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Original Research

Inflammatory and immune responses to a 3-day period of downhill running in active females

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Abstract: Exercise-induced muscle damage (EIMD) is accompanied by inflammatory and immune responses. However, due to the repeated bout effect, there will probably be less EIMD. Hence, the purpose was to investigate inflammatory and immune responses over a three-day period of downhill running in active females. Eleven moderately trained healthy females performed three 60-minute bouts of downhill running in -13.5% grade, separated by 24 hours, at a speed eliciting 70–80% of their $VO2_{peak}$ on level grade. Delayed onset muscle soreness (DOMS), range of motion (ROM) and maximum knee isotonic strength (1RM) were measured pre- and two-hour post every bout. Blood variables, including CBC, serum lactate dehydrogenase (LDH), creatine kinase (CK), myoglobin (Mb), IL-10, IL-6 and Monocyte chemoattractant protein-1 (MCP-1) were measured at 1 hour before the first bout and two hours after every bout. Data was analysed by repeated measure ANOVA ($P<0.05$). Although CK, LDH, Mb, IL-10, IL-6, MCP-1, total leukocyte count, monocytes and neutrophils increased significantly following the first bout, CK, LDH, Mb, IL-10, monocytes and neutrophils were only significantly higher following the third bout compared to the baseline (all $P<0.05$). Moreover, IL-10 and IL-6 decreased following the second and third bouts compared to the first bout ($P<0.05$). In comparison with the baseline, lymphocytes decreased after the second bout, DOMS increased following the second and third bouts, 1RM decreased following the first and second bouts (all $P<0.05$). ROM showed no significant difference. The three-day period of downhill running did not exacerbate EIMD and inflammatory response was partly attenuated.

Key words: Eccentric exercise; Cytokine; Muscle damage; Repeated bout effect; MCP-1; IL-10.

Introduction

Unaccustomed eccentric exercise induces a phenomenon known as exercise-induced muscle damage (EIMD), characterized by increased serum muscle proteins (Mb, LDH, CK) activity, stiffness and muscle soreness, swelling, decreased flexibility, loss of force production (1), muscle ultra-structure disruption, increased proteolytic enzyme activity and inflammation (2). Most athletes take part in periodic training in their programs, with phases of functional overreaching or short-term intensified training to enhance performance (3). Also, tournament participation may require athletes to perform eccentric muscle actions associated with the sport several times on the same or subsequent days (4). These acute and competitive training periods, especially in activities by eccentrically biased contractions, may cause EIMD.

However, there is another phenomenon known as the repeated bout effect, whereby a previous eccentric exercise bout can promote an adaption that restricts muscle damage, inflammation and loss of function if a similar bout of exercise is performed following a recovery period (4). The repeated bout effect is a protective mechanism (5). According to the cellular theory for this mechanism, there is less inflammatory response following the second bout of exercise, and this might be the reason for the maintenance or return of muscle function following the second bout compared to the first bout of eccentric exercise (4).

Inflammation is an essential adaptive response required for muscular adaptation and repair (6). Leukocyte infiltration into tissues during inflammation plays a central role in innate immunity. Upon irritant infliction, local macrophages and other cells sense the insult and produce a panel of inflammatory mediators, such as cytokines and chemokines, which stimulate the nearby microvasculature and attract large numbers of leukocytes to migrate across the vascular wall and infiltrate into tissues. At the inflammatory site, leukocytes perform phagocytosis and also release powerful tissue-damaging reagents to destroy anomalous cells (7). Acute skeletal muscle damage repair is a tightly-regulated process, which mainly comprises muscle inflammation, regeneration, and angiogenesis (8). The transition from the inflammatory to the muscle-repair phase depends on the attenuation of pro-inflammatory signals (9). An excess of macrophage pro-inflammatory cytokines may delay repairs (10). Interleukin-10, as a cytokine synthesis inhibiting factor, is one of the most important immune-

regulating cytokines. As an anti-inflammatory cytokine, its major function is to suppress monocyte/macrophage production of cytokines and chemokines (11). IL-10 can inhibit the production of pro-inflammatory cytokines and chemokines, such as IL-6, TNF-α (11), IL-1β (9), MCP-1 (12, 13).

Many studies survey damaging, inflammatory and immune responses during repeated bouts of eccentric exercise, e.g., after a seven-day eccentric training period on the elbow flexors, none of the muscle damage indicators or inflammatory indicators demonstrate exacerbation during Days 2 to 7 (14). Moreover, after two bouts of eccentric cycling separated by 14 days, there were no significant changes in maximal voluntary isometric strength, DOMS and CK after the second bout (15). Also, anti-inflammatory cytokines increased while pro-inflammatory cytokines decreased after the second run of two bouts of downhill running separated by 14 days (13).

However, there were limitations in previous studies, including prolonged intervals between bouts (13, 16) that are not feasible in real training (14), applying small muscle groups such as elbow flexors or knee extensors often as resistance exercise (4, 14, 17), and often using male subjects (4, 13, 14). Therefore, the purpose of this study was to investigate inflammatory and immune responses during a three-day period of downhill running in active females. The main questions were as follows: How would be change in the pro/anti-inflammatory responses during the time-course of the training? And how would be the repeated bout effect on EIMD during a three-day period of downhill running in active females?

Materials and Methods

Subjects

The subjects were 11 healthy to moderately active (at least 150 minutes of moderate to vigorous intensity physical activity per week (18)) Iranian females (students of Razi University, Kermanshah, Iran). They were randomly recruited among eligible volunteers. The sample size was chosen in accordance with similar studies (4, 13). The selection criteria for subjects were as follows: age 20–30 years, non-smoker (19); non-alcoholic (20); no regular use of any hormonal therapy, anti-inflammatory medications, anti-oxidants (19, 21)or supplementations (20); free from any known metabolic, cardiovascular, immune diseases (22, 23); no history of leg injury (13); no regular record of downhill running or eccentric exercise as part of their normal training (22); regular menstruation (between 22 to 36 days) (24-26). All subjects were informed about the risks, possible discomforts and procedures of the study prior to signing a written consent. The study was approved by the human research ethics committee of Razi University.

Initial testing

Ten days prior to the first bout in a morning session, every subject was sent to a sport lab for measurement of the baseline anthropometric characteristics (weight, height, BMI, %Body fat, …) by bioelectric impedance analysis technique (ZEUS 9.9 Plus, Jawon Medical Co., Korea), estimation of $VO2_{peak}$ and maximum isotonic strength.

$VO2_{peak}$ and running speed

All subjects completed a 20-m shuttle run test to predict $VO2_{peak}$ (27). Leger's protocol and formula were used (28). The test was administered in a sports hall (temperature 19–21°C). It involved running between two lines set 20 m apart at a pace dictated by recording emitting tones at appropriate intervals. Velocity was 8.5 $km.h^{-1}$ for the first minute, which increased by 0.5 km.h-1 every minute thereafter. The test score achieved by the subject was the number of 20 m shuttles completed before the subject either withdrew voluntarily from the test or failed to be within 3 m of the end lines on two consecutive tones (29). During the test, each subject wore a heart rate monitor (Beurer heart rate monitor, pm90, Germany). Maximum heartbeat was recorded and used to determine the appropriate running intensity and, therefore, speed for the downhill run (27). The test results were accepted as $VO2_{peak}$ if the score was ≥19 on the Borg scale and HRmax was within ±20 beats of Karvonen method (13, 19). Seventy-five percent of the maximum speed level acquired in shuttle run test was used as the subject speed on the treadmill during the downhill run.

Maximum isotonic strength

The maximum isotonic muscle strength of knee extensors was assessed by 1RM test through a trial and error procedure (30). The test was administered on a knee extensor chair with the range of motion adjusted 90◦ to 0◦ for knee extension. First, the subjects performed the general warm-up, including 10 minutes of cycling, a specific warm-up set of 8–10 repetitions at approximately 75% of the predicted 1RM in isotonic leg extension exercise, followed by another set of 3–5 repetitions at 85% of the predicted 1RM. Subsequent lifts were single repetitions of progressively heavier weights until the subject failed. The test continued until the concentric 1RM was determined (31). The number of sets ranged from three to five. The rest of the intervals between sets was two to five minutes (30).

Experimental protocol

Prior to training, subjects were told to train normally (3), ingest a normal mixed diet, be well hydrated (19), refrain from any strenuous exercise for 48 hours prior to the period (23), refrain from caffeine at least 12 hours before the first bout of training and throughout the three-day period (16, 32) and refrain from any additional methods to aid recovery (5). Subjects were asked to eat a light breakfast at least two hours prior to each bout (22, 33). Water was given ad libitum throughout the bout, with no other beverage or food being allowed (3).

Every subject was allotted a lab for a three-day training session in the follicular phase of the menstrual cycle (self-reported). This was to minimize the effects of different sex hormone concentrations. This phase has been described as an appropriate time to evaluate the effects of exercise in women (34, 35). The three bouts were administered between 8 a.m. to 12 p.m.(33, 36). Each subject should do the exercise at almost the same time in each of the three days (33).

Table 1. The recorded heart rate during each downhill running bout.

	Bout 1	Bout 2	Bout 3	F
Heart rate (beat.min^{-1})	153±11.3	154±11.33	152.5±11.07	3.387

*P< 0.05 significant difference among bouts

Downhill running exercise

The training included three bouts of downhill running on a treadmill (h/p/COSMOS, pulsar, Germany) separated by 24-hour rest intervals. At the beginning of each run, subjects warmed up for five minutes by running on a level grade by increasing the velocity to the predetermined speed. The treadmill was then lowered to -13.5% grade and subjects ran for 50 minutes at a velocity equal to 70–80% $VO2_{peak}$ on level grade. In the end, subjects cooled down for five minutes by decreasing speed and levelling grade (19).

Blood sampling and analysis

In this study, each subject performed three downhill running exercise bouts every 24 hours and blood samples were taken at the beginning (8 a.m.) and 2 hours after each bout (basal, first, 24 h and 48 h) (13, 23). Blood samples were to measure Total leukocyte count (TLC), Differential leukocyte count (DLC) and serum concentrations of CK, LDH, Mb, IL-10, IL-6, MCP-1. 10 ml blood was drawn from an ante cubital vein. Then, 2 ml whole blood was collected in EDTA tubes. The blood was transported quickly to the biochemical lab (less than 30 minutes) and used to assess TLC and DLC. In the same day, Complete blood count (CBC) test was conducted by automated cell counter and DLC was conducted by preparing blood smear for microscopic test within two hours after collection. 8 ml whole blood was collected in serum separator tubes (Z serum Sep Clot Activator, 9ml), which remained at room temperature for 30 minutes, and was then centrifuged for 10 minutes at 5,000 rpm. The serum obtained was stored in multiple aliquots at -21°C until it was analysed. ELISA kits were used to assess cytokines (eBioscience Co, USA) and myoglobin (DRG® Myoglobin). Serum activity of CK and LDH was determined by enzymatic colorimetric assay.

Practical tests and DOMS

Practical tests—including 1RM and ROM as well as DOMS—were conducted one hour before and two hours after every bout. Blood samples were obtained before these tests to preclude any potential acute effects of the test on blood parameters (37).

ROM of knee joint (in degrees) at flexion movement was measured by a goniometer (4). The perception of DOMS was assessed by a visual analogue scale (34) of pain (3, 4). Subjects were asked to report the degree of muscle soreness of gluteus (38), anterior thigh muscles (5, 38) and posterior lower leg muscles (5) after coming down a step as high as 30 cm. Each subject came down for four times from the step alternatively changing the leading leg between attempts. After each attempt, they indicated their soreness on a 10-cm Visual analogue scale (VAS). A score of 0 corresponded to a perception of 'no soreness', whereas a score of 10 indicated a feeling of 'very very sore'. The mean of 4 soreness ratings was calculated and used for comparative analysis (5).

Statistical analysis

It was a quasi-experimental study with one-group pre-test-post-test design. Data is expressed as mean ± SD. All statistical analyses were performed with IBM SPSS Statistics Version 23. The normal distribution was assessed by the Shapiro Wilk test. All dependent variables were analysed using repeated measures ANOVA, where significant main effects were found, a Bonferroni post-hoc test was performed to evaluate mean differences. Statistical significance was set at P<0.05.

Results

Physical characteristics of subjects were as follows: age = 24.80 ± 3.26 (y); height = 165.08 ± 5.14 (cm); mass = 56.88 ± 3.01 (kg); body mass index = 20.90 ± 1.24 (kg.m^{-2}); percentage of body fat = 24.35 ± 3.98 (%); VO_{2peak} = 41.48± 2.01 (ml.kg^{-1}.min^{-1}); running speed at 70-80 % VO_{2peak} on level grade = 7.85 ± 0.28 (km.h^{-1}). The recorded heart rate during each bout is presented in "Table 1".

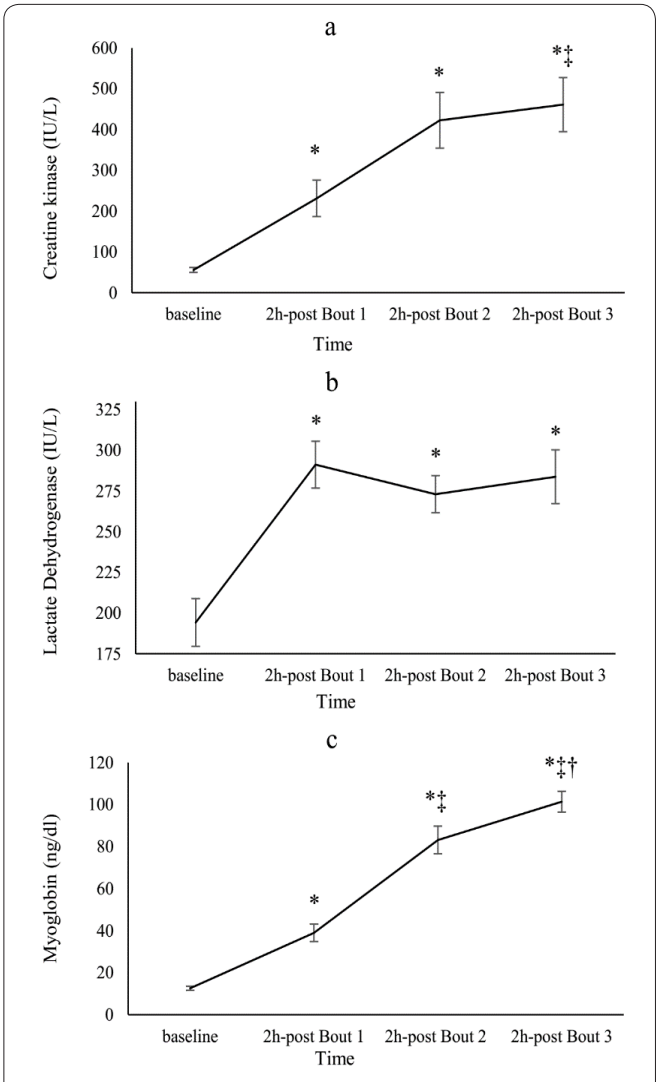

Figure 1. Values of CK (a), LDH (b) and Mb (c) during the three-day period of downhill running. * Significant difference form baseline; ‡ Significant difference from 2h-post Bout1; † Significant difference from 2h post Bout2 (P<0.05).

There was a significant increase in serum activity of CK, LDH and Mb two hours after each bout when compared with the baseline (P<0.05). CK and Mb were significantly higher after the third bout compared to two hours after the first bout (Figure 1; P<0.05).

TLC was significantly increased two hours after the first and second bouts compared to the baseline, while neutrophil and monocyte counts were significantly increased two hours after all the bouts compared to baseline and the peak increase for all was two hours after the first bout (P<0.05). Lymphocyte count was significantly reduced two hours after the second bout compared to baseline (Table 2; P<0.05).

Serum concentrations of IL-10 were significantly elevated two hours after each bout compared to the baseline and were significantly higher two hours after the first bout compared to the second and third bouts (P<0.05). The serum concentrations of MCP-1 and IL-6 were significantly elevated two hours after the first bout compared to the baseline. Furthermore, serum IL-6 was significantly higher two hours after the first bout compared to the second and third bouts (Figure 2; P<0.05).

The maximum isotonic strength in knee extensor muscles was reduced during the three-day period of training while this reduction was significant only two hours after the first bout to two hours after the second bout compared to the baseline (P<0.05). In addition, a significant increase was displayed prior to the third bout compared to two hours after the second bout (Table 3; P<0.05).

There was no significant difference in the ROM values (P>0.05).

The delayed onset muscle soreness as a manifest feature of EIMD was significantly increased in anterior thigh muscles two hours after the second and third bouts (P<0.05). Also, DOMS of gluteus and posterior lower leg significantly increased two hours after the second bout until two hours after the third bout compared to the baseline (Table 3; P<0.05).

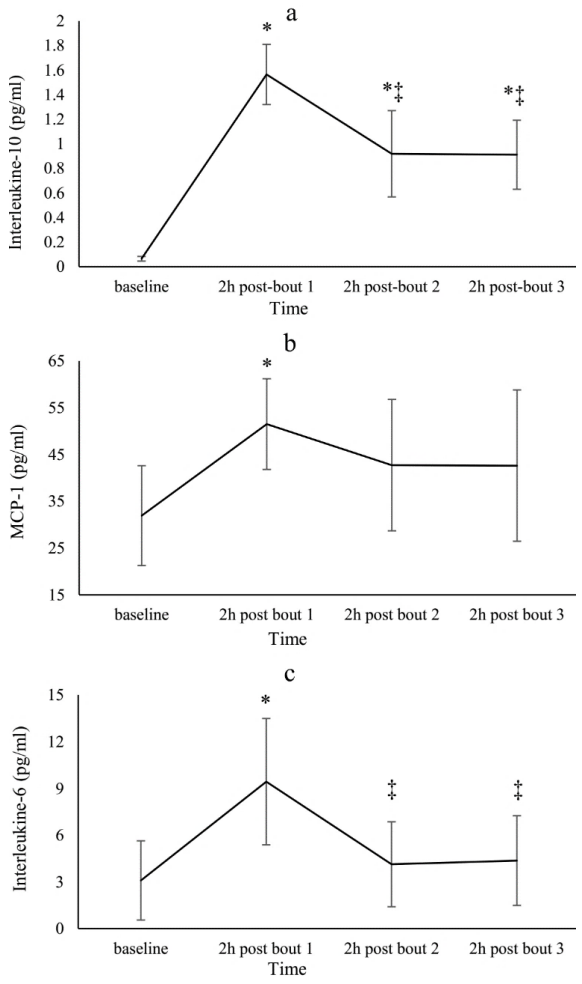

Figure 2. Values of IL-10 (a), MCP-1 (b), IL-6 (c) during the three-day period of downhill running. * Significant difference form baseline; ‡ Significant difference from 2h-post Bout1 (P<0.05).

Discussion

In the present study, the main questions were as follows: How would be change in the pro/anti-inflammatory responses during the time-course of the training? And how would be the repeated bout effect on EIMD during a three-day period of downhill running in active females? The main findings were as follows: 1) Performing a three-day downhill running induced muscle damage due to rise in serum activity in CK, LDH, Mb,

Table 2. Values of total leukocyte count, neutrophil, monocyte and lymphocyte counts during the three-day period of downhill running.

	Bout 1		Bout 2	Bout 3	F
	Baseline	2h post	2h post	2h post	
TLC (10^3/mm^3)	6.25±1.15	10.50±3.97*	7.90±1.57*	8.58±2.38	7.759
Neutrophil (10^3/mm^3)	4.10±1.03	6.30±1.30*	5.49±1.17*	5.72±1.39*	10.035
Monocyte (10^3/mm^3)	0.07±0.05	0.55±0.28*	0.46±0.19*	0.48±0.16*	14.258
Lymphocyte (10^3/mm^3)	2.20±0.59	1.80±0.65	1.39±0.37*	1.73±0.47	5.527

*P< 0.05 significant difference from baseline

Table 3. Values of one-repetition maximum and DOMS of measured muscle groups during the three-day period of downhill running.

	Bout 1		Bout 2		Bout 3		F
	Pre	2h post	Pre	2h post	Pre	2h post	
1RM (kg)	96.90±19.40	89.13±21.50*	89.38±23.68*	78.50±25.07*	83.57±24.04†	79.13±21.20	7.520
DOMS(cm):							
Gluteus	0.18±0.19	1.16±1.28	3.19±2.68	3.99±2.90*	4.02±2.87*‡	4.30±3.14*	14.186
Anterior thigh	0.42±0.36	1.83±1.67	3.84±3.33	5.16±3.48*	4.64±3.32	5.10±3.44*	11.311
Posterior lower leg	0.30±0.30	1.07±0.97	2.83±2.90	3.78±3.05*	4.21±3.56*	4.55±3.65*‡	11.705

*P < 0.05 significant difference from pre-bout 1; ‡P < 0.05 significant difference from 2h post-bout 1; †P < 0.05 significant difference from 2h post-bout 2.

increase in DOMS and decrease in maximum isotonic strength; 2) inflammatory and immune responses were observed due to increasing in TLC, monocytes, neutrophils and decrease in lymphocytes as well as increase in serum concentrations in IL-10, MCP-1 and IL-6; 3) the highest rise in TLC, neutrophils, monocytes, and all measured cytokines was after the first bout; 4) the highest rise in DOMS was after the second and third bouts; 5) the lowest isotonic strength was after the second bout, while there was no significant difference after the third bout compared to the baseline and even prior to the third bout, there was a significant increase in strength compared to post-second bout that might imply strength recovery.

In this study, serum concentrations of intracellular muscle proteins, including CK, LDH and Mb, as muscle damage indicators (1)were significantly increased during the training period. The peak levels of CK and Mb were two hours after the third bout, nearly 48 hours following the first bout. The peak level for LDH was two hours after the first bout. In similar studies, serum levels of CK and LDH significantly increased during Days 3–6 by performing a seven-day eccentric training period (14); also, serum levels of CK and Mb increased significantly immediately one hour after a three-day period of intensified running or cycling. Moreover, CK difference remained significant at 14 hours after the period (3).

Indeed, performing a three-day eccentrically biased endurance exercise as downhill running could induce high stress on tissues due to the high force-to-activation ratio (20). Muscle damage might be initiated by mechanical stress occurring when the muscle lengthens and generates braking forces when the foot strikes the ground to maintain balance against gravity (23). This stress may cause disturbance of sarcomere structure from Z-line to M-line (39), rupture both the sarcolemma and sarcoplasmic reticulum membranes and activate stretch sensitive Ca^{2+}-channels that disturb calcium homeostasis (40). The accumulation of Ca^{2+} within sarcoplasm can activate the phospholipase-prostaglandin pathway and the calpain proteolytic pathway (1). Calpains and phospholipase A_2 induce intracellular proteolysis (40)and sarcolemma breakdown (17)respectively, which lead to intracellular muscle molecule release, such as Ck, to circulation (17). The muscle damage persists or intensifies for two to three days following initial damage due to secondary damage and inflammatory response that accompanies the increased leukocyte recruitment to damage muscles and increased cytokine activity. Recruited neutrophils and monocytes/macrophages cause oxidative stress by phagocytic activity and lead to more damage (23).The difference between value and time course of intracellular proteins in this study and previous studies using eccentric exercises could depend on gender (41), age, physical activity level, intensity, duration and type of exercise, clearance rate from lymph to circulation (42)and blood sampling time (4).

DOMS had developed from the first to the third bout while it was only significant two hours after the second bout to two hours after the third bout, i.e., during 24–48 hours after the first bout. Previous studies using consecutive days of eccentric exercises, reported similar results on DOMS with respect to developing a course of time (3, 4, 14). The peak value for anterior thigh muscles was at 24 hours following the first bout, and for glutes and posterior lower leg muscles, it was at 48 hours after the first bout. The peak value for each group muscles is presented in Table 3 in which, according to the distribution of pain VAS scores (43), mild to moderate pain was felt. Exercise-induced muscle pain results from an inflammatory response when hyperalgesic mediators like TNF-α, IL-1β, prostaglandins and superoxide anion radicals are produced in large amounts and stimulate peripheral endings of nociceptive fibers (9). It results in swelling, infiltration of inflammatory cells and breakdown of myocellular components (1).The gradual increase of DOMS was concomitant with a gradual decrease in TLC and DLC. The recruited leukocytes were probably redistributed to target tissues, which were a reason for gradual fall in leukocytes from circulation concomitant with the increase in DOMS.

Strength loss and recovery are commonly used as one of the most valid and reliable indirect markers of EIMD (1). In this study, isotonic strength in knee extensor muscles significantly decreased two hours after the first bout to two hours after the second bout compared to the baseline. The lowest value was observed at two hours after the second bout, i.e., about 24 hours after the first bout. It showed 19% strength loss. There was no significant difference on the third day compared to the baseline. However, before the third bout, the value showed significant rise compared with two hours after the second bout, which may show strength recovery. The previous studies reported consistent strength loss of about 17.5% (44) and 18% (5) respectively by using downhill running protocols. Following eccentric exercises, several factors lead to muscle force reduction, including excitation-contraction coupling failure, disturbance in muscle force-bearing structures (5), immune cell infiltration, such as neutrophils, for even 48 hours after the initial injury to muscle damage sites, which result in oxidative stress (45).

After the training period, there was no significant difference in ROM of knee joint ($P>0.05$). This finding requires further discussion since numerous studies have reported a significant decrease in this variable after eccentric exercises (1). However, it should be noted that most studies which have reported ROM decrease used high intensity, low-repetition resistance-like eccentrics on certain group muscles such as elbow flexors (14, 46, 47). The protocol of this study was low-intensity high-repetition aerobic-like eccentrics. However, few studies consistent with this study reported no significant difference in ROM (4, 16). On the other hand, some studies following downhill running protocols reported significant differences or strong trend to reduction in ROM of knee and ankle joints by applying high-speed video recording to analyse ROM of joints in the gait cycle (48, 49). In the present study, a goniometer was used to assess ROM of knee joint in flexion movement lying in prone position. It is obvious that the method of measurement is a key point in these findings.

According to data, systemic immune response to exercise was clear. TLC, neutrophils and monocytes increased significantly following exercise with the peak values two hours after the first bout for all, while lymphocytes displayed significant decrease only two hours after the second bout compared to the baseline

($P<0.05$). Previous studies reported similar results. A study reported a significant increase in TLC, neutrophils and monocytes immediately and four hours after a bout of downhill running whereas lymphocytes showed no significant difference (10). In the other study, following two bouts of downhill running separated by 14 days, a significant increase was displayed for neutrophils and monocytes during 2–4 hours and 4–12 hours respectively following each bout. There was no significant difference between bouts (38).

The significant increase of TLC, neutrophils and monocytes was concomitant with an acute systemic cytokine response, which could recruit leukocytes. The initial rapid increase in blood neutrophils and monocytes following exercise is regulated by factors such as catecholamines, cortisol and blood flow increase (50) as well as IL-6 and MCP-1 as regulators of recruiting neutrophils (50) and monocytes (51)respectively. Subsequent to downhill running, macrophages and neutrophils are infiltrated to skeletal muscle tissue, which may provide an important factor of oxidative stress and inflammatory reaction following EIMD (51). Towards the end of the period, the gradual decrease of leukocytes, neutrophils, monocytes might be due to redistribution of leukocytes from blood to tissues as well as attenuated immune response to inflammation and damage. On the other hand, the decrease in lymphocytes following the second bout was likely due to increase in other leukocytes' subsets or deficit of mature cells that can be recruited, as well as the redistribution of lymphocytes from circulation to organs (52).

The inflammatory response was expected following muscle damage. IL-6, IL-10 and MCP-1 showed a significant increase following the first bout. Similarly, it has been reported an increase in IL-6, IL-10 and MCP-1 within 12 hours after the two bouts of downhill running separated by 14 days (13).

IL-6 is the first cytokine present in circulation during exercise, whose concentrations depend on exercise intensity and duration, the mass of recruited muscles, endurance capacity (53), muscle-glucose uptake and plasma adrenalin (54). IL-6 is released from skeletal muscle during exercise (53)whereas monocytes and macrophages are suggested to be the main source of IL-6 following exercise (10). Indeed, IL-6 is an inflammatory cytokine with pro- and anti-inflammatory effects that exerts anti-inflammatory effects by inhibiting the production of TNF-α and stimulating the production of IL-1ra and IL-10 (53). IL-10 is a cytokine produced during stress conditions that can limit the synthesis of inflammatory mediators (55). IL-10 is up-regulated by several factors, including arachidonic acid derivations, TNF-α, IL-6, interleukine-12, type-I interferon's, glucocorticoids, adrenaline, prostaglandin E2, hydrogen peroxide, in monocytes/macrophages or in T cells during stress conditions (55). On the other hand, MCP-1 is produced by numerous cells in damaged muscle tissue such as myofibers, myogenic cells, infiltrating inflammatory cells, muscle resident macrophages, capillary endothelial cells and fibroblasts as well as circulating monocytes and bone marrow cells (8) affected by oxidative stress, cytokines or growth factors (56). The key role of MCP-1 is to recruit monocytes/macrophages to the site of inflammation in damaged muscles (8).

In this study, although there was significant increase two hours after the first bout in all measured cytokines, serum IL-10 was only significantly higher compared to baseline following the second and third bouts. However, IL-10 showed significant decrease following the second and third bouts compared to two hours after the first bout. Serum IL-6 and MCP-1 showed no significant increase following the second and third bouts, although IL-6 showed significant decrease following the second and third bouts compared to two hours after the first bout. Interestingly, it has been reported an increase in IL-10 (95%) versus a decrease in IL-6 (50%) and MCP-1 (10%) during 12 hours after the second bout compared to the first bout of downhill running (13).

Indeed, it seems that pro-inflammatory signaling was moderated. Anti-inflammatory IL-10 inhibits production of pro-inflammatory cytokines such as IL-6 in macrophages (11). In addition, IL-10 could interfere with MCP-1 by inhibiting the production of MCP-1 as well as by generating functional decoy receptors for MCP-1 on monocytes, which act as molecular sinks and scavengers for MCP-1 chemokine (12). Furthermore, anti-inflammatory IL-10 has antioxidant and anti-hyperalgesic effects, which act by diminishing leukocyte recruiting, hyperalgesic cytokines production (such as TNF-α and IL-1β), as well as suppressing superoxidase anion, NADPH oxidase, prostaglandins and neuronal nociceptive sensitization (9).

In conclusion, inflammatory response to tissue damage is crucial. The transition from the inflammatory to the repair phase depends on the attenuation of pro-inflammatory signals concurrent with enhancement of anti-inflammatory signals. Indeed, what is known as repeated-bout effect and its related adaptations partly return to attenuation of inflammatory responses. Then, this study results confirmed this notion; pro- and anti-inflammatory cytokines responses, as well as changes in leukocyte counts following the second and third bouts (compared to the first bout), tended to moderate inflammatory responses and return to baseline levels. In addition, there was moderate soreness in muscles following the three-day downhill running concomitant with strength recovery on the third day. Moreover, continuous increase in serum levels of intramuscular proteins implied the clearance rate increased from tissue to circulation. Finally, early adaptation to exercise could be seen due to the repeated bout effect.

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