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Original article

The effect of fitness level on cardiac autonomic regulation, IL-6, total antioxidant capacity, and muscle damage responses to a single bout of high-intensity interval training

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Abstract

Purpose: The aim of this study was to investigate the influence of the cardiorespiratory fitness level on the response to high-intensity interval training (HIIT) with an individually adjusted running speed of the same relative intensity. The evaluation focused on acute cardiorespiratory response, postexercise cardiac autonomic modulation (heart rate variability (HRV)) and biochemical markers of inflammation, oxidative stress, and muscle damage.

Methods: Thirty participants were divided into 3 subgroups: well trained, moderately trained, and untrained. All the participants performed 30 min HIIT composed of 6 × 2 min interval exercise with work-to-relief ratio = 1 and work intensity 100% of individual velocity at maximal oxygen consumption (VO_{2max}). Acute cardiorespiratory variables, postexercise HRV, lactate, interleukin-6 (IL-6), total antioxidant capacity (TAC), creatine kinase, and myoglobin up to 4 h after HIIT were monitored.

Results: The differences in relatively expressed cardiorespiratory variables (heart rate, VO_2) during HIIT were at most *moderate*, with the most pronounced between-group differences in absolute VO_2 values. The disruption of the postexercise HRV was the most pronounced in untrained individuals, and this difference persisted 1 h after HIIT. The highest postexercise IL-6 and TAC concentrations and the lowest changes in creatine kinase and myoglobin were revealed in well-trained individuals.

Conclusion: The higher fitness level was associated with the less pronounced postexercise cardiac autonomic changes and their faster restoration, even when there were similar acute cardiorespiratory responses. These findings were simultaneously accompanied by the higher postexercise IL-6 and TAC concentrations and less significant changes in muscle damage biochemical markers in well-trained individuals.

Keywords: Creatine kinase; Heart rate variability; Inflammation; Myoglobin; Total antioxidant capacity; Training status

1. Introduction

High-intensity interval training (HIIT) is a method that emphasizes bioenergetics adaptations for more efficient energy transfer within the metabolic pathways by using predetermined intervals of exercise and relief periods.[1] The larger amount of time spent at high exercise intensities is the reason for the exceptional maximal oxygen consumption (VO_{2max}) increase and anaerobic metabolism enhancement after HIIT.[2] The HIIT prescription is a challenging process because a number of factors have to be considered (e.g., duration and intensity of work and relief intervals, or work-to-relief ratio).[3] Simultaneously, the principle of individualization has to be taken into account. Cardiorespiratory fitness certainly plays an important role in acute responses as well as in long-term adaptation to exercise. However, the impact of the fitness level on cardiorespiratory and cardiac autonomic responses after HIIT is less known[3,4] and is therefore the object of investigation presented within this paper.

Heart rate variability (HRV) is considered a tool for cardiac autonomic regulation assessment and provides information about fitness level or readiness to perform, particularly when possible changes in day-to-day HRV data are assessed.[5] HRV has also been shown to be useful in exercise load evaluation[6] or postexercise recovery monitoring.[7] Because cardiac autonomic modulation represents only one part of the complete response to exercise, other aspects possibly associated with fitness level were also considered. Exercise-induced

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inflammation may play an important role in metabolic and musculoskeletal adaptation to exercise,[8] and, therefore, interleukin-6 (IL-6) was analyzed. It has been reported that IL-6 has a beneficial effect on insulin-stimulated glucose disposal and fatty acid oxidation.[9] Whereas there is some evidence that HIIT significantly elicits the postexercise IL-6 response,[10,11] the influence of the fitness level has not been completely established.

Strenuous exercise also causes excessive production of free radicals, which may lead to oxidative stress. This imbalance between production of free radicals and an adequate antioxidant defense is involved in various pathologic states but also has a beneficial role in the immune processes, cellular signaling pathways, and mitogenic response.[12,13] The complex relationship between oxidative stress and exercise is also influenced by mode, intensity, and duration of exercise.[14] Another determinant of exercise-induced oxidative stress can be considered nutrition, particularly antioxidant intake.[13] Oxidative stress is naturally accompanied by the upregulation of endogenous antioxidant defenses. Because a direct relationship between exercise intensity and changes in total antioxidant capacity (TAC) has been previously reported,[15,16] the TAC analysis was included in our consideration.

The high-intensity character of HIIT can potentially lead to muscle fiber impairment, which may be perceived by athletes as delayed-onset pain, soreness, and weakness. This exercise-induced muscle damage is described by certain changes at the cellular level, including disruption of the sarcolemma or sarcotubular system, distortion of the contractile components of myofybrils, cytoskeletal damage, and extracellular matrix changes, and it is consequently manifested by increases in the concentration of intracellular enzymes and muscle proteins such as creatine kinase (CK) and myoglobin (Mb) in plasma.[17,18] The effect of the fitness level on structural muscle damage after individually adjusted HIIT has not been precisely described.

The aim of this study was to investigate the influence of cardiorespiratory fitness level on the response to the HIIT intervention with an individually adjusted running speed of the same relative intensity. The evaluation focused on the acute cardiorespiratory response, postexercise cardiac autonomic modulation and biochemical markers of inflammation, oxidative stress, and muscle damage to provide complete insight into the physiological responses to HIIT. The study results might be of interest to sports researchers as well as athletes looking to properly prescribe the exercise load, because individualization is one of the fundamental prerequisites of an effective training process.[19]

2. Materials and methods

2.1. Participants

Thirty young healthy individuals (Table 1) participated in this study. They were deliberately approached and chosen to match the specification of the study subgroups as well as all other requirements (i.e., no acute or chronic diseases, no smoking, no medication or dietary supplements). The 3 study subgroups were formed as follows: well trained (WT; regular sports training with the aim of preparing for official competitions; endurance or sport games athletes), moderately trained (MT; sports activities at the recreational level, no participation in any official competitions), and untrained (U; no intentional sports activities). The experimental protocol was approved by the Ethics Committee of the Ostrava University and is in accordance with the Declaration of Helsinki. All participants were fully informed about the research requirements in advance and provided their written informed consents.

Table 1
Basic study groups characteristics and preintervention graded exercise test results (mean ± SD).

	WT ($n=11$)	MT ($n=10$)	U ($n=9$)
Age (year)	24.18 ± 1.80	22.60 ± 1.20	24.44 ± 2.54
Height (cm)	180.55 ± 5.66	180.50 ± 5.57	180.89 ± 6.26
Weight (kg)	76.26 ± 6.38	80.14 ± 8.89	80.64 ± 14.41
Body fat (%)	10.45 ± 3.34	13.78 ± 1.94	14.31 ± 5.61
EA (h/week)	12.00 ± 5.89	6.05 ± 2.22	Null
VO_{2max} (mL/kg/min)	61.39 ± 3.63	53.46 ± 2.80	47.21 ± 3.98
vVO_{2max} (km/h)	19.55 ± 1.57	17.44 ± 0.86	14.66 ± 0.82
$v_{inc.t.}$ (km/h)	21.03 ± 1.53	19.26 ± 0.69	16.28 ± 1.15
HR_{max} (bpm)	192.73 ± 4.63	198.90 ± 3.91	196.22 ± 9.11
VT_2 (bpm)	178.09 ± 6.32	177.20 ± 11.62	175.78 ± 12.26
VT_2 (%VO_{2max})	85.27 ± 3.35	80.90 ± 4.12	77.50 ± 7.17
GXT duration (min:s)	12:58 ± 1:13	11:16 ± 0:39	8:06 ± 1:05
RPE (points)	18.00 ± 1.91	18.30 ± 0.78	18.22 ± 0.79

Abbreviations: EA = self-reported intentional exercise activity; GXT = graded exercise test; HR_{max} = maximal heart rate; MT = moderately trained; RPE = Borg's rating of perceived exertion; U = untrained; $v_{inc.t.}$ = peak incremental test speed; $VO_{2\,max}$ = maximal oxygen consumption; $vVO_{2\,max}$ = minimal running speed required to elicit VO_{2max}; VT_2 = second ventilatory threshold; WT = well trained.

2.2. Research design

The participants visited the laboratory on 3 separate occasions at 1−2 week intervals. During this time they performed a maximal treadmill test followed by an interval exercise session and a control session. The sequence of the exercise and control sessions was chosen at random. All sessions were performed in the morning and were conducted by the same researchers in a thermally controlled laboratory.

2.3. Preliminary testing

All the participants were informed about the experimental procedure during the first laboratory visit. At this time they also filled out a short questionnaire about physical activity, acute or chronic diseases, and the use of dietary supplements or medication. An anthropometric assessment and body composition analysis (BC-418 Segmental Body Composition Analyzer; Tanita, Tokyo, Japan) then followed.

To determine the maximum aerobic power (VO_{2max}) and the minimal running speed required to elicit VO_{2max} (vVO_{2max}), participants performed a graded exercise test (GXT) to voluntary exhaustion. The GXT protocol began with a 3 min run at 8 km/h. The speed consequently increased to 12 km/h, following the exercise intensity and increasing by 1 km/h every minute up to voluntary exhaustion. The inclination remained 0. The measurement was interrupted when the participant refused to continue, in spite of verbal encouragement.

Expired air was continuously monitored for an analysis of O_2 and CO_2 concentrations during the GXT by the use of a breath-by-breath system (ZAN 600 Ergo; nSpire Health GmbH, Oberthulba, Germany). Before each test, the gas analyzer was calibrated in accordance with the manufacturer's instructions. The ambient conditions were automatically recorded by a ZAN 600 Ergo and maintained by air conditioning at 21°C. It was declared that the participants had reached their VO_{2max} when at least 2 of the following criteria were met: (1) a plateau in the VO_2 or an increase less than 2.1 mL/kg/min despite the increasing running load; (2) a final respiratory exchange ratio higher than 1.10; or (3) an attainment of 95% of the age-predicted maximal heart rate (HR_{max}). The VO_{2max} was based on the highest average oxygen consumption measured over a 30 s period. Gas-exchange measurements were also used to quantify the second ventilatory threshold (VT_2), which was defined as the point at which ventilation increased disproportionately to oxygen consumption.[19] Apart from the ventilatory curve, the V-slope technique[20] and the respiratory exchange ratio were also considered for the VT_2 determination. The final incremental test speed ($v_{inc.t.}$) reached at the end of the test and at the VO_{2max} were calculated according to Kohn et al.[21] The heart rate (HR) was measured using a Polar Electro chest belt (Polar Electro Oy, Oy, Finland). The perceived exertion was rated on the Borg scale (Borg Rating of Perceived Exertion)[22] immediately after GXT.

2.4. HIIT intervention

The second and third visits to the laboratory consisted of the interval exercise intervention or rest in the case of the control session. Participants always came to the laboratory between 7:00 a.m. and 9:00 a.m. after a night of fasting (i.e., no breakfast was consumed).

Participants performed the HIIT intervention (total duration, 30 min), which consisted of the following parts: (1) 5 min warm-up at a speed of 50% vVO_{2max}; (2) interval exercise: 6×2 min, work-to-relief ratio = 1, work intensity 100% vVO_{2max}, passive 2 min recovery; (3) cool-down: 3 min at 5 km/h. Ventilatory parameters and HR were monitored during the exercise. The perceived exertion was rated on the Borg scale[22] immediately after HIIT. For the control trial, data collection was identical to the HIIT trial, but the exercise intervention was replaced with 30 min of rest.

2.5. Recovery monitoring

The participants remained resting in the laboratory for *post hoc* testing to assess the recovery process. HRV was measured in the supine position before and after the exercise and 1 h, 2 h, 3 h, and 4 h after the exercise intervention. Blood samples were collected in the sitting position from the antecubital vein before and immediately after the exercise and 2 h and 4 h after the exercise intervention.

Fluid and food ingestion during each testing session was standardized. Accordingly, each participant was provided with carbohydrate-rich, low-fat food (plain sponge biscuits, 240 g; 75.0 g carbohydrates, 11.0 g protein, and 4.9 g fat per 100 g; 390 kcal per 100 g) and 1.5 L of sweet mineral water (21.4 kcal per 100 mL).

2.6. HRV analysis

The last 5 min epochs of the 10 min supine resting electrocardiograph were analyzed using VarCor PF8 (Dimea Group Ltd, Olomouc, Czech Republic). This diagnostic system enables a routine short-term HRV evaluation with respect to the Task Force of the European Society of Cardiology[23] findings and recommendations. Electrocardiography was sampled at 1000 Hz, and the accuracy of the measurements was 1 ms. The R-R data were visually validated before analysis (i.e., assessment for stationary, ectopic, or missing data or aberrant beats). Ectopic beats were excluded.

According to Plews et al.'s[24] recommendation, the vagally derived HRV parameter rMSSD (the square root of the mean sum of the squared differences between R-R intervals in milliseconds) was used in this study for postexercise cardiac autonomic modulation assessment. HRV analysis was limited to rMSSD because it reflects vagal activity[23] and has a much greater reliability than other spectral indices,[25] particularly during free-running ambulatory conditions.[26]

2.7. Venous blood sampling and blood analysis

The blood sample was allowed to clot for 30 min and subsequently centrifuged at 2000 *g* for 10 min to separate the serum. The blood serum was consequently divided into three 1-mL aliquots, which were frozen at −70°C until analysis. The S-Monovette system (Sarstedt AG & Co., Nümbrecht, Germany) was used for blood sample collection.

Blood samples were analyzed for high-sensitive IL-6, TAC, CK, Mb, and lactate. The IL-6 concentrations were measured using a high-sensitivity Quantikine ELISA kit (R & D Systems, Minneapolis, MN, USA) on a DSX device (Dynex Technologies Inc., Chantilly, VA, USA). TAC and CK were measured by the AU 2700 device (Beckman Coulter, Inc., Brea, CA, USA). Mb was measured by the Unicel Dxi 800 instrument (Beckman Coulter, Inc.). Lactate concentration was assayed using the enzymatic method and the polychromatic endpoint technique measurement. The analysis of IL-6, TAC, CK, Mb, and lactate revealed intra-assay coefficients of a variation of 4.4%, 4.8%, 5.7%, 3.8%, and 1.66, respectively.

2.8. Statistical analysis

Collected data were checked to detect outliers and to verify sampling distribution (Shapiro-Wilk test; $p < 0.05$). The outliers were removed and not included in the statistical analysis. The data were log-transformed using the natural logarithm if a non-normality or heteroskedasticity was revealed. Data are presented as mean ± SD. The standardized changes in mean (effect size (ES)) and 90% confidence limits (CL) were calculated for the between-group as well as for the between-time points changes. Threshold values for ES statistics were <0.2 (*trivial*), ≥0.2 and <0.6 (*small*), ≥0.6 and <1.2 (*moderate*), ≥1.2 and <2.0 (*large*), ≥2.0 and <4.0 (*very large*), and ≥4.0 (*nearly perfect*). The exact probabilities were expressed, and the magnitude of the difference was also evaluated qualitatively as follows: 25%−75%, *possibly;* 75%−95%, *likely;* 95%−99.5%,

Table 2
Comparison of the GXT results.

	WT *vs.* MT	WT *vs.* U	MT *vs.* U
VO_{2max}	−1.93 (−2.55, −1.30) *Most likely large*	−4.09 (−5.12, −3.07) *Most likely nearly perfect*	−2.12 (−3.23, −1.01) *Very likely very large*
vVO_{2max}	−1.26 (−1.84, −0.67) *Most likely large*	−3.19 (−3.82, −2.57) *Most likely very large*	−2.97 (−3.76, −2.18) *Most likely very large*
$v_{inc.t.}$	−1.11 (−1.66, −0.56) *Very likely moderate*	−3.30 (−4.02, −2.59) *Most likely very large*	−4.35 (−5.55, −3.15) *Most likely nearly perfect*
HR_{max}	1.17 (0.53, 1.82) *Very likely moderate*	0.66 (−0.54, 1.87) *Unclear*	−0.59 (−1.99, 0.80) *Unclear*
VT_2	−1.25 (−2.09, −0.41) *Very likely large*	−2.32 (−3.87, −0.76) *Very likely very large*	−0.79 (−1.97, 0.39) *Unclear*
GXT duration	−1.08 (−1.62, −0.53) *Very likely moderate*	−3.99 (−4.85, −3.14) *Most likely very large*	−5.08 (−6.48, −3.68) *Most likely nearly perfect*

Note: The between-group differences are expressed as standardized (Cohen) difference of the mean (90%CL) and rating of the difference (% chance of higher/trivial/lower differences).
Abbreviations: CL = confidence limits; GXT = graded exercise test; HR_{max} = maximal heart rate; MT = moderately trained; U = untrained; $v_{inc.t.}$ = peak incremental test speed; $VO_{2\,max}$ = maximal oxygen consumption; $vVO_{2\,max}$ = minimal running speed required to elicit $VO_{2\,max}$; VT_2 = second ventilatory threshold; WT = well trained.

very likely; and >99.5%, *most likely*.[27] The smallest worthwhile change or difference is considered 0.2 of the between-individual standard deviation. If the chance of higher or lower differences was >5%, then the true difference was assessed as *unclear*. Statistical analyses were performed using the statistical spreadsheet[28] or SPSS Version 23 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Preintervention GXT

The differences in the GXT variables were mostly *moderate* to *large* between WT and MT groups, *very large* to *nearly perfect* between WT and U groups, and *very large* to *nearly perfect* between MT and U groups (Table 2).

3.2. Acute cardiorespiratory response to HIIT

All participants successfully completed the HIIT trial as prescribed. The standardized between-group differences of the cardiorespiratory response to the HIIT intervention are presented in Table 3. Most of these differences were *unclear* (mean HR and rating of perceived exertion). The between-group differences in the mean VO_2 were *very likely moderate* (WT *vs.* MT), *most likely very large* (WT *vs.* U), and *very likely large* (MT *vs.* U). If VO_2 was expressed as percentage of the individual VO_{2max} with relief intervals excluded, the WT group differed *likely moderately* from the MT and U groups. The HR and VO_2 dynamics can be viewed in Fig. 1.

3.3. Postexercise HRV measures

The square root of the mean sum of the squared differences between R-R intervals (ln rMSSD) values *most likely* decreased after the HIIT intervention in all 3 groups, with the most pronounced decrease in the U group (-3.81 ± 0.74, -3.23 ± 0.75, and -4.54 ± 1.14 for WT, MT, and U, respectively). Ln rMSSD subsequently increased according to the 1 h post exercise observation but remained *very likely largely* (WT; -1.32 ± 0.70), *very likely moderately* (MT; -0.88 ± 0.45), and *most likely very largely* (U; -2.58 ± 0.75) decreased in relationship to the pre-exercise level. The ln rMSSD changes 2 h, 3 h, and 4 h after HIIT were *trivial* to *small* (or *unclear*) in all 3 study

Table 3
The characteristics of the response to the HIIT intervention (mean ± SD) and the between-group differences (warm-up and cool-down excluded).

	WT	MT	U	WT *vs.* MT	WT *vs.* U	MT *vs.* U
Mean HR (bpm)	162.9 ± 9.5	165.3 ± 12.5	166.5 ± 10.8	0.21 (−0.64, 1.06) *Unclear*	0.34 (−0.44, 1.12) *Unclear*	0.10 (−0.58, 0.77) *Unclear*
Mean HR (%HR_{max})	84.5 ± 4.4	83.0 ± 5.1	84.9 ± 4.1	−0.32 (−1.10, 0.46) *Unclear*	0.08 (−0.62, 0.77) *Unclear*	0.33 (−0.32, 0.98) *Unclear*
Mean VO_2 (mL/kg/min)	40.1 ± 3.1	36.6 ± 3.2	32.6 ± 3.3	−1.11 (−1.85, −0.38) *Very likely moderate*	−2.51 (−3.43, −1.59) *Most likely very large*	−1.27 (−2.13, −0.40) *Very likely large*
Mean VO_2 (%VO_{2max})	65.4 ± 4.1	68.7 ± 6.3	68.6 ± 3.1	0.66 (−0.22, 1.54) *Unclear*	0.69 (0.04, 1.35) *Likely moderate*	0.02 (−0.58, 0.62) *Unclear*
Mean VO_2 (%VO_{2max}) *Relief intervals excluded*	76.5 ± 4.2	81.1 ± 7.5	80.3 ± 3.2	0.90 (−0.07, 1.88) *Likely moderate*	0.79 (0.15, 1.44) *Likely moderate*	−0.07 (−0.65, 0.51) *Unclear*
$Lactate_{end}$ (mmol/L)	12.6 ± 3.9	11.6 ± 4.3	13.2 ± 3.4	−0.24 (−0.99, 0.51) *Unclear*	0.13 (−0.55, 0.81) *Unclear*	0.33 (−0.33, 0.98) *Unclear*
Mean RER	0.97 ± 0.03	0.97 ± 0.04	0.96 ± 0.04	−0.04 (−0.82, 0.73) *Unclear*	−0.14 (−0.95, 0.67) *Unclear*	−0.08 (−0.81, 0.66) *Unclear*
RPE	15.6 ± 2.3	17.1 ± 1.2	16.5 ± 1.9	0.62 (0.07, 1.17) *Likely moderate*	0.37 (−0.28, 1.01) *Unclear*	−0.53 (−1.59, 0.54) *Unclear*

Note: The between-group differences are expressed as standardized (Cohen) difference of the mean (90%CL) and rating of the difference.
Abbreviations: CL = confidence limits; HIIT = high-intensity interval exercise; HR = heart rate; $Lactate_{end}$ = lactate concentration immediately after HIIT; MT = moderately trained; RER = respiratory exchange ratio; RPE = rating of perceived exertion; U = untrained; VO_2 = oxygen consumption; WT = well trained.

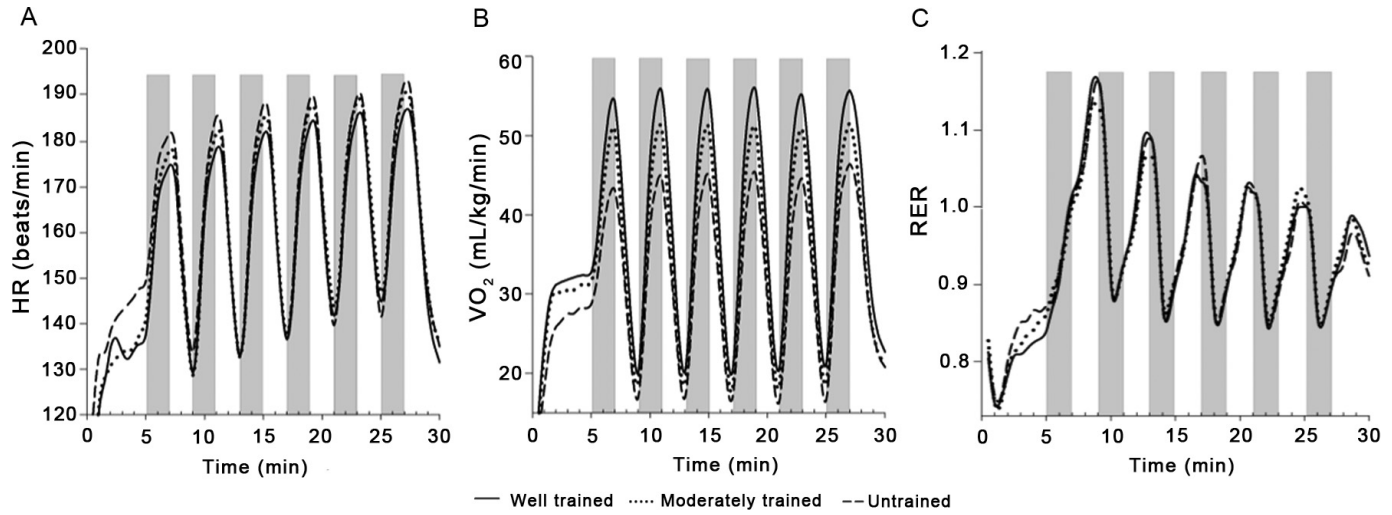

Fig. 1. The HR (A), VO_2 (B), and RER (C) dynamics during the HIIT intervention (mean data). The gray shaded areas highlight the high-intensity intervals. HIIT = high-intersity interval training; HR = heart rate; RER = respiratory exchange ratio; VO_2 = oxygen consumption.

groups (Fig. 2). The ln rMSSD changes in the control trial were mostly *small* or *unclear*.

3.4. IL-6

The post exercise IL-6 concentration increases were *most likely very large* in the WT and MT groups (ES ± 90%CL: 2.64 ± 0.85; 2.79 ± 0.76) and *most likely large* in the U group (1.67 ± 0.65). The IL-6 changes 2 h and 4 h after the exercise intervention were mostly classified as *unclear* (Fig. 3A).

3.5. TAC

There were *most likely very large* (WT: 2.10 ± 0.46) or *large* (1.88 ± 0.43 and 1.60 ± 0.21 for the MT and U groups, respectively) TAC increases immediately postexercise. The *most likely large* increase was monitored for all study groups 2 h after exercise (1.33 ± 0.46, 1.30 ± 0.25, and 1.33 ± 0.27 for WT, MT, and U, respectively), and the *very/most likely moderate* increase was measured at 4 h (1.14 ± 0.35, 0.79 ± 0.48, and 1.14 ± 0.34 for WT, MT, and U, respectively) after the exercise (Fig. 3B).

3.6. CK

The postexercise CK changes were evaluated in all the study groups as *most likely small* (0.33 ± 0.07, 0.48 ± 0.09, and 0.34 ± 0.05 for WT, MT, and U, respectively). These exercise-induced changes remained *likely* or *very likely small* in the MT and U group 2 h (0.42 ± 0.14 and 0.27 ± 0.10 for MT and U, respectively) and 4 h (0.58 ± 0.29 and 0.34 ± 0.11 for MT and U, respectively) after the exercise, whereas the exercise-induced changes in the WT group were *likely trivial* (Fig. 3C).

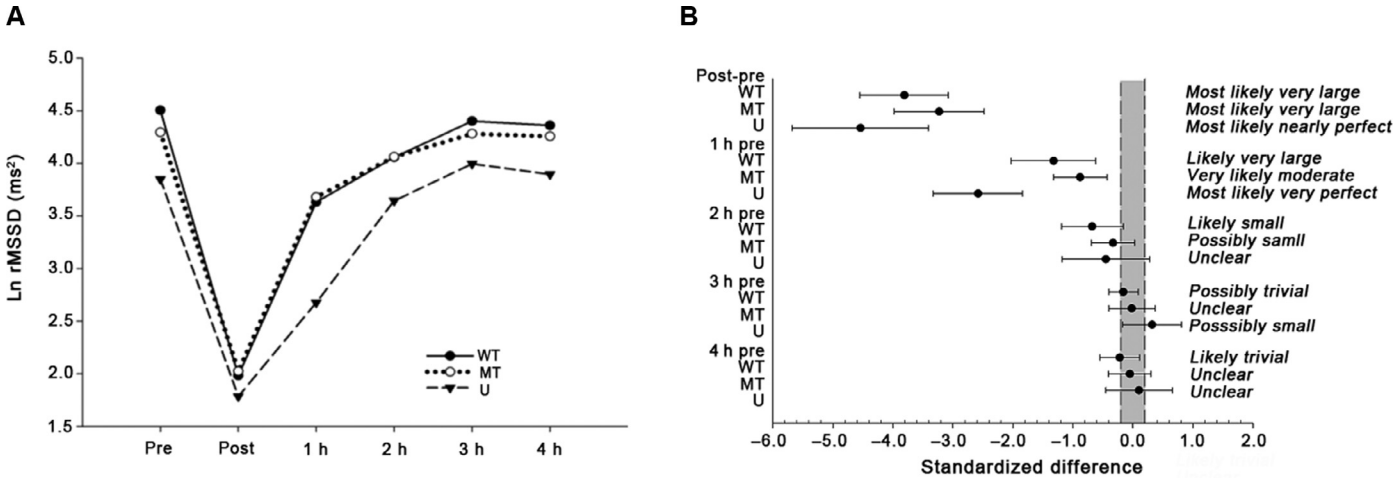

Fig. 2. (A) The ln rMSSD changes after the HIIT intervention (mean); (B) expressed as standardized difference (90%CI) from the pre-exercise values. The gray shaded area indicates the smallest worthwhile change. CI = confidence interval; HIIT = high-intensity interval training; ln rMSSD = square root of the mean sum of the squared differences between R-R intervals; MT = moderately trained; U = untrained; WT = well trained.

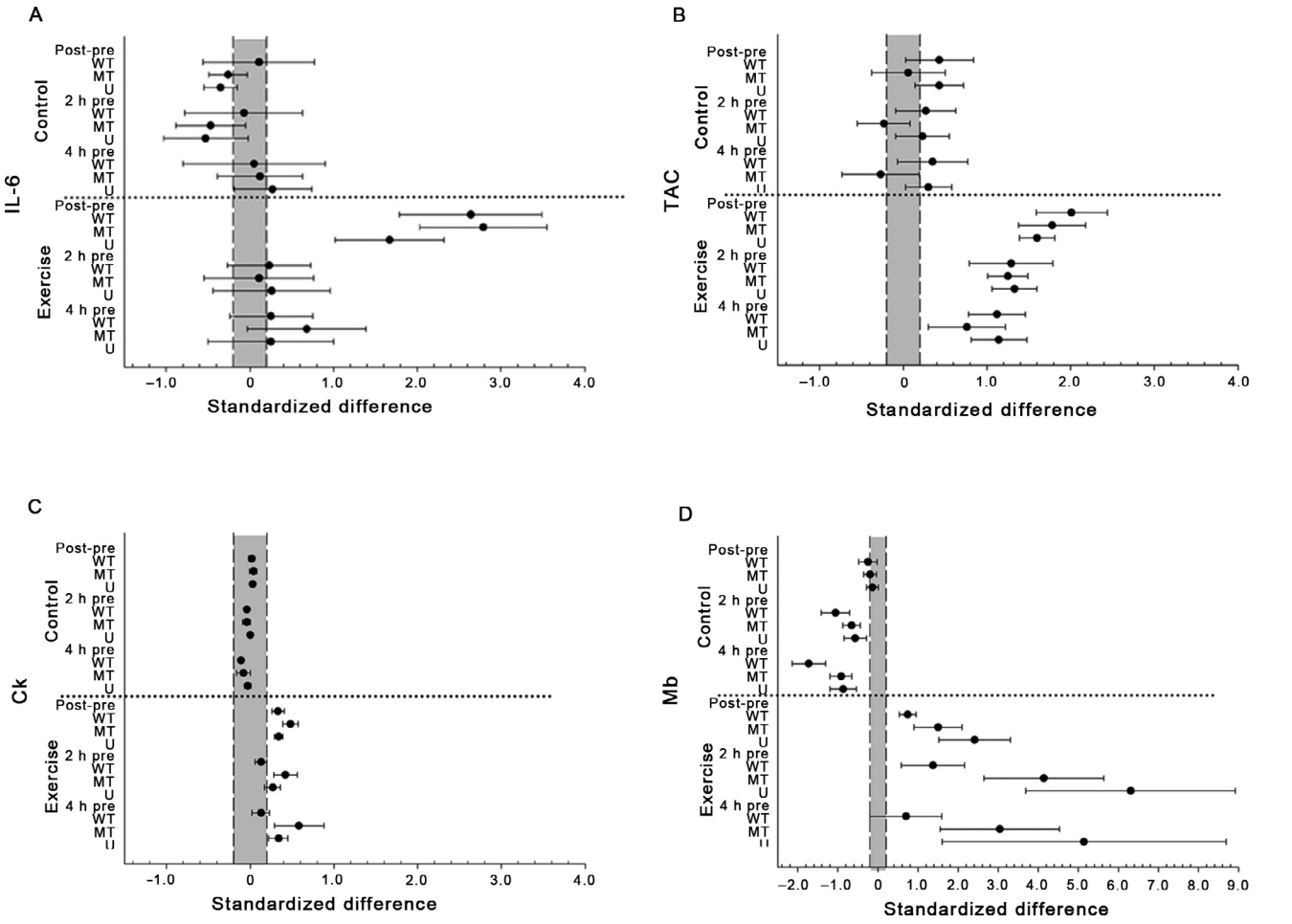

Fig. 3. The IL-6 (A), TAC (B), CK (C), and Mb (D) changes after high-HIIT and control trial expressed as standardized difference (90% confidence interval) from the pre-exercise values. The gray shaded areas indicate the smallest worthwhile changes. CK = creatine kinase; HIIT = high-intensity interval training; IL-6 = interleukin-6; Mb = myoglobin; MT = moderately trained; TAC = total antioxidant capacity; U = untrained; WT = well trained.

3.7. Mb

Mb increased immediately after the exercise *most likely moderately* in the WT group (0.74 ± 0.21), *most likely largely* in the MT group (1.50 ± 0.60), and *most likely very largely* in the U group (2.41 ± 0.89). Mb concentration further increased in 2 h postexercise samplings in all the study groups, with the most pronounced change in the U group (1.37 ± 0.79, 4.14 ± 1.50, and 6.31 ± 2.62 for WT, MT and U, respectively). The Mb concentration changes remained *very likely nearly perfect* 4 h after exercise in the U group (5.14 ± 3.55), whereas the Mb changes were classified as *likely moderate* (0.70 ± 0.89) in the WT group and as *most likely very large* (3.04 ± 1.49) in the MT group (Fig. 3D).

The control trial revealed *unclear* or *trivial* to *small* differences in most of the analyzed biochemical markers (Fig. 3). The absolute values of IL-6, TAC, CK, and Mb are presented in Table 4.

Table 4
Biochemical markers characteristics in response to HIIT (mean ± SD).

	Pre-exercise	Post-exercise	2 h post-exercise	4 h post-exercise
IL-6 (ng/L)				
WT	0.95 ± 0.23	1.62 ± 0.35	1.01 ± 0.35	1.02 ± 0.27
MT	0.94 ± 0.29	1.83 ± 0.60	0.98 ± 0.43	1.17 ± 0.30
U	0.96 ± 0.34	1.61 ± 0.45	1.06 ± 0.30	0.97 ± 0.22
TAC (mmol/L)				
WT	1.64 ± 0.06	1.78 ± 0.09	1.73 ± 0.09	1.72 ± 0.06
MT	1.62 ± 0.06	1.75 ± 0.09	1.71 ± 0.07	1.67 ± 0.09
U	1.61 ± 0.07	1.75 ± 0.07	1.73 ± 0.06	1.70 ± 0.06
CK (μkat/L)				
WT	4.17 ± 1.76	5.11 ± 1.73	4.64 ± 1.71	4.74 ± 1.71
MT	2.71 ± 1.63	3.51 ± 1.60	3.40 ± 1.52	3.73 ± 1.47
U	2.65 ± 1.99	3.44 ± 1.94	3.41 ± 1.94	3.63 ± 1.94
Mb (μg/L)				
WT	30.88 ± 1.36	41.72 ± 1.33	56.71 ± 1.81	45.21 ± 1.82
MT	26.67 ± 1.24	37.84 ± 1.27	70.11 ± 1.68	54.23 ± 1.73
U	29.03 ± 1.10	37.80 ± 1.16	57.87 ± 1.40	50.97 ± 1.60

Abbreviations: CK = creatine kinase; IL-6 = interleukin-6; Mb = myoglobin; MT = moderately trained; TAC = total antioxidant capacity; U = untrained; WT = well trained.

4. Discussion

The presented investigation of the fitness level impact on acute and post exercise responses to HIIT revealed several major findings. First, disruption of the post exercise cardiac autonomic modulation was the most pronounced in the group of untrained individuals, and this difference obviously persisted 1 h after the HIIT intervention. Second, well and moderately trained athletes demonstrated greater post exercise IL-6 and TAC increases than untrained individuals. Third, the CK and Mb increases after HIIT were less pronounced in well trained athletes when compared with moderately trained or untrained individuals. The most pronounced between-group differences in acute cardiorespiratory response were observed in the mean absolute VO_2 values. However, these differences were negligible when mean VO_2 as well as mean HR were expressed relatively (percentage of maximal values). Taking all these facts into consideration, if HIIT is individually adjusted according to fitness level, a similar acute cardiorespiratory response but different exercise-induced physiological changes might be expected after HIIT. These findings can be interpreted to indicate that a training prescription has to take into consideration the fitness level, suggesting the need for some additional exercise loading for well-trained athletes (e.g., longer training duration, more work intervals, higher relative exercise intensity) to result in a similar physiological adaptation.

4.1. High-intensity interval exercise intervention

The fundamental purpose of HIIT was to allow athletes to accumulate a substantially longer time at high intensity close to VO_{2max} than during high-intensity constant-load exercise.[29] The magnitude of the cardiorespiratory load during HIIT can be modulated through the manipulation of a number of variables (e.g., work and relief intensity and duration). HIIT design creation should certainly target the required physiological adaptation. The expected acute physiological effect of HIIT with long intervals, such as employed within this study, can be characterized as increased demands on the cardiopulmonary system and oxidative muscle fibers with a large anaerobic glycolytic energy contribution and a certain degree of neuromuscular load.[3] Fig. 1C shows that the anaerobic glycolytic pathway was clearly stimulated (crossing the 1.0 level) within most of the work intervals in this study. The intergroup differences were negligible, which is an expected response because the exercise intensity was identical. Accordingly, vVO_{2max} was assessed via a GXT before the HIIT prescription to appropriately calibrate the individual exercise intensity. Nevertheless, the *post hoc* data analysis revealed that prescribed vVO_{2max} was slightly different when vVO_{2max} was expressed relative to the peak incremental test speed (WT = 92.96%; MT = 90.55%; U = 91.89%). These exercise intensity differences might, however, be considered negligible, whereas the absolute running speed differed *largely/very largely* between the study groups.

The group of well-trained athletes covered the HIIT intervention with the highest mean VO_2 (mL/kg/min). However, when these values were expressed relative to the individual VO_{2max}, the between-group differences in the mean VO_2 (%) were not as pronounced (Table 3). Of note, when VT_2 is considered, the mean VO_2 was at the VT_2 level in the MT group (100.3% of VT_2) or slightly above VT_2 in the U group (103.6%), whereas the mean VO_2 in the WT group remained below VT_2 (89.7% of VT_2). This fact might explain why the lowest Borg rating evaluation of the HIIT intervention was in the WT group. Therefore, it is apparent that the fitness level has to be taken into consideration in HIIT prescription. For example, a slightly higher relative speed (i.e., more than 100% vVO_{2max}) can be employed for well-trained athletes. The additional finding might also be the fact that the expected maximal cardiorespiratory response (VO_{2max}) was not achieved for a substantial amount of time (Fig. 1 and Tables 1 and 3) despite the high neuromuscular load (running speed). This can be ascribed to too short a duration of work intervals, passive recovery, and/or low work-to-relief ratio.

The HR dynamics during HIIT showed an almost coincident progress in all the study groups (Fig. 1), especially when HR was expressed relatively (Table 3). The between-group HR differences were smaller than the differences in VO_2, and all of them can be classified as *unclear* owing to great interindividual HR variability. The limitation of the use of HR for intermittent exercise has to also be mentioned.[30] Even if the work intervals lasted 2 min (i.e., the shortest duration considered sufficient for the steady-state maintenance),[19] this limitation is likely the reason why the ventilatory parameters corresponded to the exercise intensity changes more closely than did HR.

4.2. Post exercise HRV

Post exercise HRV is considered a marker of exercise intensity because the anaerobic contribution appears to be of primary importance in determining the level of parasympathetic reactivation.[31] The time course of this cardiac autonomic recovery reflects the restoration of cardiovascular homeostasis, which is an important component of overall recovery.[32] The assessment of consequent HRV after exercise might be influenced by several determinants, such as blood pressure regulation, baroreflex activity, or metaboreflex, which drive sympathetic withdrawal and parasympathetic reactivation.[33]

As presented by Stanley et al.,[32] the post exercise suppression of cardiac parasympathetic activity is manifest in individuals at all fitness levels. The fitness level-induced differences are, however, expected in the magnitude of the reduction of this postexercise cardiac parasympathetic modulation as well as in the speed of restoration. Specifically, the higher the fitness level, the lower the post exercise cardiac autonomic suppression and the shorter the time needed for recovery.[34,35]

The post exercise ln rMSSD decrease was the most apparent in untrained participants when compared with the well-trained and moderately trained groups. This difference remained clear 1 h after exercise cessation. The ln rMSSD changes from the pre exercise level were consequently mostly *trivial* to *small* for the monitored period of 2−4 h after exercise in all the study groups. Despite the findings of Seiler et al.,[35] who compared "highly trained" individuals (VO_{2max}: 72 ± 5 mL/kg/min) with "trained" individuals (VO_{2max}: 60 ± 5 mL/kg/min; the equivalent of our well trained group), no substantial differences in post exercise

cardiac autonomic modulation were found between moderately and well trained individuals. This interstudy comparison signifies that the difference in the cardiorespiratory fitness level between the well trained and moderately trained groups would have to be in all probability much more pronounced to cause a substantial difference in postexercise cardiac autonomic restoration.

4.3. IL-6

The exercise-induced IL-6 increase and its direct relationships to exercise intensity and duration have been previously observed.[36] Study results concerning the impact of training status are far more inconclusive.[37] For example, the IL-6 response to acute exercise was blunted after 6 weeks of HIIT in a study by Croft et al.[38] This conclusion is, however, expected because Croft et al.[38] used an exercise at the same absolute intensity, and thereby the relative intensity decreased. When the same relative intensity was employed for the assessment of impact of a certain training program on the IL-6 response to exercise, plasma IL-6 release was reduced[39] or unchanged.[40]

In contrast to these longitudinal studies, the presented controlled cross-sectional data revealed that the post exercise IL-6 increase was the most substantial in well trained and moderately trained athletes when compared with untrained individuals. This might be explained by the fact that apart from certain proinflammatory effects, IL-6 possibly plays an important role in skeletal muscle metabolism, including glucose homeostasis maintenance and activation of glycogenolysis in the liver and lipolysis in adipose tissue, with the aim of providing muscle with the increased energy demanded during exercise.[37] All these effects could be considered a demonstration of enhanced adaptation to exercise and therefore associated with a higher fitness level. It follows that the evidence of the beneficial effect of IL-6 in response to exercise might be supported by the presented results.

4.4. TAC

Increased TAC after exercise, as well as the close relationship between exercise-induced oxidative stress and production of antioxidant species, is a consistent finding in scientific research.[16] Similarly to the IL-6 response, TAC increased *most likely* in all study groups, with the most substantial TAC increase observed in the well trained individuals. Unfortunately, the study protocol does not enable an assessment of the genuine cause of this fact because the oxidative stress was not directly measured. A definitive conclusion cannot, therefore, be made. The most pronounced TAC increase in well trained athletes can be attributed to both (1) the exercise intervention, which caused the greatest oxidative stress in well trained individuals, and (2) the antioxidant defense *per se*, which is developed the most in well trained individuals. However, growing evidence exists that high-intensity exercise, particularly interval training, may induce beneficial redox homeostasis alterations (including TAC enhancement) and greater health benefits than low- to moderate-intensity continuous exercise.[41,42]

4.5. Muscle damage markers

Muscle damage naturally accompanies strenuous or unaccustomed exercise. Its severity depends on multiple factors, such as the type of contractions[43] or the exercise intensity.[44] Mechanical deformation of muscle fibers may initiate specific adaptation to exercise.[45] This hypothesis has been, however, challenged because muscle rebuilding (e.g., hypertrophy) can be initiated without any discernible damage.[46]

The exercise-induced changes in CK were *trivial* to *small* up to 4 h after HIIT. This is not surprising because the peak CK activity was previously observed several days after exercise.[47] The only between-group difference within the monitored postexercise period can be seen in the direction of change development (i.e., a downward trend in well trained individuals), whereas the CK level in moderately trained and untrained individuals remained constant.

More noticeable post exercise changes were observed in Mb, particularly in moderately trained and untrained individuals 2 h and 4 h after the HIIT cessation. The lowest Mb response was observed in well trained athletes. Taking all these facts together, the exercise-induced muscle damage indirectly assessed by the postexercise CK and Mb changes was the lowest in well trained athletes, as expected in all probability owing to their enhanced adaptation to exercise.

5. Conclusion

The presented controlled cross-sectional study highlights the importance of fitness level in assessing for the HIIT prescription and expected exercise-induced response. Despite the running speed having been relatively identical and individually adjusted to the current training status, substantial post exercise between-group differences in various physiological variables were observed, and therefore a different exercise-induced adaptation as well as recovery course might be expected. The higher fitness level was associated with the less pronounced postexercise cardiac autonomic changes and their faster restoration, even when there were similar acute cardiorespiratory responses to HIIT among the study groups. These findings were simultaneously accompanied by the higher postexercise IL-6 and TAC concentrations and less significant changes in muscle damage biochemical markers in well trained individuals.

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Competing interests

The author declares that he has no competing interest.

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