Heart Rate Variability After Sprint Interval Training in Cyclists and Implications for Assessing Physical Fatigue

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Abstract
Hebisz, RG, Hebisz, P, and Zaton, MW. Heart rate variability after sprint interval training in cyclists and implications for assessing physical fatigue. J Strength Cond Res 36(2): 558–564, 2022—This study evaluated the time- and frequency-domain indexes of heart rate variability (HRV) during sprint interval exercise test (SIXT) and identify the onset of fatigue by HRV concurrent with changes in average (Pavg) and peak (Ppeak) power output, total oxygen uptake (Vo2tou), and blood hydrogen (H+) and lactate (La-) concentrations. Twenty-seven cyclists performed 4 sets of SIXT in which each set consisted of four 30-second maximal sprints interspersed with 90 seconds of low-intensity cycling. Each set was separated by 25–40 minutes of recovery. Before beginning each set, HRV was analyzed by time (mean normal-to-normal RR intervals [RRNN], SD of normal-to-normal RR intervals [SDNN], and square root of the mean squared differences between successive normal-to-normal RR intervals [RMSSD]) and frequency (total spectral power [T] and very low- [VLF], low- [LF], and high-frequency [HF] spectral power) domain methods. Pavg, Ppeak, and Vo2tou were recorded in each set, and H+ and La- were measured after each set. RRNN, SDNN, and VLF decreased in the second set, whereas all time and frequency indexes of HRV decreased in the third and fourth set. Pavg and H+ decreased, while Vo2tou increased in the fourth set. Ppeak decreased in the second, third, and fourth set. Correlations were found between the changes in the time and frequency indexes of HRV with H+, La-, and Vo2tou. The results indicate that HRV does not reflect the onset of physical fatigue in SIXT as observed in Pavg and no correlation was found between the changes in HRV with Pavg and Ppeak.

Key Words: fatigue, maximal sprints, the time and frequency domain indexes of HRV, power output

Introduction
Heart rate (HR) is modulated by the balance of the parasympathetic and sympathetic divisions of the autonomic nervous system (ANS). The increase in HR that occurs from rest to lower-intensity exercise is first attributed to a decrease in parasympathetic activity known as vagal withdrawal. As exercise intensity increases, various neural mechanisms increase sympathetic tone to induce a further rise in HR (40). Other stimuli such as emotional arousal can modulate HR, in which anger or mental stress can increase sympathetic activity and therefore HR (30). A popular and noninvasive measure of ANS function and its response to various stressors is the variation in the time interval between heartbeats (RR) otherwise known as HR variability (HRV). This measure and several related indexes are considered to reflect changes in autonomic regulation (primarily parasympathetic nervous system activity) and can be analyzed by time- and frequency-domain methods (3).

Although HRV is typically evaluated during rest, recent research suggests that measuring HRV kinetics in response to exercise stressors may have considerable potential as a predictor of aerobic fitness and exercise performance and in monitoring training-induced fatigue of elite athletes (41,42). Heart rate variability assessed after the cessation of exercise has also recently been used as a marker of parasympathetic reactivation to indicate postexercise recovery status (33) and as an indicator of training load (exercise intensity and time) (23,24). After acute exercise, time-domain measures such as mean normal-to-normal RR intervals (RRNN) and the SD of normal-to-normal RR intervals (SDNN) are lower than pre-exercise values. These changes are accompanied with significantly modulated HRV frequency-domain measures, from very low-frequency spectral power (VLF) to high-frequency spectral power (HF), and have been observed in both untrained (37) and trained (7) individuals. Michael et al. (33) indicated that low-frequency spectral power (LF) is dependent on both sympathetic and parasympathetic ANS activity and that the ratio of low-frequency to high-frequency spectral power (LF/HF) is indicative of sympathovagal balance where one system dominates over the other. Changes in the above HRV frequency-domain parameters are greater after longer and more intense efforts and indicate a reduction in parasympathetic nervous system activity (25,43). The recovery of parasympathetic system activity is believed to be dependent primarily on an exercise intensity-dependent combination of metabolite accumulation in blood and skeletal muscle as well as changes in blood plasma and resultant arterial-baroreflex stimulation (43). The postexercise reactivation of parasympathetic system activity relative to exercise intensity has been observed in studies involving both athletes (9) and inactive cohorts (44). In the former, Buchheit et al. (9) observed an increased delay in parasympathetic recovery after a series of maximal all-out sprints.
compared with moderate-intensity continuous running. In the latter, Stuckey et al. (44) reported reduced parasympathetic reactivation after 4 repeated sprint intervals when compared with a single sprint interval, although total recovery time in both protocols was above 60 minutes. Several authors have accredited the prolonged recovery of parasympathetic activity (primarily vagal reactivation) after higher-intensity exercise because of the greater anaerobic energy contribution in these types of efforts (9,29).

Research on parasympathetic nervous system reactivation after exercise has focused primarily on the postexercise period after a single dose of high-intensity interval training exercise (9,24,44). Less is known about ANS modulation when assessed by the time- and frequency-domain indexes of HRV particularly after sprint interval training, in which maximal sprints are repeated in sets separated by low- or moderate-intensity exercise of an extended duration. Investigating the effects of such maximal efforts can enhance knowledge on the time course of parasympathetic nervous system reactivation and correlate such data with training duration (such as the number of completed sprint repetitions). In addition to using HRV as an indicator of training load (23,24), several authors have suggested that HRV indexes can be used to assess fatigue status (14,28,38). However, these studies treated fatigue as accumulated physical fatigue induced by overreaching or overtraining during periods of several months, and in 1 case, the occurrence of a fatigue state was not accompanied by a decrease in total power output in a 5-minute maximal cycling test (38). Furthermore, the remaining studies either did not analyze pre-training and post-training differences in power and work output or quantified fatigue only by subjective ratings of perceived exertion which have certain limitations (17,28,41). For these reasons, research on the effects of repeated maximal sprints (4 sets of the sprint interval exercise test [SIXT]) on HRV recovery dynamics and correlations with various physiological measures of fatigue, including changes in power output, can provide a springboard of important data. It is possible that HRV can be used to predict power output before the execution maximal sprint and therefore serve as an important indicator of future exercise performance (12). More recent studies have suggested that baseline cardiac autonomic function, determined on the basis of LF and HF spectral analysis and its acute response to all-out interval exercise, can elucidate the individual physiological responses (workload and peak oxygen uptake) to high-intensity interval training (26).

The aim of this study was to therefore evaluate the time- and frequency-domain indexes of HRV during a SIXT and identify the onset of fatigue by HRV concurrent with changes in average and peak power output, total oxygen uptake, and blood hydrogen and lactate concentrations. Research involving repeated maximal sprints is particularly warranted as it is a common training protocol in sports that involve recurring bouts of high-intensity and low-intensity exercise such as in cross-country mountain biking (MTB). Depending on the competitive MTB subdiscipline, uphill sections can last several dozen seconds or even 20–30 minutes and the actual duration of the race can be several minutes (cross-country eliminator races—XCE), approximately 1 hour 30 minutes (cross-country Olympic races—XCO), or even 4 hours 30 minutes (cross-country marathon races—XCM) at the World Cup level. A SIXT that could emulate the structure of real-world MTB competition could provide practical findings on repeated sprint performance and postexercise recovery dynamics in this sporting domain.

It was hypothesized that HRV would decrease across when performing repeated sets of maximal sprints and that HRV-associated indexes would strongly correlate with the onset of physical fatigue.

Methods

Experimental Approach to the Problem

The study would examine HRV indexes that correlate with the onset of physical fatigue during a set of repeated maximal sprints in a SIXT. The use of HRV and HR data to predict fatigue could allow the coach or conditioning specialist to prescribe an optimal training stimulus by structuring training load to an individual’s HRV changes compared with determining fatigue only after a decrease in power output, which induces the risk of overtraining.

Subjects performed 4 sets of SIXT in which each set consisted of four 30-second maximal sprints interspersed with 90 seconds of low-intensity cycling. Each set was separated by 25–40 minutes of recovery. A SIXT protocol was adopted to emulate cross-country MTB competition where multiple maximal efforts (uphill sections) are interspersed with low- and moderate-intensity cycling (downhill sections) over a period of several hours. For the purposes of this study, HRV indexes by time- and frequency-domain methods were measured before beginning each set, average power output, peak power output and total oxygen uptake during the test, and blood hydrogen and lactate concentrations after completing each set.

Subjects

The study involved 27 well-trained MTB cyclists (21 males and 6 females). All had at least 3 years of competitive MTB experience with yearly training time between 420 and 630 hours. Group anthropometric characteristics and fitness level are presented in Table 1.

The study design was approved by the University School of Physical Education in Wroclaw, Poland (chaired by Prof. Marek Mędr; on July 11, 2013) and performed in accordance with the Declaration of Helsinki. All the cyclists were over 18 years of age (age range: 18–34 years) and provided written informed consent to participate in the study after being informed about the study’s methods, procedures, benefits, and risks.

Procedures

The tests described below were performed in controlled laboratory conditions at the Exercise Laboratory at the University School of Physical Education (PN-EN ISO 9001:2001 certified). Subjects refrained from any training and strenuous physical exercise 48 hours before study outset. Subjects first completed an incremental exercise test (IXT) to determine fitness level and after 48 hours of rest performed a SIXT for the purposes of the study.

Table 1

| Group | Age (y) | Height (cm) | Mass (kg) | V̇O₂max (ml kg⁻¹ min⁻¹) | Pmax (W) |
|-------|---------|-------------|-----------|-------------------------|----------|
|       | 22.3 ± 6.1 | 178 ± 7.4 | 69.7 ± 9.9 | 56.4 ± 8.2 | 344.6 ± 66.5 |

*V̇O₂max — maximal oxygen uptake in the incremental exercise testing; Pmax — maximal aerobic power in the incremental exercise testing.
†Data are presented as mean ± SD.
Testing was performed at the end of the preparatory season approximately 7–21 days before the beginning of the racing season.

**Incremental Exercise Test.** Testing was performed on a Cyclus 2 cycle ergometer (RBM elektronik-automation GmbH, Leipzig, Germany) that was adjusted and calibrated before each trial. Starting workload was set at 50 W and increased by another 50 W every 3 minutes until volitional exhaustion was reached. If a subject was unable to complete an entire 3-minute stage, 0.28 W was subtracted for each missing second to determine individual maximal aerobic power (Pmax). Respiratory gas exchange was measured on a breath-by-breath basis with a Quark gas analyzer (Cosmed, Milan, Italy). The device was calibrated before each test with a reference gas mixture of carbon dioxide (5%), oxygen (16%), and nitrogen (79%). Tidal air was collected by wearing a face mask and analyzed to determine oxygen uptake (V̇O₂), carbon dioxide excretion (V̇CO₂), and minute pulmonary ventilation (VE). Absolute and relative (per kg of body mass) V̇O₂max was calculated based on the composition of expired air and minute ventilation. All measures were averaged over 30-second intervals.

**Sprint Interval Exercise Test.** This test was performed on the same Cyclus 2 cycle ergometer. Braking resistance was set to an individual fixed torque of 0.8 Nm kg⁻¹. The test was preceded by a 20-minute warm-up during which the subject cycled for 5 minutes at an intensity corresponding to 40% Pmax (as determined in the IXT) and then for 15 minutes at 50% Pmax. An active 10-minute cool-down was then performed by cycling at 10% Pmax followed by 5 minutes of passive recovery spent sitting on the ergometer with arms resting on the handlebars. The test proper involved 4 sets, with each set consisting of four 30-second maximal sprints interspersed with 90 seconds of low-intensity cycling. The each set was separated by 25–40 minutes of recovery in which (a) the first 2 minutes involved low-intensity cycling; (b) then, moderate-intensity cycling at 50% Pmax was continued until blood pH returned to at least 7.35, resulting in individual recovery times from 18 to 33 minutes (blood pH was continually measured with a RAPIDLab 348 blood gas system—Siemens Healthcare, Germany); and (c) at the end of the recovery, the subject sat passively on the ergometer with arms resting on the handlebars for 5 minutes before commencing another SIXT set. Heart rate was continually monitored, and a visual representation of the SIXT protocol across the maximal sprints and recovery is presented in Figure 1.

RR intervals were recorded in the last 2 minutes of the 5-minute passive recovery when the subject was sitting on the ergometer just before beginning another set of sprints. A V800 cardiofrequencimeter (Polar, Oy, Finland) integrated with Kubios HRV Standard software (KubiosOy, Kuopio, Finland) was used to analyze HRV by time and frequency domains. Time-domain measures included the mean normal-to-normal RR intervals (RRNN), SD of normal-to-normal RR intervals (SDNN), and the square root of the mean squared difference between successive normal-to-normal RR intervals (RMSSD). For the frequency domain, spectral analysis was performed using Fast Fourier Transformation to obtain very low-frequency spectral power (VLF; 0.003–0.05 Hz), low-frequency spectral power (LF; 0.05–0.15 Hz), high-frequency spectral power (HF; 0.15–0.4 Hz), total spectral power (T), and the LF/HF ratio as a measure of sympathovagal balance.

Power output (relative to body mass through individualized braking resistance) was continually recorded by the ergometer and used to calculate average power output (Pavg) and peak power output (Ppeak) for each set. Respiratory function was evaluated using the same procedures and equipment as in the IXT. Gas exchange was measured from the beginning of each set until 2 minutes after set termination. Total oxygen uptake for each set (VO₂tou) was determined as the amount of oxygen uptake across the four 30-second sprints and 90-second recovery components. Arterialized capillary blood was collected by squeezing the finger to draw blood into a capillary tube 3 minutes after the fourth sprint in each set to determine hydrogen (H⁺) and lactate (La⁻) concentrations with a RAPIDLab 348 blood gas analyzer (Siemens Healthcare, Erlangen, Germany) and LP400 photometer (Dr. Lange, Burladingen, Germany), respectively.

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**Figure 1.** Illustration of the sprint interval exercise test protocol concomitant with subject heart rate (HR), heart rate, VO₂tou = total oxygen uptake, Pavg = average power output, Ppeak = peak power output, H⁺ = blood hydrogen concentrations, La⁻ = blood lactate concentrations, HRV = heart rate variability.
Statistical Analyses

The data set was analyzed with the Statistica 13.1 software package (Statsoft). Arithmetic mean values and SDs were calculated for all measures. Data were screened for outliers with the three-sigma method, and all measures (3 subjects) outside of 3 SDs of the mean were removed. The normality of the data distribution was then examined with the Shapiro-Wilk test. One-way repeated-measures analysis of variance (ANOVA) with Scheffe’s test post hoc was used to compare the means across each of the 4 sets. To estimate the magnitude of effect sizes, the eta-square ($\eta^2$) was calculated for 1-way ANOVA with repeated measurements.

Correlation analysis was performed by first calculating the absolute difference for each variable between the SIXT sets for each subject. The differences between the first and second set, first and third set, first and fourth set were determined to obtain 72 values in total (3 values for each variable of the 4 sets. To estimate the magnitude of effect sizes, the eta-square ($\eta^2$) was calculated for 1-way ANOVA with repeated measurements.

Results

The 1-way repeated-measures ANOVA indicated significant differences for RRNN ($F = 37.57; p = 0.000$), SDNN ($F = 20.95; p = 0.000$), RMSSD ($F = 7.36; p = 0.000$), VLF ($F = 8.56; p = 0.000$), LF ($F = 9.86; p = 0.000$), HF ($F = 5.61; p = 0.002$), and $T$ ($F = 10.37; p = 0.000$) across the sets. Post hoc analysis showed significant differences for RRNN ($F = 1.70; p = 0.0176$) (Table 3).

Comparative analysis showed that $d_{V_O_{2tou}}$ weakly correlated with $d_{SDNN}$, $d_{VLF}$, and $d_{HF}$ with moderately with $d_{RRNN}$. In turn, $d_{HF}$ was moderately correlated with $d_{RRNN}$, $d_{SDNN}$, and $d_{RMSSD}$, whereas $d_{La^+}$ weakly correlated with $d_{SDNN}$ and moderately with $d_{RMSSD}$. No significant correlations were observed for $d_{Ppeak}$ or $d_{Pavg}$ with any of the between-set differences in HRV or between $d_{HF}$, $d_{La^+}$, and any of the between-set differences for any of the frequency-domain indexes of HRV (Table 4).

Discussion

The present results showed a positive correlation between the changes in the time-domain indexes of HRV with $H^+$ and $La^+$ concentrations yet a negative correlation between the changes in the time-domain (RRNN and SDNN) and frequency-domain (VLF and $T$) indexes of HRV with $V_{O_{2tou}}$. Between-set comparisons showed increasingly greater reductions in SDNN, RMSSD, and HF with repeated maximal sprints (quantified as the number of completed SIXT

Table 2

| Heart rate variability indexes during the last 2 minutes of passive recovery component before each sprint interval exercise test set.*† |
|---|
| **Pre-1st set** | **Pre-2nd set** | **Pre-3rd set** | **Pre-4th set** | **ES** |
| 
| Time domain (ms) |
|---|
| RRNN | 544.7 ± 67.7 | 492.9 ± 49.6§ | 473.4 ± 36.6§ | 471.2 ± 37.4§ | 0.62 |
| 95% CI: U | 573.3 | 516.1 | 488.8 | 457.9 | 486.9 |
| SDNN | 20.6 ± 11.0 | 12.6 ± 8.3§ | 8.9 ± 5.5§ | 8.2 ± 4.8§ | 0.48 |
| 95% CI: U | 25.2 | 15.9 | 11.3 | 6.6 | 10.2 |
| RMSSD | 13.9 ± 14.0 | 7.6 ± 6.1 | 5.2 ± 4.1§ | 4.6 ± 2.6§ | 0.24 |
| 95% CI: U | 19.9 | 8.0 | 9.2 | 5.0 | 5.7 |
| Frequency domain (ms²) |
|---|
| VLF | 39.5 ± 43.7 | 18.6 ± 20.6§ | 11.5 ± 12.7§ | 10.7 ± 15.0§ | 0.27 |
| 95% CI: U | 57.9 | 21.0 | 16.9 | 6.1 | 17.0 |
| LF | 266.6 ± 289.7 | 187.0 ± 301.6 | 65.0 ± 92.0§ | 55.9 ± 64.2§ | 0.30 |
| 95% CI: U | 388.9 | 144.3 | 103.8 | 26.2 | 83.0 |
| HF | 53.5 ± 68.8 | 34.0 ± 91.6 | 12.8 ± 23.5§ | 7.6 ± 10.1§ | 0.20 |
| 95% CI: U | 82.6 | 24.5 | 22.7 | 2.9 | 11.9 |
| T | 154.6 ± 204.9 | 411.7 ± 67.8 | 141.3 ± 37.1 | 106.5 ± 41.9 | 0.31 |
| 95% CI: U | 359.8 | 366.7 | 239.7 | 407.1 | 74.2 |
| LFHF | 6.3 ± 3.4 | 9.4 ± 7.1 | 8.4 ± 6.7 | 8.8 ± 6.7 | 0.05 |
| 95% CI: U | 8.0 | 4.7 | 12.4 | 6.3 | 11.2 |
| *RRNN = mean normal-to-normal RR intervals; SDNN = SD of normal-to-normal RR intervals; RMSSD = square root of the mean squared difference between successive normal-to-normal RR intervals; VLF = very low-frequency spectral power; LF = low-frequency spectral power; HF = high-frequency spectral power; T = total spectral power; LFHF = ratio of low-frequency to high-frequency spectral power.†Data are presented as mean ± SD with effect sizes (ES) and 95% confidence intervals: upper | lower (95% CI: U).‡p < 0.05. §Significantly different from the first set. ||Significantly different from the second set.
sets), suggesting reduced parasympathetic nervous system activity, primarily in vagal tone, and/or prolonged parasympathetic reactivation in trained MTB cyclists. These findings support the notion that total exercise time is a determinant of postexercise parasympathetic recovery and HRV-related measures (5,21,23,24,44). Interestingly, the changes in the time- and frequency-domain indexes of HRV registered in the second, third, and fourth sets were significant only in relation to the first set. No significant changes in the indexes of HRV were observed when comparing the second set with the third and fourth sets (excluding LF) or when comparing the third set with the fourth set, suggesting a finite limit of HRV kinetics after repeated maximal sprints and high-intensity exercise (23,24).

Previous studies have posited that the changes observed in the time- and frequency-domain indexes of HRV can be used to assess fatigue status in trained subjects (41). Although early research attributed fatigue exclusively to the accumulation of H⁺ (19), later findings have suggested several cause-and-effect models (1,4,35). These include the inability of the heart to deliver sufficient oxygenated blood to working muscle including cardiomyocytes resulting in a depletion of phosphocreatine (10), the onset of metabolic acidosis (36), and the excess accumulation of ADP and phosphate (2,8). However, Fitts (16) and Westerblad et al. (45) suggest that low pH resulting from high concentrations of H⁺ is the principal factor responsible for muscle fatigue. We observed H⁺ concentrations exceeding 80 nmol·L⁻¹ in all 4 sets, far above normal resting values (38–44 nmol·L⁻¹) and indicative of metabolic acidosis (15,22,39). As had been indicated by other researchers, the accumulation of H⁺ can reduce parasympathetic nervous system activity (13,43) and that parasympathetic reactivation can exceed 1 hour after sprint interval training protocol similar to one used in this study (44). In our study, H⁺ decreased only after the fourth set with no significant changes in La⁻ across successive sets in the SIXT, suggesting that the changes in the time-domain indexes of HRV may depend on several factors independent of exercise intensity and the post-exercise accumulation of metabolites in blood and skeletal muscle. One possible explanation is the increase in blood plasma volume with additional sets of maximal sprints (43), which was observed in a previous study that applied an identical SIXT protocol (20).

The literature defines fatigue as the inability to maintain a prescribed exercise intensity or a reduction in power output (6,34), with fatigue in short-duration sprints (<10 seconds) quantified as a decrease in both peak power and mean power (18,32). We observed a reduction in Ppeak in the second, third, and fourth sets, whereas Pavg decreased only in the fourth set. Although this study involved 30-second and not 10-second sprints, the applied exercise protocol appears to be insufficient in significantly decreasing average power output. This finding challenges the prognostic significance of the time-domain indexes of HRV in the assessment of fatigue during repeated maximal sprints particularly in well-trained athletes. It is possible that the active recovery component between each set was sufficient in duration to eliminate the onset of peripheral fatigue (8,11). Another reason may lie with the high aerobic fitness level of the recruited subjects. Girard et al. (18) reported that trained athletes do not show large decreases in power output during repeated sprint (<10 seconds) exercise, whereas Menaspà et al. (31) showed that elite cyclists generate similar levels of power output during repeated sprint exercise regardless of the preceding mode of exercise (10 minutes of nonvariable cycling at 93% HRmax vs. 10 minutes variable cycling at an intensity approximately 93% HRmax). Other factors that may have modulated the onset of fatigue in certain

### Table 3

|                | 1st set | 2nd set | 3rd set | 4th set | ES       |
|----------------|---------|---------|---------|---------|----------|
| Ppeak [W]      | 1,253.7 | 1,154.4 | 1,146.8 | 1,139.9 | 0.28     |
| 95% CI: U      | ± 315.0 | ± 294.6 | ± 302.4 | ± 295.4 |          |
| Pavg [W]       | 604.0   | 621.2   | 604.8   | 596.6   | 0.27     |
| 95% CI: U      | ± 120.8 | ± 120.3 | ± 118.7 | ± 117.1 |          |
| V̇O₂tou [L]    | 19.06   | 19.41   | 19.70   | 20.11   | 0.17     |
| 95% CI: U      | ± 3.94  | ± 4.11  | ± 4.01  | ± 4.24  |          |
| H+ [mmol·L⁻¹] | 89.32   | 85.71   | 85.21   | 81.42   | 0.30     |
| 95% CI: U      | ± 9.07  | ± 8.33  | ± 9.06  | ± 11.06 |          |
| La⁻ [mmol·L⁻¹] | 17.70   | 17.69   | 17.83   | 17.06   | 0.07     |
| 95% CI: U      | ± 2.33  | ± 1.85  | ± 1.87  | ± 2.53  |          |

*Ppeak = peak power output; Pavg = average power output; V̇O₂tou = total oxygen uptake; H⁺ = blood hydrogen concentration; La⁻ = blood lactate concentration.

### Table 4

**Pearson’s correlations coefficients (r) for the between-set differences of heart rate variability indexes and physiological, biochemical, and physical measures.**

|                | dV̇O₂tou | dH+  | dLa⁻  | dPpeak | dPavg   |
|----------------|---------|------|-------|--------|---------|
| dRNN           | -0.40†  | 0.33†| 0.03  | 0.04   | 0.02    |
| dSDNN          | -0.27†  | 0.35†| 0.29† | -0.04  | 0.03    |
| dRMSSD         | -0.17   | -0.36| 0.43† | -0.06  | 0.02    |
| dVLF           | -0.25†  | -0.01| -0.09 | -0.11  | -0.23   |
| dLF            | -0.23   | 0.20 | 0.08  | 0.10   | -0.09   |
| dHF            | -0.22   | -0.03| 0.21  | -0.09  | -0.09   |
| dT             | -0.26†  | 0.16 | 0.09  | 0.04   | -0.12   |
| dLF/dHF        | 0.08    | 0.02 | -0.23 | 0.03   | 0.06    |

*d suffix = difference between values obtained in the first and the second set, first and the third set, and first and the fourth set calculated for each subject and them for the entire sample; RNN = mean normal-to-normal RR intervals; SDNN = SD of normal-to-normal RR intervals; RMSSD = square root of the mean squared difference between successive normal-to-normal RR intervals; VLF = very low-frequency spectral power; LF = low-frequency spectral power; HF = high-frequency spectral power; T = total spectral power; LEF/HF = ratio of low-frequency to high-frequency spectral power; V̇O₂tou, total oxygen uptake; H+ = blood hydrogen concentration; La⁻ = blood lactate concentration; Ppeak = peak power output; Pavg = average power output.

†p < 0.05.
individuals external to a disrupted acid-base balance including sodium-potassium pump inhibition and a decrease in membrane excitability reduced motor unit recruitment and firing rate (18) and a greater depletion of muscle glycogen stores (1). Nonetheless, the above results coupled with the lack of correlation between Ppeak or Pavg and the between-set differences in any of the HRV indexes might indicate that the development of fatigue in this scenario cannot be measured by HRV.

As previously mentioned, relatable research treated fatigue as overreaching during the training macrocycle and that some studies analyzed changes in performance measures (17,41) or, that those did, reported no change in power output with regard to changes in HRV (38). Only 1 study in the literature observed a reduction in IXT-obtained maximal power during an overload training period concurrent with changes in HRV, although these measures were averaged across a 7-day period and therefore not comparable with acute pre-exercise or post-exercise values (27). As a result, there is limited evidence supporting the relationship between HRV and fatigue other than fatigue induced by a significant acid-base disturbance such as after exercise with a large anaerobic glycolytic component. Hence, it may be that changes in HRV are more likely the result of significant work performed and not indicative of the onset of physical fatigue after repeated sets of maximal sprints (9,24,27,44).

Practical Applications
Changes in the time- and frequency-domain indexes of HRV do not reflect the onset of physical fatigue in SIXT, as in 3 subsequent sets, no change in Pavg was observed and no correlation was found between changes in the time- and frequency-domain indexes of HRV with Ppeak or Pavg.

Based on our results, the development of fatigue before a set of maximal sprints cannot be predicted by observing HRV during SIXT in trained cyclists.

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