects, a third study was performed after an additional 3–4 months.

The effects of  $\beta$ -adrenergic receptor blockade was assessed in two groups of experiments. In 10 subjects, after the baseline studies, the study was repeated on a later day after IV bolus of 0.2 mg/kg propranolol (Inderal, ICI) in order to block acutely  $\beta$ -adrenergic receptor-mediated effects (Table 1).

In 12 subjects, the study was repeated after 6 days of 0.6 mg/kg p.o. propranolol (Inderal, ICI) t.i.d. in order to block chronically  $\beta$ -adrenergic receptor mediated effects (Table 1).

### Controlled Respiration

The effects of controlled respiration were tested in 16 subjects both at rest and during tilt. These patients were studied first while breathing spontaneously, and on a separate day while breathing at 20 acts/min, following a metronome. Respiratory movements were monitored with an impedance respirometer (Cardioline) and recorded on FM tape. Seven of these patients breathed through a mouthpiece connected to a spirometer (Jaeger) in order to quantify their tidal volume. All of these patients had been well acquainted with the laboratory through 1–3 training sessions, during which they learned to breathe quietly and to adjust their tidal volume to the increased respiratory rate. During spontaneous and controlled breathing, transcutaneous  $P_{CO_2}$  and  $P_{O_2}$  (Sensor Medics) were monitored in 5 patients.

### Systemic Arterial Pressure (Invasive Studies)

In 7 additional subjects, a microminiature Millar tip pressure transducer (03 French) was inserted percutaneously into the radial artery of the nondominant arm with a Seldinger technique.

Calibrations of the transducer, both in absolute values of millimeters of mercury and millivolts, were performed just before the insertion of the catheter and at the end of the recording procedure. After suitable amplification, arterial blood pressure and ECG were continuously recorded at rest and during tilt on FM tape. These studies were the last part of a 24-hour protocol for suspected hypertension, based on a continuous ambulatory recording of direct high-fidelity arterial pressure and ECG.<sup>20</sup>

### Animal Experiments

Twelve mongrel dogs (25–35 kg body weight) were used for this part of the study. They were lightly anesthetized with thiopental sodium (10 mg/kg, IV) (Farmotal, Farmitalia) and, after the skin had been infiltrated with 2% xylocaine (Byk Gulden), a cut-down was

TABLE 1. Effects of Age and  $\beta$ -Adrenergic Blockade on the Response of R-R Interval Variability to Tilt

	Arterial pressure		R-R interval (msec)	R-R variance (msec <sup>2</sup> )	Low-frequency component		High-frequency component		LF:HF ratio
	Systolic (mm Hg)	Diastolic (mm Hg)			Normalized power	Frequency (Hz Eq)	Normalized power	Frequency (Hz Eq)	
<b>A. Rest</b>									
20-30 yr (n = 30)	116 ± 2	73 ± 2	834 ± 34	4097 ± 361*	58.2 ± 3.3	0.11 ± 0.01	24.0 ± 2.0	0.24 ± 0.02	3.62 ± 0.70
30-45 yr (n = 10)	116 ± 4	79 ± 3	931 ± 39	2581 ± 356*	62.2 ± 6.3	0.10 ± 0.01	26.3 ± 4.3	0.27 ± 0.02	3.69 ± 1.23
45-60 yr (n = 17)	118 ± 3	78 ± 2	926 ± 27	1354 ± 205	49.9 ± 4.9	0.09 ± 0.01	32.0 ± 4.6	0.27 ± 0.01	2.58 ± 0.61
Acute $\beta$ -adrenergic blockade, 20-30 yr (n = 10)	103 ± 3†	70 ± 3	1076 ± 26†	8255 ± 1676†	50.9 ± 5.0	0.12 ± 0.01	32.7 ± 4.3	0.26 ± 0.02	1.74 ± 0.31
Chronic $\beta$ -adrenergic blockade, 20-30 yr (n = 12)	106 ± 2†	66 ± 2†	1115 ± 59†	7180 ± 1712†	39.4 ± 5.7†	0.12 ± 0.01	53.4 ± 5.4†	0.26 ± 0.02	1.07 ± 0.35†
<b>B. Tilt</b>									
20-30 yr	111 ± 1	79 ± 2	687 ± 14‡	2642 ± 217‡	89.7‡ ± 1.4	0.09 ± 0.01	7.5‡ ± 0.9‡	0.29 ± 0.03	20.79 ± 3.68‡
30-45 yr	109 ± 4	78 ± 3	719 ± 44*‡	2160 ± 253**	83.7‡ ± 4.6*‡	0.09 ± 0.01	11.4‡ ± 2.8*‡	0.29 ± 0.06	17.30 ± 8.19‡
45-60 yr	113 ± 3	77 ± 2	767 ± 24‡	1212 ± 213	75.7‡ ± 4.6‡	0.08 ± 0.01	16.2‡ ± 3.4‡	0.23 ± 0.02	12.61 ± 3.21‡
Acute $\beta$ -adrenergic blockade, 20-30 yr	100 ± 4†	72 ± 4	929 ± 24†‡	3009 ± 361‡	74.1‡ ± 7.0†‡	0.09 ± 0.01	13.0‡ ± 2.5†	0.22 ± 0.02	7.48 ± 1.77†‡
Chronic $\beta$ -adrenergic blockade, 20-30 yr	97 ± 2†‡	69 ± 4†	951 ± 35†‡	4067 ± 664†‡	66.7‡ ± 5.2†‡	0.11 ± 0.01	24.0‡ ± 4.4†‡	0.22 ± 0.02	3.78 ± 0.93†‡

\*Significantly different contrast ( $p < 0.05$ ).†Value during  $\beta$ -adrenergic blockade significantly different ( $p < 0.05$ ) from value obtained in unblocked conditions in the young age group.‡Value during tilt significantly different from value at rest ( $p < 0.05$ ).

performed in the region of the femoral artery. A catheter was introduced into it, advanced to the aorta, and secured with a suture. The wound was closed, and the catheter was exteriorized at the base of the neck.

After baseline studies, 5 dogs underwent bilateral stellectomy. By sterile techniques, they were anesthetized with thiopental sodium (30 mg/kg, IV) followed by a continuous infusion of fentanyl citrate (6.2  $\mu$ g/kg, IV) and droperidol (0.2 mg/kg, IV) (Leptofen, Carlo Erba). The animals were paralyzed with small (0.1 mg/kg, IV) intermittent doses of succinylcholine (Wellcome) and artificially ventilated with a positive-pressure pump (Harvard). A thoracotomy was performed in the fourth left intercostal space, the left stellate ganglion and its branches were excised, and the thoracotomy was repaired. Seven to 15 days later, under similar anesthesia and through a right thoracotomy in the fourth intercostal space, the right stellate ganglion was excised as well.

Aortic pressure was measured with the implanted catheter using a pressure transducer (Statham Instruments). Aortic mean pressure was obtained with an RC filter with a 2-second time constant. The electrocardiogram (lead II) was obtained with subcutaneous silver electrodes and an AC amplifier. Heart rate was monitored continuously with a cardiometer triggered by the R-wave.

Respiratory movements were monitored with a pneumatic belt connected to a pressure transducer (Statham Instruments). Data were recorded on a multi-channel FM tape recorder (Racal Store 7) and played back on a direct-writing recorder (Brush Gould).

Experiments were performed after a postoperative period long enough to allow complete recovery of the dogs from the operation, as judged by their normal behavior, body temperature, and hematocrit. Five to 7 days usually were sufficient after the implantation of the pressure catheter, while 10-15 days were given after the thoracotomy. While the trained dogs were lying quietly on the recording table, aortic blood pressure, ECG (lead II), and respiratory movements were recorded continuously for 20-30 minutes of control, followed by an infusion of nitroglycerin (32  $\mu$ g/kg per min, IV) for 15-20 minutes, in order to excite sympathetic activity.<sup>21</sup>

#### Data Analysis

Off-line analysis was performed on DEC MNC 11/23 and PDP 11/24 minicomputers. ECG, respiration, and pressure data were played back from the FM tape and digitized at 300 samples/sec per channel. The principles of the software for data acquisition and analysis have been described previously.<sup>13,20,22</sup>

Briefly, stationary sections of data both at rest and

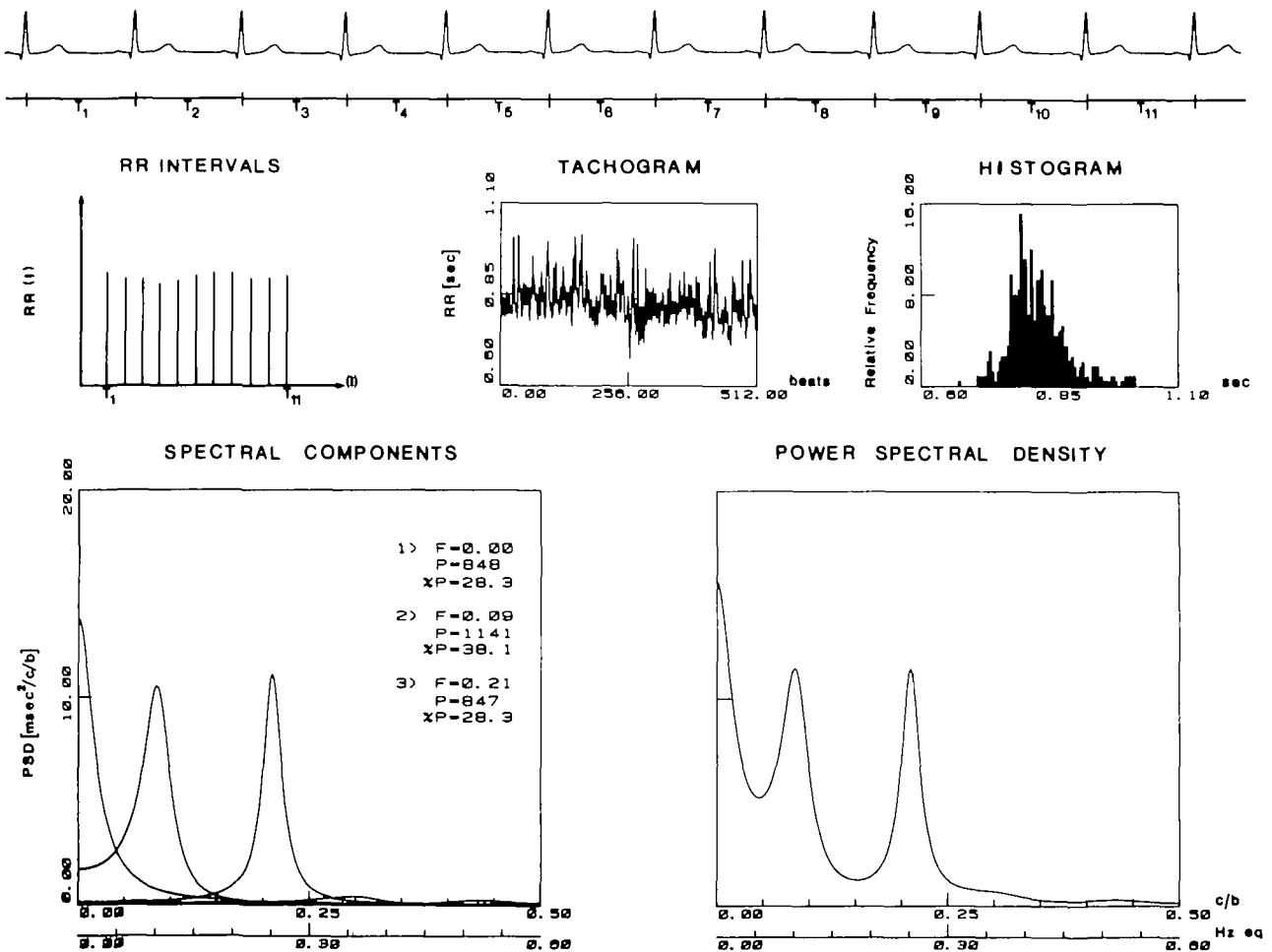


FIGURE 1. Schematic outline of the computer analysis of R-R variability. From the surface ECG (top trace), the series of R-R intervals is calculated as a function of the beat number. This gives rise to the tachogram and, from it, simple statistics are first computed, such as mean, variance, and the relative frequency distribution, i.e., the histogram. Following this, spectral analysis is performed: Individual spectral components are automatically determined, together with their center frequency and associated power, i.e., area. Finally, the autospectrum is computed and plotted. Inset of left lower panel:  $F$  = frequency in cycles/beat;  $P$  = power in  $\text{msec}^2$ ;  $\%P$  = fractional power. In this, as well as in the other R-R interval variability autospectra, the power spectral density (PSD) units should be multiplied by  $10^3$ .

during tilting of appropriate length were selected for analysis. As schematically represented in Figure 1, the computer program first calculates the interval tachogram, i.e., the series of  $N$  consecutive R-R intervals, and saves them in memory. From sections of tachogram of 512 interval values, simple statistics (mean and variance) of the data are computed. This length of the tachogram has been selected as a best compromise between the need for a large time series, in order to achieve greater accuracy in the computation, and the need to obtain stationary recordings, which would be easier for short time periods. As an example, for an average heart rate of about 70/min, 512 successive R-R intervals would amount to approximately 7.5 minutes. The computer program automatically calculates the autoregressive coefficients necessary to define the power spectral density estimate (see the Appendix). An important feature of the program is that it also calculates the model that provides the best statistical estimate and prints out the power and frequency of

every spectral component. Each spectral component is presented in absolute units, as well as in normalized form, by dividing it by the total power less the DC component, if present. This component can also be recognized in the graphs of the autospectra on the decaying part of the curve near the origin of the abscissa. Only components greater than 5% of the total power were considered significant. Stationarity was assessed either by pole diagram analysis (see the Appendix) or by verifying that a difference of less than 5% was present between autospectral components calculated in the two successive 256 beat series constituting the whole series of 512 beats.

In this study, the duration of the periodical phenomena in the variability signal was measured as a function of cardiac beats, rather than seconds. As an example, a four-beat periodical component is represented with a frequency of 1:4, i.e., 0.25 cycle/beat. However, this frequency is easily converted into hertz equivalents (Hz Eq) by dividing it by the average R-R interval

length. For instance, if the average R-R length were 1000 msec, this would correspond to 0.25 Hz Eq. In the figures, both units are indicated, whereas, in the text, only hertz equivalents are used.

De Boer et al<sup>23</sup> have compared different methods of spectral analysis of the heart rate variability signal and found that the analysis of the interval series that represents heart period as a function of the beat number not only provides comparable results to methods that represent heart period as a function of time, but is even better suited to the study of the relationship between heart period and arterial pressure on a beat-by-beat basis.

After synchronous acquisition and appropriate calibration, a similar procedure is used to compute the spectrum of the arterial pressure data for both systolic and diastolic values.<sup>20</sup>

Spectral analysis of the impedance respiratory signal was also performed to assess the effects of metronome breathing on respiratory waveform.

### Statistics

Data are presented as means  $\pm$  SE. Student's *t* test was used to determine the significance of the differences between rest and tilt.

Analysis of variance with Scheffé test was used to assess the effects of age and of  $\beta$ -adrenergic receptor blockade, as well as the effects of controlled breathing.

Differences were considered significant at  $p < 0.05$ .

Regression analysis was used to test the effects of age on R-R interval duration variance.<sup>24</sup>

## Results

### Age Dependency of Heart Rate Variability

R-R interval duration variance demonstrated a significant age dependency as it decreased with increasing age both at rest and during passive upright tilt (90°) (Figure 2). This effect appeared significantly represented by a curvilinear, i.e., exponential, relationship ( $r = 0.70$ ,  $p < 0.001$  at rest, and  $r = 0.60$ ,  $p < 0.001$

during tilt). Therefore, subjects are subdivided into three age groups of, respectively, 20–30 ( $n = 30$ ), 30–45 ( $n = 10$ ), and 45–60 ( $n = 17$ ) years (Table 1). Furthermore, to account for the possible large differences in total power of individual autospectra, variance data are presented in absolute units, whereas spectral components are normalized by dividing each component by total power (less the DC component, if present).

Table 1 indicates that, at rest, there was a slight tendency for mean R-R interval to increase with age, while R-R variance in the young age group was significantly greater ( $4097 \pm 361$  msec<sup>2</sup>) than both in the mid ( $2581 \pm 356$  msec<sup>2</sup>) or in the old age group ( $1354 \pm 205$  msec<sup>2</sup>).

### Effects of Tilt on Heart Rate Variability

Figure 3 depicts a representative example, in a young subject, of the effects of tilting on the time series of R-R intervals, i.e., the tachogram, as well as on the computed autospectrum. The small fluctuations of the instantaneous R-R values around the mean that were present both at rest and during tilt, when analyzed in their nonrandom components, provided two drastically different autospectra. At rest, there were two major spectral components at low ( $\sim 0.1$  Hz Eq) and high ( $\sim 0.25$  Hz Eq) frequency. The normalized area of the low-frequency component (calculated automatically, see "Materials and Methods" and the Appendix) was slightly predominant ( $58 \pm 3\%$ ) with an average low frequency:high frequency (LF:HF) ratio of  $3.6 \pm 0.7$  (Table 1). Notice that only about 85% of total variability is represented by the sum of the LF and HF components (Table 1) since in the individual subjects, smaller components could also be present.

During tilt, the majority of R-R variability was represented by a largely predominant LF component ( $90 \pm 1\%$ ). However, an HF component was also present ( $9 \pm 1\%$ ) although at times very small (Figure 3), with an average LF:HF ratio of  $21 \pm 4$ . As shown in Table 1, heart period during tilting underwent a signifi-

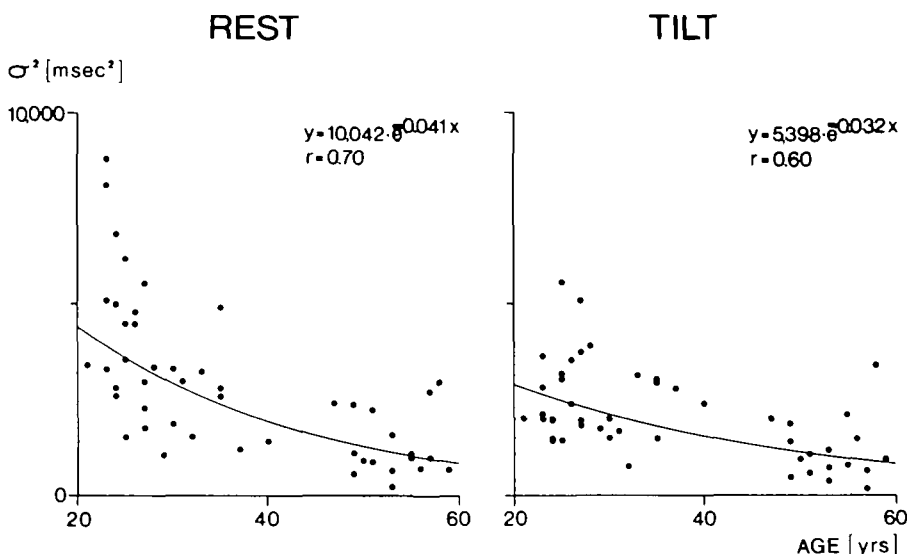


FIGURE 2. Relationship between R-R interval variability, expressed as variance ( $\sigma^2$ ), and age in the study population at rest and during 90° upright passive tilt. Notice that in both cases there is a significant exponential relationship ( $p < 0.001$ ,  $n = 48$ ).

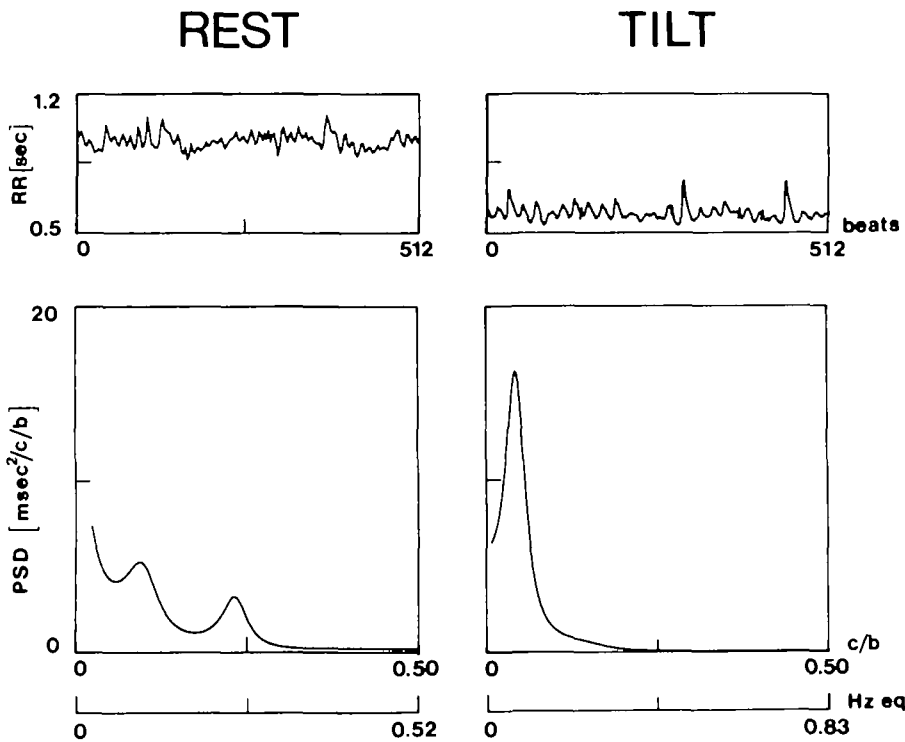


FIGURE 3. R-R interval series, i.e., tachogram at rest and during passive upright 90° tilt. On the auto-spectra (bottom panels), two clearly separated low- and high-frequency components are present at rest. During tilt, the low-frequency component becomes preponderant.

cant reduction, as expected. However, there was no significant correlation between the increases in LF component and in heart rate induced by tilting ( $r = 0.19$ ).

Autospectra of R-R variability in individual subjects remained remarkably constant when repeated over time. As shown in Table 2, there was no difference ( $p > 0.05$ ), at rest and during tilt, in the results obtained in two or three different recording sessions with an interval up to 1 year.

**Age Dependency of the Response to Tilt**

During tilt in the old age group, mean R-R was significantly longer, and variance smaller, than both in the mid and young age groups (Table 1).

The effects of tilt on the autospectra of R-R interval variability when examined in the three different age groups demonstrated only minor differences, as LF component was smaller and HF component slightly greater in the old age group (Table 1). This difference, however, was no longer apparent in the LF:HF ratio, which was not significantly different in the three age groups both at rest and during tilt (Table 1).

**Effects of Controlled Respiration**

The effects of controlled respiration were examined in 16 young subjects that were first studied while breathing spontaneously (Figure 4, top panels) and, subsequently, while breathing following a metronome (Figure 4, bottom panels). Respiratory frequency, in

TABLE 2. Reproducibility of the Effects of Tilt on R-R Variability

	R-R interval (msec)	R-R variance (msec <sup>2</sup> )	Low-frequency component		High-frequency component	
			Normalized power	Frequency (Hz Eq)	Normalized power	Frequency (Hz Eq)
<i>Rest</i>						
First study (n = 10)	885 ± 43	3264 ± 464	62.0 ± 5.0	0.11 ± 0.01	28.5 ± 3.3	0.26 ± 0.02
Second study (n = 10)	858 ± 32	3508 ± 766	61.4 ± 4.6	0.10 ± 0.01	29.0 ± 3.7	0.25 ± 0.02
Third study (n = 4)	914 ± 34	4201 ± 781	56.1 ± 2.6	0.12 ± 0.01	31.3 ± 3.8	0.31 ± 0.03
<i>Tilt</i>						
First study (n = 10)	672 ± 24*	2612 ± 528	89.5 ± 1.4*	0.09 ± 0.01	6.0 ± 0.5*	0.30 ± 0.04
Second study (n = 10)	676 ± 33*	1955 ± 303	89.2 ± 2.2*	0.10 ± 0.01	6.9 ± 0.07*	0.26 ± 0.03
Third study (n = 4)	700 ± 25*	3381 ± 250	87.0 ± 2.0*	0.10 ± 0.01	7.7 ± 1.3*	0.27 ± 0.02

Average delay in time between the first and second study: 138 days (range 7–365). Average delay in time between the second and third study: 127 days (range 90–150).

\*Value during tilt significantly different from value at rest ( $p < 0.05$ ).

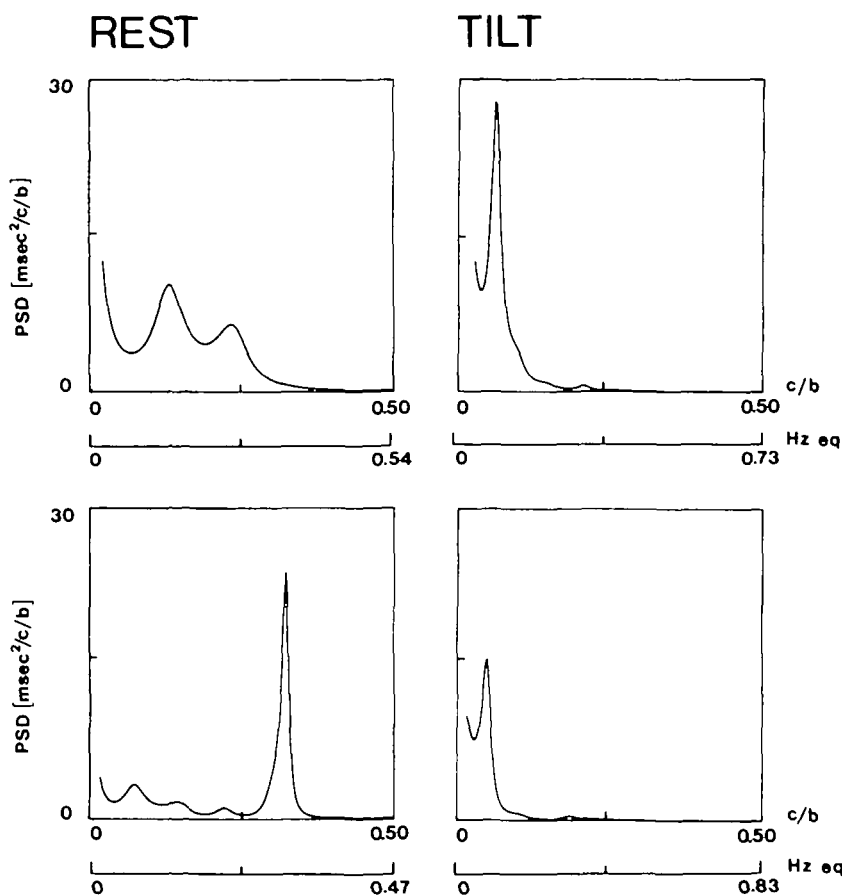


FIGURE 4. Representative example of autospectra of R-R interval variability during spontaneous breathing (top panels) and during controlled respiration, at 20/min (bottom panels). Note that, with controlled respiration at rest, the relative power of the high-frequency component becomes predominant.

the latter condition, was set at 20/min, i.e., 0.33 Hz, in order to separate better the low-frequency from the high-frequency respiratory component.

This maneuver modified the respiratory waveform, as demonstrated by spectral analysis: during spontaneous respiration, nonstationarities in the signal are reflected by a diffuse profile of the spectrum, whereas during metronome breathing, the near sinusoidal shape of the respiratory waveform is reflected by a single narrow component in the spectrum. In either condition, the HF component of the spectrum of R-R variability coincided with the main respiratory frequency. The LF component never coincided with a major component of the spectrum of the respiratory waveform.

During controlled respiration, at rest, there was a significant reduction of the LF component with a concomitant increase in the HF component (Figure 4, bottom; Table 3). The LF:HF ratio was significantly reduced from  $2.5 \pm 0.3$  to  $0.7 \pm 0.1$ . As the subjects were allowed to adjust their tidal volume, during spontaneous respiration the tidal volume ( $n = 7$ ) was  $552 \pm 98$  ml, and slightly less ( $533 \pm 76$  ml) during controlled breathing at 20/min. During metronome breathing, there was only a small but not significant change in transcutaneous  $P_{CO_2}$  and  $P_{O_2}$  (respectively,  $42 \pm 2$ ,  $83 \pm 4$  mm Hg during spontaneous and  $38 \pm 2$ ,  $79 \pm 6$  mm Hg during controlled breathing,  $p > 0.05$ ). During tilt, the HF component was de-

TABLE 3. Effects of Metronome Breathing on R-R Variability ( $n = 16$ )

	R-R interval (msec)	R-R variance ( $msec^2$ )	Low-frequency component		High-frequency component		
			Normalized power	Frequency (Hz Eq)	Normalized power	Frequency (Hz Eq)	
<i>Rest</i>							
Free resp frequency Hz	$0.26 \pm 0.02$	$884 \pm 27$	$4095 \pm 436$	$56.2 \pm 3.9$	$0.11 \pm 0.01$	$27.3 \pm 2.7$	$0.24 \pm 0.02$
			*		*	*	
Control resp frequency Hz	$0.33$	$934 \pm 29$	$4220 \pm 541$	$28.9 \pm 4.3$	$0.11 \pm 0.01$	$51.6 \pm 4.4$	$0.33 \pm 0.01$
<i>Tilt</i>							
Free resp frequency Hz	$0.26 \pm 0.03$	$678 \pm 21^\dagger$	$2647 \pm 308^\dagger$	$86.4 \pm 2.9^\dagger$	$0.10 \pm 0.01$	$7.8 \pm 1.4^\dagger$	$0.25 \pm 0.02$
			*		*	*	
Control resp frequency Hz	$0.33$	$705 \pm 22^\dagger$	$2119 \pm 428^\dagger$	$71.6 \pm 5.3^\dagger$	$0.10 \pm 0.01$	$15.3 \pm 3.0^\dagger$	$0.31 \pm 0.02$

\*Significant difference ( $p < 0.05$ ).

†Value during tilt significantly different from value at rest ( $p < 0.05$ ).



creased while the LF component was increased, as expected; however, this LF increase was less pronounced than during spontaneous breathing (Figure 4). Consequently, the increase in LF:HF ratio induced by tilt was smaller during controlled respiration ( $8.3 \pm 1.6$ ) than during spontaneous breathing ( $16.0 \pm 3.0$ ).

Furthermore, during tilt, tidal volume was slightly smaller with controlled respiration than with spontaneous breathing ( $574 \pm 100$  and  $657 \pm 48$  ml, respectively), and no significant change ( $p > 0.05$ ) was observed in transcutaneous  $P_{CO_2}$  or  $P_{O_2}$  (respectively,  $39 \pm 4$ ,  $87 \pm 5$  mm Hg with spontaneous and  $43 \pm 3$ ,  $82 \pm 4$  mm Hg with metronome breathing).

#### $\beta$ -Adrenergic Receptor Blockade

The effects of  $\beta$ -adrenergic receptor blockade were assessed in two sets of experiments. Acute  $\beta$ -adrenergic receptor blockade was obtained in 10 young subjects by IV bolus of 0.2 mg/kg of propranolol that reduced significantly heart rate and arterial blood pressure (Table 1). Under these conditions, R-R interval variance was significantly increased from control (Figure 5; Table 1), but normalized autospectral components were not modified significantly (Table 1). Dur-

ing tilt, there was a significantly lesser reduction in R-R interval and a smaller increase in the LF component and in the LF:HF ratio (Figure 5; Table 1).

Chronic  $\beta$ -adrenergic receptor blockade was obtained in 12 young subjects with 0.6 mg/kg p.o. propranolol t.i.d. for 6 days, which reduced significantly resting heart rate and arterial blood pressure. The effects on resting R-R interval variability, like acute blockade, were characterized by augmented variance (Figure 5; Table 1). However, marked changes were observed in the resting autospectra: LF was smaller, HF greater, and hence, LF:HF significantly reduced, compared with controls (Table 1; Figure 5). During tilt, the LF component was reduced and HF component increased, compared with control. Thus, during chronic  $\beta$ -adrenergic receptor blockade, LF:HF ratio increased to only  $3.78 \pm 0.93$ , which was smaller than that observed in the young age group without  $\beta$ -blockade during tilt ( $20.79 \pm 3.68$ ) but was similar to their resting value ( $3.62 \pm 0.70$ ).

#### Simultaneous R-R and Blood Pressure Variabilities

In a group of 7 normotensive subjects (20–60 years old) in whom systemic arterial pressure was recorded with direct high-fidelity techniques (see "Materials

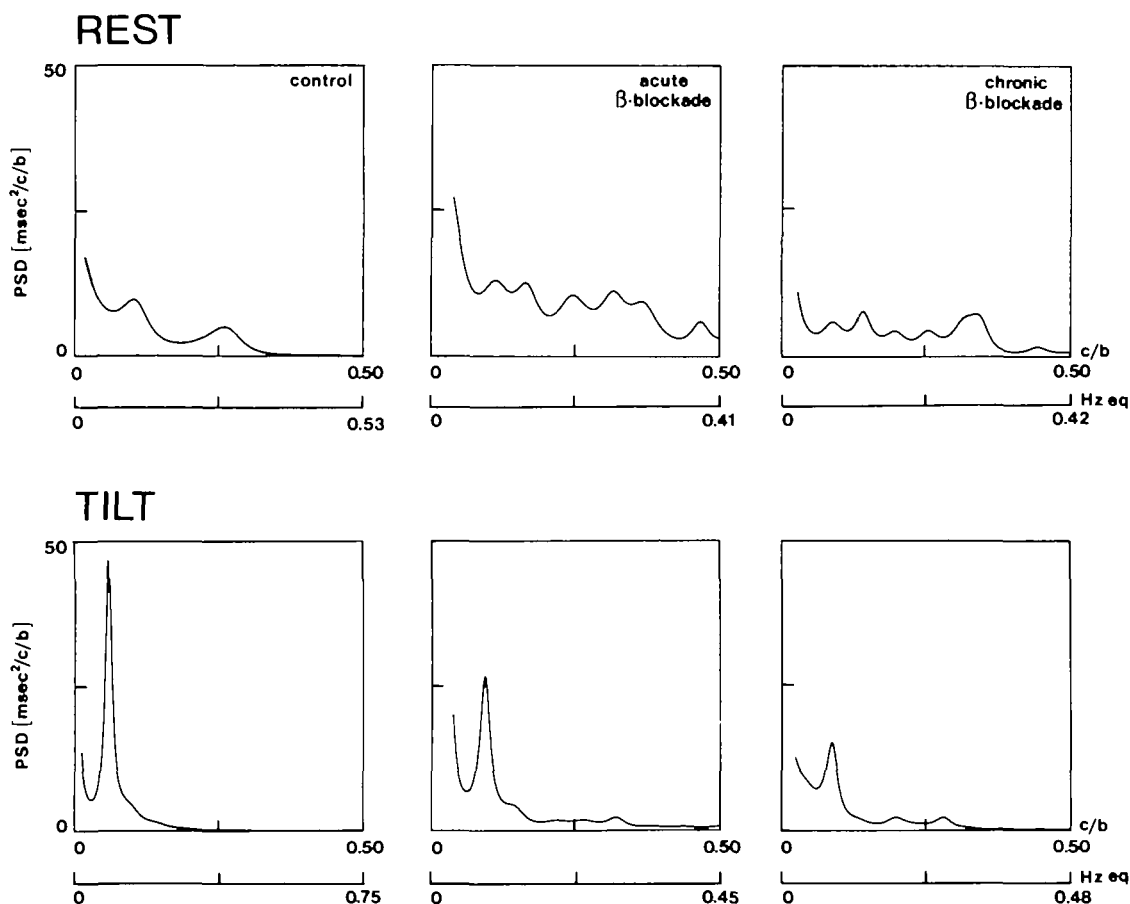


FIGURE 5. Autospectra of R-R interval variability of a young subject at rest (top panels) and during tilt (bottom panels) under control conditions (left panels) and during acute (0.2 mg/kg IV propranolol, middle panels), and chronic (0.6 mg/kg p.o. propranolol t.i.d.)  $\beta$ -adrenergic receptor blockade. Note the progressive reduction in the relative power of the low-frequency component during tilt.

and Methods") simultaneously with the ECG, the analysis of the variability of systolic and diastolic beat-by-beat values was also performed.

Systolic arterial pressure variability was characterized by small oscillations in the time series of beat-by-beat values (Figure 6), resulting in a tracing comparable to the interval tachogram.

Moreover, the autospectra of systolic arterial pressure variability demonstrated two major components, respectively, a LF of  $41 \pm 8\%$  and HF of  $19 \pm 5\%$ , with a relative ratio of  $2.8 \pm 0.7$  at rest. During tilt, the LF component became largely predominant ( $73 \pm 8\%$ ), with an increase in the LF:HF ratio of  $17 \pm 5$ . Spectral analysis of diastolic blood pressure gave similar results (Table 4). It should be appreciated that the simultaneous autospectra of R-R variability (Figure 6; Table 4) provided results resembling those obtained from the analysis of arterial pressure variability, with two components recognized at the same frequencies, with similar relative ratios.

As shown in Figure 7, cross-spectral analysis of R-R and blood pressure variability confirmed this evaluation by showing coherence only between the same LF and HF components both at rest and during tilt. The phase difference between R-R and arterial pressure variability was approximately  $0^\circ$  at HF. At LF, the phase presented different patterns in rest and in tilt conditions: In the former case, the phase had a linear relation with frequency, whereas, in the latter case, it oscillated around a fixed value. In both cases, the phase corresponding to the central frequency of the LF peak was about  $60^\circ$ , i.e.,  $\frac{1}{6}$  of the entire  $360^\circ$  cycle. As LF corresponds to a period of about 10 beats, the calculated phase difference amounted to  $\frac{1}{6}$  of that period, i.e., to a delay of 1.7 beats, with pressure leading.

#### R-R and Arterial Pressure Variability in Conscious Dogs

To assess more directly the effects of sympathetic innervation on heart rate variability, we performed experiments on a group of conscious dogs before and after bilateral stellectomy.

As shown in Figure 8 and Table 5, under control conditions R-R variability was almost solely repre-

sented by a HF respiratory-linked component, whereas a very small ( $8 \pm 2\%$ ) LF component was present in only 50% of the animals. Similarly (Table 5), the autospectra of both systolic and diastolic blood pressure variabilities demonstrated a major HF component. During moderate hypotension ( $-9 \pm 2\%$  from  $85 \pm 3$  mm Hg mean arterial pressure) obtained by a continuous infusion of IV nitroglycerin ( $32 \mu\text{g}/\text{kg}$  per minute), heart rate increased  $47 \pm 9\%$  from  $82 \pm 5$  beats/min as a consequence of sympathetic activation.

Under those conditions, not only was there a significant reduction of total power of R-R variability, but also the HF component of the autospectrum was reduced to  $42 \pm 3\%$ , whereas, in all animals, a LF component of similar power ( $36 \pm 6\%$ ) became evident. Similar changes were observed in arterial pressure autospectra (Table 5). Bilateral stellectomy did not significantly modify arterial pressure, heart rate, or R-R or pressure variability, as expressed both by variance or by their autospectra (Figure 8; Table 5). The IV infusion of nitroglycerin, repeated at the same dose, reduced arterial pressure and increased heart rate to a similar extent (Table 5). The response of R-R variability, however, was modified. Although total power was reduced to a value similar to that observed in the intact animals, only a HF component could be observed in the autospectrum. Conversely, the increase in LF components of pressure autospectra was essentially preserved (Table 5).

#### Discussion

This study in man examines the beat-to-beat oscillations which characterize heart rate and arterial pressure under various steady state conditions, in the hypothesis that the quantitative information provided by the spectral analysis of these oscillations reflects the interaction between sympathetic and parasympathetic regulatory activities.

Various approaches have been proposed to evaluate the contributions of parasympathetic and sympathetic discharges<sup>25</sup> to the heart rate variability. Parasympathetic activity has been clinically inferred from peak-to-peak variations of the heart period either in the clinical laboratory environment<sup>26</sup> or with Holter moni-

TABLE 4. Effects of Tilt on Simultaneous R-R Interval and Arterial Pressure Variabilities (n = 7)

	Mean value	Variance	Low-frequency component		High-frequency component	
			Normalized power	Frequency (Hz Eq)	Normalized power	Frequency (Hz Eq)
<i>Rest</i>						
R-R	$748 \pm 20$ msec	$1403 \pm 571$ msec <sup>2</sup>	$41.2 \pm 8.7$	$0.12 \pm 0.01$	$19.4 \pm 5.3$	$0.33 \pm 0.03$
SAP	$125 \pm 5$ mm Hg	$20 \pm 3$ mm Hg <sup>2</sup>	$46.7 \pm 7.2$	$0.11 \pm 0.02$	$27.5 \pm 9.0$	$0.32 \pm 0.03$
DAP	$70 \pm 5$ mm Hg	$15 \pm 3$ mm Hg <sup>2</sup>	$48.5 \pm 11.7$	$0.10 \pm 0.01$	$31.1 \pm 7.7$	$0.35 \pm 0.04$
<i>Tilt</i>						
R-R	$633 \pm 29^*$ msec	$1483 \pm 643$ msec <sup>2</sup>	$73.4 \pm 8.6^*$	$0.09 \pm 0.01$	$7.4 \pm 1.9^*$	$0.33 \pm 0.02$
SAP	$129 \pm 6$ mm Hg	$47 \pm 10^*$ mm Hg <sup>2</sup>	$78.7 \pm 4.1^*$	$0.09 \pm 0.01$	$9.2 \pm 2.1$	$0.32 \pm 0.03$
DAP	$74 \pm 4$ mm Hg	$29 \pm 7^*$ mm Hg <sup>2</sup>	$73.3 \pm 2.5^*$	$0.08 \pm 0.01$	$9.9 \pm 1.7^*$	$0.35 \pm 0.03$

SAP = systolic arterial pressure; DAP = diastolic arterial pressure.

\*Value during tilt significantly different from value at rest ( $p < 0.05$ ).

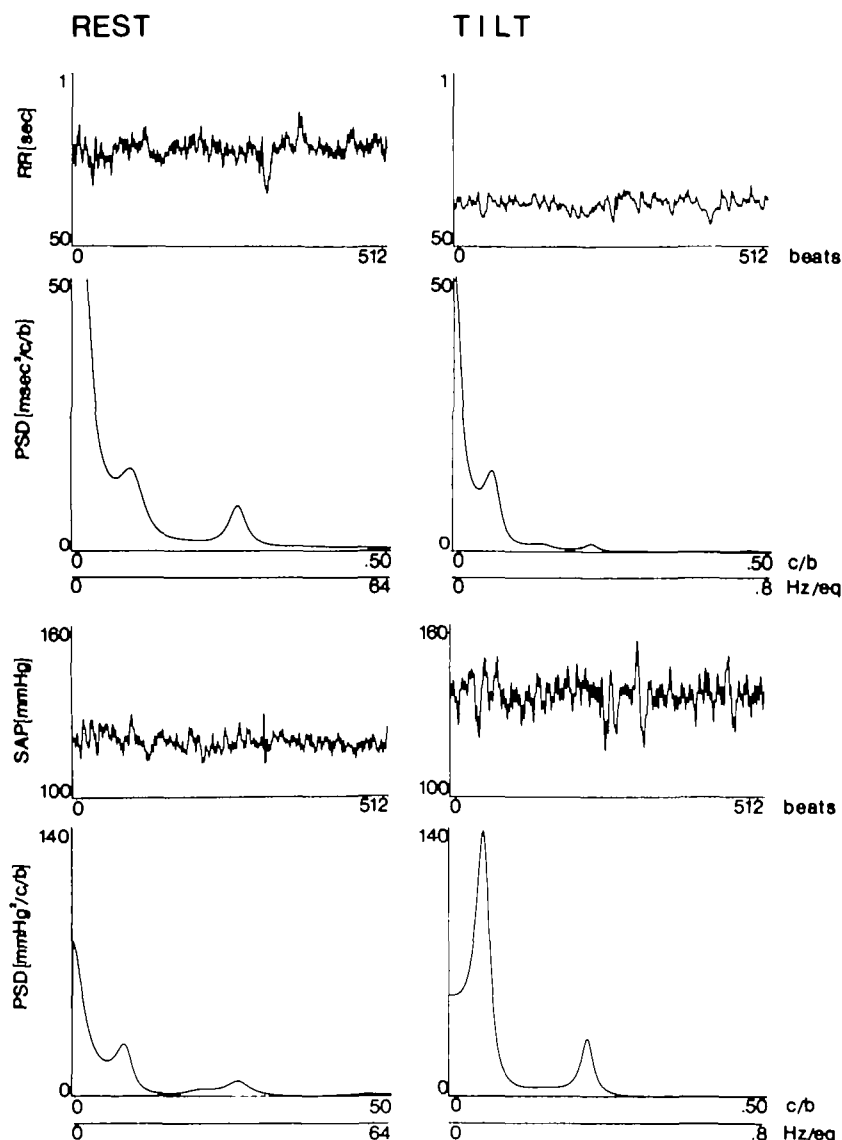


FIGURE 6. Simultaneous analysis of R-R interval and systolic arterial pressure variabilities in a young subject. Note the reduction in average R-R interval and the slight increase in average systolic arterial pressure on passing from rest to tilt. The two components of the autospectrum of systolic arterial pressure variability are similar to those of R-R interval variability at rest and during tilt.

toring.<sup>27</sup> A different approach has been used by others<sup>10,12,15</sup> who utilized spectral techniques to assess the frequency components of the heart rate variability signal. A possible major advantage of spectral analysis is the observation that changes in the sympathetic activity to the heart can also be recognized, and hence, some index of the instantaneous balance between sympathetic and vagal activity can be obtained.<sup>15</sup> It should be noted that, since heart rate variability signal is a pseudorandom phenomenon, previous studies using the fast Fourier transform to compute the power spectrum have some technical limitations (see the Appendix). They include the deterministic nature of the algorithms used, which, in principle, are applicable only to periodical phenomena,<sup>16</sup> the need of windowing the data, and the difficulty in defining with certainty the relative power of the various spectral components.

In the present study, some of these limitations were avoided because the autoregressive algorithms, which are applicable to nonperiodical phenomena,<sup>28</sup> computed automatically the number and relative power of the

various spectral components. Moreover, there was no need for windowing or filtering the data.<sup>13,22</sup>

#### Spectral Analysis of Heart Rate Variability and Parasympathetic Activity

In our analysis, we observed at rest two consistent major spectral components, a LF at  $\sim 0.1$  Hz and an HF at  $\sim 0.25$  Hz. The LF component seems to correspond to the Mayer waves,<sup>5,17</sup> while the HF component is synchronous with the respiration and has been considered as a quantitative evaluation of respiratory arrhythmia.<sup>15</sup> Since the HF component disappears after atropine, it could represent a clinically useful index of vagal activity.<sup>15</sup>

In our study, during spontaneous quiet breathing at rest, a relatively small area was found to be associated with the HF component. However, Pomeranz et al<sup>15</sup> found a predominant HF component in 8 subjects who breathed following a metronome at 15/min. A similarly enhanced HF component was found in this study in 16 subjects who breathed following a metronome at

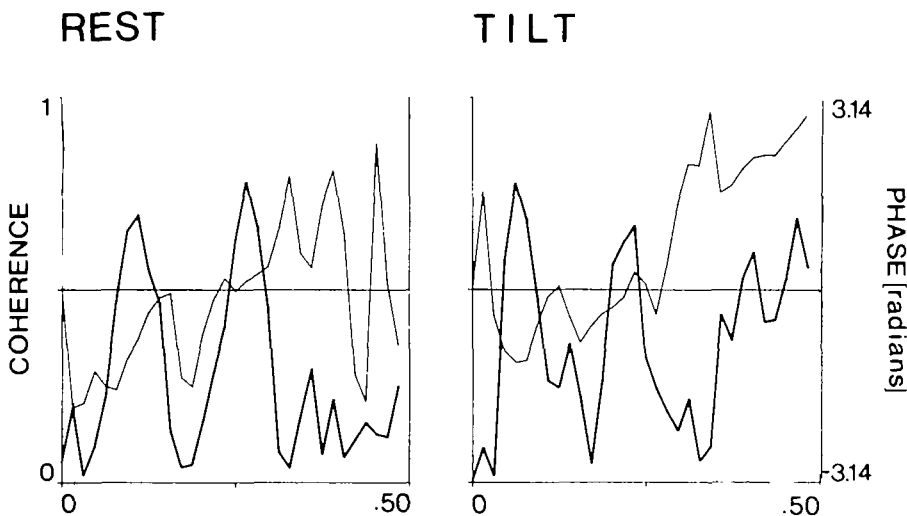


FIGURE 7. Cross-spectral analysis (coherence, thick lines; phase, thin lines) between R-R interval and systolic arterial pressure variabilities computed from the data of Table 4. Note that, both at rest and during tilt, only two spectral components show a coherence greater than 0.5 in both conditions. Phase is about 0° at the respiratory component and negative, i.e., pressure leads R-R interval, at the 0.1-Hz component.

20/min. The same subjects showed a predominant LF component when breathing spontaneously. Changes in the amount of respiratory arrhythmia and, hence, of the HF component could reflect changes in volume, as well as in frequency of respiration.<sup>29,30</sup> Important modulatory mechanisms could also be initiated by changes in arterial Po<sub>2</sub> and Pco<sub>2</sub>, and thereby changes in neural efferent activities,<sup>31</sup> as well as by variations in arterial pressure and consequent alterations in baroreceptor input.<sup>32</sup>

In this study, we could not define the exact mecha-

nisms responsible for the observed increase in HF component with controlled breathing, as tidal volume, transcutaneous Po<sub>2</sub> and Pco<sub>2</sub>, and arterial pressure did not change significantly.

However, an important role is likely to be played by the marked change in respiratory waveform,<sup>33</sup> acting either through a more efficient stimulation of lung receptors<sup>34</sup> or through fluctuations induced in arterial pressure.<sup>32,35</sup> In either case, the increased HF component of R-R variability would indicate that during metronome breathing there is enhanced vagal tone.

TABLE 5. Effects of Moderate Hypotension on Simultaneous R-R Interval and Arterial Pressure Variabilities in Conscious Dogs

	Mean value	Variance	Low-frequency component		High-frequency component	
			Normalized power	Frequency (Hz Eq)	Normalized power	Frequency (Hz Eq)
<b>Part A</b>						
Intact (n = 8) control						
R-R	755 ± 62 msec	58237 ± 17754 msec <sup>2</sup>	4 ± 2	0.14 ± 0.01	63 ± 6	0.24 ± 0.02
SAP	119 ± 18 mm Hg	217 ± 112 mm Hg <sup>2</sup>	19 ± 4	0.12 ± 0.02	60 ± 4	0.23 ± 0.03
DAP	71 ± 3 mm Hg	161 ± 71 mm Hg <sup>2</sup>	15 ± 5	0.10 ± 0.03	64 ± 4	0.24 ± 0.03
Nitroglycerin (32 µg/kg per min)						
R-R	521 ± 37* msec	7513 ± 3941* msec <sup>2</sup>	36 ± 6*	0.12 ± 0.01	42 ± 4*	0.26 ± 0.03
SAP	104 ± 5* mm Hg	45 ± 19 mm Hg <sup>2</sup>	55 ± 4*	0.09 ± 0.01	27 ± 5*	0.27 ± 0.06
DAP	66 ± 2 mm Hg	50 ± 21 mm Hg <sup>2</sup>	59 ± 7*	0.10 ± 0.01	22 ± 5*	0.25 ± 0.03
<b>Part B</b>						
Stellectomy (n = 5) control						
R-R	790 ± 41 msec	42786 ± 14370 msec <sup>2</sup>	3 ± 2	0.10 ± 0.01	61 ± 5	0.23 ± 0.01
SAP	137 ± 10 mm Hg	67 ± 28 mm Hg <sup>2</sup>	8 ± 3	0.11 ± 0.01	72 ± 4	0.25 ± 0.01
DAP	76 ± 3 mm Hg	78 ± 22 mm Hg <sup>2</sup>	8 ± 4	0.09 ± 0.01	63 ± 7	0.23 ± 0.02
Nitroglycerin (32 µg/kg per min)						
R-R	617 ± 19* msec	3491 ± 1696* msec <sup>2</sup>	0 ± 0 <sup>†</sup>	Unmeasurable	76 ± 8 <sup>†</sup>	0.24 ± 0.02
SAP	110 ± 7* mm Hg	16 ± 4 mm Hg <sup>2</sup>	38 ± 8*	0.08 ± 0.01	46 ± 10*	0.26 ± 0.02
DAP	73 ± 4 mm Hg	24 ± 13 mm Hg	44 ± 6*	0.09 ± 0.01	37 ± 7*	0.25 ± 0.02

SAP = systolic arterial pressure; DAP = diastolic arterial pressure.

\*Value during nitroglycerin infusion significantly different from control (p < 0.05).

†Significant difference (p < 0.05) between value in intact and denervated animals.

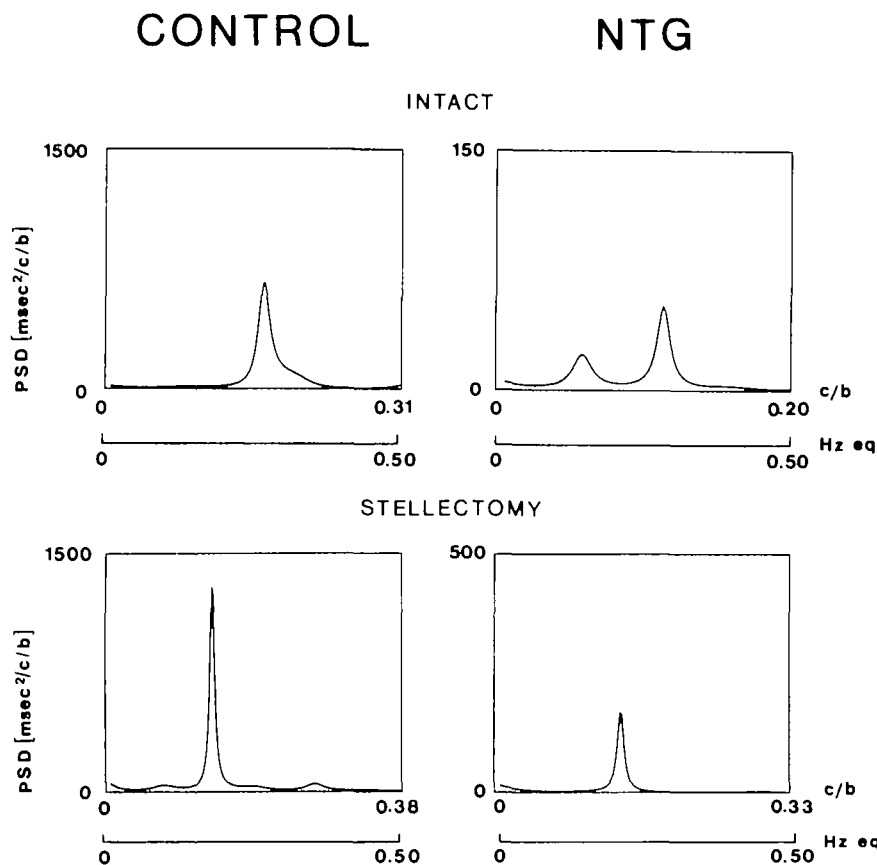


FIGURE 8. Autospectra of R-R interval variability of a conscious healthy dog in control conditions (left panels) and during an IV infusion of nitroglycerin to excite the sympathetic outflow. The top panels were obtained with cardiac nerves intact, the bottom panels after full recovery from bilateral stellectomy. Note the presence of a large low-frequency component during nitroglycerin infusion only in the neurally intact situation.

### Spectral Analysis of Heart Rate Variability and Sympathetic Activity

The possibility that spectral analysis could provide an index of sympathetic activity is less well established. In animal studies, sympathetic activity has been considered either to play no role<sup>10</sup> or else to be instrumental in the genesis of the LF rhythm.<sup>12,36</sup> Furthermore, electroneurographic recordings in dogs<sup>25</sup> suggest that sympathetic activity might also modulate respiratory arrhythmias. This apparent discrepancy of results is probably a consequence of the differences in the preparations used, i.e., anesthetized and acutely decerebrated cats<sup>10</sup> as opposed to conscious dogs.<sup>12</sup>

In our study, we tested the hypothesis that spectral analysis of heart rate variability could provide an assessment of sympathetic tone, by planning experiments in man in which sympathetic activity was either increased functionally or blocked pharmacologically. Enhanced sympathetic drive to the heart, as obtained by an orthostatic stimulus, was constantly associated with a marked increase in the LF and with a decrease in the HF component of the autospectrum. We selected a passive change of posture in order to minimize the influence on heart rate exerted by the muscular effort of active standing.<sup>37</sup>

Acute  $\beta$ -adrenergic receptor blockade with intravenous propranolol, besides reducing heart rate, had a marked effect on R-R variability. At rest variance increased, while the relative contribution of LF and HF components remained relatively unchanged. During

tilt, there was a significant reduction of the increase of the LF component and of the LF:HF ratio. Similar blunting effects exerted by acute  $\beta$ -adrenergic receptor blockade were observed during active standing by Pomeranz et al.<sup>15</sup>

After chronic  $\beta$ -adrenergic receptor blockade, there was, at rest, not only an increase in R-R interval variance similar to that observed with acute blockade, but also a significant effect on spectral components. Thus, LF and LF:HF were significantly smaller, and HF was significantly greater than under control conditions. During tilt, the increase in LF component and in the LF:HF ratio was markedly reduced; indeed, the numerical value of the ratio was similar to that observed under resting control conditions without  $\beta$ -blockade.

It might be pertinent to recall that Wallin et al<sup>38</sup> demonstrated, with electroneurographic techniques in hypertensive subjects, that chronic  $\beta$ -adrenergic blockade was effective in reducing sympathetic efferent activity, whereas acute blockade was ineffective.

As to the effects of metronome breathing on LF, this component was reduced at rest, and its increase during tilt was blunted, possibly as a consequence of the inhibitory effect on sympathetic rhythms produced by pulmonary afferent activity.<sup>34</sup>

As to the effects of aging, it was progressively associated with a reduced R-R variability as expressed by variance, and during tilt with a smaller activation of LF and a smaller reduction of HF. However, the changes in LF:HF ratio during tilt were not significantly different among the various age groups. This approach

would suggest that aging is characterized by a new equilibrium between the two sections of the visceral nervous system rather than by alterations limited to the sympathetic outflow.<sup>39,40</sup>

#### Simultaneous Variability of R-R Interval and Arterial Pressure

An additional major observation of this study is that the autospectra of R-R interval and of both systolic and diastolic arterial pressure beat-to-beat variabilities were similar at rest and showed parallel changes with tilt. The presence in arterial pressure recordings of an HF respiratory component has been traditionally interpreted as a mechanical consequence of respiration, which could act directly on intrathoracic vessels or indirectly through changes in stroke volume<sup>7,41</sup> and heart period.<sup>36</sup> Sympathetic modulation of arterial smooth muscle is probably too slow to follow the 0.25-Hz respiratory frequency.<sup>42</sup> Obviously, these beat-to-beat pressure changes could affect R-R interval through complex reflex adjustments,<sup>43</sup> among which baroreflexes could have a paramount importance.<sup>44</sup>

The LF components correspond to the well-known Mayer<sup>5</sup> waves, a phenomenon that, although described in quite artificial experimental conditions, seems to pertain to normal human subjects<sup>8</sup> as well. Various theories, including myogenic oscillations, central rhythms, feedback mechanisms, and "resonance" disturbances, have been advanced for their interpretation.<sup>17</sup>

As to the effects of tilt, arterial pressure beat-by-beat variability, as expressed by variance, increased significantly for both systolic and diastolic values. Furthermore, the LF oscillatory components increased significantly and to an extent similar to that observed in the autospectrum of R-R interval, whereas HF components were, in both autospectra, markedly reduced.

In this respect, it should be mentioned that an increase in arterial pressure variability has been described also in essential hypertension,<sup>45</sup> and a greater LF component has been documented in daytime as opposed to nighttime recordings in ambulatory patients.<sup>46</sup> Both conditions are possibly characterized by a higher sympathetic activity.

Cross-spectral analysis of systolic arterial pressure and R-R interval variabilities indicated that a high degree of coherence existed between the fluctuations of these two variables both in recumbency and during tilt. In correspondence to the HF component, arterial pressure and R-R interval changes occurred in phase, whereas each LF pressure change preceded R-R interval oscillation by about two beats.

Although this cross-spectral analysis provides no direct insight into the mechanisms linking heart period and arterial pressure oscillations, with their possible neural and non-neural components,<sup>12,47</sup> it supports the conclusion that similar information on oscillatory rhythms can be obtained from both invasive and noninvasive studies, not only at rest, but also during augmented sympathetic activity.

In a previous study by De Boer et al,<sup>47</sup> only a small HF component was observed in the autospectrum of

diastolic arterial pressure measured in sitting subjects either with direct or indirect plethysmographic clamp method.<sup>48</sup> Technical differences in arterial pressure recording and the intermediate level of gravitational stimulation induced by sitting should account for the difference in our data.

#### Conscious Dogs

More direct information on the role of cardiac sympathetic nerves was derived from a group of experiments in conscious dogs before and after bilateral stellectomy. In the conscious dog, the LF components are, under control conditions, either absent<sup>33</sup> or variable<sup>12,36</sup> and small (Table 5). An excitation of sympathetic activity as induced by moderate hypotension obtained with phentolamine<sup>36</sup> or, as in our experiments, with nitroglycerin, was associated with an increase in LF components. This increase was no longer present after bilateral stellectomy, which, on the other hand, did not modify the LF and HF components characterizing the arterial pressure variability (Table 5). Hence, in conscious dogs that had fully recovered from surgery, it was possible for the first time to dissociate the LF components present in the heart rate and arterial pressure autospectra by selectively interrupting the cardiac sympathetic loop.

#### Changes in LF:HF Ratio as Markers of Changes in Sympatho-Vagal Balance

It has been a rather simple but traditional working hypothesis that conditions which increase sympathetic activity decrease vagal tone and vice versa.<sup>48</sup> However, in electrophysiological experiments, recordings of the sympathetic and vagal discharges, directed to the heart, indicated both a reciprocal<sup>50</sup> and a nonreciprocal<sup>31</sup> organization.

The actual balance between these two outflows is likely to have paramount importance not only under such physiological conditions as gravitational stimuli, exercise, and changes in central command,<sup>51</sup> but also during disturbances such as arrhythmias,<sup>52</sup> myocardial ischemia, arterial hypertension,<sup>53</sup> or alterations in the renin-angiotensin system.<sup>12</sup>

It has recently been suggested that spectral analysis of R-R interval variability might reflect this balance.<sup>15</sup> In our study, the LF:HF ratio appeared to be a convenient index of such interaction. Indeed, the simultaneous increase in vagal and decrease in sympathetic mechanisms produced by metronome breathing were reflected by a reduction of LF:HF ratio at rest and by a blunting of the increase induced by tilt. Furthermore, LF:HF ratio appeared to follow the reductions of sympathetic activity produced by acute and chronic  $\beta$ -adrenergic receptor blockade both at rest and during tilt. Clinical research into those states characterized by a disturbance in the neural regulatory mechanisms will probably reveal in the near future whether this approach will have true pathophysiological significance.

#### Appendix

The basic assumption underlying the proposed signal processing methods is that heart rate and systolic and diastolic

blood pressure values fluctuate cycle by cycle, even in stable conditions, around a given mean value. Beat-to-beat heart rate and arterial blood pressure variability signals are obtained first from the original biological signals as a discrete time series  $y(k)$  where  $k$  is the progressive number of detected events.

Variability signals are intrinsically pseudo-random and can be considered as the realization of a stochastic process  $y(k)$  which is the output of a linear time-invariant system driven by white noise  $w(k)$ , which constitutes the random component of the model and is fully characterized by a mean value which is zero for simplicity of notation and a variance  $\lambda^2$  [i.e.,  $w(k) = WN(O, \lambda^2)$ ] according to the model

$$w(k) \rightarrow \boxed{H(z)} \rightarrow y(k),$$

where  $H(z)$  is the transfer function in the complex variable  $z$ , which is determined in a quantitative way and is the model of the signal-generating mechanism. A simple structure of input/output relation is given by the autoregressive (AR) modelization

$$y(k) = \sum_{i=1}^p a(i)y(k-i) + w(k)$$

where  $a(i)$  are the  $p$  unknown parameters of identification. More complex models are described by Box and Jenkins.<sup>28</sup> Obviously,

$$H(z) = \left( 1 - \sum_{i=1}^p a(i) \cdot z^{-i} \right)^{-1}$$

which relates the transfer function with the identification coefficients. Hence, given  $N$  samples of variability signals, the problem is to estimate  $H(z)$  or, more precisely, the vector  $\theta$  of parameters

$$\theta = [a(1), a(2), a(3) \dots a(p), \lambda^2]$$

The algorithm applied for such an identification is the Batch least squares method via Levinson-Durbin recursion.<sup>18</sup>

Two tests are used to check the validity of the assumed model. The first one is Anderson's test, which measures the whiteness of the prediction error: The identification is not accepted if the test is not satisfied within 5% confidence interval. After fulfilling Anderson's test, the order of the model is chosen as the one which minimizes Akaike's final prediction error (FPE) figure of merit.<sup>54</sup> In this way, the model is completely determined by order  $p$  and vector  $\theta$ .

Power spectral density (PSD) estimation  $P(f)$  is obtained from the following relation

$$P(f) = \lambda^2 / \left| 1 - \sum_{i=1}^p a(i) \exp(-j2\pi fi) \right|^2$$

where  $f$  is the frequency and the sampling period is unitary. The resulting PSD satisfies the criterion of maximum entropy<sup>16</sup> and presents many advantages in respect to the methods based on classical Fourier analysis (FFT algorithms),<sup>18</sup> namely, a more consistent and smoother spectral estimation, a spectral resolution which is independent of the number  $N$  of samples, and the possibility of avoiding windowing procedures.

Another important advantage of AR modeling with respect to the more traditional techniques is the possibility of decomposing the autocorrelation function and, hence, its transform in the frequency domain (i.e., the power spectrum density) in single components by applying the residuals theorem.<sup>55</sup> In this way, the AR spectrum also provides the individual spectral components in terms of center frequency and of the corresponding power in absolute, fractional (see Figure 1), and normalized

values. The spectrum is calculated on 512 consecutive cardiac beats; stationarity has been tested in two ways: (1) Calculation of mean values and variance of variability signals on consecutive records, by verifying that the values are inside a confidence level of 5%. (2) construction of the pole diagram of the autoregressive model, together with the confidence interval in the pole estimation, by verifying that consecutive records are characterized by poles which are inside the fitted confidence interval (wide-sense stationarity). The spectra which are presented in this paper satisfy both of the preceding conditions.

With the AR spectral estimation presented in this paper, it is possible to improve the statistical consistency, even of very low frequency components (less than 0.02 Hz Eq) which correspond to oscillations in the signal having a period comparable or superior to the considered number of cardiac beats (512). These rhythms are always present in the signals and may have an important physiological meaning, the analysis of which is outside the aim of the present paper. The method of variability signal processing presented has the advantage of decreasing the estimation variance of their power.

The multivariate analysis (cross-spectra and phase spectra) between R-R interval and systemic arterial pressure variabilities are obtained through the calculation of the complex cross-spectrum  $P_{xy}(f)$  where  $x(k)$  and  $y(k)$  are two synchronous discrete time series.

$$P_{xy}(f) = X(f) \cdot Y^*(f) / N = G_{xy}(f) \exp[j\phi_{xy}(f)]$$

where  $X(f)$  and  $Y(f)$  are the discrete Fourier transforms of the series (\* denotes the complex conjugate),  $G_{xy}$  is the amplitude cross-spectrum, and  $\phi_{xy}$  is the phase spectrum. The squared coherence  $k^2$  is then obtained as

$$k^2_{xy}(f) = G_{xy}(f)^2 / [P_x(f) \cdot P_y(f)].$$

The cross-spectrum is calculated via FFT algorithm, using a triangular window (Bartlett method)<sup>56</sup> on successive overlapping records of 64 samples each. Results are averaged over the whole set of 512 samples. The applied segmentation is a compromise between frequency resolution and estimated consistency.<sup>57</sup>

Squared coherence has values between 0 and 1 and should be considered as analogue to the squared correlation coefficient  $r^2$  in linear regression analysis. Values over 0.5 indicate a significant phase link between R-R interval and pressure variability signals. Thus, only spectral components with high coherence demonstrate a stable phase-shift between instantaneous R-R interval and arterial blood pressure. Its value can be evaluated as a function of frequency.

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