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Critical analysis of autoregressive and fast Fourier transform markers of cardiovascular variability in rats and humans

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The autonomic nervous system plays an important role in physiological and pathological conditions, and has been extensively evaluated by parametric and non-parametric spectral analysis. To compare the results obtained with fast Fourier transform (FFT) and the autoregressive (AR) method, we performed a comprehensive comparative study using data from humans and rats during pharmacological blockade (in rats), a postural test (in humans), and in the hypertensive state (in both humans and rats). Although postural hypotension in humans induced an increase in normalized low-frequency (LFnu) of systolic blood pressure, the increase in the ratio was detected only by AR. In rats, AR and FFT analysis did not agree for LFnu and high frequency (HFnu) under basal conditions and after vagal blockade. The increase in the LF/HF ratio of the pulse interval, induced by methylatropine, was detected only by FFT. In hypertensive patients, changes in LF and HF for systolic blood pressure were observed only by AR; FFT was able to detect the reduction in both blood pressure variance and total power. In hypertensive rats, AR presented different values of variance and total power for systolic blood pressure. Moreover, AR and FFT presented discordant results for LF, LFnu, HF, LF/HF ratio, and total power for pulse interval. We provide evidence for disagreement in 23% of the indices of blood pressure and heart rate variability in humans and 67% discordance in rats when these variables are evaluated by AR and FFT under physiological and pathological conditions. The overall disagreement between AR and FFT in this study was 43%.

Key words: Fast Fourier transform; Autoregressive model; Hypertension; SHR; Pharmacological blockade; Tilt test

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Introduction

The homeostasis of the cardiovascular system is efficiently maintained under control by feedback mechanisms that act to maintain perfusion pressure to the organs within a relatively narrow range of variation. The autonomic nervous system plays an important role in blood pressure regulation, producing adjustments in heart rate and myocardium contractility by altering sympathetic and parasympathetic activities to the heart, and peripheral vascular resistance and blood volume by constriction of arterial and venous vessels, respectively (1). These effects occur during each cardiac cycle and can be assessed by the variations in R-R intervals of the electrocardiogram.

In the early 70's, spectral analysis was applied to investigate cardiac interval time series (2,3) and to de-

scribe the rhythmic fluctuations that occur in the band of the respiratory frequency or high frequencies (Hering waves) and in lower frequencies (Mayer waves). In 1981, pharmacological studies showed for the first time that these variations were associated with the autonomic nervous system and hormonal mechanisms (4), suggesting a relationship between the spectral components and control mechanisms of the cardiovascular system. Taken together, the spectral analysis components facilitated the physical interpretation of the mechanisms involved in the regulation of the cardiovascular system. Later, these findings lent support to the sympathovagal balance hypothesis (5), whereby the normalized power of the low-frequency (LFnu) component of heart rate variability was associated with the sympathetic modulation and the high-frequency (HFnu) component was associated with parasympathetic modulation. The ratio between these two components established an index of the autonomic influence to the heart. This hypothesis was validated during postural changes (or orthostatic test) that impose perturbation on the cardiovascular system, revealing potential applications to assess physiological and physiopathological conditions.

The evaluation of autonomic markers on the basis of spectral analysis can be obtained by parametric (autoregressive model, AR, or autoregressive moving average) or non-parametric (fast Fourier transform, FFT) methods. In clinical research, both spectral techniques have been applied for the evaluation of the autonomic indices in myocardial infarction (6), Chagas disease (7) and diabetes (8), and in the validation of noninvasive blood pressure monitoring (9). However, a consensus regarding which is the most appropriate method for power spectral density interpretation of the cardiovascular system time series has not been established (10-12). The task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology established the standardization of measurement, physiological interpretation, and clinical use of power spectral analysis (10), and the advantages and disadvantages of both parametric and non-parametric methods have been described by others (12).

Table 1. Demographic data of the normotensive and hypertensive subjects studied.

	Normotensive	Hypertensive	
		Mild	Severe
Subjects (N)	16	8	17
Gender, M/F (% male)	9/7 (56%)	4/4 (50%)	10/7 (59%)
Age (years)	25 ± 3	38 ± 8*	52 ± 8*#
Body weight (kg)	66 ± 9	63 ± 5	78 ± 14*#
Height (m)	1.69 ± 0.09	1.61 ± 0.03	1.62 ± 008*
BMI (kg/m^2)	22.6 ± 2.9	24.2 ± 1.4	30.2 ± 3.6*#
Ambulatory blood pressure			
SBP (mmHg)	115 ± 7	139 ± 16*	168 ± 23*#
MBP (mmHg)	85 ± 8	96 ± 11	126 ± 14*#
DBP (mmHg)	71 ± 9	75 ± 11	105 ± 12*#
Heart rate (bpm)	64 ± 10	71 ± 16	75 ± 12

BMI = body mass index; SBP = systolic blood pressure; MBP = mean blood pressure; DBP = diastolic blood pressure. *P < 0.05 compared to the normotensive group; #P < 0.05 compared to mild hypertensive patients (one-way ANOVA).

In view of these considerations, we investigated the agreement of the AR and FFT methods regarding the extraction of autonomic markers during 1) postural changes (tilt test) in healthy volunteers, 2) pharmacological blockade of the autonomic nervous system to the heart in normal control rats, 3) in patients with arterial hypertension, and 4) in an experimental model of hypertension (spontaneously hypertensive rats, SHR).

Material and Methods

Animal model and subjects

All experimental procedures followed institutional guidelines for the care and use of laboratory animals (rats) and protocols used here were approved by the Institutional Review Board of the Medical School, University of São Paulo, São Paulo, SP, Brazil (humans).

Animals. Ten male normotensive Wistar rats and ten 18-week-old male SHR (Medical School, University of São Paulo) were fed standard laboratory chow and water *ad libitum* while housed (2-3 per cage) in a temperature-controlled room (22°C) with a 12-12-h dark-light cycle.

Human subjects. Sixteen normotensive volunteers and 8 subjects with mild hypertension and 17 subjects with severe hypertension were included in the study. Exclusion criteria were: age of less than 20 or more than 50 years, diabetes mellitus, cerebrovascular, aortic, or cardiac disease, smoking habit, and body mass index greater than 35 kg/m^2 (Table 1). Hypertensive patients had their medication regimens changed with the progressive withdrawal of β-blocker, angiotensin-converting enzyme inhibitors and central α-blocker over a 2-week period.

Data acquisition and analysis

Experimental model. Under anesthesia with 30 mg/kg pentobarbitone sodium administered *ip*, polyethylene catheters (PE-10, 0.28 mm ID, 0.61 mm OD, Biocorp Australia, Australia) filled with heparin solution were inserted into the abdominal aorta and inferior vena cava through the left

femoral artery and vein, respectively. The free ends of the cannulas were tunneled subcutaneously and exteriorized in the nape. Blood pressure was measured in conscious freely moving rats at least 24 h after the surgical procedures by connecting the arterial cannula to a pressure transducer (Statham P_{23}Dd, Puerto Rico). The blood pressure waveform signal from the transducer was fed to an amplifier (GPA-4 model 2, Stemtech, Inc., USA), and to a 10-bit analog-to-digital converter (DataQ Instruments, Inc., USA) for measurement on a beat-to-beat basis at a sampling rate of 2.0 kHz.

Human subjects. All patients were instructed not to consume alcohol or caffeine 24 h before the protocol. During all experimental conditions, blood pressure was measured with a digital photoplethysmograph, which provides accurate beat-to-beat systolic and diastolic values (Finapress, Omeda 2300, Monitoring Systems, USA). This device uses a photoplethysmographic finger cuff to assess blood pressure continuously using the vascular unloading principle. Heart rate was monitored by electrocardiography (ECG Amplifier, model 13-4615-64, Gould, USA). The blood pressure waveforms and ECG signals were recorded on a beat-to-beat basis (DataQ Instruments) at a sampling rate of 1.0 kHz.

Signal edition and artifact correction and rejection

Blood pressure waveforms were used to extract beat-by-beat time series of pulse (PI), and systolic (SBP) and diastolic (DBP) blood pressure. R-R interval time series were derived from ECG recordings. The signal pre-processing and spectral analysis were conducted by the same observer. Peak pressure is reported as SBP and the minimum pressure is reported as DBP. The tagged points were reviewed and edited if required after visual inspection with a special signal editor developed for Matlab (MATLAB 6.0, Mathworks, USA) (13). Pulse interval or R-R interval was calculated as the difference between the beginning and end points of the cycle (t_1-t_0).

Blood pressure and pulse/R-R interval variability

Time-domain analysis consisted of calculating mean PI or R-R intervals and SBP and DBP, followed by their variability as the variance from their respective time series. In the frequency-domain analysis, both AR and FFT methods were used to evaluate blood pressure and pulse interval and R-R interval variability. Both time- and frequency-domain parameters were determined by software. The spectral bands for humans (very low-frequency (VLF): 0.0-0.04 Hz; LF: 0.04-0.15 Hz; HF: 0.15-0.4 Hz) and rats (VLF: 0.0-0.2 Hz; LF: 0.2-0.8 Hz; HF: 0.8-2.8 Hz) were defined according to literature references (10,14). Spectral power for VLF, LF and HF bands was calculated by power spectrum density integration within each frequency band. The power density of each spectral component was calculated both as absolute values and as normalized units (nu) (10,15). The power at LF and HF for pulse interval was normalized and represents the relative value of each power component in proportion to the total power minus the VLF component. Data are reported as nu. The sympathovagal balance was defined by the LF/HF ratio. The LF component of the R-R and pulse intervals and of blood pressure variability was considered to be a marker of efferent sympathetic cardiac and vascular modulation, respectively, whereas the HF component of the R-R or pulse interval variability reflects respiratory-driven vagal modulation to the sinoatrial node (4,16).

For frequency-domain analysis, the whole 10-min time series of blood pressure and pulse or R-R intervals were evaluated under basal conditions using parametric and non-parametric methods, described in detail below.

Autoregressive method. Time series were analyzed using the Linear Analysis software (LA, version 8.5) (17), kindly provided by Alberto Porta (Department of Technologies for Health, Galeazzi Orthopaedic Institute, University of Milan, Italy). Time series were divided into segments of 300 beats that overlapped by 50%. A linear de-trending procedure was used, the spectra of each segment were calculated via the Levinson-Durbin recursion, and the order of the model was chosen according to Akaike's criterion.

Fast Fourier transform method. The linear trend was removed before the analysis of power spectral density obtained by FFT, using Welch's method. For the signal obtained in rats, we considered 10 min of data resampled at 13.8 Hz (first 2^{13} points) and segments of 2^{11} points with a Hanning window and 50% overlap. For the signal obtained in humans, we considered 10 min of data resampled at 5.0 Hz (first 2^{11}) and segments of 2^9 points with a Hanning window and 50% of overlap.

Experimental protocols

Tilt test. An established protocol currently used by Montano et al. (18) was adopted. During the entire experimental procedure, the normal volunteers (N = 8) were monitored by blood pressure and ECG measurements. Head-up tilt testing was performed with the subject in a non-sedated, post-absorptive state. After heart rate and blood pressure measurement under basal conditions (10 min), the subject was positioned upright at an angle of approximately 80 degrees for a maximum of 20 min. If syncope or pre-syncope with hypotension developed during the test, the table was lowered to the supine position.

Autonomic nervous system blockade. The experimental protocol was similar to that used in a previous study performed in our laboratory (19,20). Briefly, a 2-day protocol was used for the evaluation of the vagal and sympathetic effects on heart rate of 1 mg/kg methylatropine (Sigma Chemical Co., USA) and 1 mg/kg propranolol (Sigma) injected *iv*. On the first day of the study, heart rate was recorded for 10 min under basal conditions and after parasympathetic pharmacological blockade with methylatropine. On the second day of the protocol, the sympathetic influence on heart rate was evaluated by propranolol injection followed by methylatropine injection to assess the double sympathetic and parasympathetic blockade in order to determine the intrinsic heart rate. The effects of pharmacological blockade on heart rate were recorded for 10 min after both methylatropine and propranolol reached their maximum effects (~3 min, *iv* injection).

Statistical analysis

Data are reported as means ± SEM. The agreement of all time- and frequency-domain data between AR and FFT was analyzed by two-way analysis of variance (ANOVA) for repeated measurements followed by the Bonferroni *post hoc* test. Demographic data were analyzed by one-way ANOVA followed by the Bonferroni *post hoc* test. Similarly, disagreement between AR and FFT was analyzed by the percentage of indices that presented statistical significance for the method factor (F1: AR *vs* FFT) in the two-way ANOVA. The level of significance was set at $P < 0.05$ in all analyses. All procedures were performed with the SigmaStat statistical software (Systat Software Inc., USA).

Results

Autonomic nervous system changes in response to postural and pharmacological blockade challenges

Normal healthy subjects (N = 8) submitted to postural challenge (orthostatic tilt test) exhibited hypotension ($P = 0.039$) accompanied by a significant decrease ($P = 0.004$) in R-R interval (Table 2 and Figure 1A). Neither method showed any significant reduction in R-R interval variance ($P = 0.372$). In addition, the FFT technique presented significantly higher values ($P < 0.001$) for R-R interval variance under both basal and tilt conditions compared to AR. Both the AR and FFT methods demonstrated a signifi-

Table 2. Data obtained for normal volunteers (N = 8) before (basal) and after (tilt) submission to the head-up tilt testing.

	AR		FFT		Statistics		
	Basal	Tilt	Basal	Tilt	F1	F2	F1 x F2
SBP							
Mean (mmHg)	129.9 ± 4.0	119.0 ± 4.6	129.9 ± 4.0	119.9 ± 4.6	0.474	0.039$^{\$\&}$	0.322
Var (mmHg^2)	13.6 ± 3.7	33.1 ± 6.6	22.5 ± 5.1	120.2 ± 53.6	0.087	0.093	0.160
VLF (mmHg^2)	1.63 ± –	2.68 ± –	14.2 ± 3.9	65.5 ± 39.4	NA	NA	NA
LF (mmHg^2)	9.3 ± 3.6	19.0 ± 4.1	3.7 ± 0.8	14.7 ± 3.1	0.045*	0.022$^{\$\&}$	0.630
HF (mmHg^2)	2.2 ± 0.4	8.0 ± 2.7	3.1 ± 0.4	7.8 ± 1.6	0.658	0.029$^{\&}$	0.612
TotP (mmHg^2)	13.0 ± 3.7	28.1 ± 5.1	20.3 ± 4.5	89.5 ± 42.6	0.135	0.107	0.236
R-R interval							
Mean (ms)	938.2 ± 65.5	736.0 ± 26.0	937.8 ± 65.1	736.0 ± 25.9	0.486	0.004$^{\$\&}$	0.413
Var (ms^2)	5992 ± 3506	2076 ± 456	7170 ± 3895	4541 ± 775	<0.001$^{*\#}$	0.372	0.089
VLF (ms^2)	587 ± –	963 ± 208	1654 ± 837	1606 ± 478	NA	NA	NA
LF (ms^2)	1376 ± 599	922 ± 276	1134 ± 466	926 ± 181	0.360	0.487	0.267
HF (ms^2)	2982 ± 1891	535 ± 204	3034 ± 1928	581 ± 228	0.321	0.230	0.941
LFnu (%)	32.6 ± 5.9	49.4 ± 9.0	47.6 ± 13.4	57.0 ± 5.7	0.207	0.225	0.414
HFnu (%)	42.1 ± 6.6	27.0 ± 7.1	55.3 ± 4.9	30.8 ± 6.7	0.055	0.003$^{\$\&}$	0.194
LF/HF	1.15 ± 0.41	6.16 ± 2.54	1.01 ± 0.34	2.68 ± 0.62	0.114	0.042$^{\&}$	0.137
TotP (ms^2)	4650 ± 2426	1817 ± 429	6021 ± 3220	3188 ± 714	0.066	0.299	0.999

Time domain variance (Var), very low-frequency (VLF), low-frequency (LF) and high-frequency (HF) powers, normalized power of LF and HF (LFnu and HFnu), sympathovagal balance (LF/HF) and total power (TotP). In the two-way ANOVA, F1 signifies the method factor (autoregression, AR, or fast Fourier transform, FFT); F2, the autonomic test factor (basal or tilt), and F1 x F2, the interaction between F1 and F2. NA is non-analyzed data (AR analysis provided only one measure). SBP = systolic blood pressure. $^{\$}P < 0.05$ between basal and tilt conditions for the FFT method. $^{\&}P < 0.05$ between basal and tilt conditions for the AR method. $^{*}P < 0.05$ between the AR and FFT methods for the basal condition. $^{\#}P < 0.05$ between the AR and FFT methods for the tilt condition.

cant increase in LF for SBP (P = 0.022), and a decrease in HFnu for pulse interval (P = 0.003) induced by the tilt test; however, only the AR method was able to show changes in HF for SBP (P = 0.029) during the orthostatic tilt test. Moreover, a significant increase (P = 0.042) in LF/HF ratio after postural change was observed only with the AR method.

Pharmacological blockade (Table 3 and Figure 2) was used to assess the modulation of the autonomic nervous system produced by sympathetic and parasympathetic activities to the heart in an experimental model. Vagal blockade with methylatropine induced a significant reduction in pulse interval (P < 0.001). However, the blockade of this cardiac sympathetic activity using propranolol did not change pulse interval values (Figure 2A) compared to those of the basal condition. A significant difference in pulse interval variance was observed under basal conditions (P = 0.030) when AR and FFT were compared. The absolute values of LF (P = 0.363) and HF (P = 0.150), with power evaluated by both AR and FFT methods, were not affected by pharmacological blockade. However, the absolute value of the LF component, estimated by AR, was lower compared to FFT (P = 0.011) under basal conditions and after methylatropine blockade. Cardiac sympathetic blockade with propranolol (Figure 2B) tended to reduce the LFnu component by FFT, without any change in the AR method results. Furthermore, HFnu oscillation was significantly increased (P < 0.001) by propranolol blockade in both methods. Parasympathetic blockade with methylatropine (Figure 2C) did not change the LFnu component of either the AR or FFT method (P = 0.468). The reduction in HFnu oscillation of PI after parasympathetic blockade with methylatropine was detected by both the AR and FFT methods (P < 0.001). In addition, AR and FFT analysis was not concordant in the values for the LFnu (P < 0.001) and HFnu (P < 0.001) components under basal and methylat-

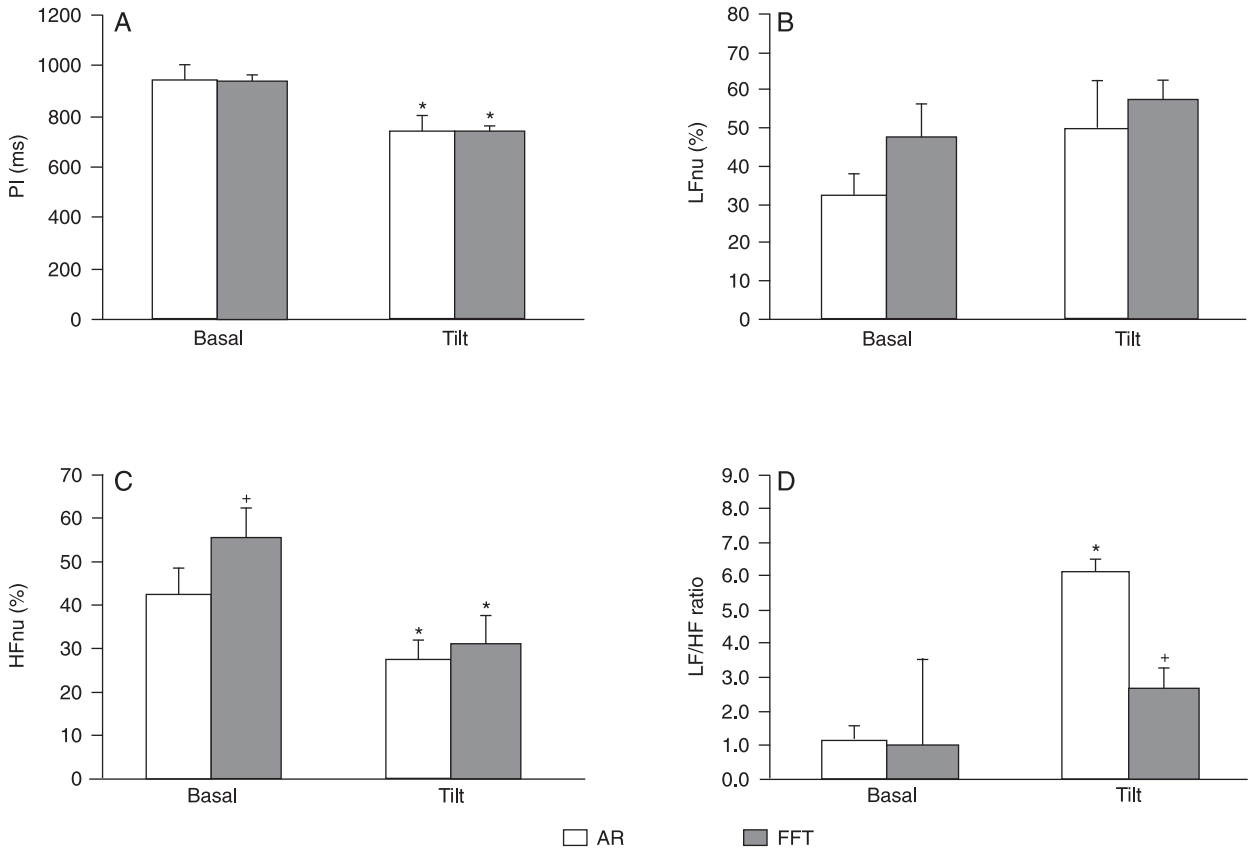

Figure 1. Mean pulse interval (PI, Panel A) and LFnu (Panel B) and HFnu (Panel C) oscillations, and LF/HF ratio (Panel D) for PI at basal condition and after tilt test in normal volunteers. LF = low-frequency; HF = high-frequency; LFnu = normalized power of LF; HFnu = normalized power of HF; AR = autoregressive method; FFT = fast Fourier transform method. *P ≤ 0.05, compared to respective basal group. $^{+}$P ≤ 0.05, FFT compared to AR.

Table 3. Time- and frequency-domain analysis of pulse interval of normotensive rats (Wistar, N = 8) submitted to pharmacological blockade.

	AR			FFT			Statistics		
	Basal	Methylatropine	Propranolol	Basal	Methylatropine	Propranolol	F1	F2	F1 x F2
Mean (ms)	169.7±4.9	141.0±4.7	177.4±2.8	169.6±4.8	138.6±2.9	177.3±2.8	0.315	<0.001$^{\&+}$	0.425
Var (ms^2)	29.8±8.2	13.7±2.9	22.1±5.0	102.6±38.0	16.4±2.4	33.9±5.9	0.030$^{\#}$	0.030^{+}	0.021
VLF (ms^2)	16.2±4.4	2.1±0.4	4.3±0.7	71.7±31.7	10.6±3.9	10.8±2.0	0.046$^{\#}$	0.043^{+}	0.094
LF (ms^2)	0.60±0.23	0.22±0.08	0.82±0.23	4.85±1.89	5.40±2.53	1.11±0.30	0.011$^{\#\$}$	0.363	0.192
HF (ms^2)	11.13±3.50	7.36±2.21	16.14±4.47	6.16±1.73	2.69±0.84	4.67±1.32	0.012*	0.150	0.042
LFnu (%)	4.61±1.42	1.63±0.23	4.65±0.90	23.76±5.01	25.75±11.45	8.81±3.04	0.006$^{\#\$}$	0.468	0.293
HFnu (%)	86.25±3.44	60.46±4.45	91.74±1.25	36.12±5.01	15.11±5.36	32.98±4.85	<0.001$^{\#\$*}$	<0.001$^{\&+}$	0.342
LF/HF	0.061±0.020	0.030±0.003	0.053±0.011	0.724±0.240	1.609±0.269	0.245±0.060	<0.001$^{\#\$}$	<0.001^{+}	<0.001
TotP (ms^2)	18.46±5.37	12.32±1.31	19.85±5.82	82.93±35.74	18.58±7.24	16.69±3.23	0.053	0.095	0.055

Time domain variance (Var), very low-frequency (VLF), low-frequency (LF) and high-frequency (HF) powers, normalized power of LF and HF (LFnu and HFnu), sympathovagal balance (LF/HF), and total power (TotP). In the two-way ANOVA, F1 signifies the method factor (autoregression, AR, or fast Fourier transform, FFT); F2, the pharmacological blockade factor (basal, methylatropine or propranolol), and F1 x F2, the interaction between F1 and F2. $^{\#}P < 0.05$ between the AR and FFT methods for basal. *P < 0.05 between AR and FFT methods for propranolol. $^{\$}P < 0.05$ between AR and FFT methods for methylatropine. $^{\&}P < 0.05$ between basal *vs* methylatropine, and methylatropine *vs* propranolol for the AR method. $^{+}P < 0.05$ between basal *vs* methylatropine, and methylatropine *vs* propranolol for the FFT method.

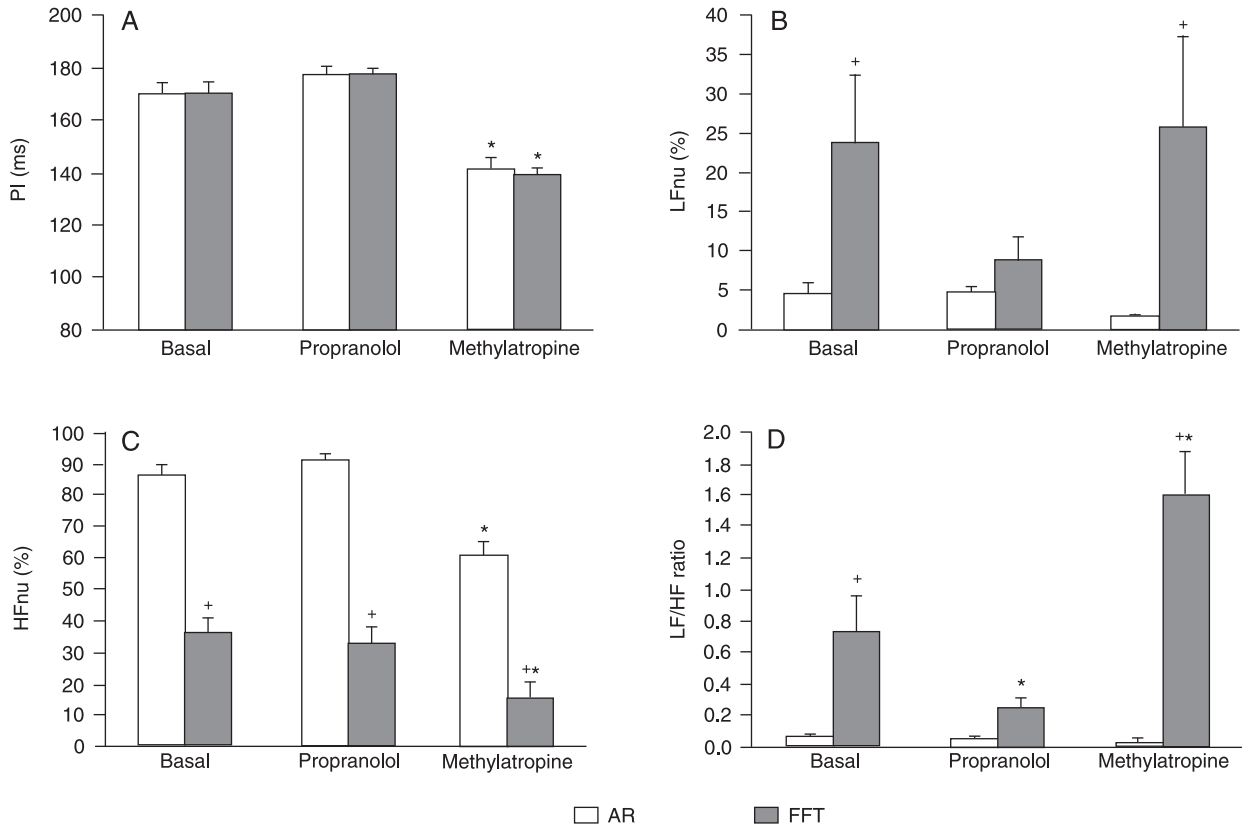

Figure 2. Mean pulse interval (PI, Panel A) and LFnu (Panel B) and HFnu (Panel C) oscillations, and LF/HF ratio (Panel D) for PI at basal condition and after pharmacological blockade of sympathetic (propranolol) and parasympathetic (methylatropine) systems in rats. LF = low-frequency; HF = high-frequency; LFnu = normalized power of LF; HFnu = normalized power of HF; AR = autoregressive method; FFT = fast Fourier transform method. *$P \leq 0.05$, compared to respective basal condition. $^{+}P \leq 0.05$, FFT compared to AR.

ropine conditions. The LF/HF ratio for pulse interval (Figure 2D) was unchanged after both sympathetic and parasympathetic blockade when evaluated by the AR technique, but was increased after methylatropine injection when evaluated by the FFT method (P < 0.001). Furthermore, the AR and FFT techniques showed a disagreement in the LF/HF ratio of the pulse interval under basal and methylatropine conditions (P < 0.001).

Autonomic nervous system evaluation in hypertensive patients and in experimental hypertension

In patients, the autonomic changes commonly associated with arterial hypertension were used to evaluate the concordance of the FFT and AR methods (Table 4). As expected, SBP values increased significantly according to the severity of arterial hypertension (P < 0.001) compared to normotensive subjects. R-R interval values under basal conditions were significantly reduced in the hypertensive groups (P = 0.038). Both spectral estimation techniques demonstrated a significant increase in the values of SBP variance (P < 0.001) according to the severity of hypertension, and R-R interval variance decreased in both hypertensive (mild and severe) groups (P = 0.004). Neither the AR nor the FFT method showed any change in the LF (P = 0.688) or HF (P = 0.817) components of SBP in any of the hypertensive groups. The total power of SBP showed a nonsignificant increase according to the severity of hypertension only in the FFT method (P = 0.459). However, both total power (P < 0.001) and variance (P < 0.001) of SBP were significantly higher in FFT than in the AR method in all groups evaluated. The LFnu component for the R-R interval showed increasing values according to the severity of hypertension (P = 0.050) by both methods, whereas the HFnu component was significantly reduced (P = 0.012). The increase in the LFnu component was accompanied by a nonsignificant increase in LF/HF ratio (P = 0.065) in both methods, and presented a statistical difference between the AR and FFT methods in normotensive subjects (P = 0.031).

Table 5 shows the time- and frequency-domain data obtained in an experimental model of hypertension (SHR). Similar to humans, basal SBP was significantly increased (P < 0.001) in SHR (N = 8) compared to normotensive Wistar rats (N = 8). Pulse interval values under basal

Table 4. Time- and frequency-domain analysis of systolic blood pressure (SBP) and R-R interval of normotensive subjects (N = 16), patients with mild hypertension (N = 8) and patients with severe hypertension (N = 17).

	AR			FFT			Statistics		
	Normal	Mild	Severe	Normal	Mild	Severe	F1	F2	F1 x F2
SBP									
Mean (mmHg)	125.0±2.8	138.6±5.7	175.0±5.8	125.0±2.8	141.7±5.3	175.0±5.8	0.140	<0.001$^{\&+}$	0.165
Var (mmHg^2)	23.2±5.1	30.1±5.6	34.4±9.5	38.4±8.1	69.0±24.8	66.8±15.7	<0.001$^{*\$}$	0.292	0.205
VLF (mmHg^2)	13.8±7.8	29.2±5.0	71.9±31.7	35.8±7.9	53.7±13.8	56.7±14.5	0.040$^{\$}$	0.791	0.429
LF (mmHg^2)	15.1±5.2	5.7±1.1	13.2±3.3	7.4±1.8	11.0±3.1	11.4±1.6	0.525	0.688	0.072
HF (mmHg^2)	3.1±0.5	3.2±0.6	2.5±1.2	3.6±0.4	3.3±1.0	3.0±1.1	0.266	0.817	0.886
TotP (mmHg^2)	21.8±5.2	27.5±6.8	33.0±9.6	34.6±7.4	51.4±13.8	53.3±14.6	<0.001$^{\#*\$}$	0.459	0.381
R-R interval									
Mean (ms)	967±37	884±72	818±30	966±37	883±71	818±30	0.963	0.038$^{\&+}$	0.999
Var (ms^2)	5320±1814	946±194	934±199	6645±2050	1615±350	1489±295	0.004$^{\#}$	0.030^{+}	0.405
VLF (ms^2)	587±-	616±186	537±158	1711±493	608±139	663±152	NA	NA	NA
LF (ms^2)	1259±325	307±92	456±159	1193±266	328±74	351±87	0.429	0.013$^{\&}$	0.741
HF (ms^2)	2748±1006	339±105	217±65	2661±1013	391±117	216±61	0.950	0.031$^{\&}$	0.952
LFnu (%)	35.8±6.0	43.5±10.3	62.1±5.1	43.4±7.6	53.0±8.7	56.7±3.6	0.339	0.050	0.251
HFnu (%)	50.5±6.4	39.1±9.2	28.7±4.1	53.8±5.2	47.6±6.3	30.1±5.1	0.126	0.012$^{\&+}$	0.627
LF/HF	1.37±0.45	2.53±1.15	4.56±1.78	1.13±0.28	1.42±0.42	2.74±0.49	0.084	0.065	0.477
TotP (ms^2)	4197±1275	1054±326	920±202	5719±1721	1352±293	1278±276	0.031$^{\#}$	0.023^{+}	0.180

Time domain variance (Var), very low-frequency (VLF), low-frequency (LF) and high-frequency (HF) powers, normalized power of LF and HF (LFnu and HFnu), sympathovagal balance (LF/HF), and total power (TotP). In the two-way ANOVA, F1 signifies the method factor (autoregression, AR, or fast Fourier transform, FFT); F2, the pathological condition factor (normal, mild or severe), and F1 x F2, the interaction between F1 and F2. NA is non-analyzed data (AR analysis provided only one measure). $^{\#}$P < 0.05 between the AR and FFT methods for normotensive subjects. *P < 0.05 between the AR and FFT methods for mild hypertensive patients. $^{\$}$P < 0.05 between the AR and FFT methods for severely hypertensive patients. $^{\&}$P < 0.05 for normal *vs* mild, and mild *vs* severe for the AR method. $^{+}$P < 0.05 for normal *vs* mild, and mild *vs* severe for the FFT method.

Table 5. Time- and frequency-domain analysis of systolic blood pressure (SBP) and pulse interval (PI) of normotensive (Wistar, N = 8) and spontaneously hypertensive (SHR, N = 8) rats.

	AR		FFT		Statistics		
	Wistar	SHR	Wistar	SHR	F1	F2	F1 x F2
SBP							
Mean (mmHg)	123.4 ± 5.7	188.9 ± 3.6	123.4 ± 5.7	188.9 ± 3.6	0.838	<0.001$^{\$\&}$	0.124
Var (mmHg^2)	12.1 ± 3.7	34.6 ± 7.5	29.7 ± 14.5	97.0 ± 13.3	<0.001$^{\#}$	0.004$^{\$}$	0.014
VLF (mmHg^2)	10.5 ± 5.7	16.0 ± 0.1	20.5 ± 11.5	50.4 ± 9.4	0.104	0.388	0.370
LF (mmHg^2)	2.36 ± 0.90	23.38 ± 7.29	4.89 ± 1.43	17.57 ± 3.10	0.217	0.012$^{\$\&}$	0.085
HF (mmHg^2)	1.47 ± 0.32	5.09 ± 1.09	1.49 ± 0.39	4.80 ± 0.39	0.117	0.009$^{\$\&}$	0.409
TotP (mmHg^2)	10.22 ± 3.96	32.60 ± 7.19	26.97 ± 12.74	72.91 ± 10.27	<0.001$^{\#}$	0.007$^{\$}$	0.018
PI							
Mean (ms)	171.5 ± 4.0	168.6 ± 3.4	172.3 ± 4.6	168.6 ± 3.9	0.714	0.597	0.096
Var (ms^2)	19.9 ± 6.4	49.6 ± 8.9	45.0 ± 15.7	150.6 ± 29.2	<0.001$^{\#}$	0.005$^{\$}$	0.026
VLF (ms^2)	7.1 ± 1.7	6.7 ± 0.4	29.5 ± 13.7	64.8 ± 17.4	0.001$^{*\#}$	0.054$^{\$}$	0.041
LF (ms^2)	0.80 ± 0.11	3.46 ± 0.52	6.77 ± 4.76	7.70 ± 1.98	0.030$^{\#}$	0.801	0.331
HF (ms^2)	12.07 ± 4.57	34.44 ± 7.79	10.77 ± 4.58	29.63 ± 5.56	0.008$^{\#}$	0.023$^{\$\&}$	0.126
LFnu (%)	8.25 ± 1.27	9.56 ± 1.68	43.17 ± 17.38	21.10 ± 6.90	0.004$^{*\#}$	0.262	0.846
HFnu (%)	85.57 ± 4.05	70.06 ± 5.07	118.24 ± 50.17	74.29 ± 8.68	0.100	0.338	0.010
LF/HF	0.099 ± 0.023	0.161 ± 0.040	0.393 ± 0.102	0.261 ± 0.049	0.010*	0.325	0.524
TotP (ms^2)	18.46 ± 5.37	41.05 ± 6.54	47.79 ± 23.30	104.47 ± 22.46	<0.001$^{\#}$	0.036$^{\$}$	0.201

Time domain variance (Var), very low-frequency (VLF), low-frequency (LF) and high-frequency (HF) powers, normalized power of LF and HF (LFnu and HFnu), sympathovagal balance (LF/HF), and total power (TotP). In the two-way ANOVA, F1 signifies the method factor (autoregression, AR, or fast Fourier transform, FFT); F2, the pathological condition factor (Wistar or SHR), and F1 x F2, the interaction between F1 and F2. $^{\$}P < 0.05$ between Wistar and SHR groups for the FFT method. $^{\&}P < 0.05$ between Wistar and SHR groups for the AR method. *P < 0.05 between the AR and FFT methods for Wistar rats. $^{\#}P < 0.05$ between the AR and FFT methods for SHR.

conditions did not differ between groups (P = 0.597) and were not changed by the method (P = 0.714). High blood pressure levels were accompanied by significant increases in SBP (P = 0.004) and pulse interval (P = 0.005) variances, shown only by the FFT method. Both the AR and FFT methods demonstrated an increase in the power of LF oscillation for SBP (P = 0.012) in SHR compared to normotensive Wistar rats. Despite the relative concordance of the two methods, AR presented different variance values (P < 0.001) and total power estimation (P < 0.001) for SBP compared to FFT in the SHR group. Similarly, for pulse interval analysis, the AR and FFT methods presented a significant difference in variance (P < 0.001), absolute (P = 0.030) and normalized (P = 0.004) values of the LF component, absolute values of HF (P = 0.008), LF/HF ratio (P = 0.010), and total estimation power (P < 0.001).

Discussion

The present study compared the agreement between the AR and FFT methods under several conditions: a physiological stimulus, postural changes induced in normal healthy volunteers or pharmacological blockade in an experimental model, and under pathological conditions, i.e., patients with mild and severe hypertension or a genetic experimental model of hypertension (SHR). Despite specific agreement between parametric and non-parametric methods in time- and frequency-domain indices, several disagreements were detected in the present study.

In the time-domain analysis, the AR and FFT methods seemed to be concordant, with the exception of differences detected in the calculation of the time variance of both PI/R-R interval and SBP under all conditions evaluated. Our data partially agree with the study of Pitzalis et al. (21) who demonstrated that the time-domain measurements may be considered to be reproducible. Most of the differences observed between the AR and FFT methods are related to the frequency-domain analysis. Considering all the frequency-domain indices evaluated, the present study found that 43% of the data did not agree. Interestingly, most of the non-concordant time- and frequency-domain data were related to protocols using experimental animals (67%); in humans, 23% of non-concordant values were found in the present study. Moreover, we observed that the LF component was not concordant between methods in any of the experimental models, i.e., pharmacologi-

cal blockade and genetic hypertension. The AR method presented 2- and 5-fold lower LFnu values for pulse interval compared to FFT in SHR and normotensive Wistar rats, respectively. Additionally, we observed significant differences in the HFnu component of the R-R interval for both normotensive and hypertensive patients. Indeed, several studies (22-24) have shown an overestimation of the HF component by the FFT method. Furthermore, a nonsignificant increase was observed in the LF/HF ratio for the R-R interval using the FFT method during the tilt test in normal healthy volunteers, whereas the AR method showed a significant increase in sympathovagal balance during the protocol involving postural changes. In the animal model of hypertension, we observed higher values of the total power for the R-R interval when analyzed by the FFT method. Pichon et al. (24) observed significant differences between the AR and FFT methods in the total power estimation for both sitting and standing positions. Moreover, these investigators showed that the power of the HF component was greater in the FFT method, suggesting a poor interchangeability in the heart rate variability indices in normal volunteers when parametric and non-parametric methods were compared. The disagreement between parametric and non-parametric methods was not limited to the analysis of heart rate variability. We were able to detect lower levels of the LF component for SBP obtained with the FFT method, compared to AR in normal healthy volunteers. Increased values of total power estimation for SBP in hypertensive patients were also observed using the FFT method. The difference in the total power estimation was consistent with the data obtained in the experimental model of hypertension (SHR) for SBP.

With regard to pathological conditions (arterial hypertension), we achieved a total of 33% non-concordant data for time- and frequency-domain indices. Indeed, Chemla et al. (25) compared both parametric and non-parametric methods and showed that FFT overestimated the LF (ms^2 and nu) component and LF/HF ratio for the R-R interval in diabetic patients, while the AR method overestimated the total power, VLF and HFnu components. These data suggested that parametric and non-parametric methods of spectral analysis were not comparable in diabetes. In hypertensive patients, Badilini et al. (26) demonstrated that, under basal conditions, the FFT method provided a lower estimation of VLF and total power for the R-R interval, but presented higher values for the LF and HF components compared to the AR method. The increase in LFnu and HFnu values for the R-R interval obtained by the FFT method compared to AR agrees with our data. We showed that the FFT method overestimated the normalized values of the HF component for the R-R interval in both normotensive and severe hypertensive subjects, and overestimated the total power for SBP only in the normotensive subjects.

The Linear Analysis software used in the present investigation for the autoregressive technique showed several undetected or missing VLF, LF and HF values in the spectral analysis of both blood pressure and PI/R-R interval variability in most of the patients and animals studied. On the other hand, all spectral components obtained with the FFT analysis were detected in animal models and patients. Similarly, Chemla et al. (25) recently reported that the use of the AR method resulted in 50% missing values of both LF and HF components. These investigators showed that changing the model order of the analysis did not modify the null or missing data observed with the AR method.

Although a consensus regarding the most appropriate method for power spectral density estimation of cardiovascular system time series has not been established (10-12), several advantages and disadvantages regarding parametric and non-parametric methods have been described. The advantages of the non-parametric (FFT) methods are: the simplicity of the algorithm employed, good reproducibility, and high processing speed. On the other hand, the advantages of parametric (AR) methods are: good performance in time series with reduced number of points, smoother spectral components, which can be distinguished independently of predefined frequency bands, easy post-processing of the spectrum with an automatic calculation of low- and high-frequency components, easy identification of the central frequency of each component, and an accurate estimation of power spectrum density even for a small number of samples in which the signal is supposed to remain stationary. The basic disadvantage of parametric methods is the need to verify the suitability of the chosen order of model. On the other hand, methods based on the non-parametric (FFT) technique require some experience in dealing with the non-stationary segments of the time series and with the overlapping and windowing to filter the power spectral density. Considering the findings of the present investigation, in which spectral analysis was affected by different species, autonomic stimulation or blockade, blood pressure, and AR or FFT methodological approach, the present investigation strongly recommends that studies or groups that intend to use spectral analysis as a window to the autonomic nervous system use their signal database of well-known experimental protocols with autonomic nervous challenges to construct and share some pairs of data and spectral “fingerprints” to compare each new finding and conclusion within and between research groups.

Experimental investigations (27) have suggested that changes in autonomic balance might be helpful to identify

high cardiovascular risk, i.e., life-threatening arrhythmias during acute myocardial ischemia. Based on this experimental evidence, in a prospective study, La Rovere et al. (28) demonstrated that time- and frequency-domain indices of heart rate variability have independent prognostic values after myocardial infarction. However, we are concerned with the use of autonomic markers derived from different spectral analysis methods to stratify cardiovascular risk. The dependence on the technique adopted and the absence of agreement between AR and FFT spectral analysis limit the direct application of these autonomic markers in clinical practice. In fact, the present study and others (21,22,24,25) have demonstrated that the AR and FFT methods are not exactly interchangeable.

Taken together, the data obtained in the present study demonstrate that the frequency-domain analysis, using parametric (AR) or non-parametric (FFT) methods do not agree in several aspects. The absence of agreement between methods extended to physiological conditions, i.e., postural changes in humans or pharmacological blockade in experimental animals. The typical changes in the autonomic markers observed during physiological conditions were not equally detected by AR and FFT. Considering the species studied, several differences were observed under basal conditions in both human and rat models when AR and FFT routines were applied for time- and frequency-domain analysis. Under pathological conditions in patients, such as arterial hypertension, both methods seem to be adequate for power spectral estimation and autonomic index estimation. However, in a genetic model of spontaneous hypertension (i.e., SHR) the parametric and non-parametric methods evaluated were not concordant. Furthermore, the present study showed that the disagreement between parametric and non-parametric methods was not restricted to the analysis of heart rate variability, but affected equally the spectral analysis of systolic blood pressure time series, and the overall disagreement between AR and FFT in this study was 43%.

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