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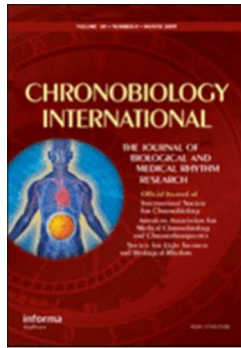
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**Time of day affects heart rate recovery and variability after maximal exercise in pre-hypertensive men**

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Keywords:	circadian rhythm, parasympathetic reactivation, diurnal variation, cardiac autonomic modulation, aerobic exercise

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**Time of day affects heart rate recovery and variability after maximal exercise in pre-hypertensive men.**

**Time of day and post-exercise recovery**

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**ABSTRACT**

Heart rate (HR) recovery (HRR) and variability (HRV) after exercise are non-invasive tools used to assess cardiac autonomic regulation and cardiovascular prognosis. Autonomic recovery is slower after evening than morning exercise in healthy individuals, but this influence is unknown in subjects with autonomic dysfunction, although it may affect prognostic evaluation. This study compared post-exercise HRR and HRV after maximal morning and evening exercise in pre-hypertensive men. Ten volunteers randomly underwent two maximal exercise tests conducted in the morning (8–10 a.m.) and evening (6–8 p.m.). HRR60s (HR reduction at 60s of recovery – prognostic index), T30 (short-term time-constant of HRR - parasympathetic reactivation marker), rMSSD<sub>30s</sub> (square root of the mean of the sum of the squares of differences between adjacent R-R intervals on subsequent 30-s segments – parasympathetic reactivation marker), and HRR $\tau$  (time constant of the first order exponential fitting of HRR – marker of sympathetic withdraw and parasympathetic reactivation) were measured. Paired t-test and two-way ANOVA were used. HRR60s and HRR $\tau$  were similar after exercise in the morning and evening ( $27\pm 7$  vs.  $29\pm 7$  bpm,  $P = .111$ , and  $79\pm 14$  vs.  $96\pm 29$  s,  $P = .119$ , respectively). T30 was significantly greater after evening exercise ( $405\pm 215$  vs.  $295\pm 119$  s,  $P = .002$ ) and rMSSD<sub>30s</sub> was lower in the evening (main factor session,  $P = .009$ ). In conclusion, in pre-hypertensive men, the prognostic index of HRR, HRR60s, is not affected by the time of day when exercise is conducted. However, post-exercise parasympathetic reactivation, evaluated by T30 and rMSSD<sub>30s</sub>, is blunted after evening exercise.

Keywords: Circadian rhythm, parasympathetic reactivation, diurnal variation, cardiac autonomic modulation, aerobic exercise.

## Introduction

Alterations in cardiac autonomic modulation are associated with higher cardiovascular risk for cardiovascular diseases (Greenwood et al. 1998). In addition, autonomic dysfunction, characterized by decreased cardiac parasympathetic modulation and increased cardiac sympathetic modulation, is present in many cardiovascular diseases (Greenwood et al. 1998). In hypertension, autonomic dysfunction is already observed before diagnosis (i.e. in pre-hypertensive individuals) (Pal et al. 2012), and gets worse with disease severity and the presence of end-organ damage (Fisher & Paton 2012).

Autonomic response after exercise can be easily measured and may be used to evaluate cardiovascular risk. Post-exercise heart rate (HR) recovery (HRR) and post-exercise HR variability (HRV) are non-invasive tools that evaluate cardiac autonomic regulation after exercise (Goldberger et al. 2006; Imai et al. 1994; Pecanha et al. 2013). Immediately after exercise cessation, HR presents a rapid fall mainly driven by a sudden reactivation of cardiac parasympathetic activity (Imai et al. 1994; Pecanha et al. 2013). Afterwards, HR gradually returns to its baseline value by the summed actions of parasympathetic reactivation and sympathetic withdrawal (Perini et al. 1989; Pecanha et al. 2013). Concomitantly, HRV, which mainly reflects parasympathetic modulation, increases progressively throughout the recovery period (Goldberger et al. 2006). Alterations in these autonomic indexes (HRR and HRV after exercise) reflect the presence of cardiac autonomic dysfunction and are considered independent predictors of cardiovascular morbidity and mortality in many populations, including hypertensive individuals (Cole et al. 1999; Nishime et al. 2000; Smith et al. 2005).

Indices of HRR and HRV can be easily assessed after a single session of exercise that is often performed at different times of the day. Nevertheless, cardiac autonomic modulation present circadian variations (Lombardi et al. 1992) that might influence HRR and HRV assessed at different times of day, influencing cardiovascular prognosis based on these

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3 indexes. In healthy subjects, HRR is reduced after evening compared with morning exercise  
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5 (Reilly et al. 1984; Cohen & Muehl 1977). However, in subjects who have cardiovascular  
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7 autonomic dysfunction, such as pre-hypertensives (Pal et al. 2012), this response might be  
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9 different. Pre-hypertensives present a greater sympathetic morning surge (Grassi et al. 2008),  
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11 and it is known that autonomic recovery is slower in the presence of higher sympathetic  
12  
13 levels (Ushijima et al. 2009). Thus, in pre-hypertensives, HRR and HRV may be slower after  
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15 morning than evening exercise, and there are no studies that have been investigated it.  
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19 Therefore, the aim of the current study was to investigate the influence of time of day  
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21 at which exercise is performed on HRR and post-exercise HRV in pre-hypertensive subjects.  
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23 The hypothesis is that, in pre-hypertensive subjects, HRR and HRV recovery are slower after  
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25 morning than evening exercise.  
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## 28 29 30 Materials and Methods

### 31 32 33 Subjects

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35 Twenty-two supposed pre-hypertensive men (systolic/diastolic blood pressure levels  
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37 between 120 and 139/80 and 89 mmHg) (Chobanian et al. 2003) aged 20 to 45 years were  
38  
39 invited to participate in the study. Besides pre-hypertension, other study criteria were: I) to  
40  
41 have neither type chronotype; and II) good quality of sleep. The exclusion criteria were: I)  
42  
43 smoking, II) obesity level equal to or greater than 2, III) practice of regular exercise  $\geq 2$  times  
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45 per week, IV) presence of known cardiometabolic diseases, V) use of medications that could  
46  
47 affect cardiovascular responses, and VI) presence of electrocardiographic abnormalities at  
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49 rest or during a maximal exercise test.  
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52  
53 The study was approved by the Ethics Committee of the School of Physical Education  
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55 and Sport under the process (2011/17), University of São Paulo, and it was registered at  
56  
57 [www.ensaiosclinicos.gov.br](http://www.ensaiosclinicos.gov.br) (RBR-3HKQG9) and conducted in accordance with the  
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3 Declaration of Helsinki and the experimental protocol followed to international ethical  
4 standards (Portaluppi et al. 2010). All volunteers signed an informed written consent before  
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7 study enrollment.  
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10 To confirm the aforementioned criteria, all volunteers reported to laboratory for  
11 preliminary exams. However, 12 did not fulfil all the criteria (in 7 pre-hypertensive diagnosis  
12 was not confirmed, 1 was obese beyond level 2, 1 had other comorbidity besides pre-  
13 hypertension, 1 had a morningness chronotype, and 2 practiced regular exercise). Thus, 10  
14 subjects initiated and completed all the study procedures, and their characteristics are  
15 described in table 1.  
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25 Insert Table 1  
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### 30 Study design

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32 The experimental protocol consisted of four visits in different days. The first two  
33 visits were used for the preliminary exams that included: i) health status and absence of  
34 regular exercise, assessed by interview; ii) pre-hypertension diagnosis confirmation, assessed  
35 by auscultatory measurement of blood pressure on two visits (Chobanian et al. 2003); iii)  
36 neither type chronotype, assessed by Horne & Ostberg's morningness and eveningness  
37 questionnaire, and scored between 42 to 58 (Horne & Ostberg 1976); iv) good quality of  
38 sleep, assessed by Pittsburgh Sleep Quality Index, and values equal to or below 5 (Buysse et  
39 al. 1989); v) obesity level lower than 2, assessed by body mass index lower than 35 kg/m<sup>2</sup>  
40 (National Institutes of Health 2000); and iv) electrocardiographic abnormalities, assessed by  
41 electrocardiogram (ECG) (EMG System do Brazil, EMG 030110/00B, São Paulo, Brazil)  
42 performed before and during a maximal cardiopulmonary exercise test (Brazilian Society of  
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3 Afterwards, the study was performed in a crossover design, which all subjects who  
4 fulfilled the study criteria underwent two maximal exercise tests: one in the morning (8-  
5 10a.m.) and the other in the evening (6-8p.m.). The tests were performed in a randomized  
6 order on two different days separated at least for 72 hours. The order of these tests was  
7 randomly assigned for each participant. For this, a blind researcher made a simple raffle to  
8 indicate the order of the experimental sessions for each subject. All subjects were instructed  
9 to have a light meal 2 hours before the test and to avoid stimulant products (such as caffeine)  
10 that might affect cardiovascular function, physical efforts and alcoholic drinks for the  
11 previous 24 hours. The maximal exercise tests were conducted on a cycle ergometer (Lode  
12 Medical Technology, Corival, Groningen, Netherland) with a graded protocol that initiated  
13 with 30W and increased 30W every 3 min until the subjects were unable to continue. In all  
14 subjects, immediately after fatigue, workload was decreased to 30W and the subjects  
15 remained pedalling for 5 min (active recovery). Respiratory rate was not controlled during the  
16 test and recovery. ECG was continuously registered (EMG System do Brazil, EMG  
17 030110/00B, São Paulo, Brazil), oxygen uptake ( $VO_2$ ) and respiratory exchanged rate (RER)  
18 were continuously measured (CPX Ultima, Med Graphics, Minnesota, USA).  $VO_{2peak}$  was  
19 determined as the highest value achieved during exercise in averages of 30s. HR was also  
20 continuously assessed during exercise and recovery by a HR monitor (POLAR 800cx,  
21 Kempele, Finland), and peak HR was considered as the highest value achieved at the end of  
22 the exercise.

#### 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 Data analysis

50  
51 HR signal was transmitted to the Polar Pro Trainer Software® (v. 5.0, Polar Inc.,  
52 Kempele, Finland). RR intervals (RRi) were automatically detected and inspected, and they  
53 were corrected by a moving average filter, according to the Polar Pro Trainer Software® (v.  
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3 5.0, Polar Inc., Kempele, Finland). Then, RRi time series were exported to Matlab<sup>®</sup> (The  
4 Math Works, Massachusetts, USA) for post-exercise HRR and HRV analysis.  
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#### 8 9 Post-exercise HRR

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11 HRR was assessed by calculating the following index: i) HRR60s – absolute heart rate  
12 reduction after 60s of recovery in relation to the peak HR (Kannankeril et al. 2004); ii) T30 –  
13 the negative reciprocal of the slope of the regression line between the natural logarithm of  
14 HR from the 10<sup>th</sup> to 40<sup>th</sup> s after exercise (Buchheit et al. 2008); and iii) HRR $\tau$  – time constant  
15 of the first order exponential fitting of the HRR curve of the five min after exercise (Pierpont  
16 et al. 2000). The two first indexes are indicative of parasympathetic reactivation (Kannankeril  
17 et al. 2004; Imai et al. 1994), while the third index is indicative of parasympathetic  
18 reactivation and sympathetic withdrawal (Pierpont et al. 2000; Imai et al. 1994).  
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#### 32 Post-exercise HRV

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34 Post-exercise HRV was calculated by the square root of the mean squared difference  
35 of successive RRi on subsequent 30-s non-overlapped segments (rMSSD<sub>30s</sub>). To smooth out  
36 any transient outliers in the rMSSD<sub>30s</sub> plots, a median filter operation was applied to the  
37 entire RRi time series (Goldberger et al. 2006). This index represents parasympathetic  
38 reactivation after exercise (Goldberger et al. 2006).  
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#### 48 Statistical analysis

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50 A power analysis was performed to determine the required sample size for the study  
51 (GPower V.3.1.5, Kiel, Germany). For a power of 80%, and alpha error of 5%, the minimum  
52 sample size required to show a difference of 8 $\pm$ 5 bpm (Buchheit et al. 2007a) in HRR60s  
53 were 7 subject.  
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Box plot was employed to verify outliers. Shapiro-Wilk test was used to assess normal or non-normal distribution of data (SPSS for windows, Illinois, USA). Paired t-test or Wilcoxon test were used to compare HRR indexes between morning and evening exercise. A two-way ANOVA for repeated measures was employed to compare rMSSD<sub>30s</sub> during post-exercise period, taking into account time of day (morning and evening) and stages (windows of 30s) as the main factors (Statsoft, Statistic for windows, Oklahoma, USA). All analysis were two-tailed and significance was accepted as  $P \leq .05$ , and data were presented as mean $\pm$ SD.

## Results

HR and rMSSD<sub>30s</sub> measured at rest before the exercise were not different in the morning and evening ( $76\pm 10$  vs.  $75\pm 8$  bpm,  $P = .835$  and  $23.72\pm 10.12$  vs.  $21.01\pm 6.60$  ms,  $P = .413$ , respectively).

Maximal workload,  $VO_{2peak}$ , RER, and exercise time ( $183\pm 37$  vs.  $185\pm 38$  watts,  $P = .341$ ;  $28.9\pm 5.9$  vs.  $27.9\pm 4.0$  ml.kg<sup>-1</sup>.min<sup>-1</sup>,  $P = .284$ ;  $1.26\pm 0.07$  vs.  $1.29\pm 0.09$ ,  $P = .285$ ; and  $1047\pm 250$  vs.  $998\pm 168$  s,  $P = .341$ , respectively) were not different in the morning and evening tests, while peak HR was significantly higher in the evening than the morning test ( $175\pm 13$  vs  $170\pm 12$  bpm,  $P = .031$ , respectively).

HRR<sub>60s</sub> and HRR $\tau$  did not differ after exercises conducted in the morning and evening ( $27\pm 7$  vs.  $29\pm 7$  bpm,  $P = .111$ , and  $79\pm 14$  vs.  $96\pm 29$  s,  $P = .119$ , respectively) (Figure 1, panels A and B). On the other hand, T<sub>30</sub> was significantly greater after exercise conducted in the evening than the morning ( $405\pm 215$  vs.  $295\pm 119$  s,  $P = .002$ , respectively) (Figure 1C).

\* Figure 1

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3 For HRV analysis, no significant interaction was found for session and time, however  
4 significant main factors were detected. Thus, rMSSD<sub>30s</sub> was lower after performing exercise  
5 in the evening than the morning (P= .009). In addition, rMSSD<sub>30s</sub> was significantly greater at  
6 all time points compared with the first 30s window.  
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14 \*Figure 2  
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## 18 Discussion 19

20 The novelty of this study is the assessment of the time of day influence on post-  
21 exercise HRR and HRV in a population expected to present autonomic dysfunction (Pal  
22 2012). The main findings were that, in pre-hypertensive men, T30 was higher and rMSSD30s  
23 recovery was slower after maximal exercise performed in the evening (6–8p.m.) than in the  
24 morning (8–10a.m.). On the other hand, HRR60s and HRR $\tau$  were not influenced by time of  
25 day.  
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33 Since morning sympathetic activation is greater in individuals with autonomic  
34 dysfunction (Grassi et al. 2008), the hypothesis of the study was that parasympathetic  
35 reactivation after exercise would be blunted after the morning exercise. However, contrary to  
36 the hypothesis, the increased T30 and slower rMSSD30s restoration observed after the  
37 evening exercise suggest that parasympathetic reactivation is slower when exercise is  
38 performed in the evening than the morning. Previous studies with healthy subjects have also  
39 observed a slower HRR after evening exercise in comparison with morning exercise (Cohen  
40 & Muehl 1977; Reilly et al. 1984), showing results in the same direction.  
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51 Three indexes were applied to analyse parasympathetic reactivation after the exercise,  
52 i.e. the quantification of the HR reduction after 60s of exercise (HRR60s) (Kannankeril et al.  
53 2004), a mathematical fitting approach to HR recovery in the first 30s of exercise (T30) (Imai  
54 2004), a mathematical fitting approach to HR recovery in the first 30s of exercise (T30) (Imai  
55 2004), a mathematical fitting approach to HR recovery in the first 30s of exercise (T30) (Imai  
56 2004), a mathematical fitting approach to HR recovery in the first 30s of exercise (T30) (Imai  
57 2004), a mathematical fitting approach to HR recovery in the first 30s of exercise (T30) (Imai  
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60 2004), a mathematical fitting approach to HR recovery in the first 30s of exercise (T30) (Imai

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3 et al. 1994), and the time to 30s HRV to recover after exercise (rMSSD30s) (Goldberger et al.  
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5 2006). Two of these indexes revealed a slower reactivation after the evening exercise (T30  
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7 and rMSSD30s), while HRR60s showed no difference. The difference in responses between  
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9 the indices may be attributed to the fact that HRR60s is more prompt to be influenced by  
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11 peak HR during exercise (Pierpont et al. 2013), while T30 have been shown not to be  
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13 influenced by peak HR (Buchheit et al. 2007b; Nakamura et al. 2009; Arduini et al. 2011).  
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15 After the evening exercise, peak HR was significantly higher than after the morning exercise,  
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17 which has already been reported in other populations (Reilly et al. 1984; Cohen & Muehl  
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19 1977) and might have affected the absolute decay of HR in the first 60s of recovery after the  
20  
21 evening exercise. It is also interesting to observe that even though HRR60s is considered a  
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23 parasympathetic reactivation index (Kannankeril et al. 2004), it also receives other influences  
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25 that might reduce its sensibility to parasympathetic reactivation (Pierpont et al. 2013) and  
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27 may help to explain its different behaviour comparing with the other parasympathetic indexes  
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29 (T30 and rMSSD30s). Therefore, as T30 and rMSSD30s are more reliable indices of  
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31 parasympathetic reactivation (less influenced by other factors) and differences between  
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33 morning and evening exercise were found in these indices, the results suggest that  
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35 parasympathetic reactivation is delayed after evening exercise (Al Haddad et al. 2011;  
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37 Boulosa et al. 2014; Buchheit et al. 2008).

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43 Regarding this slower vagal reactivation after evening exercise concerns may arise  
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45 about a possible influence of the higher peak HR achieved at this time of day on this  
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47 response. In fact, higher evening peak HRs were found in 9 out of the 10 subjects, suggesting  
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49 this is really an effect of time of day. This higher evening peak HR might reflect a greater  
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51 activation of sympathetic activity during evening exercise in comparison with morning  
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53 exercise (Maciel et al. 1986). It is known that high sympathetic activity blunts  
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55 parasympathetic reactivation (Ushijima et al. 2009). Thus, the higher sympathetic activation  
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3 achieved during evening exercise may be the mechanism responsible for both the higher peak  
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5 HR and the delay in parasympathetic reactivation after the evening exercise.  
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8 Concerning the sympathetic withdrawal after exercise, it occurs mainly during the  
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10 slow phase of recovery, after two minutes (Imai et al. 1994; Perini et al. 1989; Pecanha et al.  
11  
12 2013). HRR at this phase is mainly influenced by exercise intensity (Imai et al. 1994). In the  
13  
14 current study, peak workload did not differ between the maximal tests conducted in the  
15  
16 morning and evening, which may explain, at least in part, the absence of difference on HRRt  
17  
18 between both times of day. In addition, this result suggests that sympathetic withdrawal  
19  
20 immediately after maximal test is not affected by time of day.  
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24 The results of the current study may have clinical implications. First, in clinical  
25  
26 research employing T30 and rMSSD30s as markers of parasympathetic reactivation after  
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28 exercise, experiments should be conducted at the same time of day to avoid circadian  
29  
30 influence. Regarding clinical practice, cut-off points of HRR for prognosis have been  
31  
32 established without taking into account the time of day in which the exercise is performed. As  
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34 HRR60s was not influenced by the time of day, it might be a good index for this  
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36 applicability. Thus, depending on the objective, the time of day at which exercise is  
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38 performed might be considered in clinical research and practice.  
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42 Despite its important implications, this study also has some limitations. Pre-  
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44 hypertensives were studied as representative of autonomic dysfunction based on previous  
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46 studies with this population (Pal et al. 2012). However, this dysfunction was not directly  
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48 assessed in the current study since a control group with healthy subjects was not included.  
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50 Nevertheless, mean HRR60s in the present study was similar to values observed by Erdogan  
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52 et al. (Erdogan et al. 2011) in pre-hypertensive subjects who showed lower values than  
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54 normotensives. In addition, as a healthy group was not included, the difference in HRR  
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56 between morning and evening cannot be attributed particularly to pre-hypertensives and no  
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3 comparison with healthy subjects could be done. Future studies should address this  
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5 comparison. Another important aspect is that a maximal test was used in the study to improve  
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7 the clinical applicability of the results because this kind of exercise is usually used for  
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9 measuring HRR as a marker of cardiovascular risk (Cole et al. 2000). This option, however,  
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11 resulted in some limitations especially because peak HR was higher after evening than  
12  
13 morning exercise. To deal with this limitation, future studies should conduct other type of  
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15 exercise, such as morning and evening submaximal exercises at the same HR. The present  
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17 study employed the classical autonomic indices of HRR used in literature. New indices have  
18  
19 been developed and the influence of time of day on them should be tested in the future. The  
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21 indices used in the present study are mainly indices of parasympathetic reactivation which  
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23 was another limitation. Future studies employing other specific tools for evaluating  
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25 sympathetic modulation may add information to literature. In addition, although the HRR  
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27 indices have shown good reproducibility (Buchheit et al. 2008; Al Haddad et al. 2011;  
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29 Boulosa et al. 2014), the reproducibility of the differences in these indices between morning  
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31 and evening still need to be assessed. Finally, it is important to highlight that this study  
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33 involved only subjects who had neither type chronotype, because it is known that HRR is  
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35 differently influenced by time of day in morningness and eveningness healthy chronotype  
36  
37 subjects (Sugawara et al. 2001). By studying neither type chronotype subjects, the results of  
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39 the present study may have a wider applicability since most of the population presents this  
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41 chronotype (Roenneberg et al. 2007).  
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47 In conclusion, in pre-hypertensive men, HRR after maximal test, evaluated by  
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49 HRR60s and HRR $\tau$ , is similar after morning and evening exercise. However,  
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51 parasympathetic reactivation, evaluated by T30 and rMSSD30s, is slower after evening  
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53 exercise.  
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10 Declaration of interest statement

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15 of interest.  
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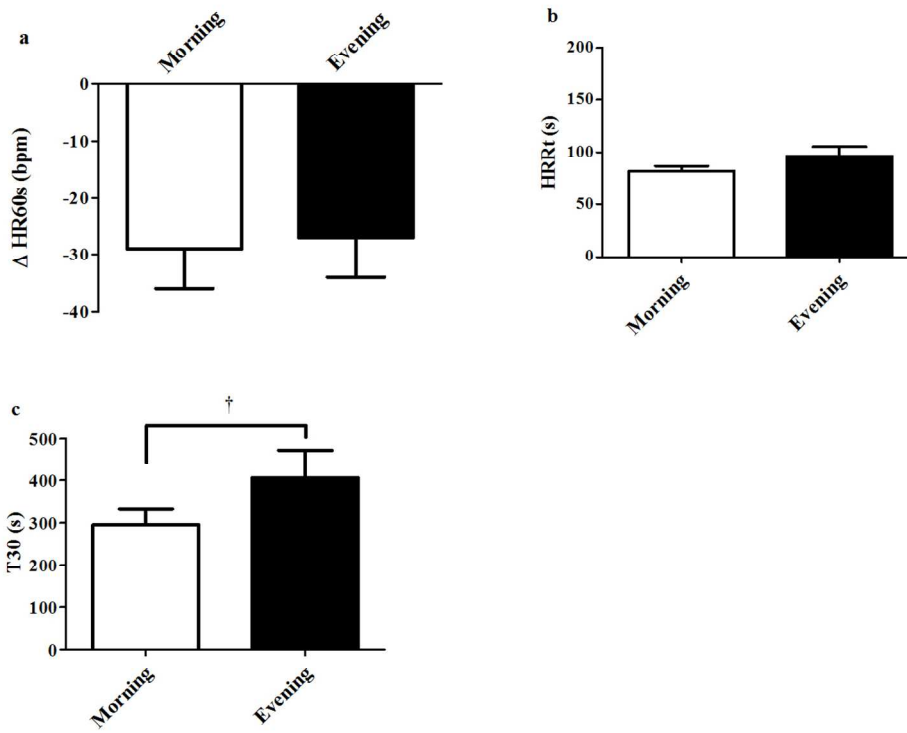
Table 1. Characteristics of the sample.

N	10
Age (years)	33±6
Height (m)	1.74± .06
Weight (kg)	84.5±12.7
Body mass index (kg/m <sup>2</sup> )	27.9±2.6
Resting systolic blood pressure (mmHg)	123±7
Resting diastolic blood pressure (mmHg)	84±5
Chronotype	52.1±5.4
Sleep quality	4.1± .5

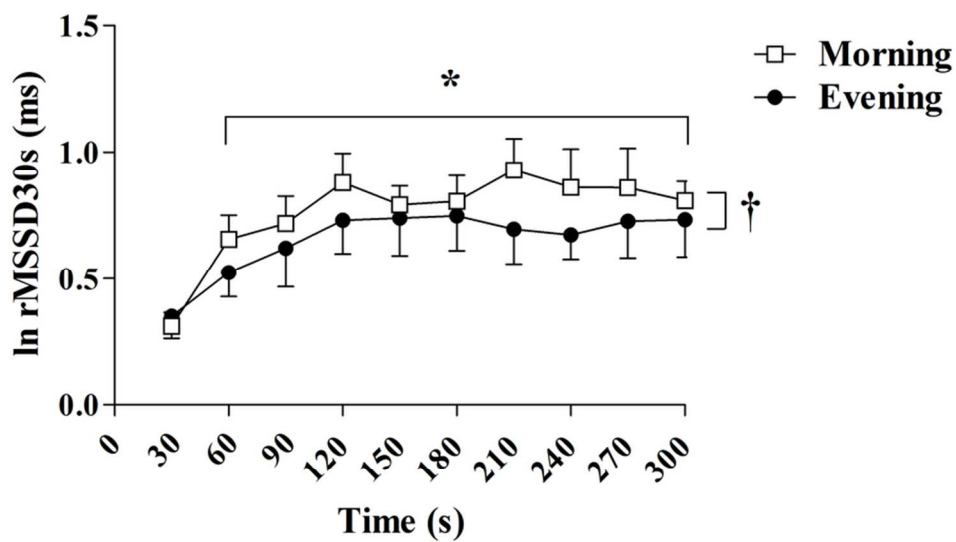
Values in mean±SD.

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3 Figure 1. Heart rate recovery indexes evaluated after maximal tests conducted in the morning  
4 (white bar) and evening (black bar) in pre-hypertensive men. Panel a - heart rate reduction  
5 after 60s of recovery (HRR60s); Panel b - mono-exponential time constant decay of heart rate  
6 in 5-min of recovery (HRR $\tau$ ); Panel c - time-constant of heart rate decay from the 10<sup>th</sup> to 40<sup>th</sup>  
7 s of recovery (T30). † Significant difference between times of day. Data showed as  
8 mean $\pm$ SD.  
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20 Figure 2. Heart rate variability assessed by the square root of the mean of the sum of the  
21 squares of differences between adjacent normal R-R intervals on subsequent 30-s non-  
22 overlapped segments (rMSSD<sub>30s</sub>) after maximal exercise tests performed in the morning  
23 (square) and evening (ball) in pre-hypertensive men. † Significant difference between times  
24 of day. \* Significant different from the first 30-s window,  $p \leq .05$ . Data showed as mean $\pm$ SD.  
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120x98mm (300 x 300 DPI)



80x48mm (300 x 300 DPI)