American Journal of Sports Science
2018; 6(2): 32-37
http://www.sciencepublishinggroup.com/j/ajss
doi: 10.11648/j.ajss.20180602.11
ISSN: 2330-8559 (Print); ISSN: 2330-8540 (Online)

Systemic Responses of Inflammation-Related Factors Following Eccentric Exercise in Humans

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To cite this article:
Anastassios Philippou, Maria Maridaki, Constantinos Psarros, Michael Koutsilieris. Systemic Responses of Inflammation-Related Factors Following Eccentric Exercise in Humans. *American Journal of Sports Science*. Vol. 6, No. 2, 2018, pp. 32-37.
doi: 10.11648/j.ajss.20180602.11

Received: October 11, 2017; **Accepted**: October 26, 2017; **Published**: March 16, 2018

Abstract: Background: Exercise-induced muscle damage is followed by muscle adaptation which has been associated with an inflammatory response and is influenced by a crucial balance between pro- and anti-inflammatory cytokines. This study investigated the pattern of systemic cytokine responses for several hours after muscle-damaging exercise. Methods: Nine healthy, young men volunteers performed 50 maximal eccentric muscle actions with each leg using the knee extensors. Serum levels of interleukin (IL)-1α, IL-2, IL-6 and IL-10, transforming growth factor (TGF)-β1 and tumor necrosis factor (TNF)-α were measured by ELISA before and at 6, 48 and 120 hours post-exercise. Results: Volunteers reported significant muscle soreness and their serum creatine kinase (CK) activity increased gradually up to 120 hrs post-exercise ($p<0.05$). Circulating levels of IL-1a remained unaltered and TGF-β1 increased slightly over time, while IL-2 showed a moderate increase 48 hrs following eccentric exercise ($p>0.05$). Levels of TNF-α and IL-10 exhibited a similar pattern of response over time, showing a nearly 50% and 100% increase, respectively, 6 hrs post-exercise, while IL-6 increase significantly 6 and 48 hrs post-exercise ($p<0.05$). Conclusion: These findings suggest that eccentric exercise might trigger a systemic, predominantly anti-inflammatory, acute cytokine response as part of the adaptation process to muscle damage, where IL-6 may be especially involved.

Keywords: Cytokines, IL-6, IL-10, Muscle Damage, TGF-β1, TNF-α

1. Introduction

Mechanical overloading and stretch of skeletal muscle that occur in eccentric muscle actions have been extensively shown to lead to muscle damage and, thus, eccentric exercise has been utilized as a well characterized model to study the contraction-induced muscle damage and its consequent responses [1-3]. Muscle damage results in structural and functional disturbances in the eccentrically exercised muscle and changes in its mechanical properties, delayed-onset muscle soreness (DOMS), loss of muscle fiber integrity, leakage of muscle proteins into the blood and an acute inflammatory response are some well-characterized responses to muscle damaging exercise [1, 4, 5].

The classical damage–inflammation–regeneration process, which has been developed to describe muscle repair and adaptation following damage, involves an acute inflammatory response and the purpose of this aseptic inflammation is primarily the repair of the damaged muscle [1, 6]. The inflammatory response induced by muscle damage is largely directed by endogenous, muscle-derived cytokines, as well as cytokines produced by a variety of cells, such as tissue-resident leukocytes, circulating leukocytes and endothelial cells. These cytokines coordinate inflammatory-related events and are thought to play an active role as pro- or anti-inflammatory regulators of muscle repair process [1, 6, 7].

In particular, a balanced regulation of pro-inflammatory and anti-inflammatory factors determines the overall route of the inflammation and repair processes in the damaged muscle. Based on their main role in muscle damage-induced inflammation, cytokines can be divided as pro-inflammatory cytokines,

anti-inflammatory cytokines or biological inhibitors of the inflammatory cytokines, and cytokines of the TGF-β family, which regulate multiple biological processes including cell growth, differentiation and apoptosis, and promote extracellular matrix (ECM) synthesis, wound healing, and tissue remodelling (for review, see [6]). Thus, pro-inflammatory cytokines such as interleukin (IL)-1 and tumor necrosis factor-α (TNF-a), as well as anti-inflammatory cytokines such as IL-10 and IL-4, are expected to interact with each other, moderating the production of an excessive inflammatory reaction and leading to an adequate repair of the damaged muscle [7, 8]. The local muscle inflammation following the disruption of fibers in the damaged muscle is accompanied by acute phase response, a systemic response which further includes the production of numerous hepatocyte-derived acute-phase proteins [6, 9].

Although most has been learned about the interactions between muscle damage-induced inflammation and repair, the role of the systemic cytokine response to muscle damage appears to be complex and still not fully defined, as differential systemic responses of inflammation-related cytokines have been reported after muscle damaging exercise [6-10]. Thus, it remains a challenge to further characterize the systemic inflammatory response in the context of potential regulatory interactions between pro-inflammatory and anti-inflammatory factors in the regulation of exercise-induced muscle damage acute responses and adaptation. Hence, the purpose of this study was to examine the systemic responses of inflammation-related cytokines to muscle damage, by monitoring their serum changes for several hours after the completion of an eccentric exercise protocol in humans.

2. Methods

2.1. Ethical Approval

All the volunteers provided a written informed consent to participate in this study, which has been approved by the University's Ethics Committee, while all experimental procedures conformed to the Declaration of Helsinki.

2.2. Subjects

Nine healthy men (age 25.7 ± 1.7 years, height 180.4 ± 1.7 cm, body mass 77.2 ± 2.7 kg, body mass index 23.7 ± 0.6) participated in the study. The participants were physically active but had not participated in any type of resistance training or regular exercise regime for at least 6 months before the study and also were unaccustomed to high-intensity eccentric exercise. These volunteers were free of any lower extremity musculoskeletal disorders and they refrained from taking any nutritional supplementations or medications throughout the experimental period. In addition, these individuals were not allowed to perform any vigorous physical activities during the entire experimental period. They were also instructed to maintain their habitual diet, while on the day prior to and the day of each blood draw to have similar meals.

2.3. Experimental Design

The participants performed a maximal eccentric exercise protocol of the knee extensors with each leg. Before and at 6, 48 and 120 hrs after the eccentric exercise, blood samples were collected from each individual volunteer. The blood sampling time-points were chosen to cover an adequate period within the acute phase response/adaptation following exercise-induced damage. In addition, muscle soreness was measured on each leg at 24, 48 and 120 hrs post-eccentric exercise protocol.

2.4. Eccentric Exercise Protocol

Subjects performed an eccentric exercise bout with the knee extensors of each leg on an isokinetic dynamometer (Cybex Norm Lumex, Inc., Ronkonkoma, NY, USA), which has been shown to result in muscle damage [3]. Briefly, before the exercise protocol the subjects completed a familiarization session in which they were acquainted with the procedure of the eccentric exercise with each leg. The exercise protocol consisted of 2 sets of 25 maximal voluntary eccentric (lengthening) muscle actions in isokinetic mode, while a 5-min break was allowed between the sets.

2.5. Muscle Soreness

DOMS was evaluated on each leg as described in details elsewhere [4]. Briefly, muscle soreness was evaluated using a visual analog scale and the participants were instructed to rate soreness upon light palpation of the entire knee extensors area, always by the same investigator and with the thigh at rest, as well as during one repetition of fully flexing and extending the knee joint. For each participant, the two values were averaged and the mean was used as the criterion score for that day.

2.6. Blood Sampling and Serum Analyses

Blood samples were withdrawn prior to and after the exercise bout (at 6, 48 and 120 hrs post-exercise). The participants were seated quietly for 30 min and 10 ml of blood were drawn. Blood samples allowed to clot at room temperature for 30 min and serum was collected after centrifugation at 4,000 RPM for 10 min at 4°C, stored frozen in 0.5-ml aliquots at -80°C and only thawed once for analysis. Serum was assayed for creatine kinase (CK) activity (Roche/Hitachi ACN 057, Mannheim, Germany) at 37°C using commercially available kit (Roche Diagnostics, Mannheim, Germany). Serum CK activity was used as indirect marker of muscle damage.

Serum interleukin (IL)-6, IL-2, IL-10, IL-1α, tumor necrosis factor-α (TNF-α), and transforming growth factor-β1 (TGF-β1) were determined by standard sandwich enzyme-linked immunosorbent assay (ELISA) protocols using commercially available kits (IL-6: Quantikine HS, R&D Systems inc., MN, USA; IL-2, IL-10 and TNF-α: Biosource Europe S.A., Nivelles, Belgium; IL-1α: Invitrogen, CA, USA; TGF-β1: Assay designs, MI, USA) according to manufacturer's instructions. The colour formation was measured by a microplate reader (Versamax, Molecular Devices, CA, USA) at 450 nm, and calculations were carried out using a SoftMax Pro software (Molecular Devices,

CA, USA). All samples were run simultaneously, analyzed in duplicate and the results were averaged. According to the manufacturers, the minimal detection limits of the assays used were 0.039 pg ml^{-1}, 0.05 U ml^{-1}, 1 pg ml^{-1}, 1 pg ml^{-1}, 3 pg ml^{-1}, 3.3 pg ml^{-1} for IL-6, IL-2, IL-10, IL-1α, TNF-α and TGF-β1, respectively, while the intra- and inter-assay coefficient of variation (CV) were as follow: 6.9% to 7.8% and 6.5% to 9.6% for IL-6, 3.6% to 5.7% and 6.3% to 7.5% for IL-2, 2.8% to 3.7% and 2.7% to 2.8% for IL-10, 3.3% to 3.9% and 3.6% to 4.3% for IL-1α, 3.7% to 5.2% and 8% to 9.9% for TNF-α, and 3.9% to 8.5% and 5.3% to 9.4% for TGF-β1.

2.7. Statistical Analysis

One-way analysis of variance (ANOVA) with repeated measures over time was used to evaluate changes in ratings of DOMS and in all serum measurements (SPSS v. 21 statistical package). A non-parametric (Friedman) test was conducted where the data had violated the assumptions necessary to run the repeated measures one-way ANOVA (e.g., data not normally distributed). Where significant F ratio was found for main effect ($p<0.05$), the means were compared using Tukey's post–hoc test, or Wilcoxon signed-rank test with Bonferroni correction for non-parametric tests. All data are presented as mean ± standard error of the mean (S.E.M). The level of significance was set at $p<0.05$.

3. Results

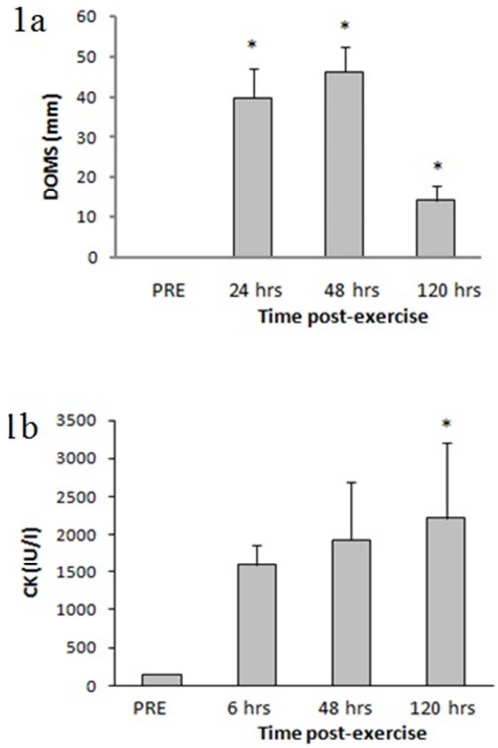

Figure 1. *Changes in muscle soreness (1a) and serum CK activity (1b) compared to pre-exercise levels (mean ± S.E.M.; n=9). Muscle soreness of the exercised muscles showed the higher ratings on day 2 post-exercise. CK activity levels were gradually increased up to 120 hrs post-exercise. Significantly different from pre-exercise; *: $p < 0.05$.*

Significant alterations in classical markers of muscle damage were observed after eccentric exercise. All participants reported significant muscle soreness in each leg post-exercise and no differences were observed between legs. The average muscle soreness ratings of both legs peaked on day 2 and remained significant on day 5 post-exercise ($p<0.05$; Figure 1a). CK activity was elevated following eccentric exercise. Specifically, its serum levels increased gradually and reached statistical significance at 120 hrs post-exercise ($p<0.05$; Figure 1b).

Standard curves of the ELISA analyses (absorbance vs concentration) of all the factors examined had a coefficient r^2 = 0.975 - 1. Circulating levels of the pro-inflammatory cytokine IL-1a remained unaltered throughout the experimental period and (Figure 2a), while IL-2 showed a moderate increase 48 hrs following eccentric exercise, but without reaching significance (Figure 2b; $p>0.05$).

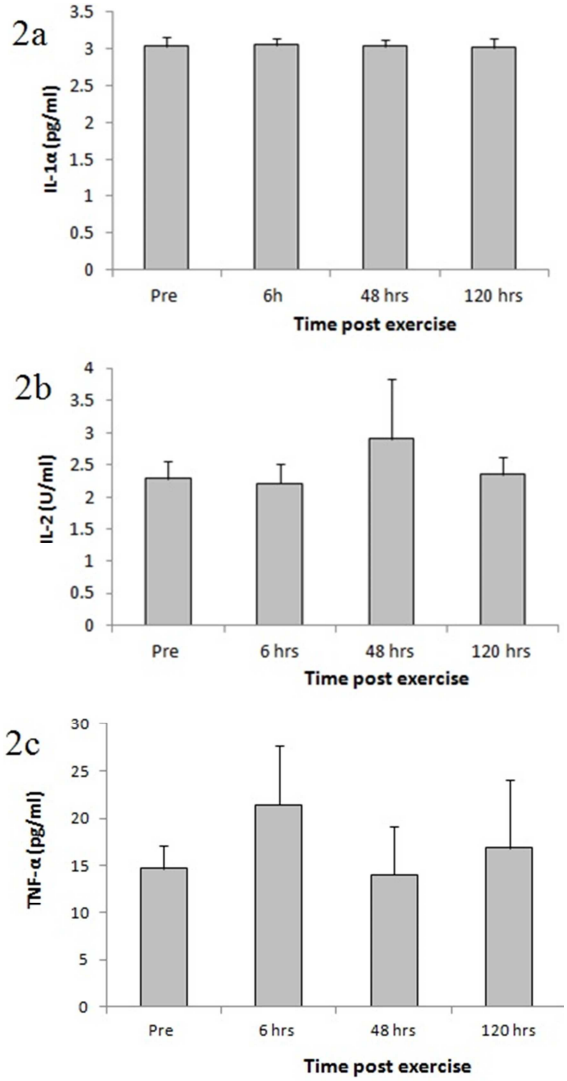

Figure 2. *Serum concentrations of the pro-inflammatory cytokines IL-1a (2a), IL-2 (2b) and TNF-α (2c) before (PRE) and after maximal eccentric exercise of both legs (mean ± S.E.M.; n=9). IL-1a levels remained unaltered throughout the experimental period, while IL-2 and TNF-α levels exhibited only mild changes over time which did not reach statistical significance (p > 0.05).*

Serum levels of the pro-inflammatory cytokine TNF-α (Figure 2c) and the anti-inflammatory IL-10 (Figure 3a) exhibited a similar pattern of response over time; In particular, IL-10 increased by nearly 100% and TNF-α by 50% 6 hours post exercise and then both decreased in a similar manner throughout the experimental period (Figures 2c and 3a).

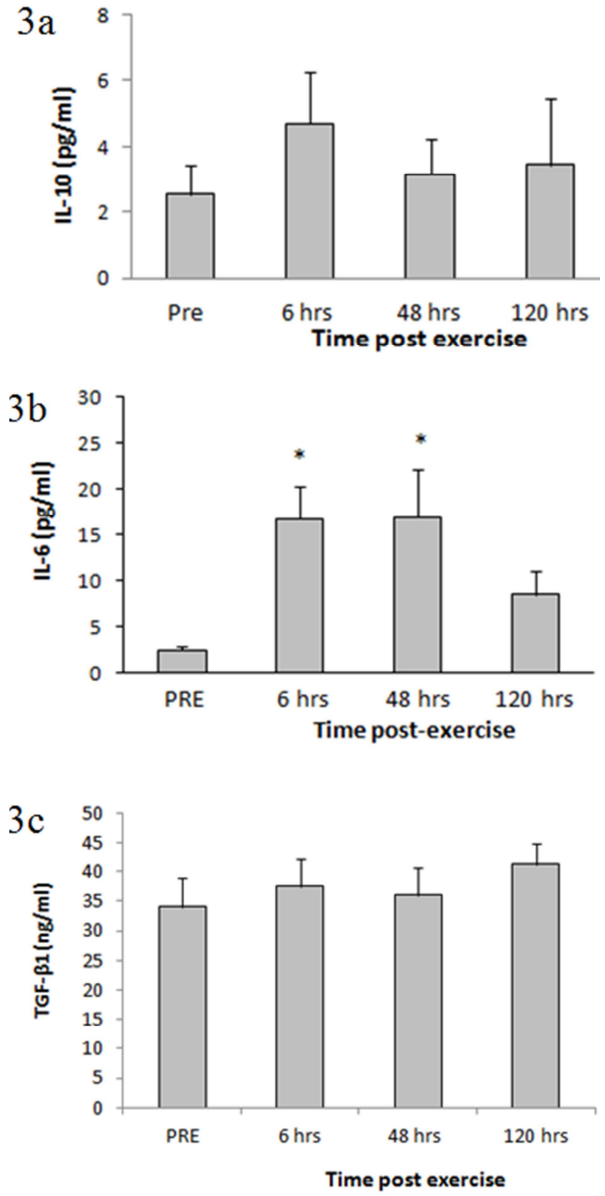

Figure 3. *Changes in the circulating levels of the anti-inflammatory cytokines IL-10 (3a), IL-6 (3b) and TGF-β1 (3c) following maximal eccentric exercise compared with the pre-exercise levels (mean ± S.E.M.; n=9). TGF-β1 increased slightly over time and IL-10 showed a nearly two-fold, though not significant, increase 6 hours post exercise. IL-6 exhibited significantly increased levels at 6 and 48 hrs post-exercise;*: significantly different from pre-exercise ($p < 0.05$).*

However, post-exercise changes of these two cytokines failed to be statistically significant ($p>0.05$) due to a large variability shown between the subjects. Circulating levels of IL-6 showed a significant increase at 6 and 48 hrs post-exercise before decreasing to non-significant levels 120 hrs after eccentric exercise ($p<0.05$; Figure 3b), while TGF-β1 increased slightly, though gradually, over time compared with the pre-exercise levels (Figure 3c).

4. Discussion

Our study examined the pattern of changes in the circulating levels of specific, inflammation-related cytokines after an eccentric exercise protocol, in order to reveal potential involvement and interactions of those pro- and anti-inflammatory factors during the acute phase response and adaptation process following exercise-induced muscle damage. The remarkable and sustained changes in the indirect markers of damage, i.e., muscle soreness and the leakage of muscle enzyme CK into the circulation post-exercise, indicated that the eccentric exercise used in this study did result in muscle damage. The main findings of our study demonstrated a mild systemic pro-inflammatory response accompanied by a stronger, acute anti-inflammatory reaction, as indicated by the changes in circulating cytokine concentrations post-exercise. Similarly to the findings of this study, previous studies have shown that severe muscle damage after eccentric exercise was not accompanied by any large changes in systemic cytokine concentrations, while an anti-inflammatory response has been associated with the adaptation process to muscle damage [5, 11].

4.1. Systemic Responses of Pro-Inflammatory Cytokines Following Muscle Damage

Damage of skeletal muscle due to eccentric exercise stimulates the local production of inflammatory cytokines, which are released at the site of inflammation by various cell types including muscle cells, macrophages, neutrophils, and fibroblasts, depending on the time course of their recruitment to the region of damage, while this local cytokine reaction is predominantly pro-inflammatory [8, 12]. However, although the pro-inflammatory responses occur within the damaged muscle and an acute-phase reaction is initiated, the release of pro-inflammatory cytokines into the circulation appears to be largely inhibited and a fully developed systemic pro-inflammatory response does not follow eccentric exercise [6, 10].

In this study, we investigated the pattern of response of the two major pro-inflammatory cytokines, IL-1 and TNF [8, 13], which have been traditionally considered as the main inducer cytokines of acute-phase reactions [9], although the cytokine-specific roles in the various phases of the inflammatory response cannot be clear [12]. Thus, these cytokines promote skeletal muscle damage or modulate its regeneration; however their role may vary with their targets as well as the type and stage of damage. Specifically, during the early inflammatory response, the activation of inflammatory M1 macrophages can promote their actions to lyse muscle cells. Besides, TNF-a and IL-1 induce most inflammatory chemokines, typically released by immune cells and function as chemoattractants for leukocytes, which in turn play an important role in the early inflammatory response following muscle damage [12, 14]. During the later stages of damage,

they can directly influence the regeneration process by modulating muscle cells proliferation and myogenic differentiation [14, 15].

In our study, serum levels of IL-1a did not change over time up to 120 hrs post-muscle damaging exercise, while circulating levels of TNF-α exhibited only a rapid increase at 6 hrs post exercise, similarly to the transient peak previously observed on day 1 after muscle-damaging downhill running [16]. Thus, in contrast to the local, prolonged production of IL-1 and TNF-a within muscle after eccentric exercise [6, 8], our findings corroborated previous reports that there is only a slight and/or transient increase in their systemic levels [9, 11, 16].

The production of TNF-a is controlled by various cytokines, such as interferon (IFN) and IL-2, while IL-6 inhibits its production [17]. Furthermore, local production of TNF-a and IL-1 can stimulate the expression of IL-6 in muscle cells [18], whose production is also up-regulated by IL-2 [9]. Hence, IL-2 appears to be a key mediator of the production of both TNF-α and IL-6. In the present study, we observed a transient, not significant increase in the systemic levels of IL-2 at 48 hrs after the eccentric exercise, which coincided with the post-exercise up-regulation of IL-6.

4.2. Systemic Responses of Anti-Inflammatory Cytokines Following Muscle Damage

Interestingly, we further found that a rapid increase in their systemic levels was the common response for TNFα, IL-6 and IL-10 while, particularly, pro-inflammatory TNF-α and anti-inflammatory IL-10 cytokines exhibited a similar pattern of response throughout the experimental period. This close association between TNF-α and IL-10 responses could reflect a feedback mechanism previously described [19], which involves IL-10 and has been associated with an inhibitory effect on TNF-a production by macrophages, thus regulating the initial inflammatory response and restricting secondary muscle damage [20, 21]. Overall, the lack of changes in the systemic levels of IL-1, along with the similar pattern of TNF-α and IL-10 changes post-exercise, may reflect the inhibitory effects of IL-10 on those pro-inflammatory cytokines, attenuating the inflammatory response to muscle-damaging exercise.

In addition, IL-6 also acts indirectly to restrict inflammation, by stimulating the production of anti-inflammatory cytokines, including IL-10 and IL-1 receptor antagonist (IL-1ra), as well as soluble TNF-a receptors [9, 22, 23]. In particular, it has been suggested that after muscle-damaging eccentric exercise, a cytokine cascade takes place, where TNF-a, IL-1, IL-6, and IL-1ra are released sequentially; TNF-a stimulates the release of the other pro-inflammatory cytokine IL-1, while both work synergistically to promote the production of IL-6, which in turn stimulates a rapid and remarkable increase of circulating IL-1ra, thus blocking IL-1 bioactivity and inducing a systemic anti-inflammatory response [9, 23, 24]. Indeed, similarly to previous findings [7, 25], in this study we observed an early and sustained increase in serum IL-6 levels after the exercise-induced muscle damage, which further supports the notion that IL-6 is a key cytokine in the acute-phase response, controlling a local or systemic inflammatory response and regulating homeostasis after an inflammatory reaction [23]. It should be noted, however, that the systemic levels and the time course of cytokines like IL-10, IL-6, L-1ra and IL-1 may be differentially affected by specific characteristics of the eccentric exercise such as its intensity and duration [8, 10, 11]. Nevertheless, high levels of IL-10 and IL-6 might be a marker of a normal regeneration and adaptation of muscle after muscle-damaging exercise [26].

In the context of skeletal muscle regeneration and adaptation, TGF-β1 has been characterized as a multipotent cytokine which inhibits myogenic differentiation [27], while it has a major influence on the remodeling of the ECM and basal membrane surrounding damaged myofibers through the period of muscle regeneration. Moreover, TGF-β1 was initially identified as a powerful chemotactic cytokine to initiate inflammation, by stimulating migration of monocytes, lymphocytes, neutrophils and fibroblasts (for review, see [28]). However, there is a growing body of evidence which reverses the role of TGF-β1 to dominantly an immune suppressor [29]. In our study, the gradual, though slight, increase of TGF-β1 systemic levels up to 120 hrs after muscle-damaging exercise might reflect its contribution, if any, to muscle remodeling- rather than inflammation-related events [28, 30].

5. Conclusions

The pattern of systemic cytokine changes observed in this study may indicate potential interactions between certain pro- and anti-inflammatory factors during the acute phase response following muscle-damaging exercise. We speculate that these interactions may result in a predominantly anti-inflammatory systemic response, which inhibits the release of pro-inflammatory cytokines into the blood. Thus, it could be assumed that anti-inflammatory cytokines may act as regulatory mediators in the cytokine network to eventually restrict progression of a systemic inflammatory stress during the acute phase and support a normal adaptation process following exercise-induce muscle damage. Nevertheless, further studies to characterize the mechanisms by which the cytokine interactions are triggered and regulated, both at the systemic and skeletal muscle tissue level, following different muscle-damaging exercise protocols remain of particular interest.

Conflicts of Interest Statement

The authors have no conflict of interest relevant to this article.

Acknowledgements

The authors are grateful to the study participants for their invaluable contribution to this research.

References

[1] Peake JM, Neubauer O, Della Gatta PA, Nosaka K, Muscle damage and inflammation during recovery from exercise. J Appl Physiol (1985) 2016; 122: 559-570.

[2] Philippou A, Bogdanis GC, Nevill AM, Maridaki M, Changes in the angle-force curve of human elbow flexors following eccentric and isometric exercise. Eur J Appl Physiol 2004; 93: 237-244.

[3] Philippou A, Maridaki M, Bogdanis G, Halapas A, Koutsilieris M, Changes in the mechanical properties of human quadriceps muscle after eccentric exercise. In Vivo 2009; 23: 859-865.

[4] Philippou A, Maridaki M, Bogdanis GC, Angle-specific impairment of elbow flexors strength after isometric exercise at long muscle length. J Sports Sci 2003; 21: 859-865.

[5] Kanda K, Sugama K, Hayashida H, Sakuma J, Kawakami Y, Miura S, Yoshioka H, Mori Y, Suzuki K, Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. Exerc Immunol Rev 2013; 19: 72-85.

[6] Philippou A, Maridaki M, Theos A, Koutsilieris M, Cytokines in muscle damage. Adv Clin Chem 2012; 58: 49-87.

[7] Smith LL, Anwar A, Fragen M, Rananto C, Johnson R, Holbert D, Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise. Eur J Appl Physiol 2000; 82: 61-67.

[8] Peake J, Nosaka K, Suzuki K, Characterization of inflammatory responses to eccentric exercise in humans. Exerc Immunol Rev 2005; 11: 64-85.

[9] Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K, Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. Exerc Immunol Rev 2002; 8: 6-48.

[10] Pedersen BK, Hoffman-Goetz L, Exercise and the immune system, Regulation, integration, and adaptation. Physiol Rev 2000; 80: 1055-1081.

[11] Hirose L, Nosaka K, Newton M, Laveder A, Kano M, Peake J, Suzuki K, Changes in inflammatory mediators following eccentric exercise of the elbow flexors. Exerc Immunol Rev 2004; 10: 75-90.

[12] Smith C, Kruger MJ, Smith RM, Myburgh KH, The inflammatory response to skeletal muscle injury: Illuminating complexities. Sports Med 2008; 38: 947-969.

[13] Figarella-Branger D, Civatte M, Bartoli C, Pellissier JF, Cytokines, chemokines, and cell adhesion molecules in inflammatory myopathies. Muscle Nerve 2003; 28: 659-682.

[14] Tidball JG, Villalta SA, Regulatory interactions between muscle and the immune system during muscle regeneration. Am J Physiol Regul Integr Comp Physiol 2010; 298: R1173-1187.

[15] Karalaki M, Fili S, Philippou A, Koutsilieris M, Muscle regeneration: Cellular and molecular events. In Vivo 2009; 23: 779-796.

[16] van de Vyver M, Myburgh KH, Cytokine and satellite cell responses to muscle damage: Interpretation and possible confounding factors in human studies. J Muscle Res Cell Motil 2012; 33: 177-185.

[17] Pedersen BK, Akerstrom TC, Nielsen AR, Fischer CP, Role of myokines in exercise and metabolism. J Appl Physiol (1985) 2007; 103: 1093-1098.

[18] Frost RA, Lang CH, Skeletal muscle cytokines: Regulation by pathogen-associated molecules and catabolic hormones. Curr Opin Clin Nutr Metab Care 2005; 8: 255-263.

[19] Strassmann G, Patil-Koota V, Finkelman F, Fong M, Kambayashi T, Evidence for the involvement of interleukin 10 in the differential deactivation of murine peritoneal macrophages by prostaglandin e2. J Exp Med 1994; 180: 2365-2370.

[20] Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, Plonquet A, Gherardi RK, Chazaud B, Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J Exp Med 2007; 204: 1057-1069.

[21] Villalta SA, Nguyen HX, Deng B, Gotoh T, Tidball JG, Shifts in macrophage phenotypes and macrophage competition for arginine metabolism affect the severity of muscle pathology in muscular dystrophy. Hum Mol Genet 2009; 18: 482-496.

[22] Petersen AM, Pedersen BK, The anti-inflammatory effect of exercise. J Appl Physiol (1985) 2005; 98: 1154-1162.

[23] Reihmane D, Dela F, Interleukin-6: Possible biological roles during exercise. Eur J Sport Sci 2014; 14: 242-250.

[24] Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK, Il-6 enhances plasma il-1ra, il-10, and cortisol in humans. Am J Physiol Endocrinol Metab 2003; 285:E433-437.

[25] Philippou A, Bogdanis G, Maridaki M, Halapas A, Sourla A, Koutsilieris M, Systemic cytokine response following exercise-induced muscle damage in humans. Clin Chem Lab Med 2009; 47: 777-782.

[26] Zembron-Lacny A, Naczk M, Gajewski M, Ostapiuk-Karolczuk J, Dziewiecka H, Kasperska A, Szyszka K, Changes of muscle-derived cytokines in relation to thiol redox status and reactive oxygen and nitrogen species. Physiol Res 2010; 59: 945-951.

[27] Bouche M, Canipari R, Melchionna R, Willems D, Senni MI, Molinaro M, Tgf-beta autocrine loop regulates cell growth and myogenic differentiation in human rhabdomyosarcoma cells. FASEB J 2000; 14: 1147-1158.

[28] Philippou A, Maridaki M, Koutsilieris M, The role of urokinase-type plasminogen activator (upa) and transforming growth factor beta 1 (tgfbeta1) in muscle regeneration. In Vivo 2008; 22: 735-750.

[29] Han G, Li F, Singh TP, Wolf P, Wang XJ, The pro-inflammatory role of tgfbeta1: A paradox? Int J Biol Sci 2012; 8: 228-235.

[30] Gao HG, Fisher PW, Lambi AG, Wade CK, Barr-Gillespie AE, Popoff SN, Barbe MF, Increased serum and musculotendinous fibrogenic proteins following persistent low-grade inflammation in a rat model of long-term upper extremity overuse. PLoS One 2013; 8: e71875.