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#### Title page

The Effects of Recovery Duration on Physiological and Perceptual Responses of Trained Runners

during four Self-Paced HIIT Sessions

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### Abstract:

Objectives: This study aimed to examine the effects of different recovery durations on self-selected running velocities, physiological responses, and ratings of perceived exertion (RPE) in a commonly used high intensity interval training (HIIT) protocol.

Design & Methods: Twelve trained runners performed an incremental treadmill exercise test to determine maximal oxygen uptake ( $\dot{V}O_2$ max) and heart rate (HRmax). In four subsequent visits, participants performed a HIIT session comprising six 4-min work intervals, in which the recovery duration between work intervals equalled either a fixed (1MIN, 2MIN, 3MIN) or a self-selected duration (ssMIN). HIIT sessions were run on a non-motorized treadmill, and were performed under isoeffort conditions.

Results: Mean running velocity was significantly higher in 3MIN compared with all other protocols, and higher in ssMIN compared with 2MIN. No significant differences in time spent  $\geq$  90% and 95%  $\dot{V}O_2max$ , or  $\geq$  90% and 95% HRmax were evident between the four protocols. RPE responses were similar across and within the protocols showing a gradual increase with each progressive interval.

Discussion: In a self-paced HIIT session of six 4-min work intervals, the length of recovery durations had a limited effect on the total physiological strain endured in the training. However, running velocities were higher when participants received the longest recovery period (3MIN).

Conclusion: Longer recovery durations may facilitate a higher external training load (faster running), whilst maintaining a similar internal training load (physiological stimulus), and may therefore allow for greater training adaptations.

Keywords: exercise, energy metabolism, cardiovascular, athletic performance

#### Introduction

High intensity interval training (HIIT) is often regarded as the most effective training modality to improve cardiorespiratory and metabolic functioning, and, in turn endurance performance. <sup>1</sup> Previously, Demarie et al.,<sup>2</sup> showed that athletes can spend up to 10 minutes per HIIT session in their 'red zone'; the intensity domain close to the maximal oxygen uptake and heart rate ( $\geq$  90%  $\dot{V}O_2$ max

and HRmax respectively). At these exercise intensities the oxygen delivery and utilization systems are maximally stressed, which may provide the most effective stimulus to enhance  $\dot{V}O_2$ max.<sup>1,3,4</sup> Even though HIIT is common practice in training regimes of endurance athletes, little is known how manipulating protocols may maximize time spend around  $\dot{V}O_2$ max per training session.

The workload of a HIIT session is determined by the exercise intensities and durations of both the work and recovery intervals, and the total of intervals performed. <sup>5,6</sup> Recovery durations within HIIT protocols are traditionally based on fixed work:recovery ratios or on the return of HR to a fixed percentage of HRmax. <sup>7,8</sup> Theoretically, work intervals interspersed with short recovery intervals maximize the physiological stimulus of a HIIT session, as subsequent work intervals will start from an elevated  $\dot{V}O_2$  and HR. However, insufficient recovery in a session can lead to premature fatigue, resulting in a reduced number of completed intervals and/or a reduction in exercise intensity in work intervals. Longer recovery between work intervals conversely, will lead to a lower  $\dot{V}O_2$  and HR at the start of subsequent intervals which may attenuate the peak values achieved during the work phases, and potentially decreasing the total exercise time performed in the 'red zone'. While longer recovery may lower the physiological strain, a delayed fatigue may allow athletes to achieve higher external work intensities (i.e. running velocity) in work intervals.

Understanding the acute response to manipulating recovery durations is important when designing HIIT sessions. Smilios et al.,<sup>9</sup> noted recovery durations of 2, 3 or 4 min did not affect the percentage of  $\dot{V}O_2$ max attained and the total time spend  $\geq$  80%, 90% and 95% of  $\dot{V}O_2$ max or HRmax during four 4-min intervals, ran at 90% maximal aerobic velocity. Although the data from the above study is informative,<sup>9</sup> it also is a prime example of most published data, as acute physiological responses are evaluated to a HIIT protocol that incorporates predefined fixed work intensities. In contrast to standardized exercise protocols, athletes measure and pace their work in training sessions on ratings of perceived exertion (RPE) and accumulated fatigue.<sup>10</sup> In self-paced HIIT, the actual work intensity per interval therewith is not a stable function of power or velocity over time, but rather the

integrative outcome of feedback from external and internal receptors, and knowledge of the session demands.<sup>11,12</sup>

While self-paced HIIT interventions have been addressed in cycling recently <sup>10,13</sup>, there is a paucity of research exploring its use in running. Recently, we and others showed that a curved non-motorized treadmill (cNMT; Woodway Inc., Waukesha, United States of America) can be a useful tool to study self-paced running in a lab setting. <sup>14,15</sup> Running on the cNMT is participant driven and provides a closer experience to overground locomotion by allowing for rapid changes of velocity, step-to-step gait variability and, most importantly, an unconsciousness decision making process to change pace.<sup>16</sup>

The aim of this study was to compare the effect of different recovery durations on the acute physiological and perceptual responses, and the accompanying running velocities in a HIIT session performed under isoeffort conditions. A theoretical trade-off was expected between the physiological stimulus (time spend  $\geq$  90% and 95%  $\dot{V}O_2$ max and HRmax) and the external stimulus (running velocity). Thus, it was hypothesised that a short recovery between work intervals would lead to an increased physiological stimulus, at the cost of a decreased running velocity throughout the HIIT session.

#### Methods

Twelve recreationally trained male runners (mean±SD: 34±11 years; 1.80±0.06 m; 74±6 kg; VO<sub>2</sub>max: 53±7 mL·kg<sup>-1</sup>·min<sup>-1</sup>) participated, providing voluntary written informed consent. The study received approval from the local ethics committee (University of Essex, UK) and was conducted in accordance with the Declaration of Helsinki.

**Experimental Design:** Participants visited the laboratory on five different occasions over a four-week period, with visits separated by a minimum of two days. In the first visit, participants performed an incremental running test and one 4-min effort on the cNMT to familiarize with this piece of equipment. In the four following visits participants performed a HIIT session on the cNMT. Participants were familiarized with the concept of using the 15-point RPE scale <sup>17</sup> and a perceived readiness scale (PR) <sup>18</sup> as a means of self-determining readiness to recommence exercise between work intervals. The participants were instructed to avoid any form of strenuous exercise 48 h before each visit.

The incremental running was performed on a motorised treadmill (Pulsar 3p, H/P Cosmos, Nussdorf-Traunstein, Germany), with the gradient set at 1%. The test started at 8 kmh<sup>-1</sup>, increasing 1 kmh<sup>-1</sup> every minute until volitional exhaustion or when at least two of the following criteria were met: 1) HR  $\geq$  90% of the age-predicted maximum; 2) respiratory exchange ratio (RER)  $\geq$  1.10; 3) stable  $\dot{V}O_2$ despite increased intensity.<sup>19</sup>  $\dot{V}O_2$ max was defined as the highest average  $\dot{V}O_2$  over a 30 sec period. HRmax was defined as the highest value obtained at the end of the test. Maximal aerobic velocity (MAV) was defined as the highest velocity (kmh<sup>-1</sup>) that could be maintained for a complete minute, or, as the velocity of the last complete stage added to the completed fraction of an incomplete stage. Gas exchange threshold (GET) was determined from a cluster of measures, previously outlined by Bailey et al.,<sup>20</sup> The running velocity corresponding to 70% of the difference ( $\Delta$ ) between the velocity at GET and MAV was then calculated, and then converted to the corresponding running

velocity on the cNMT.<sup>15</sup> Participants were then instructed to run one 4-min effort on 65% MAV on the cNMT, which would result in a (calculated) exercise intensity of 92.5% VO<sub>2</sub>max.<sup>15</sup>

Over the next four visits, participants performed a HIIT session comprising six 4-min work intervals, separated by either 1, 2, 3-min or a self-selected recovery duration (1MIN, 2MIN, 3MIN, ssMIN) in a counterbalanced order. Prior to each HIIT session participants performed a 6-min warm-up at 70%  $\Delta$ GET on the cNMT, followed by a 9-min break.<sup>20</sup>

Participants were instructed to maintain the highest average running velocity across the work intervals of each session, and to finish the HIIT session on a RPE  $\geq$  17. To avoid poor pacing participants were instructed (but not restricted) to target a velocity of 65% MAV in the first interval. Continuous feedback was available on elapsed time and running velocity during the work intervals. In the recovery intervals, participants were free to select either walking or standing. RPE was obtained immediately after every work interval, and PR was scored every 45 sec during recovery in 1MIN, 2MIN and 3MIN, but only in ssMIN did this indicate the start of a work interval (when participants scored '4' on the PR scale, indicating 'adequate recovery').<sup>18</sup> In ssMIN, participants were blinded to elapsed recovery time.

Breath-by-breath  $\dot{V}O_2$  data were linearly interpolated to 1-sec values, and were then fitted from the onset to the end of each work interval using a mono-exponential growth curve. The mean response time (MRT) was calculated using the formula below.

 $\dot{V}O_2(t) = \dot{V}O_2$ baseline +  $A\dot{V}O_2 \cdot (1 - e - t/\tau)$ 

In this,  $\dot{V}O_2(t)$  represents the  $\dot{V}O_2$  at a given time (t);  $\dot{V}O_2$ baseline the mean  $\dot{V}O_2$  of the last 30 sec before the start of each repetition;  $A\dot{V}O_2$  the amplitude of the  $\dot{V}O_2$  response ( $\dot{V}O_2$  plateau –  $\dot{V}O_2$ baseline); and  $\tau$  the time constant for the model. Similar calculations were performed for the analyses of HR kinetics

During the incremental running test and the four HIIT sessions, heart rate and running cadence were measured continuously at 1 Hz using a Garmin HR monitor and a telemetric foot pod (Garmin 910XT, Garmin Ltd., Schaffhausen). Respiratory parameters were obtained breath by breath, using open circuit spirometry (Oxycon Delta, Jaeger, Höchberg). The physiological responses to the HIIT sessions were indexed for  $\dot{V}O_2$ max and HRmax. Running velocity was sampled at 4 Hz in the accompanying cNMT product software (Woodway Curve 1.5 Software v2.1).

Data were analysed using SPSS Software (SPSS 23.0; IBM Corporation, Armonk, NY, USA). Only the physiological measurements obtained *during* the work intervals were analysed. Mean differences between protocols in physiological parameters (exercise time  $\geq$  90% and 95%  $\dot{V}O_2$ max and HRmax, average  $\dot{V}O_2$  and HR in work intervals, during the last minute of the work intervals, and 30 sec before the start of work intervals) were assessed using one-way repeated measures analysis of variance (ANOVA). A two-way repeated measures (protocol × interval) ANOVA was conducted to examine differences in RER, running velocity and RPE (Tukey's post hoc tests where necessary). Pearson correlations were used to establish the relationship between exercise time 90% and 95%  $\dot{V}O_2$ max and HRmax. Significance was set at p < 0.05.

#### Results

A difference in mean running velocity was found between HIIT protocols. Post-hoc analysis showed that participants ran faster in 3MIN compared to 1MIN, 2MIN and ssMIN (p<0.01). Further, the mean running velocity in ssMIN was higher compared to 2MIN (p=0.001). Subtle fluctuations in running velocities were apparent in all protocols across work intervals (see table 1). RPE responses were similar across and within the protocols (interaction effect p=0.36, see table 1), and participants rated the last interval an RPE score of  $\geq$  19, verifying isoeffort conditions. Table 1 further depicts the mean RER per interval for each experimental protocol. A significant interaction effect was evident (p=0.004), with a higher RER in intervals 4 - 6 in 1MIN compared with 2MIN and 3MIN.

#### >> table 1 around here <<

During the recovery intervals 6 participants walked on all occasions, and 6 participants stood still each time. There was no difference in the  $\dot{V}O_2$  / HR kinetics according to activity in the recovery period (data not shown). Experimental outcomes for  $\dot{V}O_2$  measures are shown in table 2. Repeated measure ANOVA showed no differences in the total exercise time  $\geq$  90% (p=0.24) or  $\geq$  95% (p=0.12)  $\dot{V}O_2$ max between protocols. Mean  $\dot{V}O_2$  before subsequent work intervals was higher in 1MIN compared to all other protocols (p<0.01), and higher in ssMIN compared to 3MIN (p=0.01). Monoexponential modelling provided an adequate fit for the  $\dot{V}O_2$  data (R<sup>2</sup> range 0.73±0.15 – 0.79±0.10). MRT was significantly slower in 1MIN compared to all other protocols, which was accompanied by a lower  $\dot{V}O_2$  amplitude. No differences were found between protocols in  $\dot{V}O_2$  plateau (p=0.29), average  $\dot{V}O_2$  during (p=0.36), or  $\dot{V}O_2$  in the final minute of the work intervals (p=0.21).

No significant differences were evident between protocols for time spent  $\ge$  90% (p=0.48) and  $\ge$  95% HRmax (p=0.39; see table 2). Baseline HR was higher in 1MIN compared to all other protocols, and lower in 3MIN compared to 2MIN and ssMIN. Mono-exponential modelling showed a very good fit for the data (R<sup>2</sup> range 0.96±0.06 – 0.99±0.01). MRT was significant slower in 1MIN compared to

3MIN and ssMIN, and slower in 2MIN than in 3MIN (see table 2). Average HR in the work intervals was higher in 1MIN compared to 3MIN and ssMIN, but did not different in the last minute between protocols (p=0.43).

>> table 2 around here <<

Across the recovery intervals in ssMIN, self-selected recovery duration averaged 100±34 seconds (see figure 1). Recovery time was significant shorter inbetween the first and second work interval, after which compared to subsequent recovery phases, the recovery duration remained constant.

>> figure 1 here<<

### Discussion

This study aimed to examine the effects of different recovery durations on self-selected running velocities and the accompanying physiological and perceptual responses. Mean running velocity was highest when participants received a longer recovery period (3MIN) between intervals, however, total time spend  $\geq$  90% and 95 % $\dot{V}O_2$ max did not differ between protocols. Similarly, time spend  $\geq$  90% and 95% HRmax but was not different between protocols.

HIIT aims to enhance the metabolic overload of a training session by maximizing the total accumulated time spent at high exercise intensities ( $\geq 90\%$   $\dot{V}O_2$ max and HRmax). In line with previous studies, the current data showed that repeated high intensity work intervals of 4 min are performed around 95%  $\dot{V}O_2$ max by recreationally trained runners, and that  $\dot{V}O_2$  in the last minute reaches values close to  $\dot{V}O_2$ max.<sup>9,21,22</sup> Repeated 4-min work intervals are often described as 'long aerobic intervals', and in line with this description the RER values in the current study were under the unit value across all intervals, highlighting the dependency on the aerobic metabolism for ATP re-synthesis (table 1). Hetlelid et al., <sup>22</sup> found the training status of participants plays an important

role in the ability to achieve a steady state even in high-intensity interval exercise. The results of the present study add to those findings, showing a decline in RER with successive high intensity work intervals, despite maintained / elevated oxygen consumption and running velocity.

Total time spend at or above 90% and 95% VO2max, the average VO2 in the work intervals and the average  $\dot{V}O_2$  in the last minute of the work intervals did not differ between protocols. Participants spend around 57% of the exercise time  $\geq$  90% and 37% of time  $\geq$  95% VO<sub>2</sub>max (table 2). These findings are in agreement with those of Smilios et al., <sup>9</sup> though subtle differences are noticeable between study outcomes. Smilios found a (non-significant) linear decrease in time spend  $\geq$  80%, 90% and 95 %VO<sub>2</sub>max with the increase of recovery duration. In contrast, a more U-shaped response was prevalent in the current study (table 2). Despite not reaching statistical significance, time  $\geq$  90% VO<sub>2</sub>max was considerably higher when participants received 3 min recovery compared with other recovery periods, and for 1MIN compared with 2MIN and ssMIN. A similar trend was found for time ≥ 95% VO2max. Basic oxygen kinetic analysis revealed no differences in VO2 plateau between protocols, despite subsequent work intervals starting from a lower metabolic rate in 3MIN and 2MIN compared with 1MIN and ssMIN. Starting intervals from an increased metabolic rate lengthened time needed to reach  $\dot{V}O_2$  plateau in 1MIN, which was accompanied by the lowest  $\dot{V}O_2$  amplitude. In line with the findings of Smilios et al.,<sup>9</sup> our results show a decrease in MRT with the longer recovery duration, with the amplitude following a contrariwise response. This relationship suggests that  $\dot{V}O_2$ kinetics adjust to regulate the oxygen supply that corresponds to the metabolic requirements of the exercise stimulus.

Heart rate monitoring has long been considered an important means to monitor exercise intensities, yet much research shows that it is neither related to systemic  $O_2$  demand nor muscular energy turnover. <sup>1</sup> We found only weak correlations between the measures of the times spent 90% and 95 % $\dot{V}O_2$ max and HRmax across the different protocols. The most notable differences in the time spent  $\geq$  90% and 95% HRmax were found between 2MIN and ssMIN (64 and 121 sec, respectively),

though the magnitude of these differences was considerably lower than the VO<sub>2</sub> measures. A heart rate plateau was found around 95% HRmax independently of recovery duration, and MRT was, as in the VO<sub>2</sub> measures, moderated by the elevation of baseline levels in 1MIN, 2MIN and ssMIN. Overall, subsequent work intervals in 3MIN started from the lowest metabolic rate, but similar times in the exercise zones were achieved because a faster MRT and higher HR amplitude (table 2). The results suggest that HR cannot inform coaches and athletes on the aerobic metabolic requirements and on the intensity of physical work performed in a HIIT session, as we showed similarities in HR plateau and average interval HR across intervals, while differences in running velocities were present between and within protocols (tables 1 and 2).

In the present study, participants were instructed to run at their highest sustainable running velocity throughout the work intervals, and to finish the sessions on a RPE  $\geq$  17. Previously, Seiler and Hetlelid<sup>23</sup> reported that well-trained male runners ran faster when the recovery duration increased from 1 to 2 min (attaining 84%  $v\dot{V}O_2max$ ), but a further increase to 4 min had no additional effect on self-selected running velocities. Laurent et al., <sup>21</sup> reported an increase in running velocity when the recovery duration was increased from 1 to 2 min and from 2 to 4 min. In line with these findings, our results show participants ran faster in 3MIN compared to all other conditions and the running velocity was higher in ssMIN compared to 2MIN. However, in contrast to the earlier findings of both Seiler and Hetlelid<sup>23</sup> and Laurent et al.,<sup>21</sup> we did not find an increase in running velocity when recovery time was increased from 1 to 2 min. In ssMIN, participants were instructed to start subsequent work intervals when they felt 'adequately recovered'. Self-selected recovery averaged 100 ± 34 seconds, similar to earlier findings of Seiler and Hetlelid<sup>23</sup>, but almost a minute shorter than was reported by Edwards et al., <sup>18</sup> in a comparable interval session. The ssMIN protocol produced the most stable pacing profile, with the difference between the fastest and slowest work interval being only 0.53±0.3 kmh<sup>-1</sup> (table 1), however, average running velocities were slower compared to 3MIN. Athletes in the present study may have been were more accustomed to a 'short' recovery

between work intervals, and therefore may not have fully utilized the opportunity of increasing their recovery duration.

Conclusion / Future research:

The use of the 'isoeffort' approach in a scientific setting shifts the decision making on interval exercise intensities towards the participant, thus increasing the external validity of the protocol. Participants in the current study rated their final interval 19.0, which indicates 'extremely hard' exercise. In previous studies, exercise intensities have been both over- and/or underestimated leading to a reduced number of completed intervals<sup>25</sup> or a session that is 'too easy' (indicated by a final RPE of 15).<sup>9</sup> While the results of the current study suggest that recovery duration has a limited effect on the total physiological strain of the training, running velocities were fastest when participants received the longest recovery period. Longer recovery durations may facilitate a higher external training load (running speed) whilst maintaining a similar internal load (physiological stimulus) in HIIT sessions, and therefore, may allow for greater training adaptations.

#### PRACTICAL IMPLICATIONS

- Coaches should take into account that a longer recovery interval (3 min) between efforts facilitates a faster running velocity, which is particularly important when the focus of the session is speed work.
- Recovery duration does not influence total metabolic load of a single training session, thus athletes can recovery for a greater period than may be traditionally thought.
- A self-selected recovery period results in the most consistent running velocity which may be of importance when athletes are working on pacing.

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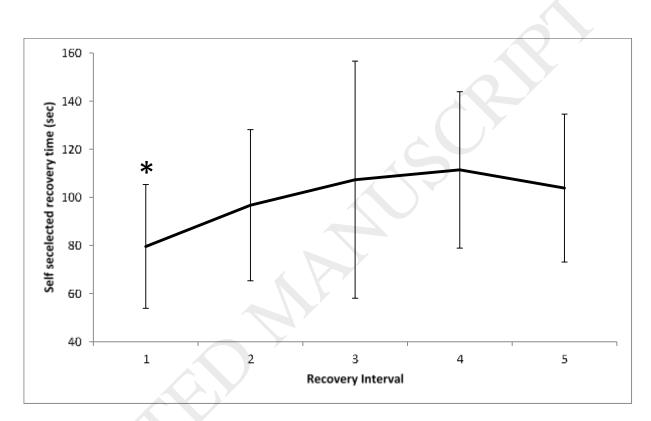
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Figure legend

Figure 1: Self selected recovery duration in subsequent recovery intervals (n=12)



\* p < 0.05 Compared to all subsequent recovery intervals

	HIIT Protocol							
	Work Interval	1MIN	2MIN	3MIN	ssMIN			
RER	1	0.95±0.05	0.97±0.08	0.96±0.03	0.96±0.07			
	2	0.99±0.05ª	0.98±0.08	0.96±0.02	0.99±0.07ª			
	3	0.96±0.06ª	0.94±0.07 <sup>a</sup>	0.93±0.02ª	0.96±0.07ª			
	4	0.96±0.05*	0.93±0.07ª	0.93±0.02	0.94±0.07ª			
	5	0.95±0.05*	0.92±0.06	0.92±0.02	0.93±0.06 <sup>a</sup>			
	6	0.95±0.04*	0.92±0.04	0.92±0.02	0.93±0.06			
RPE (au)	1	14.6±1.9	15.0±1.7	14.1±2.0	15.1±1.4			
	2	16.3±1.5ª	16.7±1.6ª	16.6±1.6ª	16.4±1.4 <sup>a</sup>			
	3	17.2±1.3ª	17.3±1.1ª	17.3±1.4ª	17.3±1.2ª			
	4	18.6±0.8ª	17.8±1.0	18.2±1.0ª	18.0±1.2ª			
	5	18.8±0.7	18.3±0.9ª	18.4±0.8	18.5±1.0ª			
	6	19.3±0.5	19.2±0.6 <sup>a</sup>	19.0±0.7ª	19.2±0.8ª			
Velocity (kmh <sup>-1</sup> )	1	11.7±0.9	12.0±1.1	11.9±1.1	11.8±0.9			
	2	11.8±1.1	11.9±1.0	12.2±1.1	12.0±1.0			
	3	11.6±1.2	11.5±1.0ª	12.1±1.1	11.8±1.1ª			
	4	11.5±1.2	11.2±1.1ª	12.0±1.1	11.7±1.1			
	5	11.4±1.3	11.1±1.1	11.8±1.0	11.6±1.1			
	6	11.5±1.3	11.3±0.9ª	12.0±1.0	11.7±1.0			

Table 1: Mean(±SD) RER, RPE and Running Velocity measured during work intervals 1 through 6 in the 1MIN, 2MIN, 3MIN and ssMIN protocol (n=12)

RER: Resiratory exchange ratio (VCO<sub>2</sub>/VO<sub>2</sub>); RPE: Ratings of perceived exertion; au: arbitrary unit

\* p < 0.05 Compared to 2MIN and 3MIN

<sup>a</sup> p < 0.05 Compared to previous work interval

Table 2: Mean(±SD) Oxygen uptake and heart rate during simulated HIIT sessions, with 1MIN, 2MIN, 3MIN or ssMIN recovery between subsequent work intervals (n=12)

	Oxygen uptake				Heart rate			
	1MIN	2MIN	3MIN	ssMIN	1MIN	2MIN	3MIN	ssMIN
exercise time ≥ 90% VO₂max / HRmax (sec)	849±341	727±388	918±232	776±335	979±257	1017±231	989±149	953±198
exercise time ≥ 95% VO₂max / HRmax (sec)	574±373	422±347	629±330	476±408	468±317	493±347	441±296	372±287
30sec baseline VO2/HR (ml.kg.min - bpm)	26.6±4.1*	18.6±4.0	17.8±5.7	20.3±5.6~	140±14*	126±15	115±14#	126±16
VO₂/HR Plateau (ml.kg.min - bpm)	50.3±6.8	49.0±6.3	51.6±7.8	50.1±6.6	177±12	177±10	176±11	175±11
Mean response time (sec)	33.1±2.6*	30.2±4.2	28.8±3.0	29.2±5.4	45.2±7.5^	40.7±4.5~	37.3±4.2	40.3±7.0
mean VO2/HR interval (%max)	90.1±8.5	87.1±5.2	91.0±6.2	89.4±7.5	90.2±3.2^	89.2±4.6	88.6±3.1	88.4±3.1
mean $\dot{V}O_2$ /HR last 60sec of interval (%max)	96.1±8.7	92.9±6.4	98.0±6.5	95.8±8.2	94.9±2.2	95.3±3.1	95.4±1.6	94.5±1.8

VO₂ = oxygen uptake; HR = heart rate; bpm = beats per minute. Recovery phases are excluded from the analysis.

\* p < 0.01 vs 2MIN, 3MIN and ssMIN ~ p <0.05 vs 3MIN ^ p < 0.05 vs 3MIN and ssMIN # p < 0.05 vs 2MIN and ssMIN