Thieme

Acute Hematological and Inflammatory Responses to High-intensity Exercise Tests: Impact of Duration and Mode of Exercise

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ABSTRACT

The purpose of this study was to investigate the hematological and inflammatory responses to 4 maximal high-intensity protocols, considering energy expenditure in each test. 9 healthy volunteers performed 4 high-intensity exercise tests of short [Wingate (WANT); Repeated-sprints (RSA)] and long durations [Continuous VO_2 test (VCONT); intermittent VO_2 test (VINT)] in a cycle-ergometer, until exhaustion. Hematological parameters and IL-6, IL-10 and creatine kinase (CK) levels were determined before (PRE), POST, 30 min, 1, 2, 12 and 24 h after the end of the protocols. Additionally, energy expenditure was determined. Leucocytes, erythrocytes and lymphocytes increased at POST and returned to PRE values at 30 min for all protocols. Lymphocytes had a second decreased at 2 h and granulocytes increased at 2 h when compared to PRE. Both variables returned to PRE values between 12–24 h into recovery. The magnitude of response for IL-6 was greater in VINT and for IL-10 in VCONT. There was no association of energy expenditure within each exercise protocol with the pattern of IL-6, IL-10 and CK responses to the exercise protocols. The present finding support that similar responses after continuous or intermittent acute protocols are observed when exercises are performed to volitional failure, regardless of the duration and mode of exercise.

Abbreviations

CK	Creatine Kinase
Hb	Hemoglobin
Ht	Hematocrit
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume
RBC	Red Blood Cells
RER	Respiratory exchange ratio
RSA	Repeated-sprints Test
VCONT	Continuous VO_2 test; long-term high-intensity exercise until exhaustion in cycle-ergometer
VINT	Intermittent VO_2 test; long-term high-intensity exercise until exhaustion in cycle-ergometer
WANT	Wingate Anaerobic Test

Introduction

The interactions between exercise, hematological system and immune function have been widely studied since the observation of exercise-induced leucocytosis, which has important implications for immunity [23, 34]. An acute bout of exercise places an important demand on the body, depending on the model, intensity and duration of the effort. Furthermore, high-intensity exercise creates muscle damage and inflammation, leading to disturbance in cellular homeostasis and discomfort, resulting in a cytokine-mediated

systemic inflammatory response [46] similar to the cytokine profile elicited after an acute episode of infection [34]. This may result in a temporary immunosuppression and can increase the susceptibility to infections [12, 27] and autoimmune disorders [48]. In addition, the hormonal and adrenergic effects [34] and the enhanced anti-inflammatory cytokine response (i. e., IL-10 production) [13] have been linked with this decrease in immune function after high-intensity exercise in active individuals.

It is known that the intensity of exercise and duration are important factors in determining the relative recruitment of the energy processes. Other factors, such as availability of oxygen and metabolic substrates, environmental factors and hormonal changes will modify the extent to which the energetic processes occurs [18] and the changes in hematological and inflammatory makers that may or may not happen. For example, an inverse relationship between post-exercise muscle glycogen and IL-6 plasma cytokine levels has been found [28, 37]. Additionally, a temporary depression of various aspects of immune function will usually last for 3–24 h after exercise [15].

Very few studies have considered the impact of duration and mode (continuous vs. intermittent) of exercise on immune function [8, 25, 26, 40, 41]. Hence, we focused in this study on surrogate outcomes as markers of inflammation and skeletal muscle recovery (i. e., leukocytes, IL-6, IL-10) after a single bout of maximal exercise, with different models (short-term high-intensity exercise and long-term high-intensity exercise) and their responses during a 24 h recovery period. To the best of our knowledge, this is the first study to compare maximal intensity protocols, including different durations and mode, and monitoring test responses for 24 h. Therefore, the aim of this study was to analyze the impact of duration and mode (continuous × intermittent) of 4 maximal protocols on hematological profiles and markers for systemic inflammation (IL-6 and IL-10). Additionally, we examined whether the 4 maximal protocols had different recovery patterns by looking at a 24 h post-exercise time-course response for IL-6 and IL-10.

Methods

Participants

The sample consisted of 9 healthy (male = 6, female = 3) university students (▶ **Table 1**). Their VO_{2max} was 3.1 ± 0.7 (L.min^{-1}; ▶ **Table 1**). None of the volunteers suffered from acute or chronic diseases or reported intake of medication, including antioxidants and nicotine abuse. All participants were healthy and not participating in regular exercise training. Participation was voluntary, and all participants signed an informed consent document before the participation in the study. The experimental methods and procedures were approved by the Ethics and Human Subjects Review Board at the Faculty of Sports Science and Physical Education, University of Coimbra. This study meets the ethical standards of the International Journal of Sports Medicine [17].

Experimental design

Participants attended 4 laboratory-based testing sessions. The testing order was randomized with a interval period of 72-h between tests [43]. During this time, all participants were encouraged to not perform intense physical exertion.

▶ **Table 1** Participant characteristics.

	Mean	Standard Deviation	Range/ Reference
Age (years)	32.22	(2.45)	26–41
Stature (cm)	174.47	(2.46)	156.3–184.2
Weight (kg)	69.20	(4.81)	47–95
IMC (kg.m^{-2})	22.47	(1.28)	19.2–28
$VO_{2máx}$ (L.min^{-1})	3.123	(0.2)	1.883–4.108
$VO_{2máx}$ (mL.kg^{-1}.min^{-1})	45.51	(4.90)	37.12–51.75

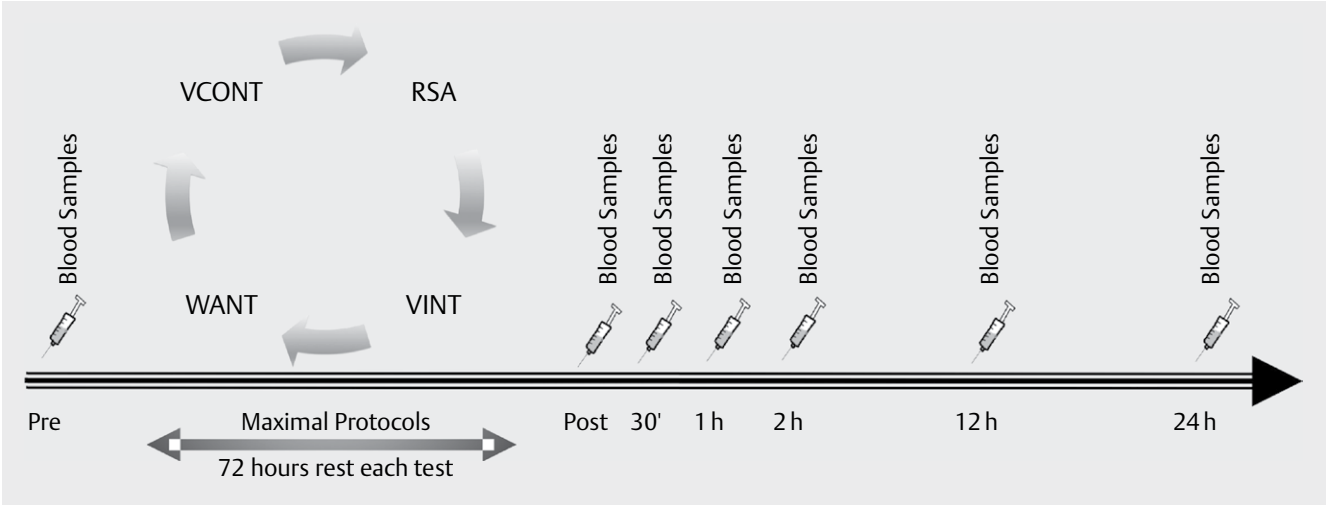
Mean ± SD of physical and blood parameters measured before all test protocols in 09 health subjects

We tested different models in a cross-sectional study (short-term high-intensity exercise and long-term high-intensity exercise, with 2 continuous and 2 intermittent protocols). In addition, to analyze the impact of duration, the aim of this study was also to analyze the impact of mode (continuous × intermittent). To do so, each participant completed 4 independent test protocols: 2 protocols of short-term high-intensity exercise in cycle-ergometer: 1) 30-s Wingate Anaerobic Test (WANT); 2) Repeated-sprints Test (RSA); and 2 protocols of long-term high-intensity exercise until exhaustion in cycle-ergometer: 3) Continuous VO_2 test (VCONT); 4) Intermittent VO_2 test (VINT). The oxygen uptake and plasma lactate accumulation were measured during the tests to determine energy system contributions and energy expenditure during exercise. Blood samples were taken at rest before (PRE), immediately post (POST), and 30 min, 1, 2, 12 and 24 h post-exercise. Blood samples were analyzed for leukocyte, lymphocyte, granulocyte and monocyte cell counts and for IL-6, IL-10 and CK plasma concentrations. An overview of the experimental protocol is presented in ▶ **Fig. 1**.

Exercise protocols

Prior to baseline measurements, participants were informed of the laboratory testing procedures. After the measurements of body mass, stature and the pre-test blood sample, participants performed the first test to exhaustion on an electronically braked cycle ergometer (Lode, Groningen, Netherlands) using an online gas collection system (Quark CPET COSMED, COSMED, Rome, Italy). Also for VCONT and VINT protocols an electronically braked cycle ergometer and the online gas collection system were used to determine VO_{2max}. During all the tests, heart rate was measured continuously using a heart rate monitor (COSMED, COSMED, Rome, Italy). VO_{2max} was determined using a voluntary criterion of exhaustion or when the following occurred: the participant reached a respiratory exchange ratio (RER) above 1.15; when oxygen uptake reached a plateau with increasing work rate; and when a heart rate close to age predicted maximal values was attained [19]. The detailed protocols are described below:

a. Wingate Anaerobic Test (WANT): participants completed a 30 s maximal effort on an electronically braked cycle ergometer at a resistance equivalent to 9 % of their body mass.



▶ **Fig. 1** Experimental design. All participants completed 4 independent test protocols. Between trials, a minimal of 72 h of interval was ensured. Before (PRE), immediately after exercise (POST), and the following recovery sessions (30 min, 1, 2, 12, 24 h), blood samples, were collected to analyzed hematological profile and muscle damage (IL-6 and IL-10). Before the experiments, participants were familiarized with the test scheme. From the first test until the end of experimentation period, the participants were instructed to avoid alcohol consumption and smoking. Other healthy habits such as good quality of sleep and food were also requested, too.

b. Repeated-sprints Test (RSA): each participant performed 10 repetitions of 10-second all-out effort with 30-second of passive recovery on cycle ergometer [1, 2].
c. Continuous VO_2 test (VCONT): participants performed an incremental test to exhaustion on an electronically braked cycle ergometer using an online gas collection system to determine VO_{2max}. Following a 5-min warm-up, the test began at 75 W with the workload increasing by 25 W every 3 min until volitional exhaustion [5]. The value recorded for VO_{2max} in the protocol corresponded to the mean of the 2 highest values achieved over a 30-second collection period.
d. Intermittent VO_2 test (VINT): participants performed an incremental intermittent test to exhaustion on an electronically braked cycle ergometer using an online gas collection system to determine VO_{2max}. Following a 5-min warm-up, the test began at 75 W with the workload increasing by 25 W every 3 min period with 1-min of passive rest, until volitional exhaustion. The value recorded for VO_{2max} in the protocol corresponded to the mean of the 2 highest values achieved over a 30-s collection period.

Blood collection

Blood samples (~12 mL) were obtained from the antecubital vein by venepuncture. The blood was collected into tubes containing EDTA and analyzed for leukocytes (WBC), erythrocytes (RBC), granulocytes, lymphocytes, monocytes, platelets, mean platelet volume (MPV), hemoglobin (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), hematocrit (Ht), and mean corpuscular hemoglobin (MCH), with a full cell count, using a blood analyzer (Beckman Coulter T660; Beckman Coulter, Inc., Miami, Florida). Whole blood Hb and Ht were used to estimate changes in plasma volume (P_V) relative to Pre, established as baseline. All measurements for erythrocyte's indices, Hb and Ht following exercise testing were corrected for changes in P_V [10]. The remaining blood was centrifuged at 4 °C for 15 min at 1500 rpm. After centrifugation, plasma was stored at − 80 °C for subsequent analyses. IL-6 and IL-10 plasma concentrations were determined by ELISA according to the manufacturer´s instructions (Invitrogen, CA). The minimum detection limit for both IL-6 and IL-10 was < 1 pg/ml. Plasma samples were assayed in duplicate. The mean coefficient variation for the duplicate analysis was 3.4 % for IL-6 and 4.0 % for IL-10.

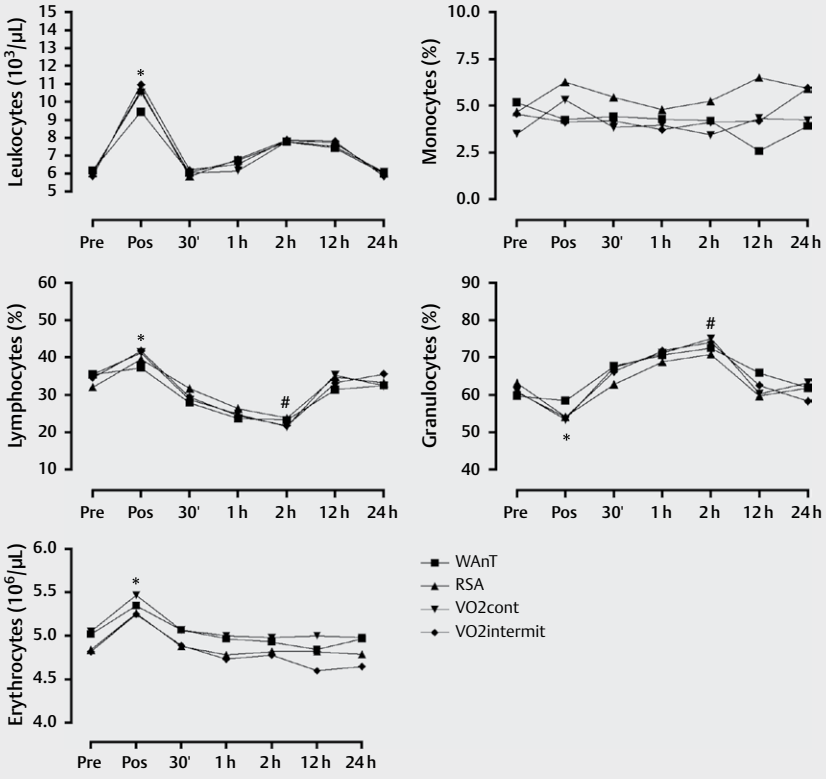
CK levels were determined using the Horiba Medical Pentra C200 analyzer (Kyoto, Japan) following the manufacturer's instructions.

Calculations of the energy expenditure and energy system contributions

In order to determine the energy system contributions and energy expenditure during exercise we measured breath-by-breath oxygen uptake (VO_2) during exercise, post-exercise VO_2 kinetics and plasma lactate accumulation (3–5 min post - pre) in each test. The total energy expenditure and the contributions of the aerobic, anaerobic lactic and anaerobic alactic metabolisms were calculated using GEDAE-LaB (GEDAE-USP, São Paulo, Brazil) software [6]

Statistical analysis

Descriptive statistics for all dependent variables at all measurement points are presented as mean and standard deviation. To describe the patterns of changes of interleukins and CK in response to different maximal exercise protocols we considered hierarchical/multilevel modeling. Individual changes are expressed as percentage changes via logarithmic transformation of the dependent variables. The dependent variables were centered on the mean of baseline outcome (measurements at PRE). To allow the interpretation of between measurement slopes we scaled time as a discrete variable ranging from zero to 7. A dummy variable was incorporated in the models to categorize the maximal exercise protocols (WANT: 0;



▶ **Fig. 2** Pre- vs. post-exercise changes of leukocytes, lymphocytes, granulocytes, erythrocytes and monocytes in the peripheral blood. * $P<0.01$ vs. pre-stage, # $P<0.05$ vs. pre-stage.

RSA: 1; VCONT: 3; VINT: 4) and was included at level 2. To explore differences in the rate of changes across the tests we included in the models interaction terms between the time terms (slopes) and maximal exercise protocol. Additionally, we considered the influence of energy expenditure within each exercise protocol (added as fixed effects on the multilevel models) on the pattern of IL-6, IL-10 and CK responses to the exercise protocols. Restricted maximum likelihood estimation was used to obtain the unknown parameters. We considered a covariance structure (first-order autoregressive) to accommodate auto-correlation of within-individual residuals (Goldstein, Healy, & Rasbash, 1994; Steele, 2008). Multilevel regression models were obtained using "nlme" package (Pinheiro & Bates, 2000), within the R statistical language (R-Core-Team, 2014).

Results

Leukocytes and erythrocytes counts increase immediately post-exercise in all protocols

There were significant increases in leukocyte and erythrocyte counts in POST for all protocols ($P<0.01$), returning to PRE values 30 min post-exercise. The variation observed for lymphocytes was multifaceted, in that a significant increase was observed in POST for all protocols ($P<0.01$), which was followed by a significant decrease 2 h after. However, between 12 to 24 h after the end the protocol the lymphocytes returned to basal values for all the protocols. Granulocytes decreased in POST in all tests, except in the WANT, and increased steadily until 2 h after exercise and returning to baseline levels between 12 to 24 h post-exercise. There were no significant changes for monocytes during the experimental design (▶ **Fig. 2**).

Mode and duration of exercise does not alter the erythrocytes indices

None of the participants showed values higher than the reference range for Hb and Ht in PRE. The hemoglobin responses for the short-duration tests (WANT e RSA) and the long-duration intermittent (VINT) test showed a significant increase ($p<0.01$) after exercise and returned to PRE values 30 min post-exercise in the WANT, RSA and VINT. No changes were detected in the hemoglobin responses for the VCONT (▶ **Table 2**). The erythrocytes (MCV, MCH, MCHC) and platelet indices (MPV) were not significantly modified for all protocols in all time-points measured (▶ **Table 2**).

The time effect on cytokine concentration is dependent on mode of exercise

Responses of interleukins and CK at the different time points by exercise protocol are summarized in ▶ **Table 3**.

▶ **Table 3** shows the multilevel regression analysis for IL-6, IL-10 and CK changes in response to exercise protocols. The patterns of IL-6, IL-10 and CK change in response to exercise are represented

▶ **Table 2** Mean and standard deviations of hematological indicators at each measurements pre- and post-exercise. Mean (SD) of Hemoglobin (g.dL-1), Hematocrit (%), Mean Corpuscular Volume (fL), Mean Corpuscular Hemoglobin (pg), Mean Corpuscular Hemoglobin Concentration (g.dL-1) and Mean Platelet Volume (fL) before (PRE) e after (POST, 30', 1 h, 2 h, 12 h, 24 h) all test protocols (n = 9).

		Before	After					
Parameters		**PRE**	**POST**	**30'**	**1 h**	**2 h**	**12 h**	**24 h**
WANT (N = 9)	Hb	14.5 (0.4)	15.5 (1.8) *	14.7 (1.6)	14.3 (1.6)	14.2 (1.5)	14.4 (1.5)	14.4 (1.1)
	Ht	44.3 (4.1)	47.6 (4.1)	45.2 (4.0)	44.2 (4.3)	43.8 (4.0)	42.8 (3.7)	44.3 (3.9)
	MCV	89.1 (2.8)	89.1 (3.3)	89.0 (3.0)	89.4 (2.9)	89.1 (2.8)	88.4 (2.6)	89.2 (2.9)
	MCH	28.4 (1.1)	28.9 (1.5)	28.8 (1.6)	28.7 (1.3)	28.9 (1.4)	29.8 (1.4)	29.0 (1.0)
	MCHC	31.8 (1.2)	32.4 (1.6)	32.3 (1.2)	32.1 (0.9)	32.4 (1.0)	33.7 (1.1)	32.9 (2.0)
	MPV	8.4 (0.8)	8.7 (0.8)	8.6 (1.0)	8.3 (0.8)	8.2 (0.7)	7.6 (0.9)	8.0 (1.1)
RSA (N = 8)	Hb	13.8 (1.2)	15.2 (1.2) *	13.8 (1.3)	13.7 (1.4)	13.7 (1.3)	13.8 (1.3)	13.7 (1.6)
	Ht	43.0 (3.8)	47.2 (3.3)	41.7 (6.9)	42.4 (3.6)	42.7 (3.6)	42.7 (3.6)	42.7 (4.1)
	MCV	88.9 (3.2)	89.8 (3.0)	88.3 (2.6)	88.7 (3.0)	88.8 (3.6)	88.6 (2.7)	89.2 (2.6)
	MCH	28.6 (1.5)	28.8 (1.3)	28.3 (1.2)	28.7 (1.4)	28.5 (1.2)	28.5 (1.5)	28.6 (1.7)
	MCHC	32.2 (1.0)	32.1 (0.9)	32.2 (0.9)	32.2 (1.0)	32.2 (0.8)	32.1 (1.2)	31.9 (1.6)
	MPV	8.3 (0.8)	8.8 (0.9)	8.6 (0.8)	8.4 (0.9)	8.1 (0.9)	7.4 (0.8)	7.7 (1.1)
V_{CONT} (N = 9)	Hb	14.5 (1.2)	14.7 (3.6)	14.6 (1.0)	14.3 (1.0)	14.3 (0.9)	14.3 (1.3)	14.4 (1.2)
	Ht	45.0 (3.5)	49.0 (3.6)	45.2 (6.9)	44.2 (3.3)	44.2 (3.0)	44.3 (3.9)	43.3 (3.8)
	MCV	89.0 (3.0)	89.5 (2.9)	89.1 (2.8)	88.4 (2.3)	88.7 (2.6)	88.5 (2.8)	88.9 (3.2)
	MCH	28.7 (1.3)	28.9 (1.2)	28.8 (1.1)	28.5 (1.3)	28.8 (1.2)	28.5 (0.9)	29.0 (1.1)
	MCHC	32.2 (0.9)	32.2 (0.8)	32.3 (0.7)	32.3 (1.1)	32.4 (1.0)	32.3 (0.8)	32.6 (1.0)
	MPV	8.4 (0.7)	8.9 (0.9)	8.4 (0.9)	8.3 (0.7)	8.1 (1.0)	7.5 (0.8)	7.9 (0.9)
V_{INT} (N = 8)	Hb	14.7 (1.2)	15.1 (1.2) *	14.0 (1.3)	13.6 (1.3)	13.6 (1.3)	13.1 (1.4)	13.4 (1.5)
	Ht	43.1 (3.4)	47.2 (3.6)	43.7 (3.5)	42.1 (3.6)	42.4 (3.7)	41.0 (3.6)	41.5 (3.7)
	MCV	89.3 (2.5)	89.7 (2.3)	89.3 (2.9)	88.8 (2.8)	88.8 (2.6)	89.0 (2.4)	89.0 (2.9)
	MCH	28.3 (1.4)	28.6 (1.2)	28.6 (1.3)	28.4 (1.5)	28.3 (1.5)	28.4 (1.6)	28.6 (1.6)
	MCHC	31.7 (0.9)	31.9 (1.0)	32.0 (0.8)	32.0 (1.0)	31.9 (1.1)	31.8 (1.1)	32.1 (1.1)
	MPV	8.4 (1.0)	8.9 (1.3)	8.3 (0.8)	8.2 (0.7)	7.8 (0.6)	7.2 (0.7)	7.4 (0.5)

Hemoglobin (Hb), Hematocrit (Ht), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Platelet Volume (MPV). * $P < 0.05$ compared to PRE

in ▶ **Fig. 3**. The exponents for intercept (WANT) and each exercise protocol indicate that participant's pre-test values of IL-6 were higher compared to the other exercise protocols. No baseline differences were present for IL-10 and CK. For IL-6 response to Wingate anaerobic test, slope values across time indicate a decrement of response between the 24 h periods of observation (5.32 pg/mL). The non-significant exponents for the measurements times by exercise protocol interaction indicate that VINT had a similar decrement in response across the 24-h period of observation. For the RSA and VCONT, IL-6 POST values were increased (2.33 and 2.16 pg/mL, respectively), but changes after 24 h values were similar to pre-test values. IL-10 values were decreased after the 24 h period in response to Wingate anaerobic test (1.94 pg/mL), particularly presenting substantial decrements 12 h after the short-term maximal effort (1.67 pg/mL). As for the other exercise protocols, the large variability suggests that no substantial change was observed across the 24 h observation period in response to exercise. A trend of acute response post-exercise was apparent for CK in response to the maximal exercise protocols (1,17 pg/mL), but no substantial differences were observed across the 24 h period of observation.

There was no association of energy expenditure within each exercise protocol with the pattern of IL-6, IL-10 and CK responses to the exercise protocols (▶ **Table 4**).

Discussion

The main results of this study demonstrate that acute changes in leukocyte subpopulations, hemoglobin and hematocrit to strenuous exercise vary slightly depending on the mode (intermittent vs. continuous) and duration of exercise. It seems that variation may be more pronounced in cytokines, IL-6 and IL-10.

Hematological changes and exercise

None of the participants showed values higher than the reference range for leukocytes, erythrocytes, hemoglobin, hematocrit and erythrocyte's indices. These are important because the stability of hematological parameters is crucial to maintain optimal performance [3]. The consistent stability of packed cell volume and hemoglobin observed could be meanwhile used as an index of general health status before and after performances [4]. Because no significant differences between genders were found, the results are discussed together.

We found significant modifications on leukocyte counts after the effort in all protocols, but, unexpectedly, the change was the same for protocols that had 30 s of duration (WANT) as for those with more than 20 min of duration, on average (VCONT and VINT). In this study all test protocols were maximal. It is well known that maximal effort has substantial effects on total WBC, probably due

▶ **Table 3** Fixed explanatory variable results based on multilevel regression analysis of IL-6, IL-10 and CK changes (%) across 24-h in response to different high-intensity exercise protocols.

	IL-6	IL-10	CK
Fixed explanatory variables			
Intercept (Wingate Anaerobic test as reference)	28.7 (38.5)	0.00 (30.5)	0.0 (21.3)
Pre- to post-exercise slope (Wingate Anaerobic test as reference)	1.3 (31.7)	27.7 (27.1)	16.2 (5.6) * *
Post-exercise to 30 min slope (Wingate Anaerobic test as reference)	−10.4 (32.6)	17.0 (27.9)	7.2 (7.0)
30 min to 1 h slope (Wingate Anaerobic test as reference)	−58.7 (33.4)[a]	−31.0 (27.9)	5.4 (7.7)
1 h to 2 h slope (Wingate Anaerobic test as reference)	−29.0 (35.1)	−16.0 (27.9)	9.5 (8.0)
2 h to 12 h slope (Wingate Anaerobic test as reference)	−138.6 (56.1) *	−51.3 (27.9) [a]	12.9 (8.2)
12 h to 24 h slope (Wingate Anaerobic test as reference)	−167.2 (46.7) * *	−66.1 (27.9) *	5.4 (8.3)
Repeated sprints test	−79.4 (36.1) *	−13.1 (30.3)	3.9 (8.6)
Continuous VO_2 test	−22.5 (34.6)	6.7 (28.9)	−1.6 (8.4)
Intermittent VO_2 test	−60.1 (34.7)[a]	−2.0 (28.9)	−0.1 (8.5)
Repeated sprints test × Pre- to post-exercise slope	84.6 (43.9) *	39.2 (41.3)	−5.0 (8.1)
Repeated sprints test × Post-exercise to 30 min slope	58.1 (48.7)	21.5 (42.6)	−5.5 (10.4)
Repeated sprints test × 30 min to 1 h slope	111.1 (49.8) *	79.3 (42.6) [a]	−3.0 (11.2)
Repeated sprints test × 1–2 h slope	88.5 (53.8) [a]	51.8 (43.9)	−7.0 (12.0)
Repeated sprints test × 2–12 h slope	12.5 (60.2)	0.6 (43.9)	−6.1 (12.6)
Repeated sprints test × 12–24 h slope	123.2 (59.2) *	29.1 (44.4)	8.6 (13.2)
Continuous VO_2 test × Pre- to post-exercise slope	77.2 (42.1) [a]	64.8 (39.6) [a]	1.4 (7.9)
Continuous VO_2 test × Post-exercise to 30 min slope	30.6 (47.7)	15.0 (41.7)	8.9 (9.9)
Continuous VO_2 test × 30 min to 1 h slope	52.4 (47.9)	67.3 (41.0) [a]	4.6 (10.8)
Continuous VO_2 test × 1–2 h slope	9.8 (48.2)	47.9 (41.0)	8.4 (11.4)
Continuous VO_2 test × 2–12 h slope	−8.8 (51.3)	−2.0 (41.0)	−0.5 (11.7)
Continuous VO_2 test × 12–24 h slope	132.3 (48.4) * *	47.7 (40.3)	−5.4 (12.0)
Intermittent VO_2 test × Pre- to post-exercise slope	79.5 (42.2) [a]	59.0 (39.6)	5.0 (7.9)
Intermittent VO_2 test × Post-exercise to 30 min slope	19.3 (46.8)	−14.2 (40.8)	2.3 (9.9)
Intermittent VO_2 test × 30 min to 1 h slope	76.2 (48.7)	47.1 (41.8)	11.2 (10.8)
Intermittent VO_2 test × 1–2 h slope	53.6 (49.0)	51.5 (41.8)	−1.6 (11.3)
Intermittent VO_2 test × 2–12 h slope	98.4 (52.5) [a]	26.2 (41.8)	5.2 (11.6)
Intermittent VO_2 test × 12–24 h slope	131.3 (51.2) * *	−25.7 (43.1)	−0.5 (11.7)

* * $P \le 0.01$, * $P \le 0.05$, [a] $P \le 0.10$

to an increase in catecholamines. This is a first mechanism that induces the recruitment of marginated leukocytes from the pool (▶ **Fig. 1**, Panel a). After a stabilization or slight decrease of WBC (in this study 30 min after exercise) the second increase in WBC may be triggered by the increase of cortisol [4], which induces the release of leukocytes from the bone marrow. In the present study, all protocols showed a tendency for a second increase in leukocytes 1 h after exercise (▶ **Fig. 2**). On the other hand, the lymphocytopenia observed post-exercise could be a result of immune cell migration [42], apoptosis [11] or both [24]. Considering the effect of 6 series of Wingate anaerobic cycle tests on lymphocyte apoptosis and migration, it was reported that post-exercise lymphocytopenia was a result of immune cell migration, particularly the suppressor T cell subset, rather than apoptosis [11]. Observations based on intensive treadmill running protocol at 80 % maximal O_2 uptake, provided evidence that the mobilized lymphocytes appeared to extravasate the peripheral blood compartment [42]. More recently, observations considering the effects of 3 consecutive days of high-intensity interval running on $CD4^+$ (helper), $CD8^+$ (cytotoxic), and $CD19^+$ (B-cells) lymphocyte cell markers of apoptosis and migration in untrained individuals who completed an exertion test and later performed 3 consecutive days of an intermittent run protocol to exhaustion were reported [24]. B-cells displayed increases in both apoptotic and migratory markers following the first 2 days of intermittent running, and the apoptotic response persisted following day 3 [24]. It is possible that the ability of exercise to provide a stimulus capable of inducing apoptosis is dependent on the duration of exercise. On the other hand, the cellular migration had the greatest influence on cytotoxic T lymphocytes following each day of the interval runs [24].

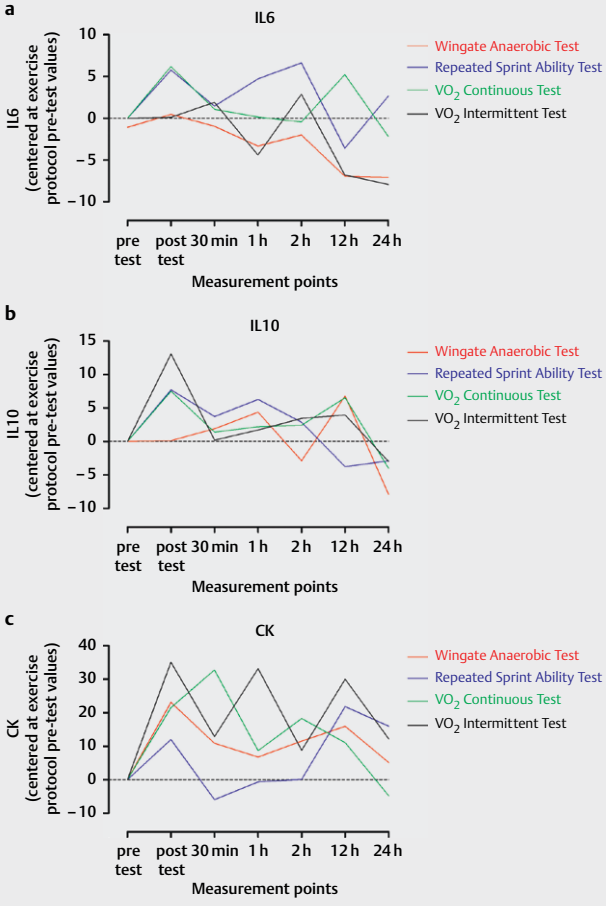
In peripheral blood, granulocytes are mainly comprised of neutrophils. Neutrophils in part regulate tissue repair by phagocytosis of damaged cells, modulate the function of macrophage infiltrators, and eliminate microbial infection [47]. Acute exercise results in a first, rapid and profound neutrophilia (increase in blood neutrophil number) followed by a second, delayed increase in blood neutrophil counts a few hours later [32]. The initial increase is likely due to demargination caused by shear stress and catecholamines, whereas the later increase may be due to cortisol-induced release of neutrophils from the bone marrow [22, 49].

IL-6 and exercise

The first report that acute exercise increased plasma IL-6 concentration was published 25 years ago [29]. It was suggested that IL-6 was produced predominantly by leukocytes as a response to exercise-induced local damage in working muscles. However, after the initial findings that circulatory blood cells were not the main source of IL-6 during exercise, researchers turned their attention to the muscle [35, 38]. In response to long-duration and high-intensity exercise, circulating levels of IL-6 increase by up to 100-fold [46]. In the current study, the levels of IL-6 increased immediately after exercise. These results suggest that, regardless of exercise mode or intensity, post-exercise increases in IL-6 may be expected. A trend of higher increase was observed for the VINT. This is probably explained by the relationship between release of IL-6 and bioavailability of carbohydrates (CHOs). Although, no association of energy expenditure within each exercise protocol with the pattern of IL-6 response to the exercise protocols was present in our study, the total energy expenditure for this test was the highest of all the protocols studied, with the predominance of CHO utilization (▶ **Table 4**). The role of glycogen availability in the contracting muscle as a primary signaling mechanism for IL-6 mRNA expression and release into the circulation is unclear [28]. However, this cytokine is a very "fuel sensing molecule" and when the higher exercise intensity leads to a faster glycogen depletion in the working muscles, plasma IL-6 concentration is enhanced [31].

Some authors have shown that more than 50 % of the variation in plasma IL-6 following exercise can be explained by exercise duration alone [36] during shorter exercise bouts [39]. In this study, the difference of total time between the VCONT and VINT tests was about 8 min and twenty seconds, on average (included the intervals time in VINT), and the lactate concentrations (VCONT = 11,2 ± 3,03 mmol/L and VINT = 12,18 ± 1,73 mmol/L, data no showed) were not different between protocols ($P \geq 0.05$). Contributions of the lactic anaerobic system and the aerobic system were higher in the VINT when compared to the VCONT ($p = 0.025$ and $p = 0.017$ respectively), indicating a higher energy expenditure and greater use of carbohydrate stores during the test. VINT is an intermittent test and the metabolic profiles of repeated-sprint performances are strongly dependent on the sprint duration and the capacity of recovery during the duration of the repeated sprints [2].

The interval workouts in VINT (1-min interval time each 3-min of test, larger test) may not have been large enough to allow for replenishing of the muscle energy supply, thus, the IL-6 increase after exercise was higher than after VCONT. Of note, the anaerobic ATP production during a single short-duration sprint (< 10 s) is provided by phosphocreatine breakdown degradation and anaerobic glycolysis [9]; however, the relative contribution of anaerobic glycolysis to the performance in subsequent sprints throughout the repeated tasks is reduced, and is partially replaced by an increase in the aerobic metabolism [45].



▶ **Fig. 3** Changes in IL-10, IL-6 and CK before (PRE) and after (POST, 30 min, 1, 2, 12, 24 h) all test protocols (n = 9).

IL-10 and exercise

Acute exercise bouts have been shown to promote an acute phase response, resulting in post-exercise cytokine levels similar to those observed during sepsis or inflammatory disease [30]. Nevertheless, the transient increase in IL-6 circulating levels during exercise appears to be responsible for a further increase in circulating levels of anti-inflammatory cytokines IL-10 and receptor antagonist 1A (IL-1ra), by stimulating the release of cortisol and decreasing the levels of TNF-α. IL-10 is known to be produced mainly by regulatory T cells but also by T helper 2 (Th2), Th1 and Th17 cells, monocytes, macrophages, dendritic cells (DCs), B cells and $CD8^+$ T cells [14]. Regardless of the source, the main function of IL-10 seems to be the down regulation of adaptive immune responses and reduction

▶ **Table 4** Energy system contributions and total energy expenditure for all protocols.

	Aerobic (kcal)	Anaerobic Lactic (kcal)	Anaerobic Alactic (kcal)	Total (kcal)
WANT	4.44 ± 0.88	10.78 ± 3.32	3.67 ± 1.46	18.89 ± 3.76
RSA	62.68 ± 18.03	8.79 ± 3.27	5.45 ± 3.91	76.92 ± 20.42
V_{CONT}	149.56 ± 72.80	8.87 ± 2.30	3.81 ± 2.28	162.23 ± 73.88
V_{INT}	196.00 ± 108.33	10.25 ± 2.22	5.22 ± 3.58	211.47 ± 111.43

of inflammation-induced tissue damage [14]. In fact, IL-10 decreases the expression of MHC molecules, the intercellular adhesion molecule 1 (ICAM1) and co-stimulatory CD80 and CD86 molecules in antigen-presenting cells [14]. Furthermore, IL-10 compromises the ability of effector T cells to sustain inflammatory responses by interfering or completely inhibiting the expression of various pro-inflammatory cytokines and other soluble mediators. Thus, IL-10 is a potent promoter of an anti-inflammatory state [7, 21, 44].

In the present study, the time effect for IL-10 concentrations was more pronounced in VCONT. IL-10 is not expressed in skeletal muscle after exercise [33]. Constitutive expression of IL-10 in skeletal muscle cells is low [33], but IL-10 production may have increased in response to the early rise in IL-6. Moreover, IL-10 production during exercise may also depend on changes in the numbers of certain T cell subsets [20]. Observations of the effects of training status and mode (sprint × endurance) on resting circulating T regulatory (Treg) cell counts and antigen-stimulated IL-10 production and the effect of an acute bout of exercise on the Treg response showed an association between high training loads (endurance) with greater resting IL-10 production and Treg cell counts than in sedentary, recreationally active and sprint-trained athletes [16]. Regardless of the exercise duration, the increase in Treg and antigen-stimulated IL-10 production reported after continuous protocols corroborate our results.

Several researchers have compared the effects of interval training vs. continuous training on hematological and inflammatory markers [19, 50, 51]. However, it is difficult to compare studies because of varying sample sizes, different exercise protocols, recovery times and different biochemical analysis techniques. In the present study, the acute response of hematological and inflammatory markers after maximal protocols was obtained from the same participants, performing all protocol tests under identical conditions.

In summary, the acute effects of maximal high-intensity exercise triggers hematological and inflammatory response that changes the normal muscle homeostasis. Particularly, the present results showed that hematological and inflammatory markers have a similar pattern of response after acute strenuous exercise tests regardless of the duration and mode (intermittent vs. continuous) of exercise. The present finding support that similar responses after continuous or intermittent acute protocols are observed when exercises are performed to volitional failure.

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Conflict of interest

No conflict of interest is declared.

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