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| Keywords:         | circadian rhythm, parasympathetic reactivation, diurnal variation, cardiac autonomic modulation, aerobic exercise |
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), rMSSD30s (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 HRRτ (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 HRRτ were similar after exercise in the morning and evening (27±7 vs. 29±7 bpm, P=.111, and 79±14 vs. 96±29 s, P=.119, respectively). T30 was significantly greater after evening exercise (405±215 vs. 295±119 s, P=.002) and rMSSD30s 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 rMSSD30s, 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
indexes. In healthy subjects, HRR is reduced after evening compared with morning exercise (Reilly et al. 1984; Cohen & Muehl 1977). However, in subjects who have cardiovascular autonomic dysfunction, such as pre-hypertensives (Pal et al. 2012), this response might be different. Pre-hypertensives present a greater sympathetic morning surge (Grassi et al. 2008), and it is known that autonomic recovery is slower in the presence of higher sympathetic levels (Ushijima et al. 2009). Thus, in pre-hypertensives, HRR and HRV may be slower after morning than evening exercise, and there are no studies that have been investigated it.

Therefore, the aim of the current study was to investigate the influence of time of day at which exercise is performed on HRR and post-exercise HRV in pre-hypertensive subjects. The hypothesis is that, in pre-hypertensive subjects, HRR and HRV recovery are slower after morning than evening exercise.

Materials and Methods

Subjects

Twenty-two supposed pre-hypertensive men (systolic/diastolic blood pressure levels between 120 and 139/80 and 89 mmHg) (Chobanian et al. 2003) aged 20 to 45 years were invited to participate in the study. Besides pre-hypertension, other study criteria were: I) to have neither type chronotype; and II) good quality of sleep. The exclusion criteria were: I) smoking, II) obesity level equal to or greater than 2, III) practice of regular exercise ≥ 2 times per week, IV) presence of known cardiometabolic diseases, V) use of medications that could affect cardiovascular responses, and VI) presence of electrocardiographic abnormalities at rest or during a maximal exercise test.

The study was approved by the Ethics Committee of the School of Physical Education and Sport under the process (2011/17), University of São Paulo, and it was registered at www.ensaiosclinicos.gov.br (RBR-3HKQG9) and conducted in accordance with the
Declaration of Helsinki and the experimental protocol followed to international ethical standards (Portaluppi et al. 2010). All volunteers signed an informed written consent before study enrollment.

To confirm the aforementioned criteria, all volunteers reported to laboratory for preliminary exams. However, 12 did not fulfil all the criteria (in 7 pre-hypertensive diagnosis was not confirmed, 1 was obese beyond level 2, 1 had other comorbidity besides pre-hypertension, 1 had a morningness chronotype, and 2 practiced regular exercise). Thus, 10 subjects initiated and completed all the study procedures, and their characteristics are described in table 1.

Insert Table 1

Study design

The experimental protocol consisted of four visits in different days. The first two visits were used for the preliminary exams that included: i) health status and absence of regular exercise, assessed by interview; ii) pre-hypertension diagnosis confirmation, assessed by auscultatory measurement of blood pressure on two visits (Chobanian et al. 2003); iii) neither type chronotype, assessed by Horne & Ostberg’s morningness and eveningness questionnaire, and scored between 42 to 58 (Horne & Ostberg 1976); iv) good quality of sleep, assessed by Pittsburgh Sleep Quality Index, and values equal to or below 5 (Buysse et al. 1989); v) obesity level lower than 2, assessed by body mass index lower than 35 kg/m² (National Institutes of Health 2000); and iv) electrocardiographic abnormalities, assessed by electrocardiogram (ECG) (EMG System do Brazil, EMG 030110/00B, São Paulo, Brazil) performed before and during a maximal cardiopulmonary exercise test (Brazilian Society of Cardiology 2010).
Afterwards, the study was performed in a crossover design, which all subjects who fulfilled the study criteria underwent two maximal exercise tests: one in the morning (8-10 a.m.) and the other in the evening (6-8 p.m.). The tests were performed in a randomized order on two different days separated at least for 72 hours. The order of these tests was randomly assigned for each participant. For this, a blind researcher made a simple raffle to indicate the order of the experimental sessions for each subject. All subjects were instructed to have a light meal 2 hours before the test and to avoid stimulant products (such as caffeine) that might affect cardiovascular function, physical efforts and alcoholic drinks for the previous 24 hours. The maximal exercise tests were conducted on a cycle ergometer (Lode Medical Technology, Corival, Groningen, Netherlands) with a graded protocol that initiated with 30W and increased 30W every 3 min until the subjects were unable to continue. In all subjects, immediately after fatigue, workload was decreased to 30W and the subjects remained pedalling for 5 min (active recovery). Respiratory rate was not controlled during the test and recovery. ECG was continuously registered (EMG System do Brazil, EMG 03011000B, São Paulo, Brazil), oxygen uptake (VO$_2$) and respiratory exchanged rate (RER) were continuously measured (CPX Ultima, Med Graphics, Minnesota, USA). VO$_2$peak was determined as the highest value achieved during exercise in averages of 30s. HR was also continuously assessed during exercise and recovery by a HR monitor (POLAR 800cx, Kempele, Finland), and peak HR was considered as the highest value achieved at the end of the exercise.

Data analysis

HR signal was transmitted to the Polar Pro Trainer Software® (v. 5.0, Polar Inc., Kempele, Finland). RR intervals (RRi) were automatically detected and inspected, and they were corrected by a moving average filter, according to the Polar Pro Trainer Software® (v.
5.0, Polar Inc., Kempele, Finland). Then, RRi time series were exported to Matlab® (The Math Works, Massachusetts, USA) for post-exercise HRR and HRV analysis.

Post-exercise HRR

HRR was assessed by calculating the following index: i) HRR60s – absolute heart rate reduction after 60s of recovery in relation to the peak HR (Kannankeril et al. 2004); ii) T30 – the negative reciprocal of the slope of the regression line between the natural logarithm of HR from the 10th to 40th s after exercise (Buchheit et al. 2008); and iii) HRRτ – time constant of the first order exponential fitting of the HRR curve of the five min after exercise (Pierpont et al. 2000). The two first indexes are indicative of parasympathetic reactivation (Kannankeril et al. 2004; Imai et al. 1994), while the third index is indicative of parasympathetic reactivation and sympathetic withdrawal (Pierpont et al. 2000; Imai et al. 1994).

Post-exercise HRV

Post-exercise HRV was calculated by the square root of the mean squared difference of successive RRi on subsequent 30-s non-overlapped segments (rMSSD30s). To smooth out any transient outliers in the rMSSD30s plots, a median filter operation was applied to the entire RRi time series (Goldberger et al. 2006). This index represents parasympathetic reactivation after exercise (Goldberger et al. 2006).

Statistical analysis

A power analysis was performed to determine the required sample size for the study (GPower V.3.1.5, Kiel, Germany). For a power of 80%, and alpha error of 5%, the minimum sample size required to show a difference of 8±5 bpm (Buchheit et al. 2007a) in HRR60s were 7 subject.
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$_{30s}$ 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±SD.

Results

HR and rMSSD$_{30s}$ measured at rest before the exercise were not different in the morning and evening (76±10 vs. 75±8 bpm, $P=.835$ and 23.72±10.12 vs. 21.01±6.60 ms, $P=.413$, respectively).

Maximal workload, VO$_{2peak}$, RER, and exercise time (183±37 vs. 185±38 watts, $P=.341$; 28.9±5.9 vs. 27.9±4.0 ml.kg$^{-1}$.min$^{-1}$, $P=.284$; 1.26±0.07 vs. 1.29±0.09, $P=.285$; and 1047±250 vs. 998±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±13 vs 170±12 bpm, $P=.031$, respectively).

HRR$_{60s}$ and HRR$_{\tau}$ did not differ after exercises conducted in the morning and evening (27±7 vs. 29±7 bpm, $P=.111$, and 79±14 vs. 96±29 s, $P=.119$, respectively) (Figure 1, panels A and B). On the other hand, T30 was significantly greater after exercise conducted in the evening than the morning (405±215 vs. 295±119 s, $P=.002$, respectively) (Figure 1C).

* Figure 1
For HRV analysis, no significant interaction was found for session and time, however significant main factors were detected. Thus, rMSSD<sub>30s</sub> was lower after performing exercise in the evening than the morning (P= .009). In addition, rMSSD<sub>30s</sub> was significantly greater at all time points compared with the first 30s window.

*Figure 2

**Discussion**

The novelty of this study is the assessment of the time of day influence on post-exercise HRR and HRV in a population expected to present autonomic dysfunction (Pal 2012). The main findings were that, in pre-hypertensive men, T30 was higher and rMSSD30s recovery was slower after maximal exercise performed in the evening (6–8p.m.) than in the morning (8–10a.m.). On the other hand, HRR60s and HRR<sub>T</sub> were not influenced by time of day.

Since morning sympathetic activation is greater in individuals with autonomic dysfunction (Grassi et al. 2008), the hypothesis of the study was that parasympathetic reactivation after exercise would be blunted after the morning exercise. However, contrary to the hypothesis, the increased T30 and slower rMSSD30s restoration observed after the evening exercise suggest that parasympathetic reactivation is slower when exercise is performed in the evening than the morning. Previous studies with healthy subjects have also observed a slower HRR after evening exercise in comparison with morning exercise (Cohen &Muehl 1977; Reilly et al. 1984), showing results in the same direction.

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

Regarding this slower vagal reactivation after evening exercise concerns may arise
about a possible influence of the higher peak HR achieved at this time of day on this
response. In fact, higher evening peak HRs were found in 9 out of the 10 subjects, suggesting
this is really an effect of time of day. This higher evening peak HR might reflect a greater
activation of sympathetic activity during evening exercise in comparison with morning
exercise (Maciel et al. 1986). It is known that high sympathetic activity blunts
parasympathetic reactivation (Ushijima et al. 2009). Thus, the higher sympathetic activation

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achieved during evening exercise may be the mechanism responsible for both the higher peak HR and the delay in parasympathetic reactivation after the evening exercise.

Concerning the sympathetic withdrawal after exercise, it occurs mainly during the slow phase of recovery, after two minutes (Imai et al. 1994; Perini et al. 1989; Pecanha et al. 2013). HRR at this phase is mainly influenced by exercise intensity (Imai et al. 1994). In the current study, peak workload did not differ between the maximal tests conducted in the morning and evening, which may explain, at least in part, the absence of difference on HRRτ between both times of day. In addition, this result suggests that sympathetic withdrawal immediately after maximal test is not affected by time of day.

The results of the current study may have clinical implications. First, in clinical research employing T30 and rMSSD30s as markers of parasympathetic reactivation after exercise, experiments should be conducted at the same time of day to avoid circadian influence. Regarding clinical practice, cut-off points of HRR for prognosis have been established without taking into account the time of day in which the exercise is performed. As HRR60s was not influenced by the time of day, it might be a good index for this applicability. Thus, depending on the objective, the time of day at which exercise is performed might be considered in clinical research and practice.

Despite its important implications, this study also has some limitations. Pre-hypertensives were studied as representative of autonomic dysfunction based on previous studies with this population (Pal et al. 2012). However, this dysfunction was not directly assessed in the current study since a control group with healthy subjects was not included. Nevertheless, mean HRR60s in the present study was similar to values observed by Erdogan et al. (Erdogan et al. 2011) in pre-hypertensive subjects who showed lower values than normotensives. In addition, as a healthy group was not included, the difference in HRR between morning and evening cannot be attributed particularly to pre-hypertensives and no
comparison with healthy subjects could be done. Future studies should address this comparison. Another important aspect is that a maximal test was used in the study to improve the clinical applicability of the results because this kind of exercise is usually used for measuring HRR as a marker of cardiovascular risk (Cole et al. 2000). This option, however, resulted in some limitations especially because peak HR was higher after evening than morning exercise. To deal with this limitation, future studies should conduct other type of exercise, such as morning and evening submaximal exercises at the same HR. The present study employed the classical autonomic indices of HRR used in literature. New indices have been developed and the influence of time of day on them should be tested in the future. The indices used in the present study are mainly indices of parasympathetic reactivation which was another limitation. Future studies employing other specific tools for evaluating sympathetic modulation may add information to literature. In addition, although the HRR indices have shown good reproducibility (Buchheit et al. 2008; Al Haddad et al. 2011; Boullosa et al. 2014), the reproducibility of the differences in these indices between morning and evening still need to be assessed. Finally, it is important to highlight that this study involved only subjects who had neither type chronotype, because it is known that HRR is differently influenced by time of day in morningness and eveningness healthy chronotype subjects (Sugawara et al. 2001). By studying neither type chronotype subjects, the results of the present study may have a wider applicability since most of the population presents this chronotype (Roenneberg et al. 2007).

In conclusion, in pre-hypertensive men, HRR after maximal test, evaluated by HRR60s and HRRτ, is similar after morning and evening exercise. However, parasympathetic reactivation, evaluated by T30 and rMSSD30s, is slower after evening exercise.
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Declaration of interest statement

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Table 1. Characteristics of the sample.

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Values in mean±SD.
Figure 1. Heart rate recovery indexes evaluated after maximal tests conducted in the morning (white bar) and evening (black bar) in pre-hypertensive men. Panel a - heart rate reduction after 60s of recovery (HRR60s); Panel b - mono-exponential time constant decay of heart rate in 5-min of recovery (HRRτ); Panel c - time-constant of heart rate decay from the 10th to 40th s of recovery (T30). † Significant difference between times of day. Data showed as mean±SD.

Figure 2. Heart rate variability assessed by the square root of the mean of the sum of the squares of differences between adjacent normal R-R intervals on subsequent 30-s non-overlapped segments (rMSSD$_{30s}$) after maximal exercise tests performed in the morning (square) and evening (ball) in pre-hypertensive men. † Significant difference between times of day. * Significant different from the first 30-s window, p≤ .05. Data showed as mean±SD.