Bout duration in high-intensity interval exercise modifies hematologic, metabolic and antioxidant responses

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ABSTRACT

Objective: This study compared hematologic, metabolic and antioxidant responses between three high-intensity interval exercise (HIIE) trials of different bout duration and a continuous exercise trial (CON), all with equal average intensity, total work, and duration.
Methods: Eleven healthy young males performed four trials involving 20 min of cycling, either continuously (49% of power at VO_2max, PPO), or intermittently with 48 10-s bouts (HIIE10), 16 30-s bouts (HIIE30) or 8 60-s bouts (HIIE60) at 100% PPO, with a 1:1.5 work-to-recovery ratio at 15% PPO. Venous blood was obtained before, immediately after, and 1 h post-exercise to evaluate hematologic, metabolic and antioxidant responses. Blood lactate concentration was measured in capillary blood during exercise, while urine lactate was measured before and 1 h post-exercise.
Results: Post-exercise leukocyte count (mean $\pm$ SD; 9.7 $\pm$ 2.8 k μL^{-1}), uric acid concentration (0.35 $\pm$ 0.10 mmol L^{-1}), glucose concentration (6.56 $\pm$ 1.44 mmol L^{-1}), and plasma volume change ($-13.5 \pm 4.4\%$) were greater in HIIE60 compared to all other trials ($p < 0.05$). One-hour post-exercise, lymphocytes decreased below pre-exercise values in all HIIE trials, and uric acid increased in the HIIE60 trial ($p < 0.05$). Urine lactate concentration 1 h post-exercise increased compared to pre-exercise only in HIIE60 (19-fold, $p < 0.001$), and this was related with the higher blood lactate concentration during exercise in that trial.
Conclusions: These findings highlight the importance of bout duration, given that shorter bouts of HIIE (30 s or 10 s) induce lower blood cell perturbations, metabolic stress, and antioxidant responses compared to the commonly used 1-min bouts, despite equal total work, duration, and work-to-recovery ratio.

1. Introduction

High-intensity interval exercise, either in the form of all-out repeated 30-s sprints (i.e., sprint interval training, or SIT) or in the form of longer (1—4 min) repeated bouts at intensities around VO_2max (HIIE), is widely used as a time-efficient and effective training strategy for improvement of exercise performance and

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enhancement of overall health.[1–4] Some previous studies have shown that an acute session of SIT or HIIE induces large alterations on hematologic profile, redox status and metabolic indices.[5–8] However, few studies have attempted to compare the effects of different HIIE protocols on these responses,[6,7] while there is little or no information for HIIE performed at an intensity around VO₂max and bout durations less than 1 min.[9,10] It is known that strenuous exercise may cause a temporary decrease in immune cell function, creating a potential "open window" for infection.[11,12] A shift in the balance between reactive oxygen and nitrogen species, oxidant biomarkers, and antioxidants[13–15] may also contribute to exercise-induced modifications of immune cell function.[16] Several studies have shown that, as the intensity and/or duration of exercise increase, so do perturbations in redox homeostasis.[17,18]

Due to the large number of variables that define a HIIE protocol, this type of exercise may elicit widely different responses.[4,19–21] Several studies have used a 10 x 1-min protocol, performed at an intensity near or at VO₂max,[2,21,22] and suggested it may be used as an alternative and more tolerable regimen than all-out sprint protocols.[23] However, little is known about the acute effects of a 10 x 1-min protocol on immune responses, with most data having been obtained during SIT. For example, a greater acute-phase leukocyte count and redox response has been reported following low-volume SIT (4 × 30 s sprints separated by 4 min of recovery), compared with 30 min of continuous cycling at 70% of peak oxygen uptake (VO₂max).[7] Another study comparing short-term (i.e., up to 3 h post-exercise) perturbations in circulating leukocytes and their subfractions (lymphocytes and neutrophils) between two HIIE protocols (4 × 30 s and 4 × 4 min) showed greater changes after the former.[9]

Antioxidant responses to high-intensity exercise have been examined in a limited number of studies, which mostly used SIT.[7,14,24] These studies showed that SIT altered blood redox status, inducing increases in antioxidants, such as uric acid[7,25] and total antioxidant capacity (TAC),[7,14] while the few HIIE studies showed a decrease in glutathione (GSH).[16] The increasing use of HIIE instead of SIT, which involves very high metabolic stress,[26–28] makes it necessary to further explore the acute effects of HIIE on immune and antioxidant markers, especially when comparing bouts lasting less than 1 min, about which there is lack of information. This is because it has recently been shown that bout duration in HIIE modifies blood lactate responses and exercise severity.[10,29] Thus, it may be hypothesized that a reduction in metabolic perturbations, as reflected in blood lactate and glucose responses[26–28,30] and plasma volume shifts,[31] may impact immune and antioxidant responses[13,32] due to their dependence on metabolic stress. Therefore, the aim of the present study was to compare the hematologic, metabolic, and antioxidant responses between three HIIEs of different bout duration (10 s, 30 s, 1 min), but equal total work and work-to-recovery ratio, and continuous exercise.

2. Methods

2.1. Participants

Eleven recreationally active healthy males (age, 28 ± 6 y; height, 1.77 ± 0.07 m; body mass, 70.4 ± 10.6 kg; VO₂max, 47.1 ± 6.6 ml·kg⁻¹·min⁻¹; mean ± SD) took part in this study after providing written informed consent and following medical screening. The study was approved by the Aretaieion Hospital Ethics Committee (B-153/04-02-2016) and was conducted in accordance with the Code of Ethics of the World Medical Association (Helsinki declaration of 1964, as revised in 2013).

An a priori power analysis using G*Power (version 3.1.9.7; Kiel University, Kiel, Germany)[33] provided a sample size of 8 as necessary based on a power of 0.80, alpha of 0.05, and correlation coefficient of 0.5 between repeated measures. In relevant studies,[7,14] the effect size regarding hematological and immune parameters ranged between medium and large. We therefore opted to use a medium effect size in the a priori power analysis for all parameters examined (partial eta squared, or η^2, of 0.137).

2.2. Experimental design

A repeated-measures design was used to examine the hematologic, metabolic, and antioxidant responses to interval and continuous exercise trials. Participants completed two familiarization sessions, followed by a session for anthropometric measurements and VO₂max assessment. Four to seven days afterwards, participants performed four 20-min cycling trials of equal mean power output [49% of power at VO₂peak (PPO)], placed one week apart in random, counterbalanced order. Participants cycled either continuously (CON) at an intensity corresponding to 49% of PPO or intermittently performing 48 × 10-s (HIIE10), 16 × 30-s (HIIE30), or 8 × 60-s bouts (HIIE60) at 100% PPO. Recovery intervals during the HIIE trials were 15, 45 and 90 s, respectively (work-to-recovery ratio of 1–1.5) and included cycling at 15% of PPO. In each trial, capillary blood lactate concentration (BLa) was measured before and during exercise, while venous blood was obtained before, immediately after, and 1 h after exercise to measure hematologic, metabolic, and antioxidant indices. Finally, urine was collected before and 1 h post-exercise for lactate measurement.

2.3. Preliminary tests and familiarization

Participants took part in four preliminary testing and familiarization sessions. The first session included 10 min of continuous (up to 80% of maximal predicted heart rate, HR) and 8 min of interval cycling exercise (30 s at 80–90% of predicted PPO and 30 s at 30 W). In the second session, body height, body mass and VO₂max were measured. VO₂max was measured using an incremental test to volitional exhaustion on an electronically braked cycle ergometer (Ergo bike premium 8, Daum electronic Gmbh, Fürth, Germany), during which power output was increased by 20–30 W per minute. Pedal cadence was set to 70 rpm. Gas exchange was measured breath-by-breath (MedGraphics ULTIMA, Metabolic System, MedGraphics Corporation, St. Paul, MN, USA), and respiratory data were averaged every 10 s VO₂max represented the mean of the highest two VO₂ measures. Peak power output (PPO) was defined as the average power output corresponding to the time interval at which VO₂max was attained. HR was continuously measured by telemetry (Polar S410, Kempele, Finland) and averaged every 5 s. The last two sessions included a submaximal graded cycling test and a 9-min HIIE bout for familiarization with HIIE.

2.4. Experimental trials

Participants were instructed to abstain from alcohol consumption, caffeine consumption, and any exercise for 24 h before each of the four experimental trials. They also recorded their habitual diet for the 24 h preceding the first trial and were asked to replicate it on the day before each subsequent trial.

For each trial, participants reported to the laboratory between 7:30 and 8:30 a.m., in a hydrated state (having drunk 0.5 L of water during the last half hour) and after an overnight fast. Each trial started with a 3-min warm-up at 15% PPO, followed by 3 min of rest. Then participants completed 20 min of either continuous or intermittent cycling at a constant cadence of 70 rpm. During the HIIE trials, power was alternated automatically by increasing or decreasing the resistance applied by the cycle ergometer. BLa was

measured before and every 5 min during exercise using a Lactate Scout + portable analyzer (EKF Diagnostics, SensLab GmbH, Leipzig, Germany), and the BLa incremental area under curve (IAUC) was calculated by applying the trapezoid rule, ignoring the area beneath resting BLa.

2.5. Blood and urine sampling and analyses

Three 10-mL samples were obtained from an antecubital vein at rest, immediately post-exercise and 1 h later. All blood samples were taken with the participant sitting for at least 15 min. Samples were allowed to clot at room temperature and were then centrifuged at 1500 *g* and 4 °C for 15 min to obtain serum. Part of the serum was used immediately for glucose determination in an ARCHITECT-ci8200 analyzer (Abbott Diagnostics Laboratories, Abbott Park, IL; Abbott 65205, Wiesbaden, Germany), while the rest was separated in multiple aliquots and frozen at −80 °C for other analyses. Another 2.5 ml of blood were obtained as above in tubes containing EDTA for full blood count, which was immediately performed using an Abbot Cell-Dyn 3700 Hematology Analyzer, and GSH measurement, which was performed later in an aliquot kept at −80 °C as described below.

Plasma volume changes (PVC) from baseline to immediately after and 1 h post-exercise were calculated according to Dill and Costill (1974)[34] using hematocrit and hemoglobin values. Serum insulin was measured on an Immulite 2000 analyzer (Siemens Healthcare Diagnostics Products Ltd., UK) using two-site chemiluminescent immunometric assay with analytical sensitivity of 2 μIU/mL. Uric acid was measured spectrophotometrically using a kit from Spinreact (Girona, Spain). GSH was measured spectrophotometrically in blood lysates as described.[35] Hemoglobin was also measured in the lysates with a kit from Spinreact (Girona, Spain) in order to express GSH per gram of hemoglobin. TAC was measured according to Janaszewska and Bartosz (2002)[36] on the basis of the scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH).

For urine lactate (ULa) determination, participants emptied their bladders into plastic beakers before and 1 h after each trial (which was necessary to maximize the urine lactate concentration)[37] after having taken 0.5 L of water within 30 min of the end of the trial to ensure reliable measurements.[38] ULa was measured as previously described.[38]

2.6. Statistical analyses

The Shapiro–Wilk test was used to assess normality, and all variables were found to be normally distributed. Data were analyzed using two-way ANOVA with repeated measures (trial x time), except for BLa IAUC, was analyzed using one-way ANOVA with repeated measures. When a significant interaction or main effect was observed, Tukey's post-hoc test was used to locate differences between means. η^2 values were used to estimate effect sizes (small, 0.01 to 0.059; moderate, 0.06 to 0.137; large, > 0.137). All statistical analyses were performed using SPSS (IBM SPSS Statistics version 23). Data are presented as mean ± standard deviation. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Metabolic responses

Metabolic responses are shown in Fig. 1. A trial effect ($p = 0.020$, $\eta^2 = 0.27$), time effect ($p < 0.001$, $\eta^2 = 0.58$) and trial × time interaction ($p < 0.001$, $\eta^2 = 0.52$) were observed for glucose. Post-hoc tests revealed that glucose increased post-exercise only in HIIE30 and HIIE60 compared to baseline ($p < 0.05$) and returned to
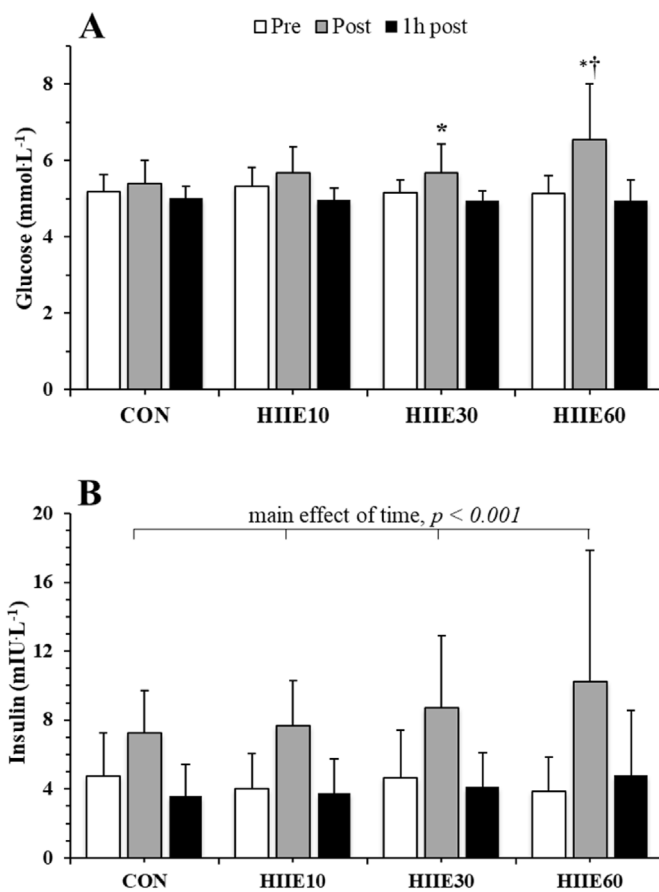

Fig. 1. Serum glucose (A) and insulin (B) before (Pre), immediately after (Post) and 1 h post-exercise (1h post). *: different from other means in the same trial ($p < 0.05$); †: different from the same time point in all other trials ($p < 0.001$).

baseline 1 h later ($p < 0.001$). The increase in HIIE60 was greater than in all other trials ($p < 0.001$). Additionally, a time effect ($p < 0.001$, $\eta^2 = 0.67$) was found for insulin, due to a post-exercise increase.

BLa IAUC is presented in Fig. 2A. BLa IAUC was higher in HIIE60 than in all other trials ($p < 0.001$), while, in HIIE10 and HIIE30, it was higher than in CON ($p < 0.05$). ULa (Fig. 2B) showed a significant trial effect ($p < 0.001$, $\eta^2 = 0.46$), time effect ($p = 0.009$, $\eta^2 = 0.50$) and trial × time interaction ($p < 0.001$, $\eta^2 = 0.47$). Post-hoc tests showed that ULa increased only in HIIE60 (about 19-fold increase, $p < 0.001$). The relationship between BLa IAUC and change in ULa was exponential (Fig. 2C), with a high coefficient of determination ($R^2 = 0.9993$), suggesting the existence of a threshold above which lactate from the circulation is excreted by the kidney.

3.2. Hematologic responses

Table 1 shows hematologic responses to each trial. Two-way ANOVA for leukocyte count revealed a significant time effect ($p < 0.001$, $\eta^2 = 0.76$) and trial × time interaction ($p < 0.001$, $\eta^2 = 0.47$). Post-hoc tests showed that leukocyte count increased post-exercise and returned to baseline 1 h later in all trials ($p < 0.05$). The increase in HIIE60 (from 6.1 ± 1.6 to 9.7 ± 2.6 k/μL) was greater than in all other trials ($p < 0.05$).

A time effect ($p < 0.001$, $\eta^2 = 0.66$) was observed for neutrophil count. Post-hoc tests revealed an elevation from baseline immediately post-exercise ($p < 0.001$) and a reduction 1 h post exercise ($p = 0.034$) without reaching baseline ($p = 0.006$). Main effects of
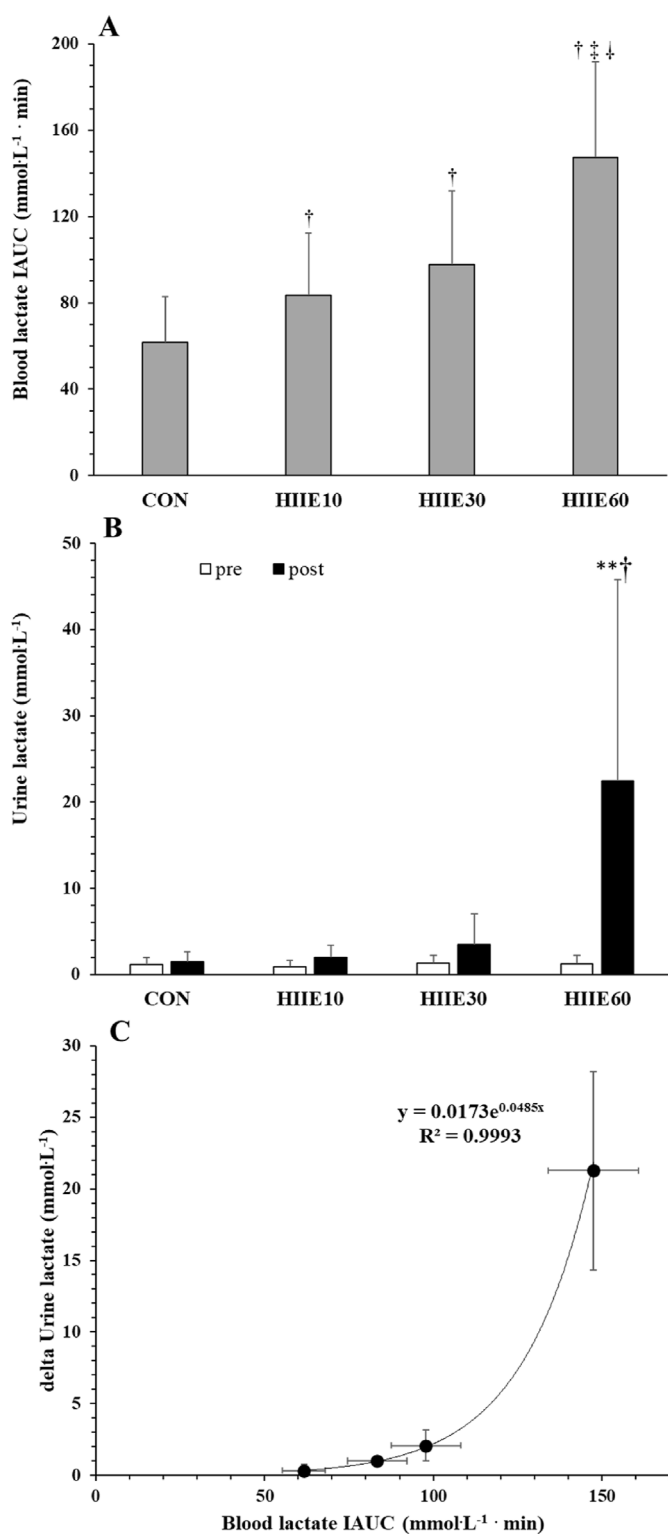
Fig. 2. Blood lactate incremental area under the curve (IAUC) during exercise (A), urine lactate pre- and 1-h post-exercise (B), and relationship between blood lactate IUC and change in urine lactate (C). **: $p < 0.001$ from pre-exercise in the same trial; †: different from the 1-h post exercise time point in all other trials ($p < 0.001$).

trial ($p = 0.002$, $\eta^2 = 0.38$) and time ($p < 0.001$, $\eta^2 = 0.77$) and a trial × time interaction ($p < 0.001$, $\eta^2 = 0.62$) were observed for lymphocyte count. Post-hoc tests showed it to be increased immediately post-exercise in HIIE30 and HIIE60 ($p < 0.001$), with

the increase in HIIE60 being higher than in all other trials ($p < 0.001$). Furthermore, lymphocyte count was reduced 1 h post-exercise compared to baseline in all HIIE trials ($p < 0.05$, Table 1). A time effect ($p < 0.001$, $\eta^2 = 0.71$) and trial × time interaction ($p = 0.002$, $\eta^2 = 0.28$) were observed for monocyte count. Post-hoc tests showed an increase only in HIIE60 immediately post-exercise ($p < 0.001$), which was higher that on CON ($p = 0.011$) and HIIE10 ($p = 0.007$). Monocyte count was reduced 1 h post-exercise, reaching baseline in all HIIE trials ($p < 0.05$). A time effect ($p < 0.001$, $\eta^2 = 0.84$) and trial × time interaction ($p = 0.016$, $\eta^2 = 0.22$) were observed for platelet count. Post-hoc tests showed that it increased post-exercise and returned to baseline 1 h later in all trials ($p < 0.05$). In addition, post-exercise platelet count in HIIE30 and HIIE60 was higher than in CON ($p < 0.05$).

PVC exhibited a trial effect ($p < 0.001$, $\eta^2 = 0.93$), time effect ($p < 0.001$, $\eta^2 = 0.52$) and trial × time interaction ($p < 0.001$, $\eta^2 = 0.68$). Post-hoc tests showed greater PVC after all HIIE protocols than after CON ($p < 0.001$). In addition, post-exercise PCV was greater in HIIE60 compared to all other trials ($p < 0.001$). Plasma volume returned to baseline in all trials 1 h post-exercise (Table 1).

3.3. Antioxidant responses

Antioxidant responses are presented in Table 2. The two-way ANOVA for uric acid revealed a trial effect ($p = 0.011$, $\eta^2 = 0.30$), time effect ($p < 0.001$, $\eta^2 = 0.54$) and trial × time interaction ($p < 0.001$, $\eta^2 = 0.42$). Post-hoc tests showed that uric acid increased 1 h post-exercise compared to pre- and post-exercise in HIIE60 ($p < 0.001$), with this value being greater than in CON and HIIE10 ($p < 0.001$). GSH and TAC showed no significant main effects or interaction.

4. Discussion

The main and novel finding of the present study was that reducing the exercise bout duration below 1 min while maintaining total work, duration, and work-to-recovery ratio decreased the metabolic strain, as reflected by lower circulating glucose, lactate and plasma volume change, compared with the longer HIIE bouts. These reduced metabolic disturbances were accompanied by lower uric acid concentrations and leukocyte cell changes.

Even though total work, intensity and total exercise and recovery duration were equal between the three HIIE trials, metabolic stress, as indicated by BLa IAUC, was 44% lower in HIIE10 than HIIE60 (Fig. 2A). This may imply a lower contribution from anaerobic carbohydrate breakdown as bout duration is decreased from 60 s to 30 s–10 s, which is in agreement with the findings of a recent study using bilateral knee extension performed in a magnetic-resonance scanner.[39] Also indicative of the lower metabolic stress when bout duration was reduced to 10 s were the serum glucose responses, which were increased from baseline only in HIIE30 and HIIE60, but not in HIIE10, possibly due to a greater hormonal response to the higher metabolic stress.[30,40] A study that compared two protocols of intermittent submaximal exercise with identical treadmill speed, work-to-recovery ratio and total work, but different bout duration (6 vs. 24 s), showed that longer bouts accelerated carbohydrate metabolism, increased plasma lactate and reduced muscle oxygen availability, as compared with shorter bouts.[41] Therefore, shorter bouts, such as in HIIE10, attenuate metabolic disturbances, and this should be taken into account when HIIE is prescribed to healthy individuals and patients such as type 2 diabetics. On the other hand, the higher metabolic stress and glucose perturbation observed in HIIE30 and HIIE60 could be a more potent stimulus for adaptations, compared to protocols of

Table 1
Hematologic responses before (Pre), immediately after (Post), and 1 h post exercise (1 h post) in each trial. Data are mean $\pm$ SD.

Trial	Time	Leukocyte count (k·μL^{-1})	Neutrophil count (k·μL^{-1})	Lymphocyte count (k·μL^{-1})	Monocyte count (k·μL^{-1})	Platelet count (k·μL^{-1})	PVC (%)
CON	**Pre**	6.3 $\pm$ 2.2	3.5 $\pm$ 1.4	2.2 $\pm$ 0.8	0.4 $\pm$ 0.2	211 $\pm$ 53	
	Post	7.8 $\pm$ 2.7*	4.5 $\pm$ 2*	2.5 $\pm$ 0.6	0.5 $\pm$ 0.2	243 $\pm$ 61	−4.5 $\pm$ 1.7
	1 h post	6.7 $\pm$ 2.9#	4.2 $\pm$ 2.4*#	1.9 $\pm$ 0.5#	0.4 $\pm$ 0.2	207 $\pm$ 50	0.0 $\pm$ 2.8
HIIE10	**Pre**	6.3 $\pm$ 1.8	3.2 $\pm$ 1	2.4 $\pm$ 0.7	0.4 $\pm$ 0.2	205 $\pm$ 56	
	Post	7.8 $\pm$ 2.6*	4.2 $\pm$ 1.7*	2.8 $\pm$ 0.9	0.5 $\pm$ 0.2	253 $\pm$ 69	−8.7 $\pm$ 3.4†
	1 h post	5.9 $\pm$ 1.6#	3.5 $\pm$ 1.3*#	1.7 $\pm$ 0.5*#	0.4 $\pm$ 0.1#	201 $\pm$ 51#	0.1 $\pm$ 2.2#
HIIE30	**Pre**	6.6 $\pm$ 2.0	3.5 $\pm$ 1.2	2.4 $\pm$ 0.8	0.4 $\pm$ 0.1	208 $\pm$ 53	
	Post	8.4 $\pm$ 2.0*	4.6 $\pm$ 1.3*	3.0 $\pm$ 0.9*†	0.5 $\pm$ 0.1	259 $\pm$ 69	−9.7 $\pm$ 3.6†
	1 h post	6.8 $\pm$ 2.0#	4.4 $\pm$ 1.6*#	1.8 $\pm$ 0.6*#	0.4 $\pm$ 0.1#	207 $\pm$ 52	−0.2 $\pm$ 2.9#
HIIE60	**Pre**	6.1 $\pm$ 1.6	3.2 $\pm$ 1	2.3 $\pm$ 0.6	0.4 $\pm$ 0.1	205 $\pm$ 56	
	Post	9.7 $\pm$ 2.6*†‡ a	4.7 $\pm$ 1.8	4.0 $\pm$ 0.9*†‡ a	0.6 $\pm$ 0.1*†‡	262 $\pm$ 60	−13.9 $\pm$ 4.1†‡ a
	1 h post	6.1 $\pm$ 1.8#	3.9 $\pm$ 1.5*#	1.7 $\pm$ 0.6*#	0.4 $\pm$ 0.2#	207 $\pm$ 57	0.3 $\pm$ 2.9#
Interaction		$p < 0.001$	$p = 0.118$	$p < 0.001$	$p = 0.002$	$p = 0.016$	

PVC: plsma volume change.
*$p < 0.05$ from pre-exercise in the same trial.
#$p < 0.05$ from post-exercise in the same trial.
†$p < 0.05$ from the corresponding value in CON.
‡$p < 0.05$ from the corresponding value in HIIE10.
a$p < 0.05$ from the corresponding value in HIIE30.

Table 2
Antioxidant responses before (Pre), immediately after (Post), and 1 h post exercise (1h post) in each trial. Data are mean $\pm$ SD.

Trial	Time	Uric acid (mmol·L^{-1})	GSH (μmol/g Hb)	TAC (mmol DPPH·L^{-1})
CON	**Pre**	0.27 $\pm$ 0.06	2.85 $\pm$ 1.02	0.84 $\pm$ 0.06
	Post	0.27 $\pm$ 0.07	3.24 $\pm$ 1.82	0.81 $\pm$ 0.11
	1 h post	0.27 $\pm$ 0.07	2.99 $\pm$ 1.16	0.80 $\pm$ 0.13
HIIE10	**Pre**	0.27 $\pm$ 0.06	3.64 $\pm$ 2.10	0.82 $\pm$ 0.12
	Post	0.28 $\pm$ 0.06	3.08 $\pm$ 1.64	0.83 $\pm$ 0.11
	1 h post	0.29 $\pm$ 0.06	3.51 $\pm$ 1.64	0.84 $\pm$ 0.11
HIIE30	**Pre**	0.29 $\pm$ 0.06	3.08 $\pm$ 1.26	0.82 $\pm$ 0.11
	Post	0.29 $\pm$ 0.06	2.57 $\pm$ 1.24	0.90 $\pm$ 0.13
	1 h post	0.32 $\pm$ 0.08	3.43 $\pm$ 1.60	0.88 $\pm$ 0.11
HIIE60	**Pre**	0.26 $\pm$ 0.06	3.07 $\pm$ 1.13	0.84 $\pm$ 0.11
	Post	0.27 $\pm$ 0.06	2.51 $\pm$ 0.77	0.85 $\pm$ 0.07
	1 h post	0.35 $\pm$ 0.10*#†‡	3.64 $\pm$ 2.77	0.84 $\pm$ 0.10
Interaction		$p < 0.001$	$p = 0.705$	$p = 0.101$

GSH: glutathione; TAC: total antioxidant capacity.
*$p < 0.001$ from pre-exercise in the same trial.
#$p < 0.001$ from post-exercise in the same trial.
†$p < 0.001$ from the corresponding value in CON.
‡$p < 0.001$ from the corresponding value in HIIE10.

lower bout duration or continuous exercise. Studies have shown that high-intensity training involving ten 60-s bouts augment muscle GLUT4 content,[22,42] reduce circulating insulin at the end of an oral glucose tolerance test,[2] decrease 24-h average circulating glucose,[22] and increase insulin sensitivity.[43] Thus, the significant effect of bout duration on glucose responses and adaptations to training is worthy of further investigation.

An interesting, but though secondary finding of the present study, related with the magnitude of metabolic stress and glycolytic contribution to energy supply, was the detection of lactate in urine. Urine lactate was increased only in HIIE60, while it remained unchanged in CON, HIIE10 and HIIE30 (Fig. 2B). The exponential relationship between BLa IAUC and ULa has been observed in other types of exercise,[38,44] and suggests that lactate is not excreted by the kidney, unless a relatively high concentration is reached in the blood. The results of the present study attest to the validity of urine lactate concentration as a novel, promising exercise biomarker during HIIE.[44]

Intense exercise is also known to increase circulating concentrations of uric acid,[7,25] a product of purine nucleotide degradation due to metabolic stress.[45,46] In the present study uric acid increased only in HIIE60, although the three HIIE protocols had the same intensity and total work. It seems that the longer duration of each bout in HIIE60 required a more sustained high rate of ATP supply, which posed a bigger threat to ATP homeostasis and favored myokinase-catalyzed AMP production, leading to increased uric acid production.[46,39] On the other hand, during the shorter bout trials (HIIE10 and HIIE30) metabolic disturbances are lower, due to the higher contribution of phosphocreatine and the reduced reliance on glycolytic energy supply, thus reducing the metabolic stress and the extent of purine nucleotide degradation.[39] Apart from being an indicator of higher metabolic stress, the elevation in uric acid levels may acts as a physiological mechanism to counteract an increased radical production by intense exercise.[47] In fact, its role to prevent exercise-induced oxidative stress is particularly important since this non-enzymatic antioxidant accounts for about two thirds of total plasma antioxidant activity.[48] Thus, a possibly a greater oxidative stress in HIIE60 may have been attenuated by uric acid. In HIIE10 and HIIE30, the lower metabolic stress did not trigger any antioxidant response, as reflected in the absence of changes in uric acid, GSH and TAC (Table 2). The lack of changes in GSH and TAC in HIIE60, may imply that uric acid alone may be sufficient to restore redox homeostasis, without the necessity to involve other antioxidant mechanisms. However, in the absence of direct oxidative stress markers, safe conclusions cannot be reached.

Another indicator of differences in exercise stress, despite the

identical total work, is PVC. Even though the exercise-induced hemoconcentration is multifactorial,[49] intensity seems to be important[50]; in agreement with this, all HIIE trials presented higher PVC than CON. However, the reduction in plasma volume was greater in HIIE60 than in the other HIIE trials, reflecting greater exercise stress-related changes, similar to those observed after a 30-s sprint.[51,52] The large decrease in plasma volume during HIIE60 may be attributed to both hemodynamic (i.e., increase in blood pressure) and osmotic changes in working muscles (e.g., from lactate increase),[31,50,51] which are both indicative of the magnitude of exercise stress.

It is well known that acute exercise elicits a substantial increase in circulating leukocytes, the magnitude of which depends on exercise intensity and duration.[12] In the present study, differences in leukocyte responses between the HIIE trials were dependent on bout duration and the concomitant variations in metabolic responses. In addition, bout duration affected changes in leukocyte sub-populations in accord with the metabolic disturbances observed. For example, there is evidence that lactate and the associated H^+ ions may influence immune function,[53] while hormonal responses related to the magnitude of exercise stress are also linked with changes in leukocyte sub-populations.[54] Lymphocytosis was observed in the HIIE30 and HIIE60 trials immediately post-exercise, before the reduction in lymphocyte count below baseline in the first hour of recovery. This biphasic response is consistent with studies using strenuous or interval exercise.[6,9,55–58] The delayed exercise-induced lymphocytopenia may occur for several hours[5,6,55] and reflects the preferential movement of lymphocyte subtypes with potent effector functions (e.g., NK cells, $\gamma\delta$ T cells, and CD8$^+$ T cells) out of the blood[11] as an integral part of the physiological stress response to exercise.[11] Reduced lymphocyte count and function is associated with immunosuppression and increased risk for infection,[12] although this remains a controversial topic.[59]

Furthermore, neutrophilia occurred immediately post-exercise in all trials, which was sustained 1 h post-exercise, confirming other high-intensity or sprint exercise studies.[5,9,57,60,61] Possibly this increase is caused by exercise-induced endogenous glucocorticoids secretion that trigger mobilisation of neutrophils from the marginal pool and bone marrow.[62,63] Monocytosis immediately post-exercise was found only in HIIE60, in line with other studies using 1-min HIIE bouts.[64] It has been suggested that monocytes mobilized by exercise may infiltrate damaged muscle tissue and differentiate into tissue-resident macrophages to facilitate repair and regeneration.[11]

One limitation of the present study was that measurements were held up to 1 h following exercise and thus potentially significant delayed antioxidant responses may have been missed. Also, there is a lack of data from female participants, who exhibit lower peak power and VO$_2$max, and may be more fatigue resistant than males. Aerobic and anaerobic fitness level may also modify metabolic, immune and antioxidant responses to short- and long-bout HIIE. Future studies should investigate the long-term responses and adaptation to HIIE training using the shortest (10 s) and longest (60 s) bout durations.

5. Conclusion

In conclusion, the present study highlights the importance of bout duration in HIIE, since shorter bouts induced lower blood cell perturbations, metabolic stress, and antioxidant responses, compared to 1-min bouts and continuous exercise, despite equal total work, intensity, duration and work-to-recovery ratio. Thus, modifying bout duration enables manipulation of metabolic stress and immune responses to HIIE, and may be used as a means of

training progression, while examination of the long-term effects of short- and long-bout high intensity interval training is warranted.

Author contributions

Gregory C. Bogdanis; Conceived and designed the analysis, Performed the analysis, Analysis and interpretation of the data, Other contribution revised the paper critically for important intellectual content, approved the final version of the manuscript to be published, George Mastorakos; Conceived and designed the analysis, Performed the analysis, Analysis and interpretation of the data, Other contribution, revised the paper critically for important intellectual content, approved the final version of the manuscript to be published, Spyridon Tsirigkakis, Collected the data, Performed the analysis, Analysis and interpretation of the data, Wrote the paper, Drafted the manuscript, Other contribution, approved the final version of the manuscript to be published, Pinelopi S. Stavrinou; Performed the analysis, Analysis and interpretation of the data, Wrote the paper, Drafted the manuscript, Other contribution, approved the final version of the manuscript to be published, Athanasios Kabasakalis; Performed the analysis, Analysis and interpretation of the data, Wrote the paper, Drafted the manuscript, Other contribution, approved the final version of the manuscript to be published, Aimilia Mantzou; Performed the analysis, Analysis and interpretation of the data, Wrote the paper, Drafted the manuscript, Other contribution, approved the final version of the manuscript to be published, Vassilis Mougios; Conceived and designed the analysis, Performed the analysis, Analysis and interpretation of the data, Other contribution, revised the paper critically for important intellectual content, approved the final version of the manuscript to be published.

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