

## CARDIOMYOPATHIES

# Circadian Profiles of Heart Rate and its Instantaneous Variability in Patients With Chronic Chagas' Disease

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**Introduction and objectives.** Impairment of the autonomic nervous system in early stages of Chagas' disease is still a matter of debate, although multiple approaches (including heart rate response to orthostatism and the Valsalva maneuver, and spontaneous variability) have been used to ascertain its occurrence. The circadian profile of heart rate and its variability have not been investigated in patients with Chagas' disease.

**Patients and Methods.** We analyzed the 24-hour heart rate by Holter recordings in 63 patients with and without ECG alterations, who had positive serological findings for Chagas' disease. These results were compared with those in 22 healthy subjects matched for sex and age. Mean 24-hour heart rate and its circadian amplitude were analyzed with Cusum analysis and nocturnal dip. In a subgroup of 45 subjects (30 with Chagas' disease and 15 healthy controls), heart rate instantaneous variability (24-hour pNN50 and r-MSSD) and circadian amplitude were also calculated by Cusum analysis.

**Results.** 24-hour and diurnal heart rates were lower in patients with Chagas' disease than in healthy subjects ( $P < .05$ ). Circadian amplitude and dip were lower in patients, but these differences did not reach statistical significance. In the subgroup of 45 subjects, the reductions in instantaneous heart rate variability (pNN50 and r-MSSD) in Chagas' disease patients were small, and circadian amplitudes were preserved, when compared with healthy subjects.

**Conclusions.** The lower heart rate in patients with Chagas' disease occurred only during diurnal activity, and instantaneous heart rate variability was preserved. These findings suggest an alteration in the sympathetic division of the autonomic nervous system. The circadian heart

rate profile, which has not been studied previously in patients with Chagas' disease, does not seem appreciably blunted in this stage of the disease.

**Key words:** Chagas' disease. Heart rate. Circadian profiles. Autonomous nervous system.

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## Perfiles circadianos de la frecuencia cardíaca y de su variabilidad instantánea en una población de pacientes con infección chagásica crónica

**Introducción y objetivos.** El compromiso autonómico en las etapas iniciales de la enfermedad de Chagas es objeto de controversia, a pesar de haberse utilizado múltiples técnicas para su análisis: la frecuencia cardíaca durante el ortostatismo, la maniobra de Valsalva o la variabilidad espontánea de la frecuencia cardíaca. El perfil circadiano de la frecuencia cardíaca no ha sido estudiado a este respecto.

**Pacientes y método.** Analizamos la frecuencia cardíaca en 24 h mediante registro Holter en 63 pacientes con serología positiva para enfermedad de Chagas, con y sin lesiones electrocardiográficas. Los resultados se compararon con los obtenidos en un grupo de 22 sujetos sanos, de edad y sexo equivalentes. Se analizó el promedio de frecuencia cardíaca de 24 h y su perfil circadiano utilizando el análisis de Cusum y la caída nocturna o «dip». En un subgrupo de 45 sujetos (30 chagásicos y 15 sanos) se calculó la variabilidad instantánea de la frecuencia cardíaca (pNN50 y r-MSSD) y la amplitud circadiana de esos parámetros utilizando el análisis de Cusum.

**Resultados.** La frecuencia cardíaca de 24 h y diurna fueron menores en los chagásicos que en los controles ( $p < 0,05$ ). Los valores de «dip» y amplitud circadiana fueron menores en los chagásicos, pero no alcanzaron diferencias significativas. En el subgrupo de 45 sujetos se encontraron, en los pacientes chagásicos, escasas alteraciones de la variabilidad instantánea de la frecuencia cardíaca (pNN50 y r-MSSD), con preservación de sus amplitudes circadianas cuando se compararon con las de los sujetos sanos.

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## ABBREVIATIONS

Cusum: cumulative sum analysis.  
 CPH: cusum plot height.  
 CH1: Chagas Group 1.  
 CH2: Chagas Group 2.  
 HR: heart rate.  
 HR24h: mean 24-hour heart rate.

**Conclusiones.** La menor frecuencia cardíaca de los pacientes chagásicos durante la actividad, con preservación de su variabilidad instantánea, sugiere una alteración de la división simpática. El perfil circadiano de la frecuencia cardíaca de estos sujetos chagásicos, que no fue estudiado previamente, no muestra una clara atenuación en esta fase de la enfermedad.

**Palabras clave:** *Enfermedad de Chagas. Frecuencia cardíaca. Perfil circadiano. Sistema nervioso autónomo.*

## INTRODUCTION

Chagas' disease, or American trypanosomiasis, is a serious public health problem in Latin America. An estimated 20 million people are infected with *Trypanosoma cruzi*, the causative agent of the condition, and 90 million individuals are at risk of infection.<sup>1</sup> Since Carlos Chagas' original description of the disease<sup>1,2</sup> and subsequent descriptions by Koberle,<sup>3</sup> alterations of other organs—megacolon and megaesophagus—have been reported in addition to the cardiac lesions, suggesting autonomic nervous system dysfunction. Numerous studies have used a variety of approaches to demonstrate autonomic involvement in the disease,<sup>4-8</sup> including analyses of heart rate response to an upright position,<sup>8</sup> exercise, chronotropic stimulation,<sup>5,9,10</sup> and the Valsalva maneuver,<sup>8</sup> administration of agents that block the autonomic nervous system,<sup>11,12</sup> and more complex analyses studying heart rate variability in terms of time (standard deviation, pNN50, r-MSSD)<sup>13</sup> or frequency, using spectral analyses.<sup>7</sup> One of our recent studies<sup>14</sup> investigated this problem using non-linear modeling techniques to analyze heart rate variability in patients with Chagas' infection who presented no myocardial damage. Despite all these efforts, impairment of the autonomic nervous system in the early stages of the disease is still a matter of debate. Although there is considerable evidence to support the early presence of dysautonomia,<sup>8,13</sup> some authors find it only in the advanced stages.<sup>6,11</sup> Furthermore, the characteristics, extent and consequences of the

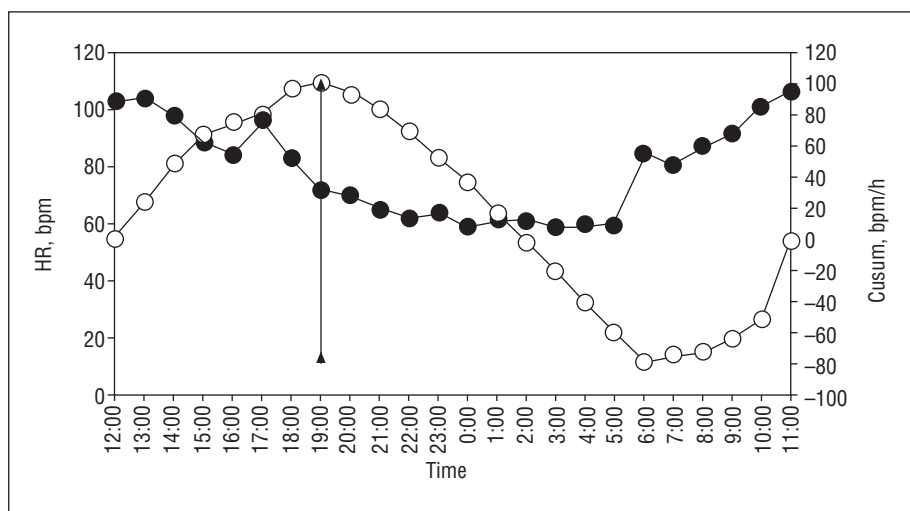
functional alterations in the autonomic nervous system have not been established, even though sympathetic and parasympathetic ganglionic alterations are known to exist in this disease.<sup>3,15,16</sup>

It is also known that changes in the 24-hour, or circadian, rhythm of blood pressure and heart rate are indicators of cardiovascular compromise<sup>17,18</sup> and markers of autonomic alterations.<sup>19</sup> Nevertheless, the circadian rhythm of heart rate in patients with Chagas' disease has not been investigated, unlike conditions such as diabetes mellitus<sup>19,20</sup> or ischemic heart disease,<sup>21</sup> in which circadian alterations have been considered a manifestation of dysautonomia.<sup>19-21</sup> This study analyzes the circadian pattern of heart rate in a population of patients with Chagas' disease who have little or no cardiac function deterioration. For this purpose, the most accurate indicators of circadian oscillation were studied in this population: the day-night dip<sup>17</sup> and the cumulative sum (cusum) analysis, used to analyze the circadian rhythm of blood pressure.<sup>22</sup> In addition, the instantaneous variability of heart rate in the time domain (pNN50 and r-MSSD)<sup>23</sup> and the circadian oscillation of this variability were measured. The purpose was to test the hypothesis that the circadian rhythm of heart rate would be altered in patients with a disease which, based on considerable evidence in the literature, is accompanied by dysautonomia. The analysis of 24-hour heart rate and variability could provide information on what type of autonomic alteration is present in the early stages of the disease.

## PATIENTS AND METHODS

### Groups Studied

The sample was composed of 85 men and women divided into 3 groups: *a*) control group with 22 subjects (10 men; mean age, 42.6±5.4 years) with negative Machado Guerreiro reaction for Chagas' disease and no clinical, radiological, electrocardiographic or echocardiographic evidence of cardiovascular disease; *b*) Chagas Group 1 (CH1), composed of 27 patients (18 men; mean age, 44.9±4.9 years) with no clinical, radiological or echocardiographic evidence of cardiovascular disease and with minimal electrocardiographic abnormalities (isolated premature supraventricular or ventricular beats); and *c*) Chagas Group 2 (CH2), composed of 36 patients (22 men; mean age, 43.6±4.9 years), with an epidemiological history and positive serology for Chagas' disease, as well as electrocardiographic abnormalities (first-degree atrioventricular block, right bundle branch block, and/or anterior fascicle, and ventricular or supraventricular arrhythmia in escape rhythm or unsustained) or echocardiographic alterations (e.g., diastolic dysfunction, mitral regurgitation, or tricuspid regurgitation) with normal overall



**Fig. 1.** Example of cusum analysis of heart rate (HR). Black circles: HR values at time points. White circles: cusum values at time points. The example shows a circadian rhythm (CPHHR) of 170 bpm/h.

left ventricular ejection fraction. All subjects had negative stress tests for myocardial ischemia.

### Parameters Analyzed

All subjects underwent 24-hour, 12-lead Holter monitoring with a Rozinn® 151 apparatus and Rozinn 718 processor. This processor allows any arrhythmias to be excluded from the time analysis. The recorders were placed on weekdays during working hours. Each recording calculated the mean 24-hour heart rate (HR24h) and the mean hourly heart rate. Subjects with gaps of more than 4 hours in the 24-hour period and gaps of more than 2 hours at night (10:00 p.m. to 6:00 a.m.) were excluded. The resulting data were used to analyze the 24-hour rhythm of heart rate using the nocturnal dip in heart rate and the cusum method.<sup>22</sup> Briefly, this method consists of plotting a chart of the cumulative differences between the time-weighted hourly means of the study variable and the weighted 24-hour mean (see Appendix). Thus, the weight of each value in the mean corresponds to the interval it covers. This plot was then used to obtain the CPH (cusum plot height) corresponding to the difference between the maximum value immediately before the nocturnal dip and the minimum value right before the rise occurring in the morning (Figure 1). The nocturnal dip in heart rate was calculated as the percent difference between the mean heart rate during the day (6:00 a.m. to 10:00 p.m.) and at night (10:00 p.m. to 6:00 a.m.).<sup>17</sup> In the most recent subgroup of 45 age- and sex-matched subjects (15 controls, 15 CH1 and 15 CH2) (control, 45±12.1 years; CH1, 45±12.7 years; CH2, 45.2±8.6 years) studied with a processor allowing a more complete analysis, we also recorded the 24-hour means, diurnal and nocturnal means, and hourly means of the pNN50 values (number of pairs of adjacent

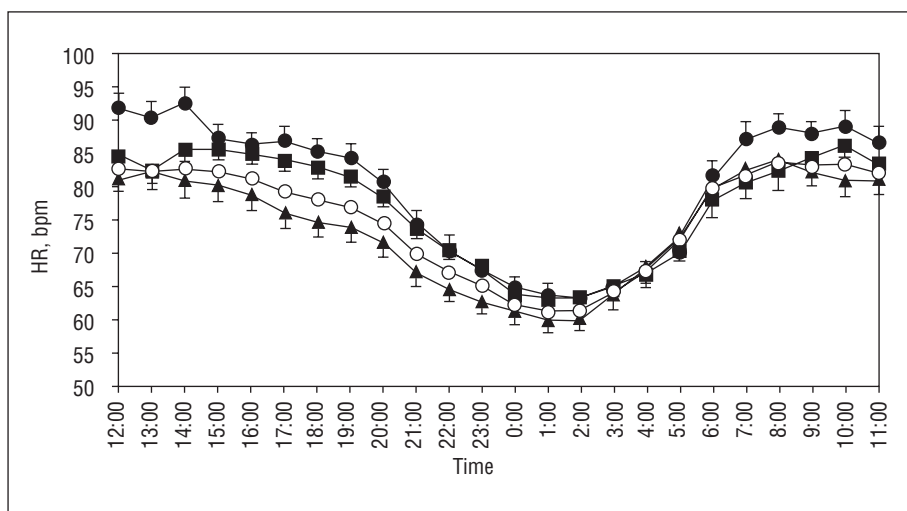
RR intervals differing by more than 50 ms in the entire recording, divided by the total number of RR intervals) and r-MSSD (square root of the mean of the squared differences between adjacent RR intervals), in accordance with the guidelines of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology<sup>23</sup> and the Sociedad Española de Cardiología (Spanish Society of Cardiology).<sup>24</sup> These hourly means were used for the cusum analysis, in which the  $CPH_{pNN50}$  and  $CPH_{r-MSSD}$  values were obtained using an approach analogous to the one used for heart rate.

### Statistical Analysis

The mean heart rates and the circadian rhythms ( $CPH_{HR}$ ) of the control subjects were compared to the values obtained in all patients with Chagas' disease (CH1+CH2) by Student's *t*-test for unpaired samples. The differences in 24-hour means among the 3 groups were analyzed using ANOVA and the Bonferroni correction. The  $CPH_{HR}$ , diurnal and nocturnal means, and dip were also analyzed among the 3 groups. In the 45-patient subgroup, in which pNN50 and r-MSSD values were calculated, the 24-hour means, diurnal and nocturnal means, and  $CPH_{pNN50}$  and  $CPH_{r-MSSD}$  values (ANOVA and Bonferroni) were similarly compared. The values obtained were expressed as mean±standard deviation (SD), unless otherwise specified. Significance was set at a *P* value of less than .05.

### RESULTS

Physiological circadian rhythm was observed in all 3 groups, although there was a trend to a lower heart rate during waking hours in the patients with



**Fig. 2.** Hourly means of heart rate in all 85 subjects studied. Black circles: healthy controls. Squares: patients in CH1 group. Triangles: patients in CH2 group. White circles: total for patients with Chagas' disease (CH1+CH2). Values are expressed as mean±standard deviation, except in the case of all patients with Chagas' disease (white circles), in which standard deviation is not shown.

Chagas' disease as compared to the controls (Figure 2).

Table 1 lists the values of HR<sub>24h</sub>, CPH<sub>HR</sub>, diurnal and nocturnal means, and nocturnal dip in the study population. The HR<sub>24h</sub> was significantly higher in the control subjects than in patients with Chagas' disease as a whole. Analysis of this difference among the 3 groups shows that the controls had a significantly higher HR<sub>24h</sub> than the CH1 and CH2 subjects, although the difference was significant only with respect to the CH2 subjects. Likewise, the CH1 patients presented a significantly higher HR<sub>24h</sub> than the CH2 group. The differences in mean heart rates were evident and significantly higher only in the waking period, with similar values in the 3 groups during the night hours. There were no significant differences in dip values between the groups. The circadian amplitude estimated by CPH<sub>HR</sub> was higher in the controls than in the CH1 or CH2 groups, though the differences were not statistically significant. Figures 3 and 4 show the instantaneous variability of heart rate (pNN50 and r-MSSD) in the subgroup of 45 subjects in whom these variables

were analyzed. The 24-hour circadian rhythms for these parameters were similar in the 3 groups, although the CH2 group showed a trend to higher nocturnal values. Table 2 indicates the 24-hour values of pNN50 and r-MSSD, as well as the CPH<sub>pNN50</sub> and CPH<sub>r-MSSD</sub> values. There were no differences, except for r-MSSD, which, as compared to the controls, was significantly lower in the CH1 group at night and significantly higher in the CH2 group during the day.

In summary, we found a lower HR<sub>24h</sub> in patients with Chagas' disease than in healthy volunteers. This lower heart rate was evident only in the daytime hours. The circadian rhythms were less pronounced in these patients, although this difference was not significant. The instantaneous variability in heart rate showed minimal alterations and was even slightly higher in the CH2 patients during the night hours.

## DISCUSSION

The main finding in this study was that patients with Chagas' disease had a lower HR<sub>24h</sub> in compari-

**TABLE 1. Heart Rate in the Total Study Population (85 Subjects)\***

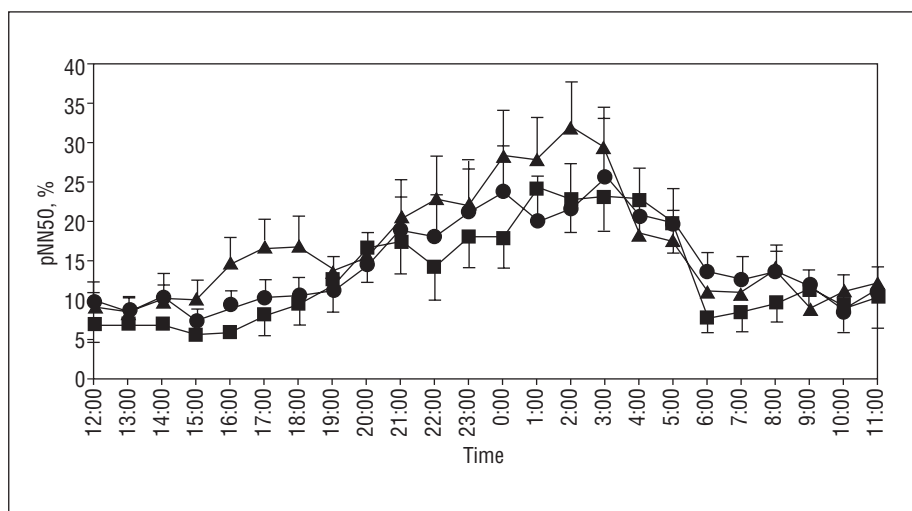
	Control (n=22)	Total Chagas (n=63)	Chagas 1 (n=27)	Chagas 2 (n=36)
HR 24 h, bpm	79.5±4.5	75.0±5.2 <sup>†</sup>	77.0±4.5	73.5±5.2 <sup>§</sup>
Diurnal HR, bpm	86.0±3.9	80.1±5.4 <sup>†</sup>	82.5±4.8 <sup>†</sup>	78.4±5.6 <sup>§</sup>
Nocturnal HR, bpm	66.3±6.9	65.2±6.1	66.4±5.5	64.3±6.4
Dip, %	22.9±7.2	18.6±5.8	19.6±4.6	17.8±6.6
CPH <sub>HR</sub> , bpm/h	101.5±34.4	95.6±27.3	99.9±27.1	92.3±27.3

\*Control indicates total healthy subjects; CPH<sub>HR</sub>, circadian amplitude of heart rate; total Chagas, total of subjects with Chagas' disease (Chagas 1 and Chagas 2); HR, heart rate; bpm, beats per minute.

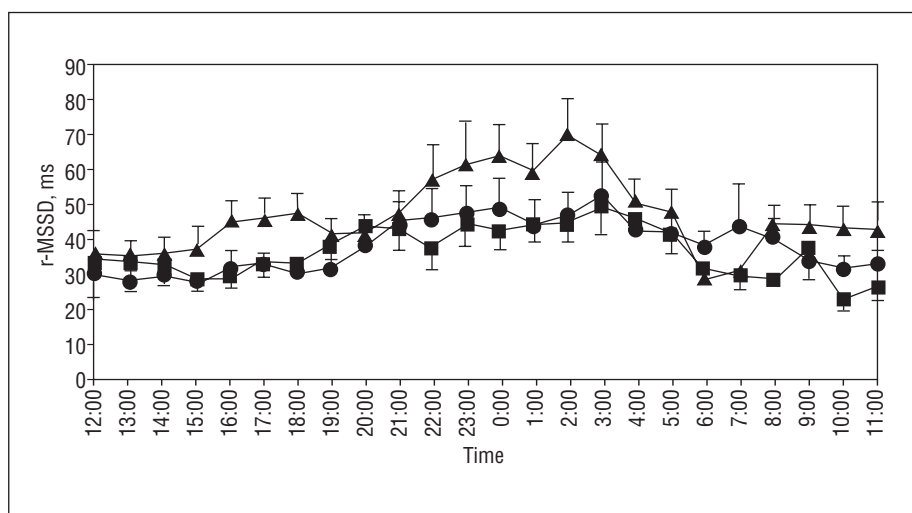
<sup>†</sup>P<.0005 with respect to control (Student's t-test for n paired samples).

<sup>‡</sup>P<.05 with respect to control (ANOVA and Bonferroni).

<sup>§</sup>P<.05 with respect to control and Chagas 1 (ANOVA and Bonferroni).



**Fig. 3.** Hourly means of pNN50 in the 45-subject subgroup. Black circles: healthy controls. Squares: patients in CH1 group. Triangles: patients in CH2 group. Values are expressed as mean±standard deviation.



**Fig. 4.** Hourly means of r-MSSD in the 45-subject subgroup. Black circles: healthy controls. Squares: patients in CH1 group. Triangles: patients in CH2 group. Values are expressed as mean±standard deviation.

son with healthy volunteers. This has already been reported in the literature<sup>5,12</sup> and is suggested to be the result of a lower sinus node<sup>12</sup> response to the autonomic stimulation or dysautonomia that accompanies

the disease.<sup>5</sup> However, it has not been established which of these factors is definitely responsible for this lower heart rate in patients with Chagas' disease. It is interesting that circadian rhythms are preserved

**TABLE 2. Instantaneous Variability in Heart Rate in the 45-Patient Subgroup\***

	Control (n=15)	Chagas 1 (n=15)	Chagas 2 (n=15)
pNN50 24 h	14.5±10.8	13.2±9.9	17.0±10.9
r-MSSD 24 h	38.2±14.1	36.49±49.2	47.3±64.8
Diurnal pNN50	11.5±6.4	9.8±8.6	13.0±8.1
Nocturnal pNN50	21.6±21.2	19.6±14.0	24.7±18.1
Diurnal r-MSSD	34.0±10.1	33.5±17.0	41.2±19.6
Nocturnal r-MSSD	46.7±26.3	42.1±19.0 <sup>†</sup>	59.0±31.0 <sup>‡</sup>
CPH <sub>pNN50</sub>	83.6±70.3	78.9±17.2	88.8±21.7
CPH <sub>r-MSSD</sub>	132.5±85.6	97.5±54.2	138.0±96.6

\*Control indicates total healthy subjects; CPH<sub>pNN50</sub>, circadian amplitude of pNN50; CPH<sub>r-MSSD</sub>, circadian amplitude of r-MSSD; see text for other abbreviations.

<sup>†</sup>P<.05 with respect to control (ANOVA and Bonferroni).

<sup>‡</sup>P<.05 with respect to control (ANOVA and Bonferroni).

in patients with Chagas' disease and, although the cumulative sum analysis showed a trend to progressive blunting of this rhythm from the controls to the CH2 group, this was not significant, suggesting that any autonomic alteration in these patients did not compromise the physiological oscillation of heart rate during the 24 hours. The same pattern was observed in the nocturnal dip in heart rate. The latter result is not surprising, since both CPH and dip are indicators of circadian rhythm, and our recent findings showed<sup>25</sup> a high correlation between these two indices, both for blood pressure and heart rate. To our knowledge, no systematic analysis of the circadian rhythm of heart rate has been previously carried out in patients with Chagas' disease. It is known that the cusum analysis gives an accurate idea of the circadian oscillation of blood pressure<sup>26</sup> and is independent of other components of the variability.

The present study also disclosed a small, inconsistent alteration in the instantaneous variability indicators pNN50 and r-MSSD (r-MSSD was slightly greater in the CH1 group than the control group, and even greater in the CH2 subjects than the controls during night hours) which, as is known, are related to the effect of the parasympathetic system on heart rate variability.<sup>27</sup> This apparently contradictory result coincides with the discrepancies reported in the literature on alterations in the instantaneous variability in patients with Chagas' disease. Some authors report that there is no instantaneous variability,<sup>28</sup> whereas others<sup>13</sup> find alterations in these parameters. These discrepancies could be related to patient age or, as mentioned in the literature, linked to potential differences in the parasite strain responsible for the infection, genetic factors, etc.<sup>29</sup> Unlike other studies,<sup>13</sup> instantaneous variability in the present study was analyzed in a subgroup of age-matched Chagas' disease patients and controls (control, 45±12.1 years; CH1, 45±12.7 years; CH2, 45.2±8.6 years) to eliminate any potential influence of age on the results. Our patients showed none of the alterations resembling megacolon and megaesophagus that suggest dysautonomia which are seen in other latitudes of the continent.<sup>29</sup>

Furthermore, our results cannot be attributed to differences in the patients' levels of daytime activity, since in this stage the disease involves no functional disability. Nor are the findings related to the effect of drugs, as the studies were conducted in patients with no or few symptoms who were not receiving drugs at that time.

Based on our analysis of the 24-hour rhythms of the instantaneous variability of heart rate in normal subjects, the heart rate also has circadian oscillation and is higher during sleeping hours than waking hours, a phenomenon already identified<sup>30</sup> and related to greater vagal tone during sleep. The patients with Chagas' disease also presented this circadian oscillation. No dif-

ferences were found in the diurnal values, and any differences in nocturnal r-MSSD values between these patients and those of the healthy controls were infrequent and inconclusive. In addition, there were no differences in the circadian amplitude values derived from the cusum analysis. This suggests that these patients presented no alterations in parasympathetic activity or in the effect of such activity on the heart rate or its variability. The 24-hour heart rate analysis shows that patients with Chagas' disease had a significantly lower heart rate than the controls during the day. Nevertheless, the nocturnal heart rates were similar in the 3 groups. This pattern, which has not been described in the literature, indicates that the circadian rhythm blunting observed in our study is linked to a decrease in daytime values rather than a lower nocturnal dip in heart rate. It is known that the increased heart rate accompanying daytime activity is related to increased adrenergic tone<sup>31</sup> and decreased vagal activity.<sup>30</sup> The attenuation of heart rate increases during daytime activity, combined with the absence of alterations in the heart rate or instantaneous variability during nighttime, suggests that the autonomic nervous system impairment seen in these patients in these initial stages (i.e., during the so-called intermediate period) is linked to an alteration in the sympathetic rather than the parasympathetic division. This appears to contradict much of the literature<sup>5,13</sup> and differs from what occurs in diabetic patients with dysautonomia, in which there is clear blunting of the circadian rhythm, both in heart rate and variability.<sup>19,20</sup> Nevertheless, our results are supported by the impaired chronotropic response described when dobutamine is given to patients with Chagas' disease<sup>9</sup> and by the morphological alterations of the sympathetic ganglia reported in this disease.<sup>15,16</sup> Nevertheless, recent scintigraphy studies indicate an alteration of cardiac sympathetic innervation in the indeterminate phase of the disease.<sup>32</sup>

In summary, any potential autonomic alteration in patients with Chagas' disease does not appear to significantly compromise the circadian rhythms of heart rate or its variability, at least during the intermediate or asymptomatic stage of the disease. The lower heart rate during waking hours indicates an alteration in the sympathetic division in these patients. The persistence of normal heart rate values during sleep, and minimal or no alterations of instantaneous variability suggest preservation of the parasympathetic division in these asymptomatic subjects. When compared to other findings reported in the literature, the difference shown by our results could be related to geographic differences in the type and progression of the disease and are in keeping with the controversy on the presence of autonomic alterations in later stages of the disease, which could be responsible for its progression. This is somewhat similar to the thrombotic and microvascular alterations that also occur in the

disease<sup>33</sup> and that have an uncertain pathophysiologic role.

## APPENDIX

According to Stanton et al,<sup>22</sup> the 24-hour mean of the heart rate (HR) is calculated as the sum of the products of the HR (bpm) intervals and the duration of the latter divided by the total duration of the 24-hour Holter recording (h):

$$\text{Mean HR}_{24h} = \frac{1}{D} \sum_{i=1}^n (\text{HR}_i)(d_i)$$

where D is the total duration of the ambulatory recording, n is the total number of intervals, HR<sub>i</sub> is the heart rate of the first interval, and d<sub>i</sub> is the first interval duration. Thus, each interval between two hourly means of heart rate is multiplied by the heart rate corresponding to this interval. The sum of these values, divided between the total time of the ambulatory recording, gives a mean 24-hour heart rate, weighted for the respective interval.

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