PARASYMPATHETIC WITHDRAWAL DURING 30-15 INTERMITTENT FITNESS TEST CORRELATES WITH ITS’ MAXIMAL RUNNING SPEED IN MALE HANDBALL PLAYERS

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ABSTRACT

Perandini LA, Chimin P, Okuno NM, Lima JRP, Buchheit M, Nakamura FY. Parasympathetic withdrawal during 30-15 Intermittent Fitness Test correlates with its’ maximal running speed. JEPonline 2009;2(1):29-39. Parasympathetic withdrawal parameters estimated during continuous graded exercise have been used as aerobic indices. The purposes of this study were to: 1) examine the pattern of parasympathetic withdrawal during an intermittent fitness test (30-15IFT) and, 2) verify the relationship among parasympathetic withdrawal parameters estimated during 30-15IFT, with its’ maximal running speed (VIFT). Thirteen male handball players performed the 30-15IFT. Heart rate variability (HRV) indices were estimated every 45 s over the 30-15IFT until 85% of maximal heart rate or 15 km.h-1, and plotted against time to estimation of parasympathetic withdrawal parameters (t, amplitude (A) and area under the curve (AUC)). Parasympathetic withdrawal was well adjusted to a mono-exponential decay curve-fitting (R² = 0.96-0.97). The parasympathetic withdrawal parameters t and A were poorly correlated with VIFT (r = 0.29-0.46, P > 0.05), whereas AUC was moderate-to-strongly correlated with VIFT (r = 0.64-0.80, P < 0.05). Therefore, AUC derived from the mono-exponential decay pattern during 30-15IFT can be considered an aerobic index of intermittent exercise.

Key Words: Exercise, Heart Rate Variability, Autonomic Control, Handball.
INTRODUCTION

Several investigations have described as a mono-exponential decay curve the parasympathetic withdrawal during progressive exercise as assessed by heart rate (HR) variability (HRV) indices (1-5). Lewis et al. (6) calculated the rate of parasympathetic withdrawal by estimating the exercise intensity associated with 50% reduction in high frequency (HF) bandwidth power of total power spectral density, adjusted by breathing frequency, during progressive exercise performed up to 85% of maximal HR (HRmax). This intensity was highly correlated with the maximal aerobic power, suggesting that vagal-related parameters could provide an indirect aerobic performance index. It should be noted that after the respiratory compensation point occurrence, HRV can increase as a result of mechanical effects of high ventilatory activity upon sinus node (7-9), irrespective of vagal influence. Therefore, stopping HRV recording at submaximal intensities seems to avoid the inclusion of this phenomenon.

The method proposed by Lewis et al. (6) for healthy young subjects utilizes breathing frequency at each stage to define the upper limit of HF bandwidth. Then, some gas analyzer is necessary, turning it less practical for coaches and practioners. Therefore, simpler HRV calculations that do not require respiratory measures to assess aerobic performance in non-exhaustive tests are warranted. This kind of methods would be of practical interest for athletic evaluation since they avoid marked fatigue, allowing cardiorespiratory fitness assessment without reaching maximal efforts for athletes (especially in prevalence of injury or overreaching) and non-athletes.

We have previously estimated the area under the curve (AUC) between HRV measures (i.e., root-mean-square of differences of successive normal RR intervals – RMSSD; and short-term standard deviation derived from the Poincaré plot – SD1) and time until achievement of 85% of HRmax in the progressive Léger 20-m shuttle run test (10). These parasympathetic withdrawal indices were well correlated with the maximal aerobic speed. Thus, similarly to Lewis et al. (6), we have provided a submaximal method to cardiorespiratory fitness assessment, although using simpler technique that does not require respiratory measures.

To date, no study was conducted to test whether parasympathetic withdrawal can be observed and also if it is related to performance in progressive intermittent protocols. The 30-15 Intermittent Fitness Test (30-15IFT) is a field test applied to team sport athletes which consists of progressive stages of running interspersed with short passive recovery (11). Besides being reliable, the maximal intermittent running velocity attained in 30-15IFT (VIFT) is shown to be related with maximal oxygen uptake (VO2max), as well with jumping and 10-m sprint abilities (11). Thus, VIFT can be considered an integrated measure of various qualities required in team sports (11), with emphasis placed on cardiorespiratory fitness (11,12).

This study sought to: (a) examine the pattern of parasympathetic withdrawal during an intermittent test (i.e., 30-15IFT) and; (b) verify the relationship among the parasympathetic withdrawal parameters, estimated using HRV indices during 30-15IFT, and cardiorespiratory fitness (i.e., VIFT). We hypothesized the occurrence of a progressive reduction in parasympathetic outflow throughout the test, and that specific parasympathetic withdrawal parameters would be significantly correlated with VIFT.
METHODS
Subjects
A group of 13 male handball players of national level (age: 23.8 ± 4.1 yr, height: 184.5 ± 0.1 cm, weight: 86.4 ± 11.3 kg, VO\textsubscript{2}max: 47.2 ± 2.0 ml.kg\textsuperscript{-1}.min\textsuperscript{-1}, seated resting HR: 72.4 ± 10.5 bpm, training experience: 12.2 ± 3.9 yr) took part in this investigation. They were in ‘general preparation’ phase for their principal competition (Brazilian Championship). Subjects provided voluntary written informed consent to participate in this study, which was approved by the University Human Ethics Review Panel. The sample size was calculated assuming a minimum value for strong correlation (r = 0.80) (13) among variables of interest, assuming β of 0.80 and α of 0.05. The estimated sample size required to accomplish this study was 9 athletes (Medcalc® v 9.2.1.0).

Procedures
Experimental protocol
The procedures were carried out in two different days, separated by a minimum of 48 h. On the first day, the first five stages of the 30-15\textsubscript{IFT} were performed in order to acclimate the subjects to the test, while on the second day players were required to perform the test until exhaustion to permit full data collection. Prior to 30-15\textsubscript{IFT}, an electrode transmitter belt was fitted to the chest of each subject, as instructed by the manufacturer, and the athletes remained 10-min in a seated position in a quiet room to evaluate the HRV at rest. Participants were allowed to breathe spontaneously but without talking, since normal respiratory rate does not result in significantly different HR-derived indices compared with controlled breathing (14). After that, they performed a standard warm-up which consisted of 10-min low-intensity running and striding. Subjects then passively rested for 5-min before the start of testing. The subjects were asked to refrain from vigorous physical activities in the 24-h period before testing. They were also instructed to remain in a fasting state in the 3-h period previous to the tests, and to not ingest beverages containing caffeine and alcohol in the previous 24-h.

30-15\textsubscript{IFT}
The 30-15\textsubscript{IFT} was conducted according to the procedures outlined by Buchheit (11). Briefly, athletes performed 30 s shuttle runs interspersed with 15 s of passive recovery, having initial velocity of 10 km.h\textsuperscript{-1}, with increments of 0.5 km.h\textsuperscript{-1} every 45 s. The 30-15\textsubscript{IFT} was performed over a 40 m shuttle distance, within which the subject had to run back and forth at a pace governed by a prerecorded beep, so that at each short beep sound the subjects should be within 3 m zones at each extremity or in the middle of the course. During the 15 s recovery period, athletes walked in the forward direction to join the closest line from where they started the next stage from a standing position. Exhaustion was defined as the inability to match the covered distance with the audio signal on three consecutive occasions. The last completed stage (\textit{V}_{IFT}) was used to predict VO\textsubscript{2}max using the equation below, proposed by Buchheit et al. (15):

\[\text{VO}_2\max (\text{ml.kg}^{-1}\text{.min}^{-1}) = 28.3 \text{ } - \text{ } (2.15 \times G) \text{ } - \text{ } (0.741 \times A) \text{ } - \text{ } (0.0357 \times W) \text{ } + \text{ } (0.0586 \times AG \times V_{IFT}) \text{ } + \text{ } (1.03 \times V_{IFT})\]

Where G stands for gender (female = 2; male = 1), AG for age, and W for weight.

Heart rate variability analysis
RR intervals recorded during 10 min rest and throughout 30-15\textsubscript{IFT} were extracted and manually edited, so that artifacts and non-sinus beats could be replaced by interpolation from adjacent normal RR intervals. The RR recording device (Polar Protrainer 4.0, Polar Electro, Kempele, Finland) is
shown to be valid when compared to standard electrocardiograms (16,17). Time domain and Poincaré plot analysis (HRV Analysis Software v1.1, Biosignal Laboratory, University of Kuopio, Finland) were carried out in the 30-15IFT at each 45 s (i.e., a complete stage duration plus its respective recovery period), since the HRV parameters used in this study account for the non-stationarity of data.

**Time domain analysis:** RR intervals were analyzed through root-mean-square of differences of successive normal RR intervals (RMSSD).

**Poincaré plot analysis:** Each RR interval was plotted as a function of the previous RR interval in a scatter gram, and the standard deviation of instantaneous beat-to-beat variability of the data (SD1) was retained for analysis.

**Parasympathetic withdrawal parameters assessment**

The SD1 or RMSSD calculated in the last 5 min at seated rest and each 45 s stage of the 30-15IFT was fitted to a first-order exponential decay curve:

\[ y = y_0 + Ae^{-x/t} \]

Where, \( y \) = RMSSD or SD1 (ms); \( A \) = amplitude (ms); \( x \) = time (s); \( t \) = time constant (s).

After fitting the SD1 and RMSSD data to a first-order exponential decay curve, the AUC (adapted from Perandini et al. (10)) were estimated through an integrate function (Microcal Origin 6.0 Northampson, USA).

![Figure 1. Scheme of amplitude (A), tau (t) and area under the curve (AUC) estimation during 30-15 intermittent fitness test.](image)

To estimate the parasympathetic withdrawal parameters (A, t and AUC; Figure 1), the SD1 and RMSSD data were calculated until the stage corresponding to 85% of HRmax was achieved (SD1 85% HRmax and RMSSD 85% HRmax), as adapted from procedures outlined by Lewis et al. (6). The parasympathetic withdrawal was evaluated until this relative intensity because after the respiratory compensation threshold (~85% of HRmax), the combination of great venous blood return with high ventilatory activity, induces exacerbated mechanical and stretch stimulus on the heart sinus node. This induced electrical phenomenon causes increased HRV independent of vagal activity (9).
Moreover, we also used the SD1 and RMSSD data until a fixed stage of 15 km.h\(^{-1}\) (SD1 15 km.h\(^{-1}\) and RMSSD 15 km.h\(^{-1}\)) to avoid best performers reaching greater AUC because of their lower HR at a given intensity (5).

### Statistical Analyses

The results were expressed as mean ± standard deviation (SD). The Gaussian distribution of data was attested by the Kolmogorov-Smirnov's test (with Lilliefor's correction). Spearman rank correlation coefficients were used to quantify the relationships between parasympathetic withdrawal parameters and \(V_{IFT}\), whereas Pearson's product moment correlations were used verify the relationships among parasympathetic withdrawal parameters. The same analysis was used to verify the association between HR\(_B\) and resting HRV with parasympathetic withdrawal parameters and performance. The level of statistical significance was set at \(P < 0.05\). All data analysis was performed using the Statistical Package for Social Sciences (SPSS), version 13.0 for Windows.

### RESULTS

The coefficients of determination (\(R^2\)) for the curve-fitting of SD1 and RMSSD until the attainment of 85% HRmax, and for SD1 and RMSSD until the attainment of 15 km.h\(^{-1}\) in the 30-15\(_{IFT}\), were all high (Table 1). The parasympathetic withdrawal parameters are shown in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>(R^2)</th>
<th>(A) (ms)</th>
<th>(\tau) (s)</th>
<th>AUC (ms.s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD1 85% HRmax</td>
<td>0.96 ± 0.07</td>
<td>31.95 ±19.35</td>
<td>37.29 ± 31.28</td>
<td>1975 ± 794</td>
</tr>
<tr>
<td>RMSSD 85% HRmax</td>
<td>0.97 ± 0.04</td>
<td>45.88 + 26.86</td>
<td>36.14 ± 24.56</td>
<td>2483 ± 997</td>
</tr>
<tr>
<td>SD1 15 km.h(^{-1})</td>
<td>0.96 ± 0.05</td>
<td>34.17 ± 18.83</td>
<td>43.18 ± 33.68</td>
<td>2416 ± 697</td>
</tr>
<tr>
<td>RMSSD 15 km.h(^{-1})</td>
<td>0.96 ± 0.04</td>
<td>45.08 ± 26.66</td>
<td>42.56 ± 30.14</td>
<td>2882 ± 879</td>
</tr>
</tbody>
</table>

The relationships between A and \(t\), with \(V_{IFT}\) were all low and non-significant (SD1 85% HRmax: A vs. \(V_{IFT}\), \(r = 0.36\), \(P = 0.22\); \(t\) vs. \(V_{IFT}\), \(r = 0.30\), \(P = 0.32\); RMSSD 85% HRmax: A vs. \(V_{IFT}\), \(r = 0.36\), \(P = 0.22\); \(t\) vs. \(V_{IFT}\), \(r = 0.46\), \(P = 0.11\); SD1 15 km.h\(^{-1}\): A vs. \(V_{IFT}\), \(r = 0.39\), \(P = 0.21\); \(t\) vs. \(V_{IFT}\), \(r = 0.36\), \(P = 0.25\); RMSSD 15 km.h\(^{-1}\): A vs. \(V_{IFT}\), \(r = 0.31\), \(P = 0.30\); \(t\) vs. \(V_{IFT}\), \(r = 0.29\), \(P = 0.34\)). In contrast, AUC estimates derived from different curve-fittings were moderate-to-strongly and significantly correlated with \(V_{IFT}\) (Figure 2). The correlations between A and AUC were also moderate (SD1 85% HRmax: A vs. AUC, \(r = 0.69\), \(P < 0.01\); RMSSD 85% HRmax: A vs. AUC, \(r = 0.70\), \(P < 0.01\); SD1 15 km.h\(^{-1}\): A vs. AUC, \(r = 0.79\), \(P < 0.01\); RMSSD 15 km.h\(^{-1}\): A vs. AUC, \(r = 0.77\), \(P < 0.01\)), suggesting that high initial HRV values imply in greater AUC linked to parasympathetic withdrawal.

The parasympathetic withdrawal parameter \(t\) was not significantly correlated with HR\(_B\) (SD1 85% HRmax: \(t\) vs. HR\(_B\), \(r = 0.04\), \(P = 0.89\); RMSSD 85% HRmax: \(t\) vs. HR\(_B\), \(r = 0.01\), \(P = 0.96\); SD1 15 km.h\(^{-1}\): \(t\) vs. HR\(_B\), \(r = 0.05\), \(P = 0.87\); RMSSD 15 km.h\(^{-1}\): \(t\) vs. HR\(_B\), \(r = 0.03\), \(P = 0.92\)) and resting HRV (SD1 85% HRmax: \(t\) vs. SD1\(_R\), \(r = -0.29\), \(P = 0.33\); RMSSD 85% HRmax: \(t\) vs. RMSSD\(_R\), \(r = -0.36\), \(P = 0.23\); SD1 15 km.h\(^{-1}\): \(t\) vs. SD1\(_R\), \(r = -0.47\), \(P = 0.12\); RMSSD 15 km.h\(^{-1}\): \(t\) vs. RMSSD\(_R\), \(r = -0.45\), \(P = 0.12\)). In contrast, A and AUC were moderate-to-strongly correlated with HR\(_B\) (SD1 85% HRmax: A vs. HR\(_B\), \(r = -0.60\), \(P = 0.03\); AUC vs. HR\(_B\), \(r = -0.67\), \(P = 0.01\); RMSSD 85% HRmax: A vs. HR\(_B\), \(r = -0.62\), \(P = 0.02\); AUC vs. HR\(_B\), \(r = -0.70\), \(P = 0.001\); SD1 15 km.h\(^{-1}\): A vs. HR\(_B\), \(r = -0.62\), \(P = 0.03\); AUC vs. HR\(_B\), \(r = -0.67\), \(P = 0.01\); RMSSD 15 km.h\(^{-1}\): A vs. HR\(_B\), \(r = -0.63\), \(P = 0.02\); AUC vs. HR\(_B\), \(r = -0.70\), \(P = 0.001\)).
**DISCUSSION**

The main findings of this study were that the parasympathetic withdrawal, as estimated by HRV during a progressive intermittent test, showed a mono-exponential decay pattern, similar to that observed during continuous protocols by Lewis et al. (6), and that the AUC presented significant correlation with $V_{\text{IFT}}$.

![Graphs showing correlation between $V_{\text{IFT}}$ and parasympathetic withdrawal parameters.](image)

Figure 2. Coefficients of correlation among $V_{\text{IFT}}$ and parasympathetic withdrawal parameters (area under the curve - AUC) estimated until 85% HRmax and 15 km.h$^{-1}$, using SD1 and RMSSD. SD1 and RMSSD were estimated every 45-s of the 30-15 Intermittent Fitness Test, plotted against time, and fitted to a mono-exponential decay equation to derive AUC.
This suggests that the parasympathetic withdrawal parameters estimated from HRV indices (SD1 and RMSSD) curve-fitting to the mono-exponential decay equation is related to cardiorespiratory fitness obtained in intermittent test. The shared variance was 41-64%. Parasympathetic withdrawal is mainly determined by the increase in breathing frequency during progressive exercise, which reduces respiratory sinus arrhythmia and consequently HRV (18). Due to non-stationarity of the RR intervals throughout the test, classical spectral analysis can not be applied in these data (19). Therefore, in the current study, the parasympathetic withdrawal during 30-15\textsubscript{IFT} was evaluated by SD1 and RMSSD indices. These two parameters were used due to not being influenced by breathing frequency (20), and because they account for the non-stationarity of RR interval data (21), typical of progressive exercise (2). The coefficients of determination obtained from SD1 and RMSSD curve-fitting into mono-exponential decay equations and the correlations of parasympathetic withdrawal parameters with \(V_{\text{IFT}}\) were all similar. It confirms the findings of Brennan et al. (22), who concluded that SD1 and RMSSD indeed represent mathematically equivalent indices. Thus, they can be used interchangeably.

The present study was the first to evaluate the parasympathetic withdrawal parameters in a field progressive intermittent protocol. The coefficients of determination of the mono-exponential decay curve-fitting of parasympathetic withdrawal (\(R^2 = 0.96 - 0.97\)) were as high as those presented by Lewis et al. (6) and Perandini et al. (10) in continuous graded exercise (\(R^2 = 0.85 - 0.90\) and 0.99, respectively). It seems that parasympathetic withdrawal shares the same pattern in continuous and intermittent progressive protocols. However, despite they have presented similar pattern, the comparison of the parameters obtained from parasympathetic withdrawal modeling is not simple. Over the 30-15\textsubscript{IFT} there might be some parasympathetic reactivation during the recovery periods (23,24) leading to a reduction of parasympathetic withdrawal rate and increased AUC, in comparison to continuous protocols. However, the degree of this influence could not be determined in this study. Hence, the relationship between autonomic indices derived from these protocols remains to be established.

During recovery periods of 30-15\textsubscript{IFT}, noises caused by transient parasympathetic reactivation occurrence could have impaired the adjustment of parasympathetic withdrawal to the mono-exponential decay equation. Though it was not clearly observable in our study, since high \(R^2\) values associated with curve fitting were found. The light forward walking during the short 15 s recovery periods could have maintained a light sympathetic activity, which could have, in turn, attenuated the eventual parasympathetic reactivation. Besides this, the time-domain and geometrical HRV indices adopted in the present study are less susceptible to fluctuations caused by parasympathetic reactivation than frequency domain derived indices, as in the latter the assumption of RR stationarity is essential to run the analysis.

The parasympathetic withdrawal parameters estimated in the current study were \(t\), \(A\) and AUC, whereas Lewis et al. (6) have estimated the rate of reduction by means of the work rate associated with a 50% reduction in vagal outflow during progressive exercise. The latter has presented a strong correlation with maximal aerobic power estimated in the progressive test (Lewis et al. (6)). However, it should be noted that the method proposed by Lewis et al. (6) utilizes respiratory rate on the calculations. Thus, it might not be of practical value for coaches and practitioners in the field (i.e., with no gas analyzer). In our study, AUC estimated solely from HRV parameters presented moderate-to-strong correlations with \(V_{\text{IFT}}\) (\(r = 0.64 - 0.80, P < 0.01\)). The correlations were found in either estimates made until 85% HRmax or 15 km.h\(^{-1}\), showing that they were not influenced by the choice of a fixed HR value (85% of HRmax). In our previous study performed in continuous graded exercise
(10), AUC estimated until 85% of HRmax have also presented a moderate correlation with maximal running speed ($r = 0.70 - 0.71, P < 0.05$).

Similarly to our previous study in continuous graded exercise, the $t$ estimated in the curve-fitting until 85% HRmax and 15 km.h$^{-1}$ presented low correlation with $V_{IFT}$ (10). It means that the time constant of the parasympathetic withdrawal decay is not related to the final velocity of the test, which is considered an integrated fitness index specific to intermittent exercise (11,25,26). Although the A presented no significant correlation with $V_{IFT}$, it was moderately correlated with AUC ($r = 0.69 - 0.79, P < 0.01$). It suggests that AUC estimation was influenced by the A of parasympathetic withdrawal decay. In addition, resting HR and HRV might also indirectly determine intermittent testing performance.

The AUC estimation in our study was the only parasympathetic withdrawal parameter that presented good correlation coefficients with $V_{IFT}$, showing that AUC can be an alternative to fitness assessment for intermittent exercise. In addition, an increase in parasympathetic outflow in absolute submaximal loads during a progressive test was observed by Leicht et al. (27) and Martinmäki et al. (28) after an intense and low-dose training, respectively. It suggests that training can increase the AUC. Indeed, we also have described the SD1 increase after short-term high-intensity training (5), suggesting that training can increase both AUC and $V_{IFT}$. This must be tested in future longitudinal investigations to establish more evidences of their possible relationship. Nevertheless, our results give support for using only submaximal stages of 30-15$V_{IFT}$ to obtain a ‘fitness index’ without the need to perform the test until exhaustion.

Although the present study has evaluated only parasympathetic withdrawal, it is reciprocally associated with sympathetic activity increases aiming to accelerate the cardiac rate and increase ejection force, and consequently the cardiac output, in order to provide blood flow to the active muscles sufficient to match with its metabolic requirement. During progressive exercise, the sympathetic predominance coincides with parasympathetic withdrawal (2,29). The sympathetic activation of muscle glycogenolysis is strongly associated with lactate and ventilatory threshold occurrences (29). Therefore, the sympa-tho-vagal balance seems to determine metabolic and physiological changes that are associated with exercise tolerance. Prolonged prevalence of parasympathetic modulation during progressive exercise can thus prevent the sympathetic-induced shift in metabolic activity to occur in the early stages, having impact on its performance.

The main limitation of the present study was the absence of reliability tests for the parasympathetic withdrawal parameters. In addition, it could not be determined in the present study the suitability of the procedures among other samples of athletes training for other disciplines and non-athletes.

CONCLUSIONS
Parasympathetic withdrawal during intermittent progressive protocols presents a mono-exponential decay pattern similar to that previously shown in a continuous one. The parasympathetic withdrawal parameters regarding rate of parasympathetic withdrawal and the amplitude of this decay were not correlated with the maximal velocity attained in the intermittent fitness test. On the other hand, the area under the heart rate variability parameters (i.e., SD1 and RMSSD) curve was moderate-to-strongly correlated with maximal velocity attained, showing that it can be used as a predictor of maximal velocity attained in progressive intermittent protocol.
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