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1 **Acute physiological responses to four running sessions performed at different intensity zones**

2 **ABSTRACT**

3 This study investigated acute responses and post 24-hour recovery to four running sessions performed  
4 at different intensity zones by supine heart rate variability, countermovement jump, and a submaximal  
5 running test. A total of 24 recreationally endurance-trained male subjects performed 90 min low-  
6 intensity (LIT), 30 min moderate-intensity (MOD), 6x3 min high-intensity interval (HIIT) and 10x30  
7 s supramaximal-intensity interval (SMIT) exercises on a treadmill. Heart rate variability decreased  
8 after all sessions, and the decrease was greater after MOD compared to LIT and SMIT ( $p<0.001$ ;  
9  $p<0.01$ ) and HIIT compared to LIT ( $p<0.01$ ). Countermovement jump decreased only after LIT  
10 ( $p<0.01$ ) and SMIT ( $p<0.001$ ), and the relative changes were different compared to MOD ( $p<0.01$ )  
11 and HIIT ( $p<0.001$ ). Countermovement jump remained decreased at 24 hours after SMIT ( $p<0.05$ ).  
12 Heart rate during the submaximal running test rebounded below the baseline 24 hours after all  
13 sessions ( $p<0.05$ ), while the rating of perceived exertion during the running test remained elevated  
14 after HIIT ( $p<0.05$ ) and SMIT ( $p<0.01$ ). The current results highlight differences in the physiological  
15 demands of the running sessions performed, and distinct recovery patterns of the measured aspects  
16 of performance. Based on these results, assessments of performance and recovery from multiple  
17 perspectives may provide valuable information for endurance athletes, and help to improve the quality  
18 of training monitoring.

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## 1 **Introduction**

2 Endurance training typically consists of various training modes differing in duration and intensity.  
3 Traditional intensity zones can be set based on individual ventilatory or lactate thresholds into low-  
4 intensity training below the first lactate threshold, moderate-intensity training between first and  
5 second lactate thresholds and high-intensity training between second lactate threshold and maximal  
6 oxygen consumption [1]. In addition to endurance intensity zones, supramaximal intensity training  
7 above the intensity of  $\dot{V}O_{2max}$  may improve maximal endurance performance [2, 3], and induce  
8 similar adaptations in the skeletal muscle oxidative capacity than traditional endurance training [3].  
9 Training intensity has effects on the cardiovascular workload, substrate utilization in energy  
10 metabolism, as well as the number and type of motor units recruited during the exercise [1], all of  
11 which may influence the type of fatigue induced and responses observed followed by the session.

12 Fatigue during endurance exercise can be stated as perceived tiredness with concurrent decrements  
13 in muscular performance and function [4]. Typically, the body needs to adjust to the growing demand  
14 of the activity performed by increasing heart rate [5-7], oxygen consumption, [5-7] and perceived  
15 effort [8] at a given workload. The autonomic nervous system responds to exercise by increasing  
16 sympathetic drive and catecholamine secretion [9], while parasympathetic activity diminishes [10].  
17 The origin of the fatigue and time frame to restore the normal function in the neuromuscular system  
18 seems to depend on the duration and the intensity of the preceding exercise [11].

19 In addition to appropriate training load, sufficient recovery is required to induce training adaptations.  
20 Resting heart rate variability (HRV) is a noninvasive measurement of the autonomic nervous system  
21 function and is suggested to provide comprehensive information about the recovery status [12].  
22 Previous research has shown that the reactivation of the parasympathetic nervous system measured  
23 as HRV after training appears to be affected most by the intensity of the session [10, 13, 14]. Full  
24 restoration after exercise at or above the first ventilatory threshold intensity can take up to 24-48  
25 hours [15]. Furthermore, individually adjusted endurance training based on the fluctuations of resting  
26 HRV has provided superior results compared to predefined training [16, 17]. A similar approach has  
27 also been examined with a heart rate-based submaximal cycling test [18]. The general assumption in  
28 submaximal tests is that increase in the power or speed at the same relative heart rate and perceived  
29 effort reflects positive training adaptation [19] and readiness to train [18].

30 Despite the potential of the resting HRV and heart rate based submaximal tests, it is unclear how well  
31 these tests reflect all aspects of recovery such as the subjective recovery or readiness of the  
32 neuromuscular system. Recently, it has been observed that the recovery timeframe of neuromuscular

1 performance and exercise-induced muscle damage may differ from that of HRV after strength training  
2 exercise [20]. Acute responses following endurance exercise also seem to differ as countermovement  
3 jump (CMJ) performance may even improve in endurance-trained athletes after high-intensity  
4 sessions [21, 22]. Additional monitoring variables may also help to contextualize whether changes in  
5 resting HRV or submaximal heart rate are due to fatigue or positive training adaptation, as similar  
6 responses may be observed in both situations [12, 23].

7 Responses to training are likely related to the intensity and duration of the preceding session and  
8 subsequently, the timeframe to recover may vary depending on the viewpoint taken. In endurance  
9 sports, heart rate assessments during rest and exercise have been studied and also utilized in training  
10 monitoring widely, while less is known about the other aspects of recovery and resemblance of  
11 different markers. The purpose of this study was to compare acute responses and post 24-hour  
12 recovery in the function of the autonomic nervous system, neuromuscular performance and  
13 submaximal running test. It was hypothesized that the acute HRV decrease is related to the intensity  
14 of the training, while CMJ performance would improve after moderate and high-intensity training  
15 sessions. In addition, it was anticipated that CMJ, metabolic and cardiorespiratory recovery would  
16 occur in the 24 hours after all sessions, but parasympathetic nervous system activity would only be  
17 fully recovered after LIT.

## 18 **Materials and methods**

### 19 Subjects

20 Twenty-five recreationally endurance-trained men, aged 20-45 years, were recruited for the study.  
21 Basic characteristics of the subjects are presented in Table 1. After being informed about the study  
22 design and possible risks and benefits of participation, subjects with an appropriate training  
23 background and health status signed a written informed consent form. One subject could not finish  
24 all the training sessions due to an injury, and, therefore, the total number of subjects was 24. After  
25 low-intensity session and high-intensity interval session, one subject did not perform the post-24-  
26 hour measurements. The study was approved by the Ethical Committee of the University ■  
27 ■, and it was conducted according to the provisions of the Declaration of Helsinki and  
28 recommendations of Harriss et al. [24].

29 **\*\*TABLE 1 ABOUT HERE\*\***

### 30 Study design

31 The study compared acute responses to and post 24-hour recovery following four different training  
32 sessions performed on a treadmill. The order of the training session was randomized by drawing the

1 sequence for each subject. After a preliminary performance testing week, training sessions were  
2 performed during the one-month study period. Before (pre), immediately after (post) and 24 hours  
3 after (post24) each session, supine heart rate variability, countermovement jumps, and a submaximal  
4 running test were performed. Additionally, perceived recovery and muscle soreness was measured at  
5 pre and post24. Subjects could continue their regular training during the study period. However, on  
6 the day before each training session, no exercise was performed and on the day before only light  
7 exercise was permitted. During the recovery phase before post24 measurements, exercising was not  
8 allowed. Subjects were advised to avoid heavy meals and caffeine 3-4 hours preceding each  
9 measurement to avoid any gastrointestinal symptoms or any other possible effects on measured  
10 variables. The structure of one training session and the measurements performed are presented in  
11 Figure 1.

12 \*\*FIGURE 1 ABOUT HERE\*\*

### 13 Preliminary examinations

14 **Incremental treadmill test:** The incremental treadmill test was performed on a treadmill  
15 (Telineyhtymä Oy, Kotka, Finland). Starting speed ( $8.2 \pm 1.1 \text{ km}\cdot\text{h}^{-1}$ ) was individually set based on  
16 obtained information of the previous performance and training background of the subjects to have at  
17 least two stages before the velocity of the first lactate threshold, and thus allow a reliable estimation  
18 of lactate thresholds. Three-minute stages were used, and speed increased by  $1 \text{ km}\cdot\text{h}^{-1}$  after every  
19 stage. Between the stages, the treadmill was stopped (15-20 s) for the fingertip blood lactate samples  
20 to be taken. Inclination was kept constant at 0.5 degrees through the whole test. Oxygen consumption  
21 was measured breath by breath (OxygonPro, Jaeger, Hoechberg, Germany) and heart rate was  
22 monitored with a Garmin Forerunner 920XT (Garmin Ltd, Schaffhausen, Switzerland).  $\dot{V}O_{2\text{max}}$  was  
23 defined as the highest 60 s average of oxygen consumption. Maximal treadmill running speed (vMax)  
24 of the test was defined as the highest completed stage speed, or if the stage was not finished, as a  
25 speed of the last completed stage ( $\text{km}\cdot\text{h}^{-1}$ ) + (running time (s) of the unfinished stage – 30 seconds) /  
26  $(180 - 30 \text{ seconds}) \cdot 1 \text{ km}\cdot\text{h}^{-1}$ . Running speed at the first lactate threshold (vLT1) and running speed  
27 at the second lactate threshold (vLT2) were determined based on the change in the inclination of the  
28 blood lactate curve during the test [19]. The first lactate threshold was set at  $0.3 \text{ mmol}\cdot\text{l}^{-1}$  above the  
29 lowest lactate value and the second lactate threshold at the intersection point between 1) a linear  
30 model between first lactate threshold and the next lactate point and 2) a linear model for the lactate  
31 points with the La increase of at least  $0.8 \text{ mmol}\cdot\text{l}^{-1}$  similar to Vesterinen et al. [19]

1 **20 m flying sprint test:** 20 m flying sprint test was performed in the indoor track. Warm-up before  
2 the test included a 10-minute low-intensity run, dynamic stretching for the lower limbs and three  
3 submaximal 50 m accelerations. Maximal running speed ( $v_{20m}$ ) was measured with two photocell  
4 gates after 30 m acceleration. Three attempts were performed with three-minutes recovery, if no more  
5 than 5 % improvement were found between the last two attempts. The best result was used in further  
6 analysis.

7 **Anthropometrics:** Fat percentage was analyzed as a sum of four skinfolds [25]. Subjects were  
8 weighed before each measurement session and the current body mass was used in  $\dot{V}O_2$  ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )  
9 calculations.

#### 10 Training sessions

11 The duration and intensity of the training sessions were pre-determined in order that they represented  
12 typical training of each intensity zone (low, moderate, high and supramaximal intensity) and to ensure  
13 it would be possible for each subject to perform the sessions. Previous studies [2, 14, 26] have also  
14 utilized similar types of training. Running speeds of the sessions were set individually based on their  
15 lactate thresholds and maximal running speed during the incremental treadmill test and the 20 m  
16 flying sprint test. A low-intensity (LIT) session was a 90-min run performed at 80 % of the speed of  
17 the first lactate threshold ( $v_{LT1}$ ). A moderate-intensity (MOD) session was a 30-min run performed  
18 at the average speed of the first and second lactate thresholds ( $(v_{LT1}+v_{LT2})/2$ ). A high-intensity  
19 interval (HIIT) session was 6x3 min with 2 min recovery performed between the second lactate  
20 threshold and maximal incremental treadmill test speed ( $(v_{LT2}+(v_{Max}-v_{LT2})/3)$ ). A supramaximal  
21 intensity interval (SMIIT) session was 10x30 s with 2.5 min recovery performed at 75 % of the speed  
22 from the 20 m flying sprint ( $v_{20m} \cdot 0.75$ ). During the recovery, treadmill speed was set at 5 km/h in  
23 both interval sessions. The submaximal running test acted as a warm-up and cool-down for the  
24 sessions. Before SMIIT, one short acceleration (15 s) to the speed of the upcoming session was  
25 performed to familiarize subjects with the treadmill velocity, and the actual session started after 2.5  
26 min recovery.

27 All training sessions were performed within-subject at the same time of the day ( $\pm 1$  h) on the  
28 treadmill (Telineyhtymä Oy, Kotka, Finland). Heart rate was measured throughout the sessions with  
29 a Garmin Forerunner 920 XT -monitor (Garmin Ltd, Schaffhausen, Switzerland). Average and peak  
30 heart rates as well as training impulse (TRIMP), based on the Edwards [27] model, were analyzed  
31 from the training sessions. In addition, at the end of each session rating of perceived exertion (RPE)  
32 were asked with the 6-20 Borg scale [28], and blood samples were drawn from the fingertip. Blood

1 lactate was analyzed with Biosen S\_line Lab+ lactate analyzer (EKF Diagnostic, Magdeburg,  
2 Germany). After each training session, subjects were given the same recovery drink (Fast Reco2)  
3 including 41 g of carbohydrates and 20 g of proteins mixed in a 500 ml of water. The recovery drink  
4 was served to ensure similar immediate nutrition for all subjects.

#### 5 Recovery measurements

6 **Heart rate variability:** Heart rate variability (HRV) was measured in a supine position with a Garmin  
7 Forerunner 920XT -monitor. Before starting three-minute data collection [29], a one-minute  
8 stabilization period was performed [30]. Subjects were able to breathe at their natural rhythm. The  
9 average natural logarithm of the square root of the mean sum of the squared differences (lnRMSSD)  
10 was calculated from the three-minute measurement period. Because the measurements were  
11 performed in the lab and not right after awakening, baseline values in each athlete were derived from  
12 pooled pre-exercise data for the four test sessions comparable to Seiler et al. [14].

13 **Countermovement jump:** Countermovement jumps were performed on a contact mat. Jump height  
14 (h) was calculated with a formula:  $h = g \cdot t^2 \cdot 8^{-1}$ , where t is the recorded flight time in seconds and g  
15 is the acceleration due to gravity ( $9.81 \text{ m} \cdot \text{s}^{-2}$ ) [31]. Subjects were advised to keep their hands on  
16 their hips and jump as high as possible. The lowest knee angle for the jump was about 90 degrees.  
17 Three attempts with 30 s recovery were performed unless an improvement of 5 % or more was found  
18 between two last jumps. The best jump of three was used in further analysis. Subjects were  
19 familiarized with the jumping technique during the preliminary tests.

20 **Submaximal running test:** The submaximal running test (SRT) was modified from the Vesterinen et  
21 al. [19] submaximal running test, and it acted as a warm-up and cool down for each training session.  
22 SRT in the current study consisted of two 5-min stages which were performed at the speeds  
23 corresponding individually to 70 % (1. stage) and 80 % (2. stage) of  $HR_{\max}$  during the incremental  
24 treadmill test. The same individually set speeds, which were calculated from the incremental treadmill  
25 test, were used in all measurements, despite possible changes in heart rate, to allow fair comparison  
26 between sessions and conditions. During SRT, heart rate (HR) was recorded (Garmin Forerunner 920  
27 XT) and oxygen consumption ( $\dot{V}O_2$ ) and respiratory exchange ratio (RER) were measured  
28 (OxygonPro, Jaeger, Hoechberg, Germany). Average of the last two minutes during the 80 % running  
29 speed was used in further analysis, as higher intensities reflect better changes in maximal performance  
30 [19]. After SRT, RPE was asked using the 6-20 Borg scale [28] and blood lactate values were  
31 analyzed from the fingertip sample.

1 **Subjective markers:** Perceived recovery was estimated on the 0-10 scale [32]. Perceived muscle  
2 soreness of the lower limbs was estimated on the 10 cm visual analogy scale where 0 represented no  
3 soreness at all and 10 represented the highest possible soreness [33].

#### 4 Statistical analyses

5 All values are expressed as mean and standard deviation (SD). Normal distribution of the data was  
6 checked with the Shapiro-Wilk test and homogeneity of the variances by Levene`s test. A one-way  
7 repeated measures ANOVA was used to compare training load variables measured during training  
8 sessions. A two-way repeated measures ANOVA was performed to examine main effects (training  
9 mode, time) and interaction (training mode x time) across measured variables. When appropriate, a  
10 Bonferroni post hoc test was used. Furthermore, in case of significant training mode x time  
11 interaction, relative changes from pre-values to post and post24 were compared between training  
12 modes using paired samples t-test with Bonferroni correction. Muscle soreness was not normally  
13 distributed even after log-transformation, so Wilcoxon signed-rank test was used for within-group  
14 comparisons and Mann-Whitney U-test for between-group comparisons. To further analyze the  
15 magnitude of observed changes, the effect size was assessed by Cohen`s D (difference of the means  
16 divided by the pooled standard deviation) [34], and after nonparametric tests by a formula:  $ES = Z/\sqrt{n}$ ,  
17 where Z is the z-score, and n are the number of observations on which Z is based. An effect size of  
18  $<0.20$  was considered trivial,  $\geq 0.20$  small,  $\geq 0.50$  medium, and  $\geq 0.80$  large [34]. Statistical  
19 significance level was set to  $p < 0.05$ . Analysis were performed with IBM SPSS Statistics v.26 -  
20 programs (SPSS Inc, Chicago, IL, USA) and Microsoft Excel 2010 (Microsoft Corporation, WA,  
21 USA).

## 22 **Results**

### 23 Training sessions

24 Results of the training sessions performed are presented in Table 2.

25 \*\*TABLE 2 ABOUT HERE\*\*

### 26 Supine heart rate variability

27 A significant main effect for the training mode ( $p < 0.001$ ), time ( $p < 0.001$ ) and training mode x time  
28 interaction ( $p < 0.001$ ) were found in lnRMSSD. lnRMSSD decreased ( $p < 0.001$ ) after LIT ( $3.8 \pm 0.5$   
29 ms vs.  $2.9 \pm 0.7$  ms,  $ES = -1.83$ ), MOD ( $4.0 \pm 0.5$  ms vs.  $1.9 \pm 0.8$  ms,  $ES = -3.13$ ), HIIT ( $3.9 \pm 0.4$  ms  
30 vs.  $2.1 \pm 0.7$  ms,  $ES = -3.12$ ) and SMIT ( $3.9 \pm 0.5$  ms vs.  $2.5 \pm 0.6$  ms,  $ES = -2.55$ ). The relative decrease  
31 was smaller after LIT when compared to MOD ( $p < 0.001$ ,  $ES = 1.35$ ) or HIIT ( $p = 0.001$ ,  $ES = 1.09$ )

1 (Figure 2). A smaller decrease was also observed after SMIT than MOD ( $p=0.009$ ,  $ES=0.90$ ) (Figure  
2 2). InRMSSD returned to baseline after all sessions at 24 hours with no differences compared to  
3 baseline within (LIT  $ES=0.15$ , MOD  $ES=-0.17$ , HIIT  $ES=0.00$ , SMIT  $ES=0.08$ ) or between sessions.  
4 Relative mean and individual changes from the baseline in the InRMSSD are presented in Figure 2.

5 \*\*FIGURE 2 ABOUT HERE\*\*

### 6 Countermovement Jump (CMJ)

7 A significant training mode x time interaction ( $p<0.001$ ) was observed in CMJ. CMJ height decreased  
8 after LIT ( $35.8 \pm 4.7$  cm vs.  $34.2 \pm 4.9$  cm,  $p=0.001$ ,  $ES=-0.34$ ) and SMIT ( $36.1 \pm 4.7$  cm vs.  $34.4 \pm$   
9  $4.1$  cm,  $p<0.001$ ,  $ES=-0.37$ ), while no difference compared to baseline was observed after MOD ( $35.3$   
10  $\pm 5.8$  cm vs.  $35.8 \pm 5.3$  cm,  $ES=0.09$ ) or HIIT ( $35.7 \pm 5.5$  cm vs.  $36.4 \pm 5.0$  cm,  $ES=0.13$ ). The relative  
11 changes after LIT and SMIT were also different compared to MOD (LIT,  $p<0.001$ ,  $ES=-1.06$ ; SMIT,  
12  $p=0.002$ ,  $ES=-1.08$ ) and HIIT (LIT,  $p<0.001$ ,  $ES=-1.33$ ; SMIT,  $p<0.001$ ,  $ES=-1.43$ ) (Figure 3). CMJ  
13 remained decreased after SMIT 24 hours after the session ( $p=0.018$ ,  $ES=-0.19$ ), while no difference  
14 was observed after other sessions (LIT,  $ES=-0.06$ ; MOD,  $ES=0.02$ ; HIIT,  $ES=-0.04$ ). Relative mean  
15 and individual changes from the baseline in the CMJ height are presented in Figure 3.

16 \*\*FIGURE 3 ABOUT HERE\*\*

### 17 Submaximal running test

18 A significant main effect for time ( $p<0.01$ ) were observed in all variables measured during the  
19 submaximal running test. In addition, significant main effect for training mode as well as training  
20 mode x time interaction was found in blood lactate ( $p<0.001$ ). Heart rate during submaximal running  
21 test increased after all sessions ( $p<0.001$ ) followed by a decrease below the baseline at 24 hours after  
22 LIT ( $p=0.001$ ), MOD ( $p=0.023$ ), HIIT ( $p=0.016$ ) and SMIT ( $p=0.011$ ). RPE during submaximal  
23 running test increased after LIT ( $p<0.001$ ), MOD ( $p=0.004$ ), HIIT ( $p=0.001$ ) and SMIT ( $p=0.002$ ),  
24 and it returned to baseline at 24 hours after LIT and MOD but remained increased after HIIT  
25 ( $p=0.048$ ) and SMIT ( $p=0.007$ ). Oxygen consumption during submaximal running test increased after  
26 LIT ( $p=0.017$ ) and MOD ( $p=0.002$ ), while no significant difference was observed after SMIT or HIIT.  
27 Oxygen consumption returned to baseline after all sessions at post24 measurements. The only  
28 between-group difference observed during the submaximal running test was in blood lactate which  
29 was higher after SMIT than any other session ( $p<0.001$ ) The absolute values and effect sizes  
30 measured during the submaximal running test are presented in Table 3.

31 \*\*TABLE 3 ABOUT HERE\*\*

## 1 Subjective markers

2 No significant main effects or interaction were observed in perceived recovery (LIT  $7.4 \pm 1.5$  vs.  $6.7$   
3  $\pm 1.7$ ,  $p=0.054$ ,  $ES=-0.42$ ; MOD  $7.0 \pm 1.5$  vs.  $7.0 \pm 1.4$ ,  $ES=-0.03$ ; HIIT  $7.3 \pm 1.5$  vs.  $\pm 7.1 \pm 1.7$ ,  
4  $ES=-0.13$ ; SMIT  $7.4 \pm 1.4$  vs.  $6.9 \pm 1.6$ ,  $ES=-0.33$ ). Perceived muscle soreness increased after LIT  
5 ( $1.5 \pm 1.3$  vs.  $2.4 \pm 1.7$ ,  $p=0.003$ ,  $ES=0.41$ ) and SMIT ( $1.7 \pm 1.9$  vs.  $2.4 \pm 1.5$ ,  $p=0.042$ ,  $ES=0.25$ ),  
6 while no change was observed after MOD ( $1.7 \pm 1.5$  vs.  $2.1 \pm 1.2$ ,  $ES=0.20$ ) or HIIT ( $1.6 \pm 1.6$  vs.  
7  $1.9 \pm 1.7$ ,  $ES=0.19$ ). No significant differences between sessions were observed in relative changes  
8 of subjective markers.

## 9 **Discussion**

10 The aim of this study was to compare acute responses and post 24-hour recovery after training  
11 sessions performed at different intensity zones. The main findings of the study were that  
12 parasympathetic reactivation measured as HRV was diminished the most after MOD and HIIT  
13 compared to LIT and SMIT. Contradictory, CMJ performance did not decrease after MOD or HIIT  
14 but acutely decreased combined with increased muscle soreness at post24 after LIT and SMIT. The  
15 main result of the submaximal running test was that all measured metabolic parameters recovered  
16 and heart rate decreased significantly at 24 hours after all sessions, despite perceived exertion being  
17 elevated after HIIT and SMIT at the respective time points. The current results highlight how  
18 physiological demands differ between training modes. Different measures of performance and  
19 recovery may induce even contradictory results illustrating the usefulness of a broad approach to  
20 endurance training monitoring.

## 21 Training sessions

22 Blood lactate, heart rate, and RPE responses to running sessions confirmed that they could be  
23 regarded as representative measures of each intensity zone. Peak and average heart rate values  
24 observed during MOD and HIIT indicated a high cardiovascular demand during these sessions.  
25 Perceived effort during SMIT was estimated by the subjects similarly as after MOD and HIIT, and  
26 despite lower heart rate values, blood lactate increased the most suggesting higher anaerobic  
27 contribution during the session. During LIT, more than double the distance was covered compared to  
28 MOD and HIIT while there was almost a fourfold increase in distance covered compared to SMIT.  
29 Although the duration or distance was not the same between the sessions, they were likely similar to  
30 the ones typically utilized by athletes [1].

31 The only unexpected response was increased blood lactate value after LIT despite the low relative  
32 intensity ( $52 \%/vMax$ ,  $80 \%/vLT1$ ), low heart rate (avg:  $70 \%/HR_{max}$ , peak:  $76 \%/HR_{max}$ ) and RPE

1 (12 on a 6-20 scale). In the study of Seiler et al. [14], no changes in blood lactate were observed after  
2 1- or 2-hour exercises performed below the ventilatory threshold. Differences compared to the present  
3 protocol were that Seiler et al. [14] had higher caliber athletes, the 1-hour session was performed on  
4 the 2-5 % incline and the longer 2-hour session was performed outside. All of these methodological  
5 differences may at least slightly influence the physiological responses compared to the present  
6 protocol. In line with the present results, Kaikkonen et al. [26] found that after a 14 km run on a  
7 treadmill at 60 %  $\dot{V}O_{2max}$  blood lactate elevated significantly compared to the control session  
8 performed at the same intensity but 3 km in distance (1.4 vs. 2.6 mmol/l) in recreational athletes. The  
9 athlete level could, therefore, be a major factor in the observed response. Because RPE and heart rate  
10 remained in target values despite elevated blood lactate levels during LIT, the training session of the  
11 present study likely illustrates a typical LIT session of a recreational endurance athlete.

## 12 Acute responses

13 Acute responses in supine HRV differed significantly between the sessions. HRV decreased less after  
14 LIT compared to MOD and HIIT despite higher TRIMP and more than the double distance covered  
15 during the session. The results are in line with previous studies [10, 14] indicating that intensity of  
16 the sessions, when performed below  $\dot{V}O_{2max}$  seems to influence the parasympathetic reactivation  
17 more than the duration of the session. Furthermore, MOD and HIIT produced quite similar acute  
18 responses in HRV supporting the theory that lactate or the ventilatory threshold may act as a lower  
19 bound for the intensity related delay in parasympathetic reactivation [14]. An unexpected finding was  
20 that a smaller decrease in HRV was also observed after SMIT than MOD, and no difference was  
21 observed in the HRV responses between LIT and SMIT despite significantly higher lactate values  
22 and RPE measured in SMIT. This is also contradictory to the results of Niewiadomski et al. [13] who  
23 found a greater decrease in HRV one hour after a supramaximal session (2 x 30 s Wingate) compared  
24 to a moderate intensity session (30 min 85 %/HRmax). In addition, Buchheit et al. [35] have suggested  
25 that the delay in parasympathetic reactivation is mainly related to the contribution of anaerobic  
26 processes. However, maximum heart rate [13], as well as mean heart rate and blood lactate levels  
27 [35] during supramaximal exercises were substantially higher compared to the present study, which  
28 may relate to a lower cardiovascular load and the sympathetic nervous system activity during the  
29 session. It should be also acknowledged that there is a wide range of anaerobic interval sessions  
30 differing in the intensity, work:relief-ratio and anaerobic glycolytic energy contribution [36]. Further  
31 studies are needed to understand how manipulation of these variables would affect the  
32 parasympathetic reactivation following anaerobic exercise.

1 Acute changes in CMJ occurred oppositely when compared to HRV responses. While no change was  
2 observed after HIIT or MOD, jump height decreased after LIT and SMIT. Previously, Boullosa et al.  
3 [21] have found improved CMJ performance after intensive running sessions in endurance-trained  
4 athletes. Because both the intensity and the work:relief-ratio of the interval exercise significantly  
5 affect neuromuscular demands of the session [36], it is somewhat expected that supramaximal  
6 intervals may induce different types of response compared to the intervals of lower intensity.  
7 Wiewelhove et al. [37] reported decreased CMJ performance after sprint interval training, while  
8 aerobic high-intensity training did not induce such an effect. Blood lactate is probably not the main  
9 contributor behind this difference as improved CMJ performance has been observed in the absence  
10 of higher blood lactate values [21] compared to the present study. In general, it is thought that  
11 neuromuscular fatigue after high-intensity exercise is mainly peripheral and caused by contractile  
12 mechanisms disturbances [11, 38], while fatigue induced by longer duration activities are mainly of  
13 central origin observed as decreased voluntary activation [11] or changes in stretch-reflex sensitivity  
14 and muscle stiffness [39]. It is plausible that different mechanisms are behind the CMJ decrease  
15 observed after LIT and SMIT, and the time needed to recover is longer after SMIT, at least in  
16 recreationally trained athletes.

17 All cardiorespiratory, metabolic and perceptual measures during the submaximal running test were  
18 quite similar between the sessions, and the responses were mainly in line with previous studies using  
19 similar types of running protocols [5, 6, 7]. Heart rate and RPE during submaximal running test  
20 increased after all sessions, with a concurrent decrease in RER indicating higher reliance on fat as a  
21 substrate despite the nature of the preceding exercise. Oxygen consumption during submaximal  
22 running test increased slightly, but still significantly only after continuous sessions. This was  
23 somewhat surprising and in contrast to the results reported by previous studies [5, 7]. However, effect  
24 sizes in acute responses remained trivial after all sessions, so any major difference between session  
25 types cannot be stated. Blood lactate during the submaximal running test remained elevated after  
26 SMIT, which was probably mainly the outcome of a higher absolute value after the exercise itself. A  
27 longer recovery period seems to be necessary for lactate clearance after such a session. Increases in  
28 body core temperature and sympathetic nervous system activity along with dehydration and a  
29 decrease in blood volume are likely the main contributors to the cardiovascular responses observed  
30 in the present study [40]. Increased heart rate may also contribute to the impaired running economy  
31 [41]. Muscle glycogen content has been shown to decrease after prolonged as well as high-intensity  
32 exercises [42], and its depletion further influences substrate utilization [43] and oxygen cost during  
33 running [44]. It is plausible that running sessions of the present study did not induce significant

1 differences in the aforementioned factors. The lack of major differences between the sessions in the  
2 submaximal running test could also be related to the time frame of the measurement, as some session-  
3 related effects may have already disappeared at the time point used in the current study (post 18-20  
4 min).

#### 5 24-hour recovery

6 Supine HRV returned to baseline in 24 hours after all sessions. Stanley et al. [15] concluded in their  
7 review, that cardiac autonomic recovery after strenuous exercise may take up to 24-48 hours. The  
8 lack of any differences within or between the sessions at post24 could possibly be related to  
9 methodological differences between nocturnal and daytime recordings. It is well known that HRV is  
10 affected by many external factors especially during the daytime [12], which makes it challenging to  
11 find significant changes in the autonomic modulation caused by a single training session. While  
12 nocturnal HRV has remained suppressed after a moderate and heavy endurance exercise [45],  
13 Niewiadomski et al. [13] measured HRV in the laboratory conditions, and found no change compared  
14 to baseline 24 hours after moderate or high-intensity training sessions.

15 Recently, it has been observed that the recovery of the neuromuscular performance and markers of  
16 muscle damage follow different patterns than HRV after strength training exercise [20]. Similar  
17 observations were found in the present study, as HRV was fully recovered at 24 hours after all  
18 sessions, but CMJ performance after SMIT remained decreased and muscle soreness was apparent  
19 after LIT and SMIT. Although it should be acknowledged that effect size of the pre-post24 change  
20 in CMJ was trivial after all sessions, it is likely that cardiac parasympathetic reactivation after exercise  
21 does not reflect all aspects crucial to recovery in endurance training, such as repletion of the energy  
22 stores and neuromuscular performance [12, 15].

23 All physiological variables measured during the submaximal running test recovered at least to  
24 baseline levels at 24 hours after all exercises with no significant differences between the sessions.  
25 However, increased perceived exertion during the submaximal running test was still apparent after  
26 both interval exercises and muscle soreness were increased after LIT and SMIT. All these changes  
27 took place despite the significantly decreased heart rate in the submaximal running test. In addition  
28 to increased muscle soreness and perceived exertion during the submaximal running test, also CMJ  
29 remained decreased at 24 hours after SMIT, which emphasizes the high neuromuscular demand of  
30 these types of sessions, which is also supported by Wiewelhove et al. [37]. It is possible that in  
31 addition to peripheral factors [11], high mechanical load of the running speed that recreational  
32 endurance athletes may be unaccustomed to further amplified the neuromuscular fatigue and time  
33 needed to recover after SMIT.

1 Like resting HRV, heart rate recordings during exercise could also be affected by multiple external  
2 factors, and a natural day to day variation during submaximal exercise can be up to 3-8 bpm [46].  
3 Taking this into account, it was interesting that the heart rate during submaximal running test  
4 decreased significantly at 24 hours after all sessions by 3-4 bpm. Previously, submaximal running  
5 tests have mainly been studied after intensive training or the competition period, while effects of a  
6 single session have been less examined. Siegl et al. [47] found heart rate decrements of 3 bpm (70  
7  $\%/\dot{V}O_{2\max}$ ) and 2 bpm (85  $\%/\dot{V}O_{2\max}$ ) with a concurrent increase of RPE two days after an  
8 ultramarathon event. Other studies have failed to show any differences in submaximal heart rate 1-4  
9 days after a 30 min high-intensity run [48] or 1-3 days after a 26 km run at an intensity of 80  $\%/\text{HR}_{\max}$   
10 [49]. One possible reason for heart rate decrease during submaximal exercise could relate to an  
11 increase in plasma volume [12], but this variable was not measured in the present study and the actual  
12 reason remains inconclusive.

13 Running economy is an important determinant of endurance performance [41], and a recovery pattern  
14 of the economy would therefore be an important aspect to examine. In the present study, oxygen  
15 consumption as well as RER during the submaximal running test returned to the baseline level at 24  
16 hours after all sessions. This was not surprising though already acute responses in the oxygen  
17 consumption could be stated as trivial after all sessions. In previous studies, running economy has  
18 recovered 24 hours after a 30 min high-intensity run [48], 26 km run at the intensity of 80  $\%/\text{HR}_{\max}$   
19 [49] as well as 48 hours after a marathon [50]. Despite exercise-induced fatigue potentially declining  
20 running economy via multiple mechanisms [41], it seems that metabolic recovery occurs quite  
21 rapidly, and running economy will not likely be impaired in the following day of a single intensive  
22 or prolonged low-intensity training session.

23 Despite clear relationships between maximal and submaximal endurance performance [19] results of  
24 the submaximal tests are sometimes complicated to interpret. Similar changes in submaximal heart  
25 rate can be a sign of a positive training adaptation [19] or negative adaptation indicating  
26 fatigue/overreaching [51]. Similarly, it may be difficult to make assertions relating blood lactate  
27 values during submaximal exercise [51]. In the present study, RPE during the submaximal running  
28 test remained elevated after both interval sessions despite the decreased heart rate and the baseline  
29 level blood lactate values. Because maximal performance was not measured in the present study, it is  
30 difficult to ascertain whether the changes in perceptual markers would be a sign of decreased maximal  
31 performance. However, Marcora and Bosio [52] found that exercise-induced muscle damage and  
32 muscle soreness may impair 30 min maximal time-trial running performance. The authors suggested  
33 that the performance impairing effect might be mediated by the increased sense of effort during the

1 time trial caused by muscle soreness [52]. Therefore, it seems reasonable to assume that higher  
2 perceptual effort at the submaximal level and increased muscle soreness may also have influenced  
3 maximal performance.

#### 4 Study limitations

5 The study population consisted of recreationally endurance-trained men and the results cannot be  
6 straightforwardly transferred to well-trained or elite-athletes. Although the physiological  
7 characteristics of each intensity zone are likely quite universal, more studies are needed to confirm  
8 the findings among high-level athlete populations and in both sexes. Because the follow-up  
9 measurements were not performed later than 24 hours after each session, all variables did not reach  
10 the baseline in all subjects. It could not, therefore, be concluded, what would have been the time  
11 frame to recover for each of the variables. Training sessions of the present study were not matched  
12 for training load but were instead chosen as one representative session type of each intensity zone.  
13 Changing session intensity or duration would possibly influence the results, so further studies are  
14 needed to grow the understanding of how manipulation of these variables would affect responses and  
15 recovery. Lastly, although trying to standardize the testing protocol and days surrounding it, there  
16 remain some aspects that may potentially influence recovery within and between individuals such as  
17 nutrition, sleep, or leisure-time activity.

#### 18 **Conclusions**

19 In conclusion, the results of the present study highlight differences in the physiological demands of  
20 the running sessions performed and distinct recovery patterns following these sessions in the  
21 measured variables of performance and training state. The delay of the parasympathetic reactivation  
22 after endurance exercise seems to relate to the intensity and cardiovascular load of the preceding  
23 session. Running sessions of a long-duration or a supramaximal intensity have high neuromuscular  
24 demands, observed as acute decreases in neuromuscular performance, and increased muscle soreness  
25 24 hours afterwards. Cardiovascular and metabolic recovery occurs rapidly, and these components of  
26 physical performance are not likely compromised 24 hours after a single intensive or a low-intensity  
27 prolonged session. Because subjective markers may give even contradictory results when compared  
28 to the objective measurements, it would be recommended to combine information from different  
29 sources, when estimating the actual recovery state and readiness to train.

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## 20 **Figure legends**

21 Figure 1. Pre, Post and Post24 measurements around each training session. Supine HRV, supine heart  
22 rate variability; SRT, submaximal running test; CMJ, countermovement jump; LIT, low-intensity  
23 session; MOD, moderate-intensity session; HIIT, high-intensity interval session; SMIT,  
24 supramaximal intensity interval session.

25 Figure 2. Mean (black line) and individual values (dots) in the relative changes compared to baseline  
26 in supine lnRMMSD. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$  significant difference compared to the baseline  
27 within session. ### $p < 0.001$ , ## $p < 0.01$ , # $p < 0.05$  significant difference in the relative change from the  
28 baseline between sessions. lnRMSSD, natural logarithm of the square root of the mean sum of the  
29 squared differences. LIT, low-intensity session; MOD, moderate-intensity session; HIIT, high-  
30 intensity interval session; SMIT, supramaximal intensity interval session.

1 Figure 3. Mean (black line) and individual values (dots) in the relative changes compared to baseline  
2 in countermovement jumps.\*\*\*p<0.001, \*\*p<0.01, \*p<0.05 significant difference compared to the  
3 baseline within session. ###p<0.001, ##p<0.01, #p<0.05 significant difference in the relative change  
4 from the baseline between sessions. CMJ, countermovement jump; LIT, low-intensity session; MOD,  
5 moderate-intensity session; HIIT, high-intensity interval session; SMIT, supramaximal intensity  
6 interval session.

7

## 8 **Table legends**

9 Table 1. Basic characteristics of the subjects (n=24)

10 Table 2. Results of four running sessions performed.

11 Table 3. Baseline values (Pre), acute responses (Post), post 24-hour recovery (Post24) and effect  
12 size of the changes (ES) in submaximal running test.

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