

# Cardiac Autonomic Modulation Evaluated by Heart Interval Variability is Unaltered but Subtly Widespread in the Indeterminate Chagas' Disease

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**Background:** Cardiac autonomic function in the indeterminate chronic form of Chagas' disease deserves better clearing-up and understanding, since the existing findings are scarce and controversial. This work analyzed the short-term heart interval variability in order to verify the cardiac autonomic modulation in indeterminate Chagas' disease subjects examined in a Brazilian endemic area.

**Methods:** Variability in time and frequency domain of 5-minute electrocardiogram (ECG) series of R-R intervals in supine and active standing positions were obtained from 18 age-, gender-, body mass index-, lifestyle-, and physical activity-matched chagasics and 18 control healthy subjects examined in Água Comprida city, MG, Brazil. Mann-Whitney test was used for analysis of the data and spread of the individual indices in both groups.

**Results:** The median of the all variability indices in the chagasic group were statistically similar ( $P = 0.17-0.87$ ) to that in the control group. A wide dispersion of the almost all individual indices values, ranging from normal to variably reduced or increased ones, was noted in the majority of the chagasics in relation to the control interquartile range, in both postural positions.

**Conclusion:** As a group, indeterminate Chagas' disease subjects showed unaltered short-term heart interval variability. Individual somewhat widespread of majority of time- and frequency-domain indices, from depressed to exacerbated ones appears to exist. This conforms to a variable cardiac autonomic modulation in this form of disease, suggesting that the majority of chagasics has no lesions, and a minority has subtle lesions of the efferent innervation-sinus node complex. (*PACE* 2007; 30:772-780)

*indeterminate Chagas' disease, cardiac autonomic function, heart interval variability*

## Introduction

Chagas' disease, caused by the protozoan *Trypanosoma cruzi* and transmitted by triatomid bugs, is an endemic chronic infection yet prevalent in almost all Latin and Central American countries, although the interruption of the vectorial transmission is in several of these. It includes an acute and long-lasting chronic stage, manifesting this last in an intermediary or terminal clinical form denominated indeterminate, and in the cardiac, digestive, or combined cardiac plus digestive terminal forms. The disease affects between 10 and 18 million people, with high morbidity and mortality in about half of the cases in

consequence of heart involvement and digestive megasyndromes.<sup>1,2</sup>

In addition to inflammatory and degenerative lesions of the myocardial and myoenteric fibers and the cardiac conducting tissue, a striking involvement of the intrinsic autonomic innervation<sup>3,4</sup> and neurotransmitters receptors at the cellular membrane<sup>5</sup> is noted in a great number of cases of chronic Chagas' heart and/or digestive disease. In the indeterminate form, only discrete to moderate focal or zonal myocarditis and/or neuroganglionitis are usually noted.<sup>4,6</sup> This form shows no clinical, electrocardiographic, cardiac, and digestive radiological manifestation, and usually has a benign course during lifetime.<sup>2,6,7</sup> It represents the most common chronic form of the disease, accounting for about 50% of the cases, and has an intriguing and still incompletely known pathophysiology and course.<sup>2,6-8</sup>

Evaluation of acute heart rate responses through different methods has repeatedly demonstrated variable impairment of parasympathetic and sympathetic modulation, principally of the first, in a high number of chagasics with isolated or combined cardiac form without heart failure,<sup>3,9-17</sup>

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and in subjects with the digestive form alone.<sup>12</sup> Time- and frequency-domain analysis of short- or long-term heart interval variability has also demonstrated autonomic dysfunction with sympathovagal imbalance in chagasics with indeterminate or cardiac forms of the disease.<sup>17-26</sup>

Studies about the indeterminate form of Chagas' disease are still scarce and the findings are apparently conflicting; some authors have observed subtle or overt autonomic dysfunctions<sup>8,17,19,22,24,25,27-29</sup> while others have not.<sup>12,30</sup> Depressed baroreflex bradycardia was also observed in the Wistar rat model of chronic *T. cruzi* infection showing neuroganglionitis similar to that in the human indeterminate form of disease.<sup>31</sup> Indeterminate Chagas' disease has possibly a peculiar heterogeneous pattern of cardiac autonomic function, with a wide range expression in different samples of subjects according the methods of evaluation employed or even in a same sample examined. These chagasics may have the cardiac autonomic function unaltered or variably affected in different phases of pathophysiological evolution.

In the present work we aimed to further investigate the momentary cardiac autonomic modulation in steady-state conditions of rest supine and active standing positions based on the spontaneous short-term heart interval variability analysis in time and frequency domains, and to verify the possibility of a widespread functional pattern in subjects with the indeterminate form of Chagas' disease, for the first time evaluated in the endemic area of the disease where they live.

## Subjects and Methods

### Study Groups

A group of 18 consecutively recruited outpatients (7 men and 11 women) aged 17-63 years (mean  $\pm$  SD: 38.5  $\pm$  12.3 years) with the indeterminate form of Chagas' disease, was compared with an age-, gender-, body mass index-matched group of 18 healthy control subjects (7 men and 11 women) aged 14-63 years (mean  $\pm$  SD: 39.0  $\pm$  12.2 years) ( $p = 0.78$ ), all living in the endemic rural area of Água Comprida city, state of Minas Gerais, Brazil, where they were examined and where triatominae infestation and transmission of the parasite has been virtually eradicated. Some lifestyle habits and physical activity patterns were also similar in the two groups of subjects. All chagasics have been regularly followed up by several years by means of periodical clinical and laboratory examination since Chagas' disease diagnosis was made.

The diagnostic criteria for the indeterminate form of Chagas' disease were based on at least two positive specific indirect immunofluorescence,

hemagglutination, and ELISA reactions made in triplicate; absence of any past or present clinical manifestation and normal resting 12-lead electrocardiogram; heart X-ray; echodopplercardiogram; and effort testing. Contrast X-ray of the upper and lower digestive tract to search for asymptomatic megaesophagus or megacolon proved to be normal in all chagasic individuals. Chagasic subjects had a past history of exposure to the vector insect of the disease. All control and chagasic subjects examined were in excellent physical and mental conditions in regular daily activities and not using any drug.

This investigation conforms with the Declaration of Helsinki and to the Brazilian Ministry of Health and was approved by the Faculty of Medicine of Triângulo Mineiro Ethics Committee of Research in Human Beings. All the individuals gave signed informed consent prior to their participation. The authors declare no conflict of interest.

### Experimental Session

Chagasic and control subjects participated in the experimental session coming from their usual activities, 2-4 hours after habitual breakfast, between 9 and 11 a.m. Subjects were instructed to avoid ingesting stimulant beverages, tea, coffee, and alcoholics, and remain without exercising, smoking, and ingesting any drug at least 24 hours before the examination.

The clinical history and a complete physical examination, including verification of arterial pressure, heart rate, weight, and height, were initially obtained and the 12-lead electrocardiogram was recorded in the supine position, in a quiet and isolated environment at room temperature (22-28°C). Next, after 10-15 minutes of rest in supine position, a continuous 5-minute electrocardiogram strip was recorded in the lead II at 25 mm/s using a conventional electrocardiograph with a sampling frequency of 250 Hz. Subsequently, the subjects were asked to actively stand up at bedside and, after 2 minutes without registration in the active orthostatic position, a new 5-minute electrocardiogram tracing was obtained. The subjects continued breathing spontaneously and regularly and had their respiratory rate monitored and counted.

### Heart Interval Variability Analysis

This analysis was performed according to the methodological standards recommended by the Task Force on Heart Rate Variability.<sup>32</sup> Each 5-minute time series of R-R intervals recorded was inspected and visually checked in a beat-to-beat basis for the presence of sinus rhythm, artifacts, and stability. The intervals were manually measured by only one reader (DC) using a magnifying

lens and employing an appropriate 5-ms precision rule. Ectopic beats when occasionally present were deleted from the series together with the preceding and succeeding intervals, without adding any new interval. Edited R-R intervals were 0.08% for all series in the chagasic group and 0.05% in the control group, for a sample of about 7200 beats in each group in both supine and standing positions.

After compilation, the intervals series were sequentially digitized one-to-one onto word processing software and archived as a text file, which was transferred to a dedicated software developed and validated in our Cardiovascular Laboratory and the Department of Electrical Engineering of the University of Brasilia, using the MATLAB version 5.03 platform (The MathWorks, Inc., Natick, MA, USA),<sup>33</sup> for processing and analysis of variability in time and frequency domain. Before analysis, residual outliers were removed for more statistical uniformity of the data series by inspecting the statistical distribution of the R-R intervals beyond the minimum and maximum ones expected for the variance of the distribution in a box-and-whiskers plot employing the software. The series from which eventual ectopic or other nonsinus beats and outliers have been removed were initially submitted to interpolation by the cubic splines method and then processed and analyzed. Considering that the R-R intervals series were recorded with the subjects in very stable conditions, without influence of any external interfering or noise factors, the series qualified for analysis showed high stationarity as estimated by the percent differences of the means and the standard deviations between each pair of three segments of the R-R intervals.

The time-domain indices provided include the mean R-R interval series and two overall variability indices reflecting the combined cardiac sympathetic and parasympathetic modulation, which are the standard deviation (SDNN) and coefficient of variation (CV: SDNN/mean) of the R-R interval series. Two other indices obtained were the pNN50 (percentage of adjacent R-R intervals that are >50 ms apart) and the r-MSSD (square-root of the averaged sum of squared differences in length between all adjacent R-R intervals), which reflects the rapid parasympathetic modulation.<sup>32,34</sup>

For the frequency-domain analysis, the nonuniform 5-minute R-R interval series without spurious beats were initially normalized and resampled at 4 Hz using the cubic splines interpolation method to accomplish equidistance and continuity of time intervals. Next, the segments were submitted to filtering with Hanning windowing to attenuate leakage side effects and then processed by the fast Fourier transform algorithm for conver-

sion of oscillating components into power spectrum. The frequency-domain indices provided include: (a) total power spectral area (0–0.50 Hz), which indicates the rate of the overall autonomic modulation; (b) absolute and relative power areas of the ultra- plus very low-frequency (0–0.04 Hz), low-frequency (0.04–0.15 Hz), and high-frequency (0.15–0.50 Hz) bands; (c) normalized or relative power areas of the low- and high-frequency bands, calculated as the absolute area of each band in relation to the sum of both absolute areas; (d) ratio of low- to high-frequency band absolute power areas, as estimate of the sympathovagal modulation balance. The low-frequency band is a marker of predominant sympathetic influence, and the high-frequency represents the nearly exclusive parasympathetic action on the sinus node.<sup>32,34,35</sup>

### Statistical Analysis

Since several variables showed skewed non-normal distribution, the medians and the spread of the heart interval variability indices of each group were compared using the Mann-Whitney test. Sample distributional or spread differences between the two groups were verified considering the indices values showed by the chagasics, below the 25<sup>th</sup> or above the 75<sup>th</sup> percentile observed in the control group, in order to identify distinctive functional patterns among the individuals in the chagasic group.<sup>36</sup> Comparison between the groups for normally distributed age, body mass index, and resting arterial pressure and heart rate, employed the Student's *t*-test. For all the tests a two-tailed *p* value less than 5% ( $p < 0.05$ ) was considered as significant. Analysis and graphic design of the data employed the SigmaStat<sup>®</sup> (Jandel Scientific, San Rafael, CA, USA) and the Prism<sup>®</sup> (GraphPad, San Diego, CA, USA) software packages.

### Results

No significant difference in the resting arterial pressure and heart rate assessed in the beginning of experimental session was found ( $p = 0.51$ ;  $p = 0.45$ ) in mean  $\pm$  SD between the chagasic ( $112 \pm 10/75 \pm 7$  mmHg;  $63 \pm 5.5$  bpm) and control ( $117 \pm 12/70 \pm 5$  mmHg;  $72 \pm 7.7$  bpm) groups. The body mass index was below  $30 \text{ kg/m}^2$  for all subjects and similar ( $p = 0.38$ ) for the chagasic ( $22.4 \pm 3.8 \text{ kg/m}^2$ ) and control ( $23.5 \pm 3.1 \text{ kg/m}^2$ ) groups.

Tables I and II show, respectively, the time- and frequency-domain indices for the chagasic and control groups in supine and standing positions. No median index value in the chagasic group was statistically different ( $p = 0.17$ – $0.87$ ) from that in the control group for each posture.

The majority of the individual indices values of the chagasics showed a wide dispersion in relation to the interquartile or extreme values ranges of

**Table I**

Median (Interquartile Range) of Time-Domain Indices of Short-Term Heart Interval Variability in Control Healthy and Indeterminate Chagas' Disease Groups of Subjects in Supine and Active Standing Positions

	Supine			Standing		
	Control group	Chagas' disease group	<i>p</i>	Control group	Chagas' disease group	<i>p</i>
N	18	18		18	18	
Mean R-Ri (ms)	899 (809–939)	880 (826–1003)	0.85	775 (705–784)	753 (693–827)	0.85
SDNN (ms)	33.1 (31.5–45.2)	34.6 (22.5–44.8)	0.60	31.5 (27.9–46.5)	31.8 (20.8–39.4)	0.40
CV (%)	4.3 (3.3–4.9)	3.7 (2.5–4.7)	0.32	4.7 (3.8–5.9)	3.8 (3.0–4.8)	0.17
pNN50 (%)	4.7 (0.25–29.4)	3.1 (0.17–17.9)	0.87	1.1 (0–2.7)	1.2 (0–4.1)	0.81
r-MSSD (ms)	28.4 (18.0–49.7)	24.6 (14.9–39.2)	0.54	16.9 (14.1–24.1)	17.7 (10.3–26.7)	0.66

*p*, significance level by Mann-Whitney test; R-Ri, R-R intervals; SDNN, standard deviation; CV, coefficient of variation; pNN50, percentage of adjacent R-R intervals that are  $\geq 50$  ms; r-MSSD, square root of the averaged sum of squared differences in length between all adjacent R-R intervals.

the respective sample distribution observed in the control group in both postures, as shown in Tables I and II, and summarized in Table III.

The box-and-whiskers plots in Figs. 1 and 2 illustrate the sample distribution of the individual indices in the time and frequency domain, respectively, for the chagasic and control groups in each position. The similarity of the median values for every index and the peculiar widespread of nearly

every index in the chagasic group as compared to the control group may be observed.

### Discussion

In the present work the cardiac autonomic modulation was evaluated in ambulatory and active subjects with indeterminate Chagas' disease and controls, for the first time in an endemic area of the disease where they live. The group of chagasics

**Table II**

Median (Interquartile Range) of Frequency-Domain Indices of Short-Term Heart Interval Variability in Control Healthy and Indeterminate Chagas' Disease Groups of Subjects in Supine and Active Standing Positions

	Supine			Standing		
	Control group	Chagas' disease group	<i>p</i>	Control group	Chagas' disease group	<i>p</i>
N	18	18		18	18	
Total power spectral area (ms <sup>2</sup> )	240 (139–348)	190 (85–406)	0.58	175 (145–405)	139 (55–298)	0.25
Low-frequency power spectral area (ms <sup>2</sup> )	62.5 (38–119)	37.0 (22–104)	0.34	67.5 (46–107)	48.0 (16–111)	0.30
High-frequency power spectral area (ms <sup>2</sup> )	37.0 (14–154)	32.0 (12–80)	0.61	18.5 (11–32.5)	16.5 (5.5–44)	0.54
Normalized low-frequency power area (%)	60.3 (47.2–69.3)	58.7 (47.7–63.0)	0.63	75.7 (70.7–82.5)	73.3 (54.1–82.0)	0.31
Normalized high-frequency power area (%)	39.6 (30.6–52.8)	41.3 (36.9–52.3)	0.63	24.3 (17.5–29.2)	26.7 (17.9–45.8)	0.30
Low-to-high-frequency power area ratio	1.55 (0.89–2.26)	1.42 (0.91–1.71)	0.63	3.12 (2.41–4.72)	2.81 (1.18–4.59)	0.31

*p*, significance level by Mann-Whitney test.

**Table III**

Proportion of Indeterminate Chagas' Disease Subjects that Showed Indices Values of Short-Term Heart Interval Variability in Supine and Active Standing Positions, within (Normal Range), Below (Downward Change), or Above (Upward Change) the Interquartile Range (25<sup>th</sup>–75<sup>th</sup> Percentiles) of the Control Group

	Supine		Standing	
	Time-domain indices	Frequency-domain indices	Time-domain indices	Frequency-domain indices
All indices within the control interquartile range	3 (16.6%)	2 (11.1%)	2 (11.1%)	0
All indices below the control 25 <sup>th</sup> percentile	4 (22.2%)	0	0	0
One or more indices exclusively below the control 25 <sup>th</sup> percentile	6 (33.3%)	5 (27.8%)	9 (50.0%)	3 (16.6%)
All indices above the control 75 <sup>th</sup> percentile	4 (22.2%)	0	2 (11.1%)	0
One or more indices exclusively above the control 75 <sup>th</sup> percentile	1 (5.5%)	3 (16.6%)	4 (22.2%)	1 (5.5%)
More than one index simultaneously below the 25 <sup>th</sup> or above the 75 <sup>th</sup> percentile	0	8 (44.4%)	1 (5.5%)	14 (77.8%)
Total	18 (100%)	18 (100%)	18 (100%)	18 (100%)

The time-domain indices considered were the SDNN, coefficient of variation, pNN50, and r-MSSD. The frequency-domain indices were the total power spectral area, absolute low- and high-frequency power area, normalized low- and high-frequency power area, and low- to high-frequency power ratio.

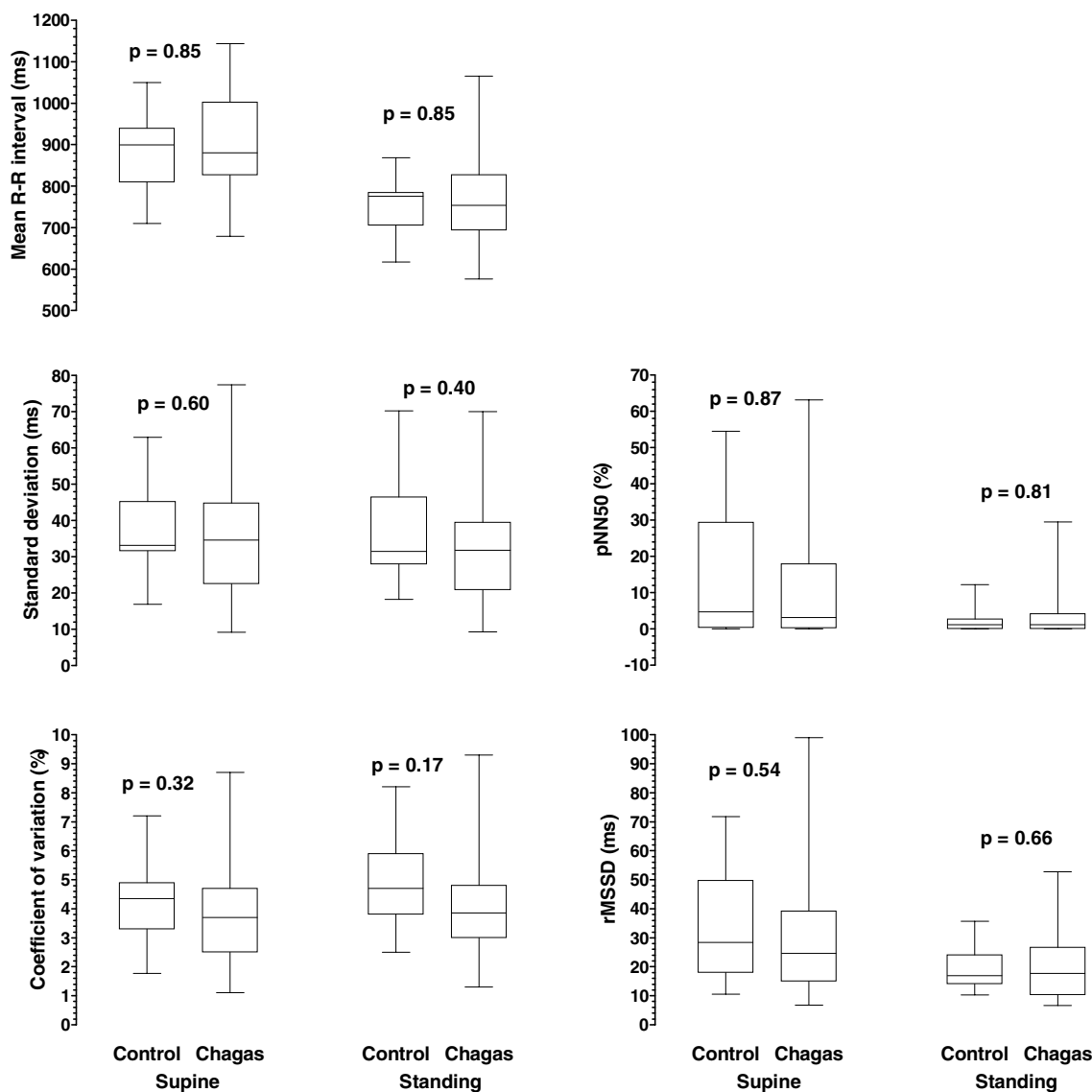
did not present impaired tonic autonomic modulation of the heart sinus node expressed by spontaneous short-term heart interval variability in the steady-state rest supine and active standing.

Similar results were observed in elderly chagasic subjects living in the same endemic area, also by employing the heart interval variability analysis.<sup>30</sup> Differing from our finding are previous observations of depressed heart interval variability in chagasics with this form of disease examined in a laboratory setting, which can explain the discrepancy.<sup>17,19,22,24,25</sup> Moreover, our analysis was based on short-lasting (5 minute) series of R-R intervals registered in controlled experimental conditions, differently from studies that used noncontrolled very long-lasting (24 hour) series based on Holter recording, which can also justify the conflicting results. Alternatively, chagasics in different studies may be infected by distinctive strains of *T. cruzi*, which may have differently influenced the pathogenicity, resulting in variable cardiac autonomic involvement and pathophysiological state and evolution.

Considering the chagasics as a group, our results also differ of observations of cardiac autonomic dysfunction in other groups of chagasics with the indeterminate form examined in labo-

ratory setting by employing study methods other than the heart interval variability.<sup>8–10,17,28,29,37,38</sup> In this case, the discrepancy may be explained considering the context of the steady-state tonic cardiac autonomic activity presently evaluated, expressed by the spontaneous heart interval variability, which is different when approaches using acute and transient cardiovascular stimuli are applied to explore phasic autonomic modulation. Disturbances in cardiac autonomic modulation of heart rate may only be expressed in stressful situations of rapid cardiovascular adjustment, as in the Valsalva maneuver<sup>8,17,37,38</sup> and under other stimuli.<sup>9,10,17,28,29</sup>

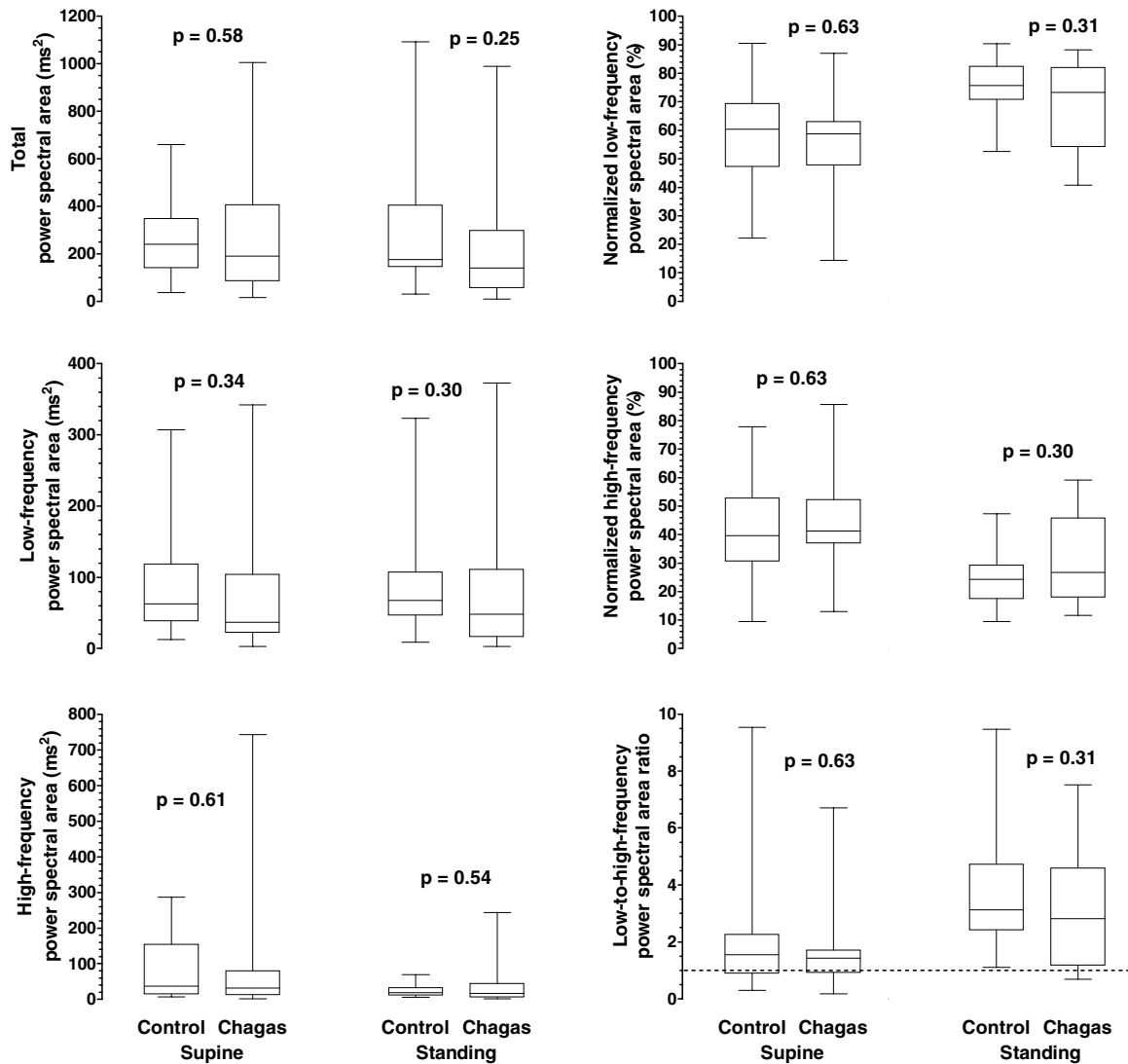
However, even when medians of different groups compared are statistically similar, differences in spread of the variables may be striking and clinically relevant.<sup>36</sup> In fact, the group of chagasics showed larger spread than the control group for most of the time- and frequency-domain indices in both postural positions. Some chagasics presented markedly or subtly depressed (downward change) or enhanced (upward change) heart period variability, beyond the interquartile or extreme values ranges showed by the control group, not detected when only medians were considered. This suggests a somewhat heterogeneous pattern of cardiac sinus node autonomic modulation of variable



**Figure 1.** Median, interquartile range, and extreme values of the time-domain heart interval variability indices of control healthy and Chagas' indeterminate disease subjects in rest supine and active standing positions. The Chagas' disease group was compared with control group by the Mann-Whitney test.

intensity in the chagasics, varying from a normal to a blunted or exacerbated pattern, denoting variable involvement of the cardiac autonomic function in the indeterminate form of Chagas' disease. Such dispersive widespread pattern of autonomic modulation had already been identified in chagasics with indeterminate Chagas' disease evaluated using the Valsalva maneuver.<sup>8</sup> Our findings did not appear to be influenced by anthropometrical and clinical confounding factors, such the large range of age of the individuals, since the groups were rigorously matched for these factors.

One possible mechanism of pathogenesis of the variable cardiac autonomic involvement is the chronic inflammatory damage of peripheral ganglia and/or neuronal fibers and perhaps of central autonomic structures, with or without neuroanglionic depopulation in different intensity or stages of evolution.<sup>3,4,31,39</sup> Depressed spontaneous steady-state cardiac autonomic modulation could appear later in the pathophysiological evolution, only when the lesions are more significant. On the other hand, exacerbated modulation may be related to the hypersensitivity phenomenon of denervated structures, in the initial phase of



**Figure 2.** Median, interquartile range and extreme values of the frequency-domain heart interval variability indices of control healthy and Chagas' indeterminate subjects in rest supine and active standing positions. The Chagas' disease group was compared with control group by the Mann-Whitney test.

the disease. In the experimental rat model of *T. cruzi* infection, exclusive ganglionic and/or neuronal inflammation without neuroganglionic depopulation associated with depressed parasympathetic impairment was observed.<sup>40</sup> Others isolate or combined mechanisms are the variable destructive binding of circulating auto-antibodies to beta-adrenergic and muscarinic cholinergic receptors<sup>5</sup> and lesion of the sinus node.

The pathophysiological and clinical significance of the cardiac autonomic dysfunction in some chagasics with the indeterminate form is a more difficult question, considering the unaltered pattern or the subtle individuals alterations as actually observed, and the good long time prognosis

of this form of the disease.<sup>2,12,15,16,24,41</sup> Impaired autonomic control of heart may be a determining or contributing factor for electrical and mechanical cardiac disturbances so common in Chagas' disease, such as arrhythmias, sudden death, and heart failure.<sup>1,4,15</sup> Additionally, inability of the moment-to-moment cardiovascular adaptation is among the possible effects that ostensive or subtle impaired cardiac autonomic modulation might variably cause.<sup>15,41</sup> Cardiac autonomic dysfunction may also be a risk factor for adverse overall and cardiovascular outcomes, since inappropriate sympathovagal balance or depressed heart interval variability have been firmly recognized to be independent markers or predictors of enhanced

cardiovascular morbidity and mortality, higher risk of arrhythmias, sudden death and other cardiac events, and of poor prognosis in different clinical conditions.<sup>32,34,35,42,43</sup> It is possible that chagasics, even with subtle autonomic dysfunction, might have more cardiac involvement and thus more careful follow up than those without any disturbance.

A possible major limitation in our study was the relatively limited samples evaluated to be confident on the validity of isolated heart interval variability measurements to characterize the cardiac autonomic function. The size of the groups, however, was approximately the minimal estimated for appropriate biological and statistical analysis and interpretation. Additionally, the data were analyzed employing nonparametric statistics and methods, which obviate the small samples evaluated and the several rightly skewed variables. Moreover, the Chagas' disease and control groups compared were rigorously matched for gender, age, body mass index, lifestyle, and physical activity. Questioning about absence of evident statistical differences in medians between the groups can not be interpreted as secure evidence of absence of possible differences, since it can be only related to the power of statistical testing.<sup>44</sup> However, the medians of the indices for the both groups were very similar and only a large individual dispersion was noted in the chagasics. Another question that can be raised refers to the power of heart interval variability analysis for to detect subtle cardiac autonomic dysfunction, since no one stimulus was

applied to induce autonomic responses. However, for analysis of the steady-state cardiac autonomic modulation the heart interval variability measurements appears to be a sensitive tool.<sup>32,34</sup> Thus, the pattern of the heart interval variability observed appears to be a meaningful finding and very improbable that can be biased.

In conclusion, the group of chagasics examined did not showed abnormality in the momentary steady-state cardiac autonomic modulation evaluated by spontaneous short-term heart interval variability analysis in time and frequency domain, both in rest supine and active standing positions. However, a widespread individual range of heart interval variability, from normal to reduced or increased ones in both postural positions was noted. This suggests a somewhat subtle heterogeneous pattern of cardiac autonomic modulation, suggesting that the intrinsic autonomic innervation involvement is absent or markedly variable in intensity in different chagasics. Such individual wide range of the cardiac autonomic function might be considered in evaluation of subjects with the indeterminate form of Chagas' disease.

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