Serum levels of interleukin-6 (IL-6), IL-10, and lactate in response to combat physical fitness test

Article *in* Sport Sciences for Health · July 2019

DOI: 10.1007/s11332-019-00567-9

3 authors, including:

Behzad Bazgir
Baqiyatallah University of Medical Sciences
27 PUBLICATIONS **109** CITATIONS
SEE PROFILE

Hossein Shirvani
46 PUBLICATIONS **120** CITATIONS
SEE PROFILE

Sport Sciences for Health
https://doi.org/10.1007/s11332-019-00567-9

ORIGINAL ARTICLE

Serum levels of interleukin-6 (IL-6), IL-10, and lactate in response to combat physical fitness test

Behzad Bazgir[1] · Alireza Delpasand[1] · Hossein Shirvani[1]

Received: 5 May 2019 / Accepted: 19 June 2019

Abstract

Purpose The aim of the study is to investigate serum levels of interleukin-6 (IL-6), IL-10 and lactate in response to combat physical fitness test.

Methods 20 volunteer (26.95 years, BMI: 22.13 ± 2.78 kg/m^2) subjects were engaged to participate the test consisted: (1) 25-yd sprint to J-hook, to 25-yd crawl, and 25-yd run through cones to 75-yd line; split (2) 75-yd casualty drag; split (3) ammo can run to grenade toss (75-yd); (4) ammo can run to end (75-yd); and (5) 650 yard endurance run. Blood was drawn before and after exercise, and circulating IL-6 and IL-10 as well as plasma levels of lactate were assessed. The levels of IL-6 and IL-10 were determined by enzyme-linked immunosorbent assay.

Results The results of the pre-test and post-test variables show: post-test heart rate and blood lactate were significantly elevated due to combat test ($p = 0.001$). Plasma interleukin 6 increased significantly after combat-related test ($p = 0.003$), but interleukin 10 had no dramatic change after the test. There were strongly significant correlations between post-test IL-6 and lactate levels ($P < 0.001$, $r = -0.591$).

Conclusion In conclusion, it suggested that IL-6 is responsive to short-term maximal power output required in combat readiness test. However, it appears that 3–4 min of high-intensity exercise induces comparatively moderate post-exercise increment in IL-6, which appears to be inadequate to activate the systemic anti-inflammatory influences that are mediated via secondary IL-6-activated up-regulation of anti-inflammatory signaling myokines such as IL-10.

Keywords Combat readiness · Inflammation · Interleukin · Military

Introduction

Combat fitness readiness is one of the inseparable elements of the armed forces. [1]. The maneuver under attack fire (MANUF) is a relevant, high-intensity and integrated test that its stages are executed by maximal power output and included combat physical requirement similar to war condition [2]. Previously, it was believed that exercise may increase interleukin-6 (IL-6) and blood lactate production. The following acute physiological response leads to muscle-derived IL-6-induced release of an anti-inflammatory cytokine/mediator, IL-10 by the leukocytes to restrict inflammatory interactions [3].

Despite the previous pointed view, comprehensive evidence is reported that during heavy physical activity such as high-intensity exercise, skeletal muscles release definite amount of IL-6 into circulation [4]. It is believed that the muscle secreted IL-6 has some metabolic roles. The response of IL-6 indicate to critical diminish in intramuscular glycogen and reliance on blood glucose as an alternative energy sources [5]. In addition, the muscle lactate shuttles (monocarboxylate transport protein) release the lactate into circulation [6]. IL-6 similar to leptin activates AMP-activated protein kinase (AMPK) in muscle and adipose tissue. The activation of AMPK impacts insulin signaling and led to more glucose uptake by active muscles [7]. Therefore, assessment of IL-6, 10 and lactate following, MANUF test could be as indicative of metabolic stress that solders endure.

Also several researches studied the response of cytokines such as interleukin 6 and 10 to high-intensity exercise and

Behzad Bazgir, Alireza Delpasand and Hossein Shirvani contributed equally to this work.

✉ Hossein Shirvani
shirvani@bmsu.ac.ir

[1] Exercise Physiology Research Center, Life Style Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran

Published online: 01 July 2019

reported contradicted results such as elevation [8–10] and no change [11, 12], and the notable issue is that in previous performed protocols, the movement pattern/type was limited to one of the resistance/power or aerobic exercise types such as cycling or running [10–12]. However, MANUF is a complete combination of war-simulated task such as sprint, crawling, tossing grenade, carrying casualties or ammo boxes [2]. Hence, it is unclear whether utilizing different movement patterns or muscle groups throughout MANUF makes any difference in interleukin 6/10 or lactate responses or not. Therefore due to lack of sufficient information about the metabolic response of MANUF test, our first aim is to measure and compare the IL-6, IL-10 and blood lactate in pre- and post-test.

IL-6, IL-10 and lactate concentration are elevated after high-intensity exercise or chest and abdominal trauma [13]. It seems that activation of monocytes to macrophages through exposure to IL-6 led to IL-10 elevation [3]. IL-6 elevation in active muscles implies intramuscular glycogen depletion due to high-intensity/volume exercise training and lactate production [5]. The promoter gene of IL-6 may contribute to the activation of IL-6 gene transcription with exercise [4]. By muscle contraction, calcium activates both NFAT and NF-κB and muscle cell increases IL-6 secretion in a p38 MAPK-dependent manner [14]. Hence, we postulate that there is a direct correlation between the ratio of IL-6 to IL-10 and lactate production.

Materials and methods

Statistical sample

The experiments were carried out in February–March 2019. In the first step, 20 army forces were randomly selected as study sample among those men who have military history more than a year. Information was provided to familiarize participants with research procedures, the benefits and potential risks. Informed consent forms of the research method, and the health and physical fitness questionnaire were completed. Height and weight measurements and familiarity with the test were performed the day before the test. At the end, 20 subjects with an average age of 26.95 years, height 176.65 ± 6.36 cm, weight 73.3 ± 7.34 kg, body mass index 22.13 ± 2.78 kg/m^2 voluntarily participated in the present study. The participant exclusion factors were history of fracture, cardiovascular disease, smoking and consuming drug.

MANUF test

The MANUF contain the tasks which are combat related. The MANUF was formed to measure ability to perform combat-related tasks and these events are representative of the movement types that are made potentially in combat. The sort of the items into a single timed event is both a combination of energy system demonstration, strength/anaerobic plus aerobic, and a measurement of military combat capability and physical fitness. During the test, subjects tracked 950 yd. The first 300 yd on a field included all MANUF tasks: performing a 25-yd sprint, J-hook turn, 25-yd low and high crawl, 75-yd casualty drag (with partner); two 75-yd ammo can (30 lb each, 1 in each hand) carry, a grenade toss and 650 yd running (Fig. 1) [2]. During testing events, the 75-yd casualty drag is a 10-yd drag, followed by a 65-yd fireman's carry. However, this was customized to reduce injury risk. Furthermore, the three push-ups that follow the grenade toss and precede the second ammo can run were removed to decrease the risk of injury.

The final 650 yd included the distance between the field and the end line. Specifically, each stage is in the following events: (1) 25-yd sprint to J-hook, to 25-yd crawl, and 25-yd run through cones to 75-yd line; split (2) 75-yd casualty drag; split (3) ammo can run to grenade toss (75-yd); (4) ammo can run to end (75-yd); and (5) 650 yard endurance run. The MANUF course is described in Fig. 1. Subjects maintained their run pace between start and course completion [2]. All protocol was approved by exercise physiology research center, life style institute, Baqiyatallah University of Medical Sciences (IR.MBSU.REC.1397.303).

Dietary and physical activity program

Subjects were requested to have a food diary before the combat testing. Subjects arrived at the testing ground at the same time of day before the tests and were requested to avoid eating or drinking (other than water) for the 2 h before the test, and to avoid alcohol, caffeine or strenuous physical activity in the preceding 24 h.

Enzyme-linked immunosorbent assays

All blood samples were gathered into K3EDTA tubes and were isolated by centrifugation (3000×*g* for 10 min).The obtained plasma was aliquoted and stored at − 80 °C until analysis. Plasma IL-6 and IL-10 levels were analyzed and measured in duplicate using high sensitivity enzyme-linked immunosorbent assay technique [15] (Quantikine HS; R&D Systems Ltd., Abingdon, UK). The IL-10 assay has a detection limit of 0.09 pg/ml and an intra-assay CV of 1.9 ± 1.7% across a range of 0.78–50 pg/ml and the IL-6 assay has a detection limit of 0.039 pg/ml and an intra-assay coefficient of variation (CV) of 3.8 ± 2.9% across the range 0.15–10 pg/ml. Lactate was measured with enzymatic essay and assay accuracy, imprecision, analytical sensitivity, linearity, and a reference interval were determined.

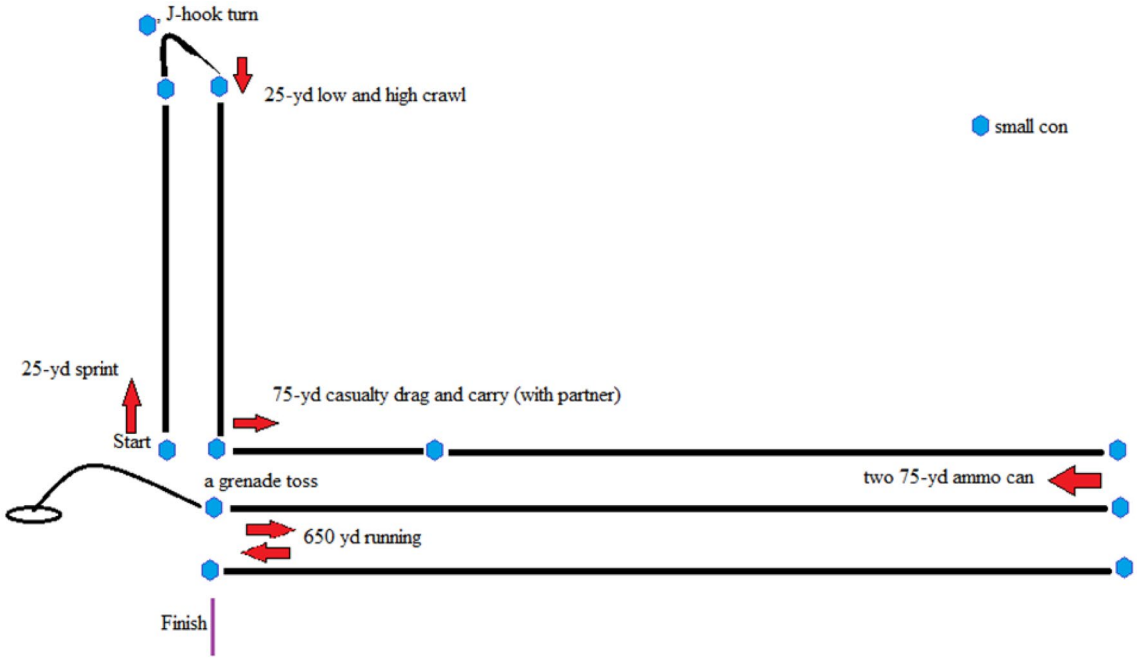

Fig. 1 The diagram of MANUF test

Statistical analysis

All data are presented as means ± standard error unless otherwise stated. Paired sample student *T* test was used to analyze the data. Statistically significant differences set at $p < 0.05$. SPSS 16.0 was used for all statistical analysis. Bivariate Pearson correlation coefficients were utilized to examine relationship between ratios of IL-6, IL-10 and IL-6 to 10 with lactate level. All data were expressed as mean ± SD and significance was set at the alpha level $p < 0.05$.

Results

Demographics

Twenty-two participants were engaged to participate; however, only 20 completed all trials and are covered in the analysis. Participants' characteristics are shown in Table 1. All individuals had at least one combat deployment before the research was operated and nearly averaged 43.1 ± 18 months of active duty service.

The results of the pre-test and post-test variables are shown in Fig. 2. Then, paired sample *t* test was used to clear out the significant differences. Within-group comparison indicated greater post-test heart rate levels as compared with pre-test value ($p = 0.001$). Also compared to pre-test, blood lactate was significantly greater in post-test ($p = 0.001$).

Table 1 Anthropometric measurements of subjects

	Mean ± SD
Age (years)	26.95 ± 3.80
Weight(kg)	73.3 ± 7.34
Height (cm)	176.65 ± 6.36
BMI (kg/m^2)	22.13 ± 2.78
Rest Heart rate	78.63 ± 12.5

BMI body mass index, *SD* standard deviation

Plasma interleukin six significantly increased after combat-related test ($p = 0.003$) but interleukin 10 had no dramatic change after the test was over. Also the ratio of IL-6 to 10 was increased after the test that was not statistically significant. The ratio of IL-6 to 10 was not significant between pre and post-test. Figure 3 shows the bivariate analysis results, the co-relationship between IL-6 and IL-10 and the ratio of IL-6 to IL-10 with lactate. There were strongly significant correlations between IL-6 and lactate in post-test ($p < 0.001$, $r = -0.52$) (Fig. 3).

Discussion

The main finding of present study demonstrated that blood lactate was significantly greater in post-test as the subjects tried to finish the test with maximal effort in approximately 3 min; hence, to supply the metabolic requirement of the

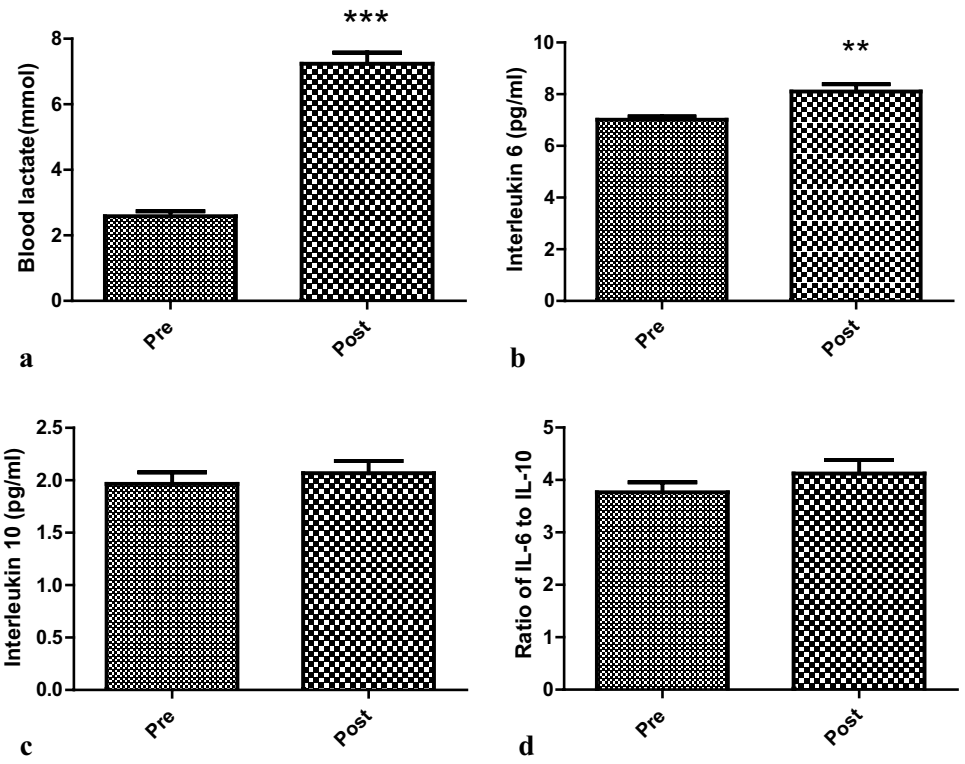

Fig. 2 Effect of combat physical fitness test on: (**a**) blood lactate, (**b**) interleukin-6, (**c**) interleukin-10, (**d**) ratio of IL-6 to IL-10. The data are shown as the means ± SD. *Significantly different with the pre-test at $p < 0.001$(***), $p < 0.01$ (**)

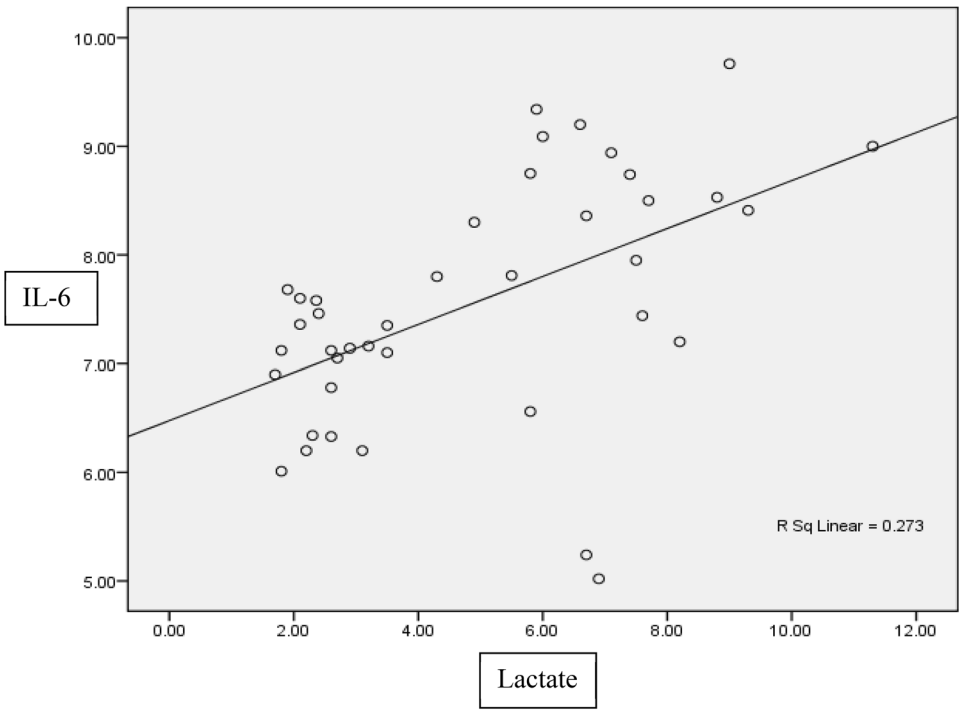

Fig. 3 The correlation between post-test lactate and IL-6

anaerobic test glycolytic/anaerobic system was utilized. Plasma interleukin six increased after combat readiness test significantly but interleukin 10 had no dramatic change after the test. In addition, strong significant correlations were observed between IL-6 and lactate in post-test; however, the ratio of IL-6 to 10 was not significant between pre- and post-test.

Studies have shown that the pro-inflammatory cytokine (IL-6) upregulated by high-intensity exercise. Cullen et al (2016) investigated the response of IL-6-related

inflammatory to high-intensity interval exercise (HIIE) and impact of exercise intensity and volume on this response. It was shown that high-intensity exercise led to a significant increase in IL-6 that this change is more than low-intensity exercise with the same duration [8]. The higher volume of regular physical activity was associated with decreased IL-6 levels and increased IL-10 levels in very healthy subjects. Thus, as a result of IL10's lack of change, the present study can be justified. Exercise may play a vital role in controlling inflammatory markers during the aging process. In addition, Dorneles et al. reported that the acute inflammatory response to interval exercise is dependent on exercise intensity. Only HIIE induced crucial changes in IL-6 levels, which may have dramatic implications in controlling low-grade inflammation [9]. The reason different change of IL-10 in this study can attribute to different training protocols. The movement pattern/type was limited to one of the resistance/power or aerobic exercise types such as cycling or running but in MANUF that there is a complete combination of war-simulated task such as sprint, carrying casualties, ammo can or crawling and mid-endurance run [2]. In addition, we found that the post-exercise elevation in IL-6 was positively correlation with blood lactate changes. It seems that activation of monocytes to macrophages through exposure to IL-6 led to IL-10 elevation [3]. IL-6 elevation in active muscles implies intramuscular glycogen depletion due to high-intensity/volume and lactate production [5]. The promoter gene of IL-6 may contribute to the activation of IL-6 gene transcription with exercise [4]. By muscle contraction, calcium activates both NFAT and NF-κB, and muscle cell increases IL-6 secretion in a p38 MAPK dependent manner [14]. In agreement with previous literature, we found that the post-exercise elevation in IL-6 was correlated with blood lactate changes, which is indicative of a dramatic metabolic reliance on carbohydrate as a substrate to end the test. This is unsurprising given that one of the critical IL-6 functions during heavy physical activity is to respond to muscle glycogen store status and facilitate metabolism of glucose [5].

Indeed our results show that blood lactate levels were significantly higher in the post-exercise than before it did. It is of note that the IL-6 response observed in this research was markedly lower than that reported following longer duration exercise such as a marathon [16]. However, IL-6 responses are noted to be smaller following cycling than exercise modes that recruited larger muscle mass, such as running [17]. Also, the subjects of the study were young (26.95 ± 3.80 years) and relatively fit; hence, given that IL-6 concentration is increased with age and decreased with physical fitness, it is probable that a greater response would have been observed in older or less fit subjects. In addition, it is possible that the similar moderate increase in IL-6 seen in our research was mainly due to the comparatively short duration of the combat fitness test [4].

IL-10 is an important immunoregulatory cytokine with multiple biological effects. It has been shown that IL-10 can decrease NF-κB activity by suppressing IKK and NF-κB DNA binding activity. Thus, the reason of no significant change of IL-10 in the present study can attribute to anti-inflammatory effects. Basically, we observed no elevation in the plasma level of IL-10, which is in contrast with studies involving more endurance exercise; for instance, 26-fold elevation in circulating IL-10 protein level and 2.7-fold raise in leukocyte gene expression have been recorded instantly following 2 h of cycling exercise [18]. Attractively, these extensive anti-inflammatory responses were accompanied by a 40-fold raise in circulating level of IL-6. Comparably, while recent evidence has proposed that IL-6 may induce the up-regulation of IL-4R in leukocytes and subsequently increment in IL-4-mediated signaling [19]. The soluble factors, PH (lactate production) and intracellular signaling pathways regulate cytokine synthesis such as IL-6 or 10 (e.g., RNA-binding proteins, microRNAs, suppressor of cytokine signaling proteins, soluble receptors).

Conclusion

In conclusion, it is suggested that IL-6 is responsive to short-term maximal power output required in combat fitness test and positively correlates with blood lactate. However, it appears that 3–4 min of high-intensity exercise induces comparatively moderate post-exercise IL- 6 increment, which appears to be inadequate to stimulate IL-10 secretion [5, 18–21]. It appears that there may be an IL-6 threshold level demanded and higher metabolic stress is required for the activation of the compensatory and positive systemic anti-inflammatory responses and an extended exercise duration is expected to be a crucial element in achieving this; however, further studies need to elucidated this.

Acknowledgements The authors acknowledge all support of the Centre of exercise physiology Baqiyatallah University of Medical Sciences, Tehran, Iran, that without which this work would not have been possible.

Funding None.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval All protocol was approved by exercise physiology research center, life style institute, Baqiyatallah University of Medical Sciences (IR.MBSU.REC.1397.303).

Informed consent Informed consent forms of the research method, and the health and physical fitness questionnaire were completed.

References

1. Kou Y-X, Wang L, ZHOU Z-l (2008) Study of combat task allocation model in multi-target attack condition. J Syst Simul 16:911–917
2. Jaworski RL et al (2015) Changes in combat task performance under increasing loads in active duty marines. Military Med 180:179–186
3. Moore KW et al (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19(1):683–765
4. Fischer CP (2006) Interleukin-6 in acute exercise and training: what is the biological relevance. Exerc Immunol Rev 12(6–33):41
5. Pedersen BK, Febbraio MA (2008) Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev 88(4):1379–1406
6. Halestrap AP (2012) The monocarboxylate transporter family—structure and functional characterization. IUBMB Life 64(1):1–9
7. Glund S et al (2007) Interleukin-6 directly increases glucose metabolism in resting human skeletal muscle. Diabetes 56(6):1630–1637
8. Cullen T et al (2016) Interleukin-6 and associated cytokine responses to an acute bout of high-intensity interval exercise: the effect of exercise intensity and volume. Appl Physiol Nutr Metab 41(8):803–808
9. Dorneles GP et al (2016) High intensity interval exercise decreases IL-8 and enhances the immunomodulatory cytokine interleukin-10 in lean and overweight–obese individuals. Cytokine 77:1–9
10. Wadley AJ et al (2016) Low volume–high intensity interval exercise elicits antioxidant and anti-inflammatory effects in humans. J Sports Sci 34(1):1–9
11. Logan GRM et al (2016) Low-active male adolescents: a dose response to high-intensity interval training. Med Sci Sports Exerc 48(3):481–490
12. Vella CA, Taylor K, Drummer D (2017) High-intensity interval and moderate-intensity continuous training elicit similar enjoyment and adherence levels in overweight and obese adults. Euro J Sport Sci 17(9):1203–1211
13. Taniguchi T et al (1999) The ratio of interleukin-6 to interleukin-10 correlates with severity in patients with chest and abdominal trauma. Am J Emerg Med 17(6):548–551
14. Pritts TA et al (2002) Proteasome inhibitors induce heat shock response and increase IL-6 expression in human intestinal epithelial cells. Am J Physiol Regula Integr Comp Physiol 282(4):R1016–R1026
15. Lassale C et al (2017) Separate and combined associations of obesity and metabolic health with coronary heart disease: a pan-European case-cohort analysis. Eur Heart J 39(5):397–406
16. Suzuki K et al (2003) Impact of a competitive marathon race on systemic cytokine and neutrophil responses. Med Sci Sports Exerc 35(2):348–355
17. Peake J, Nosaka KK, Suzuki K (2005) Characterization of inflammatory responses to eccentric exercise in humans. Exerc Immunol Rev 11:64–85
18. Nieman DC et al (2006) Blood leukocyte mRNA expression for IL-10, IL-1Ra, and IL-8, but not IL-6, increases after exercise. J Interferon Cytokine Res 26(9):668–674
19. Mauer J et al (2014) Signaling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. Nat Immunol 15(5):423
20. Abbasi A et al (2013) Changes in spontaneous and LPS-induced ex vivo cytokine production and mRNA expression in male and female athletes following prolonged exhaustive exercise. Exerc Immunol Rev 19:8–28
21. Goodyear LJ, Kahn BB (1998) Exercise, glucose transport, and insulin sensitivity. Annu Rev Med 49(1):235–261