Muscle damage and inflammation during recovery from exercise

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ABSTRACT

Unaccustomed exercise consisting of eccentric (i.e., lengthening) muscle contractions often results in muscle damage characterized by ultrastructural alterations in muscle tissue, clinical signs and symptoms (e.g., reduced muscle strength and range of motion, increased muscle soreness and swelling, efflux of myocellular proteins). The time course of recovery following exercise-induced muscle damage depends on the extent of initial muscle damage, which in turn is influenced by the intensity and duration of exercise, joint angle/muscle length and muscle groups used during exercise. The effects of these factors on muscle strength, soreness and swelling are well characterized. By contrast, much less is known about how they affect intramuscular inflammation and molecular aspects of muscle adaptation/remodeling. Although inflammation has historically been viewed as detrimental for recovery from exercise, it is now generally accepted that inflammatory responses—if tightly regulated—are integral to muscle repair and regeneration. Animal studies have revealed that other cell types including mast cells, eosinophils, CD8 and T regulatory lymphocytes, fibro-adipogenic progenitors and pericytes also help to facilitate muscle tissue regeneration. However, more research is required to determine whether these cells respond to exercise-induced muscle damage. A large body of research has investigated the efficacy of physicotherapeutic, pharmacological and nutritional interventions for reducing the signs and symptoms of exercise-induced muscle damage, with mixed results. More research is needed to examine if/how these treatments influence inflammation and muscle remodeling during recovery from exercise.

Key words: eccentric exercise, strength, soreness, swelling, leukocytes, cytokines

INTRODUCTION

Exercise-induced muscle damage has been a topic of intense focus in exercise and sports science research for more than 30 years. It is a condition characterized by transient ultrastructural myofibrillar disruption, loss of muscle strength and power, delayed onset muscle soreness (DOMS), swelling, reduced range of motion of the affected limb, systemic efflux of myocellular enzymes and proteins (e.g., creatine kinase, CK; myoglobin), or a combination of these (39). The mechanical alterations and metabolic stress associated with exercise-induced muscle damage stimulate various cell types that comprise skeletal muscle to initiate subsequent tissue repair and remodeling (39). Specifically, satellite cells (muscle stem cells) interact with inflammatory cells (e.g., neutrophils, macrophages, T lymphocytes, mast cells), vascular cells (e.g., pericytes, endothelial cells) and stromal cells (e.g., fibroblasts) interact with each other within the extracellular matrix (ECM) of skeletal muscle (39) (Figure 1). The dynamics of these intercellular interactions determine the effectiveness and time course of recovery from muscle damage.

The processes of muscle damage and inflammation have been extensively reviewed elsewhere (1, 12, 39, 86, 107). In this mini-review, we adopt a different perspective on the following issues: (i) the basic mechanisms, characteristics and time course of exercise-induced muscle damage, (ii) the time course and role of intramuscular inflammation during recovery from exercise-induced muscle damage and (iii) effective treatments for exercise-induced muscle damage and intramuscular inflammation. The themes of connective tissue regeneration (54) and muscle protein synthesis (63) are addressed in other reviews within this Highlighted Topic on Recovery from exercise.

EXERCISE-INDUCED MUSCLE DAMAGE: UNDERSTANDING THE BASICS

It is well accepted that ultrastructural disruptions in muscle, decrements in muscle strength, DOMS and efflux of muscle enzymes are greater, and the recovery of these indices is slower after eccentric (i.e., lengthening) versus concentric (i.e., shortening) muscle contractions (49, 72). Concentric muscle contractions do not cause exercise-induced muscle damage (49), but exercise-induced muscle damage is evident after isometric contractions at a long muscle length and eccentric muscle contractions—even at low intensity (15). Various mechanisms likely account for the loss of strength after eccentrically-biased exercise, which is considered to be the best indicator of exercise-induced muscle damage (23). These mechanisms are outlined in the following theoretical model (39). Mechanical strain during eccentric exercise causes half sarcomere non-uniformity and overstretching of sarcomeres beyond filament overlap, leading to 'popped sarcomeres'. These alterations likely directly reduce force production and overload sarcolemma and t-tubule structures. In turn, these events cause opening of stretch–activated channels, membrane disruption and excitation–contraction coupling dysfunction. Ca^{2+} entering the cytosol through stretch–activated channels and/or permeable sections of the sarcolemma may stimulate calpain enzymes to degrade contractile proteins or excitation–contraction coupling proteins, resulting in prolonged loss of muscle strength (39).

Although DOMS is also a common symptom of muscle damage, the precise mechanisms responsible for DOMS remain somewhat uncertain. It is commonly believed that micro-trauma of myofibers and subsequent inflammation cause DOMS. However, mechanical hyperalgesia occurs in rat muscle 1–3 d after eccentric muscle contractions, without any apparent microscopic damage of the muscle or signs of inflammation (34). Two pathways are

involved in inducing mechanical hyperalgesia after eccentric muscle contractions: (i) activation of the B2 bradykinin receptor–nerve growth factor (NGF) pathway and (ii) activation of the COX−2−glial cell line-derived neurotrophic factor (GDNF) pathway. It appears that these neurotrophic factors are produced by muscle fibers and/or satellite cells (65). These agents may induce DOMS directly by stimulating muscle nociceptors. Alternatively, they may act indirectly by binding to extracellular receptors, and inducing secretion of neurotrophins from muscle fibers, resulting in nociceptor stimulation and DOMS (39). It is likely that DOMS is associated more with inflammation in the ECM, rather than myofiber damage and inflammation (23).

The biological significance of exercise-induced disruption of sarcomeric, membrane and ECM structure in muscle remains under debate (39). Early morphological observations of streaming of Z-disks (29, 72), widening of perimyseal areas between fascicles, and separation of myofibers from one another within fascicles (101) were interpreted as evidence of muscle damage. However, more contemporary theories propose that eccentric exercise does not damage muscle fibers per se (118), and that Z-disk streaming, smearing and disruption may instead represent muscle remodeling and adaptation (117). Regardless of their precise biological significance, these ultrastructural changes in muscle after exercise are sensitive to differences in mechanical load/contraction mode (31, 72), and correlate with changes in muscle function (31, 48).

Time Course of Exercise-Induced Muscle Damage

The number of muscle fibers showing disruption of normal myofibrillar banding patterns is increased immediately after eccentric exercise (31). Disruption of Z-disks and sarcomeres appears to peak between 1 and 3 d after exercise (21, 30, 72, 117), but may remain elevated

up to 6–8 d after exercise (30, 43, 117). There is a temporal association between the extent of loss of muscle strength after exercise, and the time required to restore muscle strength back to normal. When muscle strength decreases by ≤20% immediately after exercise, it is usually restored within 2 d after exercise (21, 59). By contrast, when muscle strength decreases by ~50% immediately after exercise—especially for the initial exposure to eccentric muscle contractions—it remains below pre-exercise values at 7 d after exercise (48, 82, 84). As shown in Figure 2, the time course of changes in muscle strength, range of motion, DOMS, limb circumference (i.e., swelling) and blood CK activity in the days after intense eccentric exercise varies. Even when recovery of muscle strength is prolonged, DOMS is resolved by around 4 d after exercise (23). Muscle swelling peaks 4–5 d after exercise, while increases in blood markers of muscle damage such as CK activity are also delayed (23). Changes in muscle strength appear to influence the magnitude and time course of changes in other markers of exercise-induced muscle damage.

Factors Affecting Recovery from Exercise-Induced Muscle Damage

The most well-known factor that influences recovery from exercise-induced muscle damage is previous muscle damage. After an initial bout of muscle-damaging exercise, muscle adapts and is protected such that the signs and symptoms of exercise-induced muscle damage are less severe, and return to normal more rapidly after subsequent bouts of exercise (80, 85, 104). This phenomenon is known as the 'repeated bout effect'. These protective effects are produced by low-intensity eccentric muscle contractions, or maximal isometric contractions at a long muscle length (15, 51) that do not cause (or only induce minor) symptoms of exercise-induced muscle damage. The repeated bout effect is also conferred to the contralateral muscles, such that the second bout of eccentric exercise performed by the

contralateral arm induces less exercise-induced muscle damage than the initial bout performed by the opposite arm (14).

Exercise-induced muscle damage is greater and/or recovery is slower after: (i) exercise performed at high versus low eccentric torque (77, 78, 81), increasing numbers of eccentric muscle contractions (7), and long versus short muscle lengths (19, 79); (ii) exercise using a single joint versus multiple joints (100); and (iii) exercise using the arms versus the legs (16, 42) and the knee flexors versus the knee extensors (16). Contraction velocity does not influence muscle strength following 30 eccentric muscle contractions, whereas loss of muscle strength is greater after 210 eccentric muscle contractions at fast (210°/s) versus slow (30°/s) velocity (11). It remains unclear whether recovery from exercise-induced muscle damage differs between men and women, partly because of variation in the age and training status of research participants, the type and intensity of exercise protocols (28). However, it does not appear that the gender difference is large. Recovery from exercise-induced muscle damage is not affected by the configuration of repetitions and sets (10), the rest interval between sets of eccentric muscle contractions (64), or performing eccentric exercise with damaged muscles (1, 76).

The greater mechanical strain associated with high- versus low-force muscle contractions and a greater number of contractions most likely causes greater damage to contractile proteins and the ECM, resulting in more severe exercise-induced muscle damage (78). Muscle contractions performed at long muscle lengths likely cause a greater degree of non-uniformity of sarcomere length, in addition to larger disruption of stretched, weaker sarcomeres and the ECM, leading to more severe exercise-induced muscle damage (79). Recruitment of fewer muscle groups—that are also smaller/weaker and more vulnerable to overstretching—could

theoretically account for why exercise-induced muscle damage is greater after single- versus multi-joint exercise. Neural control is different between eccentric and concentric or isometric muscle contractions, such that untrained individuals are usually unable to fully activate their muscles during maximal eccentric muscle contractions. Motor unit discharge rate is also lower during eccentric compared with concentric muscle contractions, mainly due to reduced spinal excitability (26). The greater exercise-induced muscle damage following arm versus leg exercise and knee flexion versus extension probably occurs as a result of differences in the level of regular mechanical loading in these muscle groups (16). Differences in exercise-induced muscle damage following a large number of fast versus slow velocity eccentric muscle contractions may arise for two reasons (11). First, faster contractions may produce greater force at longer lengths which, as explained above, increase the risk of damage to contractile proteins. Second, faster contractions may activate fewer cross-bridges that are capable of producing force, and may thereby increase the amount of mechanical stress per active cross-bridge. This effect may be exacerbated in fast-twitch muscle fibers as the number of muscle contractions increases (11).

ACUTE RESPONSES AND RESOLUTION OF INFLAMMATION AFTER EXERCISE-INDUCED MUSCLE DAMAGE

The term ‘inflammation’ is often used loosely, without definition or any value statement as to whether it is a ‘good’ or ‘bad’ process. In the context of sports medicine, ‘inflammation’ encompasses clinical, physiological, cellular and molecular changes within injured tissue (98). Historically, muscle inflammation following exercise-induced muscle damage has sometimes been considered as a detrimental process associated with tissue damage, pain and delayed

recovery (109). However, this broad viewpoint does not take account of the many and varied aspects of inflammation. The notion that inflammation is a key process underlying muscular repair and regeneration is now gaining acceptance (12, 106). Under non-pathophysiological conditions (e.g., after exercise-induced muscle damage) intramuscular inflammation is a tightly coordinated and dynamic process that eventually leads to adaptive remodeling and return to homeostasis (12, 106).

The primary focus of this section of our review is on muscle inflammation following exercise-induced muscle damage in humans, based on histological evidence and quantification of cytokine mRNA and protein in muscle. We also refer to key animal and cell culture studies, because these investigations offer essential insights into the regulation and consequences of intramuscular inflammation. Considering that the kinetics of muscle-immune interactions are a key aspect for the functional recovery of the muscle (12, 106), we have also focused on the time course of inflammation in muscle after eccentric exercise. Although inflammation is intimately linked with myogenesis and remodeling of the ECM, we have not discussed these topics in detail here, because they are covered in another review by Mackey and Kjaer in this issue of the journal (54).

Time Course of Muscle Inflammation in Humans after Exercise

The accumulation of inflammatory cells (leukocytes) in the muscle tissue, as identified by histological observations, is considered a cardinal sign of exercise-induced muscle damage. A number of human studies involving various types of 'muscle-damaging' exercise have provided evidence for the accumulation of leukocytes in the muscle tissue (86). Leukocytes have been observed in muscle biopsy samples after intense, high-volume and/or unaccustomed resistance exercise (3, 18, 21, 57, 84), downhill running (59) and long distance

running/ultra-endurance exercise involving running (60). Controversy exists as to whether the local inflammatory responses are caused by repeated muscle biopsies rather than by exercise (58, 114). Yet, there is also evidence indicating that any inflammation arising from the biopsy procedure itself is minor compared with inflammation resulting from exercise (82).

As shown in Figure 1, leukocytes may start to accumulate in the exercised muscle immediately after exercise. An early accumulation of radiolabeled leukocytes—primarily neutrophils located in micro-blood vessels in the muscle tissue—has been observed between 1 to 24 h after eccentric exercise (84, 94). Histological examinations typically show that leukocytes accumulate in the extracellular space within the muscle 24 to 48 h after exercise (3, 35, 82). Specific evidence for neutrophil accumulation of neutrophils beyond 24 h post-exercise is limited (56, 90, 103, 104). This may be partly due to methodological difficulties in detecting neutrophils (82, 83), or more likely, because neutrophils rapidly disappear from the regenerating muscle (109). Increased numbers of monocytes/macrophages are observed more consistently in human skeletal muscle at later time points of recovery, such as 48 h to 7 d and beyond (43, 56, 57, 60, 82). At the same time point during recovery from exercise, some individuals display substantial leukocyte accumulation, whereas others present with very few leukocytes in muscle (Figure 3A). Figure 3A summarizes the findings of many studies that have examined histological evidence of leukocyte invasion in muscle after exercise.

These observations support the notion that neutrophils and monocytes are mobilized into the circulation following exercise-induced muscle damage (69, 71, 88). Subsequently, they transmigrate into the muscle where they break down damaged muscle tissue through phagocytosis and by releasing proteolytic enzymes (e.g., elastase, myeloperoxidase), reactive oxygen and nitrogen species (109) (Figure 1). In a previous study (70), we investigated the

time-course of changes in the transcriptome of skeletal muscle after endurance exercise involving moderate muscle damage (cycling followed by running), as indicated by increases in plasma myoglobin concentration and CK activity (71). These data suggested an early migration of leukocytes into the muscle and immune activation 3 h post-exercise (70). Furthermore, substantial transcriptional activity, functionally related to the presence of leukocytes, immune-related signaling and adaptive remodeling of the intramuscular ECM was evident until 96 h after exercise (70).

Tissue-resident leukocytes such as macrophages, may also become activated after exercise, in addition to (or potentially even in the absence of) the recruitment and accumulation of blood-borne monocytes (3, 56, 57, 84, 86, 103, 104). Notably, about 4 to 7 d following severe exercise-induced muscle damage (discussed below), leukocytes also invade the intracellular space of exercised muscle tissue (18, 82, 84) (Figure 1). There is little evidence that severe myofiber necrosis occurs even in response to intense voluntary eccentric exercise (119). Significant necrosis does occur in muscle following electrically-stimulated contractions (21). The pattern of myofiber recruitment during muscle contractions may therefore determine the extent of necrosis (21). Alternatively, segmental myofiber necrosis may occur without affecting the whole myofiber (86). In such 'severe' cases, leukocytes have been observed in the muscle tissue even 3 weeks after post-exercise (84, 95).

Coupled with histological evidence of leukocyte infiltration, exercise-induced muscle damage is also associated with increased expression of cytokine/chemokine mRNA and protein in muscle (86). The time course of changes in the expression of various cytokines and chemokines in muscle after exercise is depicted in Figures 3B (mRNA level) and 3C (protein level). Considerable attention has focused on changes in interleukin (IL)-6, C-X-C motif ligand

8 (CXCL8; also known as IL-8) and C-C motif chemokine ligand 2 (CCL2; also known as monocyte chemotactic protein (MCP)-1) mRNA expression, predominantly in two time windows at 1–4 h and at 24 h after exercise. Compared with mRNA expression, much less is known about changes in cytokine/chemokine protein expression in muscle after exercise. The functions of cytokines and chemokines in repair of muscle damage after exercise are summarized in Table 1 and discussed further below.

Effects of Muscle Workload on Muscle Inflammation and Linkages with Functional Recovery of the Muscle

The data from human studies that have investigated muscle inflammation after various types of exercise suggest that leukocyte accumulation in muscle is a gradual process that depends on the extent of muscle damage (40, 86). As discussed earlier, the assessment of muscle function by measuring force-generating capacity (i.e., strength) is considered a reliable and valid method for the degree of muscle damage (23, 31, 48). Studies that have both analyzed the presence of leukocytes in muscle biopsy samples and measured changes in muscle strength indicate an association between muscle function and leukocyte accumulation, as well as other histological observations (86). For the following brief review of these studies, we have used the scheme to assess muscle damage based on the decline in muscular strength, as well as the time of recovery to regain full strength, as proposed by Paulsen et al (86).

Evidence for leukocyte accumulation in response to 'mild' exercise-induced muscle damage (i.e., a decrease in muscle function <20% and full recovery within 2 d) is limited (86). Despite recovery of muscle function within 2 d, Crameri et al (21) observed an accumulation of $CD68^+$ macrophages in the endomysium and perimysium regions of muscle after unilateral,

maximal eccentric muscle contractions of the knee extensors. Conversely, Malm et al (59) reported no signs of leukocyte inflammation 48 h after downhill running. While muscle function had returned to baseline at this time point (i.e., 2 d post-exercise), DOMS was observed, together with increases in blood granulocytes and serum CK activity (59). This latter finding agrees with the concept that DOMS is a common symptom of muscle damage, but is not necessarily related to the accumulation of leukocytes among myofibers (82, 86, 119).

Most studies reporting moderate exercise-induced muscle damage (i.e., reduction of >20% in muscle strength and recovery within 7 d) also observed accumulation of leukocytes in the muscle (3, 38, 82, 86). Together with a large inter-individual variation in response to maximal eccentric exercise, Paulsen et al (82) reported that the intracellular infiltration of leukocytes into myofibers was typically observed in 'high responders' who showed the most pronounced and prolonged decrease in muscle function. In accordance with previous findings (94), Paulsen et al (82) also showed that leukocyte accumulation correlated with delayed recovery of muscle function.

Leukocyte accumulation is a consistent finding in studies that reported 'severe' exercise-induced muscle damage, as characterized by a decrease in force-generating capacity of more than 50%, and a recovery of more than 1 week (18, 43, 82, 84). In response to severe damage, the greatest number of leukocytes in the muscle was observed at the same time-points at which there were indications of segmental myofiber necrosis (18, 43, 82, 84). Collectively, leukocyte accumulation in muscle has been a relatively consistent finding in response to moderate to severe muscle damage, typically induced by maximal eccentric exercise across a large range of motion (86).

Compared with information on leukocyte responses, fewer studies have systematically

compared intramuscular cytokine expression after exercise at different muscle workloads (86). There are reports that protein expression of CCL2 (38, 40) and C-X-C motif chemokine 10 (CXCL10; also known as interferon γ-induced protein 10, IP-10) in muscle is greater after eccentric versus concentric exercise. Although Malm et al (59) observed greater loss of muscle strength and higher plasma CK activity after downhill running at −8° versus −4°, there were no significant changes in interleukin (IL)-6, IL-1β or leukemia inhibitory factor (LIF) protein expression after either exercise trial. Other studies measuring cytokine expression in muscle after eccentric exercise or more traditional resistance exercise did not measure changes in muscle strength as an indirect marker of muscle damage. At present, therefore, there is insufficient evidence to establish definitively that the magnitude of intramuscular cytokine expression after exercise is related to the extent of muscle damage.

Leukocyte Functions and Mechanisms Underlying Muscle-Immune Interactions during Muscle Regeneration/Recovery

Increasing evidence from studies in rodents shows that multiple immune cell types interact with the muscle and are critical for all stages of muscle repair and regeneration (12, 106) (Figure 1). The initial pro-inflammatory response to muscle damage is dominated by the accumulation of neutrophils and pro-inflammatory macrophages. Neutrophils contribute to muscle injury and impair muscle remodeling and functional recovery after contraction-induced injury in mice (74, 91). However, the high cytotoxicity and capacity of neutrophils to lyse muscle cells is reduced when neutrophils are co-cultured with macrophages (73). Neutrophils modify the cytotoxicity of macrophages, such that fewer macrophages are required to lyse muscle cells (73). These findings demonstrate that neutrophils and

macrophages interact with each other during the pro-inflammatory stage of muscle injury.

Pro-inflammatory macrophages strongly express the cell surface molecule CD68, and have traditionally been referred to as M1 macrophages. These cells have subsequently been characterized more specifically as $Gr1^{high}$ or $Ly6C^{pos}CX3CR1^{lo}$, based on their pattern on receptor expression (24, 96). Both neutrophils and M1/$Ly6C^{pos}CX3CR1^{lo}$ macrophages are important for the removal of cell debris through phagocytosis and reactive species production (2, 73) (Figure 1). However, excess production of reactive species by these inflammatory cells can exacerbate muscle damage (73).

Reducing or blocking certain muscle inflammatory responses interferes with muscle regeneration and subsequent adaptive remodeling. For example, depletion of neutrophils in mice prior to muscle injury compromises muscle regeneration, possibly as a result of impaired neutrophil-mediated macrophage recruitment (105). Blocking the recruitment of monocytes to injured muscles (52), or treatment of injured muscle with the anti-inflammatory cytokine IL-10 at the initial pro-inflammatory phase, also impairs muscle regeneration (24, 89).

Another key concept underlying the benefits of tightly regulated inflammation in muscle is that macrophages display marked and dynamic phenotype plasticity during muscle regeneration (2, 97, 112). This phenotypic plasticity of macrophages is primarily dependent on their tissue environment (106). In particular, the shift in phenotype from pro-inflammatory M1 to anti-inflammatory M2 macrophages (also characterized as $Gr1^{low}$ $Ly6C^{neg}CX3CR1^{hi}$ cells (112)) is central for the transition from a pro- to an anti-inflammatory response, and the resolution of inflammation (12, 106). Known signals that regulate this M1- to M2-macrophage transition include the phagocytosis of cell debris (2), IL-10 (24), and AMP-activated protein kinase (AMPK)-α (66).

As part of the concept that muscle inflammation is co-regulated with muscle regeneration (106), M1 macrophages interact with proliferating satellite cells, whereas M2 macrophages interact with differentiating satellite cells (97). Satellite cells and injured muscle recruit monocytes/macrophages through chemotactic factors such as fractalkine (CX3CL1) (13) and CCL2 (52). M1 macrophages secrete pro-inflammatory cytokines (e.g. TNF-α, IL-1β and IL-6) as well as secretory leukocyte protease inhibitor (12). They also attract more inflammatory cells and stimulate satellite cell proliferation (106). M2 macrophages, characterized by strong expression of CD163 (97), produce anti-inflammatory cytokines (e.g., IL-10), transforming growth factor (TGF)-β1 (2) and insulin-like growth factor (IGF)-1 (53). M2 macrophages primarily attenuate inflammation, rescue satellite cells from apoptosis and stimulate their proliferation, promote muscle regeneration and the synthesis of connective tissue (13, 24, 106).

Although most attention to date has focused on neutrophils and macrophages, other cell types including mast cells, T lymphocytes, eosinophils, fibro-adipogenic progenitors and pericytes also play important roles in muscle tissue regeneration (Figure 1). Mast cells secrete various chemoattractants and tryptase; in turn, these factors increase myoblast proliferation and reduce myoblast differentiation (27). T regulatory cells express chemokine receptors and secrete various anti-inflammatory factors including IL-10 and TGF-β. Similar to mast cells, T regulatory cells stimulate myoblast proliferation and expansion of the satellite cell pool. They also suppress myoblast differentiation and ECM proteins that induce fibrosis (8, 9, 113). CD8 T cells facilitate muscle regeneration by stimulating the secretion of CCL2 and recruiting or $Gr1^{lo}$ or M1-like macrophages into muscle (121). Eosinophils secrete the anti-inflammatory cytokine IL-4, which in turn stimulates fibro-adipogenic progenitors to initiate myoblast

differentiation and necrosis (36). Lastly, type 2 pericytes secrete various growth factors that enhance myoblast differentiation, while also stimulating satellite cell quiescence (5, 44). With the exception of pericytes (41) and CD8 T lymphocytes (25, 60), little is currently unknown about whether exercise alters the number and/or activity of these cells in muscle.

Table 1 outlines the mechanisms of action of exercise-responsive cytokines (i.e., IL-6, CCL2, interferon-γ), the pro-inflammatory cytokines TNF-α and IL-1β, and the anti-inflammatory cytokine IL-10 in muscle cells. All of these cytokines enhance myoblast proliferation, whereas their effects of myoblast differentiation vary. This variation depends partly on the cell signaling pathways that these cytokines activate (e.g., p38 MAPK or NFκB) (107), and whether the cytokines influence the activity of myogenic factors such as IGF-1 (6, 102). Cytokines such as TNF-α may regulate muscle cells during early phases of muscle regeneration through their effects on pro-inflammatory macrophages. In the later phases, TNF-α may influence muscle cells more directly by binding its receptors on the cells (107). The expression of other cytokines such as CXCL8 (IL-8), IL-7 and leukemia inhibitor factor also increases in muscle after exercise. Although the functions of these cytokines are less well known, evidence exists that CXCL8 enhances myoblast differentiation (92), IL-7 stimulates satellite cell proliferation and migration (33) and leukemia inhibitor factor increases myoblast proliferation (47).

Although animal studies have provided key insights into the complex regulatory mechanisms underlying muscle-immune interactions, it is important not to draw direct comparisons between these studies and recovery of exercise-induced muscle inflammation in humans (86). Many of these animal models involved rather non-physiological muscle actions or non-physiological techniques to manipulate inflammation (e.g., injection of

bupivacaine, snake venom and $BaCl_2$; freeze injury and crush injury). The extent of muscle damage in animal studies is also arguably more severe than muscle damage resulting from exercise.

Changes in Intramuscular Inflammation following Muscle Adaptation

In addition to investigations on the time course of muscle inflammation after exercise, several studies have examined adaptive changes in muscle inflammation after training and repeated bouts of eccentric exercise. Gordon et al (32) compared the early recovery responses to acute resistance exercise in trained versus untrained arm muscles in the same individuals following unilateral arm resistance exercise training. Training reduced the transcription of genes involved in monocyte recruitment, whereas it enhanced the transcription of genes involved in the switch from a pro- to an anti-inflammatory macrophage phenotype following acute resistance exercise (32). Other evidence indicates that the expression of some chemokines such as CCL2 is upregulated (25, 38), whereas NFκB DNA-binding activity is downregulated (115) in muscle following adaptation to repeated bouts of eccentric exercise. These findings suggest that after muscle adaptation, pro-inflammatory responses to exercise are dampened. Conversely, the greater responsiveness of CCL2 (for example) following muscle adaptation may enhance processes involved in muscle tissue repair, such as myoblast proliferation (116). Through these mechanisms, the immune system may improve the efficiency of muscle regeneration following injury.

It is important to emphasize that the sequence and timing of the stages of muscle inflammation are critical for efficient muscle regeneration and recovery (12, 106). Mounting an initial pro-inflammatory response to muscle injury is required for all subsequent phases of

inflammation that are part of the recovery process involving satellite cell activation and muscle regeneration (12, 106). However, the interactions between leukocytes and skeletal muscle must be tightly regulated to avoid prolonged inflammation, excessive tissue damage and fibrosis (12, 106).

STRATEGIES FOR ENHANCING RECOVERY FROM MUSCLE DAMAGE AND INFLAMMATION

A range of physiotherapeutic, nutritional and pharmacological strategies have been evaluated to investigate their effectiveness in restoring muscle function, relieving muscle soreness and reducing intramuscular inflammation after exercise. Some individual studies have reported benefits of these strategies for recovery from exercise-induced muscle damage. However, the results of systematic reviews and meta-analyses that have combined all of these studies reveal no major or consistent advantages from applying many of these strategies. Massage, wearing compression garments and cold water immersion consistently improve muscle soreness (37, 62, 93). Compression garments and cold water immersion also enhance recovery of muscle strength (50, 62). Research on the effects of cherry juice and polyphenols has produced some promising preliminary results, but more work is required to strengthen this evidence (4, 67). Research on other physicotherapeutic (e.g., vibration therapy, neuromuscular stimulation, intermittent pneumatic compression, low-intensity exercise, muscle warming), nutritional (e.g., protein, fish oil) and pharmacological (e.g., non-steroidal anti-inflammatory drugs) interventions has either yielded inconsistent or null findings. Accordingly, it is not possible to make definitive conclusions about their benefits as treatments for exercise-induced muscle damage.

In contrast with the body of literature on treatments for exercise-induced muscle damage, much less attention has focused on treatments for intramuscular inflammation—at

least in humans. One study has demonstrated that massage after exercise reduces NFκB p65 accumulation, IL-6 and TNF-α protein expression in muscle after exercise (22). Most research demonstrates no benefits of non-steroidal anti-inflammatory drugs for treating intramuscular inflammation after exercise (111). However, recent research suggests that non-steroidal anti-inflammatory drugs can improve muscle regeneration through other mechanisms such as activation of satellite cells (55). Quercetin supplementation does not influence NFκB, COX2 or cytokine mRNA expression in muscle after exercise (75). Compared with low-intensity cycling (i.e., 'active' recovery), cold water immersion also does not alter leukocyte infiltration or cytokine mRNA expression in muscle after resistance exercise (87). The results of several animal studies reveal that supplementation with grape seed-derived proanthocyanidolic oligomer (PCO) before or after muscle injury enhances resolution of inflammation and promotes muscle regeneration (45, 46, 68). Further research is warranted to determine whether such benefits also occur in humans after exercise-induced muscle damage.

THEORETICAL AND PRACTICAL CONSIDERATIONS

The time course of recovery following exercise-induced muscle damage depends on the extent of initial muscle damage, which in turn depends on various factors including the intensity and duration of exercise, joint angle/muscle length and muscle groups used during exercise. The effects of these factors on muscle strength, soreness and swelling are well characterized. By contrast, much less is known about how they affect molecular aspects of muscle adaptation/remodeling and intramuscular inflammation. Variability between high- and low-responders to muscle-damaging exercise is frequently reported in the literature (23). More systematic, well-controlled studies are needed to identify the precise source(s) of such

variability. Further human research is also warranted that encompasses all of the mechanisms that have been proposed to lead to the loss of strength, muscle soreness and other symptoms of muscle damage (e.g., increased stiffness, swelling) after exercise, and how these are associated with ultrastructural changes, especially in the ECM.

Data have been accumulating over the past decade or so suggesting that muscle inflammation in response to acute injury and in otherwise healthy skeletal muscle is part of the functional recovery of the muscle. Blocking muscle inflammation in response to exercise-induced muscle damage in healthy young individuals may interfere with functional recovery and adaptive processes in muscle (61). Further research is required to establish whether anti-inflammatory counter-measures are beneficial under conditions of excessive or chronic low-grade inflammation (e.g., in the elderly) as suggested by the findings of Trappe et al (110). Considering the current lack of human data on how ageing affects the interplay of skeletal muscle with immune cells during recovery from exercise, this represents an important gap in our knowledge that needs to be addressed. Furthermore, more time-course-dependent investigations following different types of exercise are required to gain a better understanding of the highly dynamic muscle-immune interactions in a physiological context.

Many treatments have been tested to determine if they help to restore muscle function and reduce muscle soreness following exercise. Perhaps with the exception of massage, cold water immersion and wearing compression garments, these treatments have not produced consistent benefits. Although evidence is lacking to support the physical benefits of some these treatments, their perceptual effects may be important for exercise recovery (20, 108). In this regard, however, a key consideration is whether by masking the perception of pain or

accelerating recovery ahead of structural remodeling, some of these treatments may actually increase risk of further muscle injury.

A number of issues related to muscle damage and inflammation warrant further investigation: (1) the effects of post-exercise recovery strategies on intramuscular inflammation; (2) similarities or differences in responses to post-exercise recovery treatments in females versus males; (3) the relationship between ultrastructural damage (e.g., Z-line streaming) and myofibril damage (e.g., necrosis); (4) the mechanical and/or biochemical factors that lead to inflammation within the ECM; (5) if and how inflammation within the ECM is related to symptoms of muscle damage (e.g. DOMS, strength loss, swelling); (6) what factors account for the large inter-subject variability for the responses to eccentric exercise-induce muscle damage and (7) whether muscle–immune interactions occur following non-damaging exercise. Thus, the factors that influence muscle damage and inflammation during recovery from exercise remain an interesting and important area for ongoing research.

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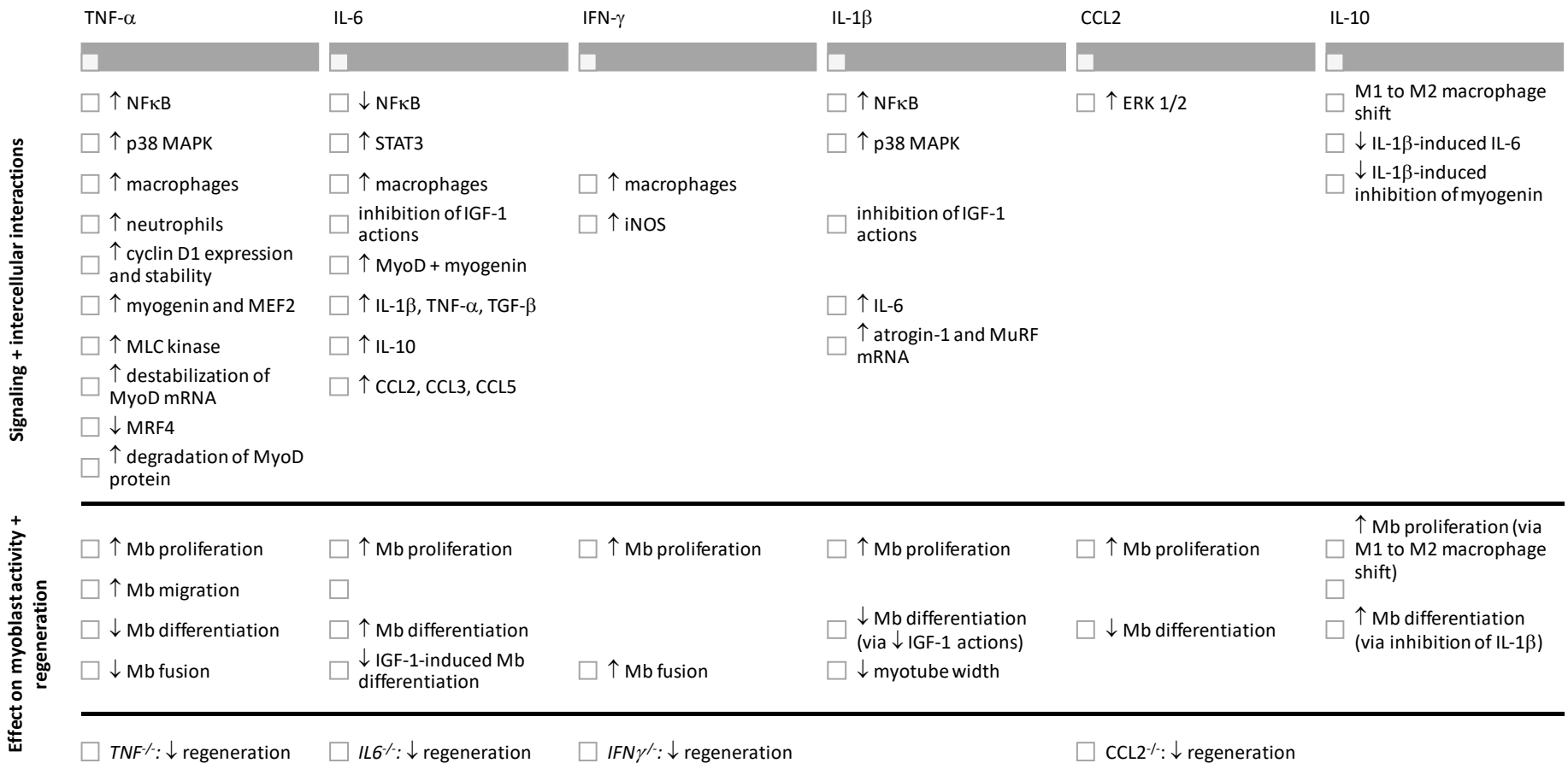
FIGURE CAPTIONS

Figure 1. Graphical illustration of the cell types within skeletal muscle that contribute to muscle-immune cell interactions and regulate muscle adaptation following exercise**.** In the early hours of the recovery period, neutrophils dominate the inflammatory cell profile, acting to clear cellular debris and propagating the inflammatory response by cytokine secretion. Mast cells also infiltrate muscle tissue, releasing histamine and chemoattractants. Between 4–24 h after muscle damage, pro-inflammatory macrophages invade muscle, secreting pro-inflammatory cytokines phagocytosing damaged tissue and initiating myoblast proliferation. After 24 h, pro-inflammatory macrophages and replaced by anti-inflammatory macrophages and CD8 and T regulatory lymphocytes. These cells secrete anti-inflammatory cytokines, recruit macrophages and stimulate myoblast proliferation and expansion of the satellite cell pool. Other stromal cells including fibro-adipogenic progenitors and pericytes are activated, and support myoblast differentiation. If these inflammatory responses are efficiently resolved, new and regenerating muscle fibers restore the ultrastructure of skeletal muscle tissue by around 7 d. Abbreviations: MPO, myeloperoxidase; RONS, reactive oxygen and nitrogen species; TNF, tumor necrosis factor; IL, interleukin; TGF, transforming growth factor; SLPI, secretory leukocyte protease inhibitor; PPAR, peroxisome proliferator–activated receptor; IGF, insulin-like growth factor; ECM, extracellular matrix; PDGF, platelet-derived growth factor; CCR, C-C motif chemokine receptor; PAF, platelet activating factor; LTB, leukotriene; NO, nitric oxide; MCP-1, monocyte chemotactic protein; VEGF, vascular endothelial growth factor.

Figure 2. Schematic illustration displaying model data for the typical magnitude and time course of changes in maximal voluntary contraction torque of the elbow flexors (MVC), range of motion at the elbow joint, swelling measured by upper arm circumference, delayed onset muscle soreness (DOMS) assessed by a visual analogue scale, and creatine kinase activity (CK) in the blood before (pre), immediately after (post) and 1–5 days after 30 maximal eccentric muscle contractions of the elbow flexors performed by healthy young men who were unaccustomed to the exercise. Data are derived from separate analysis published elsewhere (23). ● = strength. ▲ = swelling. △ = soreness. ○ = range of motion. ■ = creatine kinase.

Figure 3. Summary of published data for the magnitude and time course of changes in leukocyte infiltration (A), cytokine/chemokine mRNA (B) and protein expression in muscle after exercise. For panel A, each symbol represents data from an individual study. The dotted line represents baseline values. Abbreviations: TNF, tumor necrosis factor; IL, interleukin, TGF, transforming growth factor; LIF leukemia inhibitory factor; IFN, interferon; G-CSF, granulocyte-colony stimulating factor; CCL, C-C motif chemokine ligand; CXCL, C-X-C motif ligand.



Information sourced from (6, 17, 24, 99, 107, 116, 120). Abbreviations: NFκB, nuclear factor κB; MAPK, mitogen-activated protein kinase; MEF2, myocyte enhancer factor 2; MLC, myosin light chain; MRF4, myogenic regulatory factor; Mb, myoblast; ERK, extracellular-regulated kinase; STAT, signal transducer and activator of transcription; IGF, insulin-like growth factor; IL, interleukin; TNF, tumor necrosis factor; TGF, transforming growth factor; IFN, interferon; iNOS, inducible nitric oxide synthase; CCL, C-C motif ligand; MuRF, muscle ring finger.

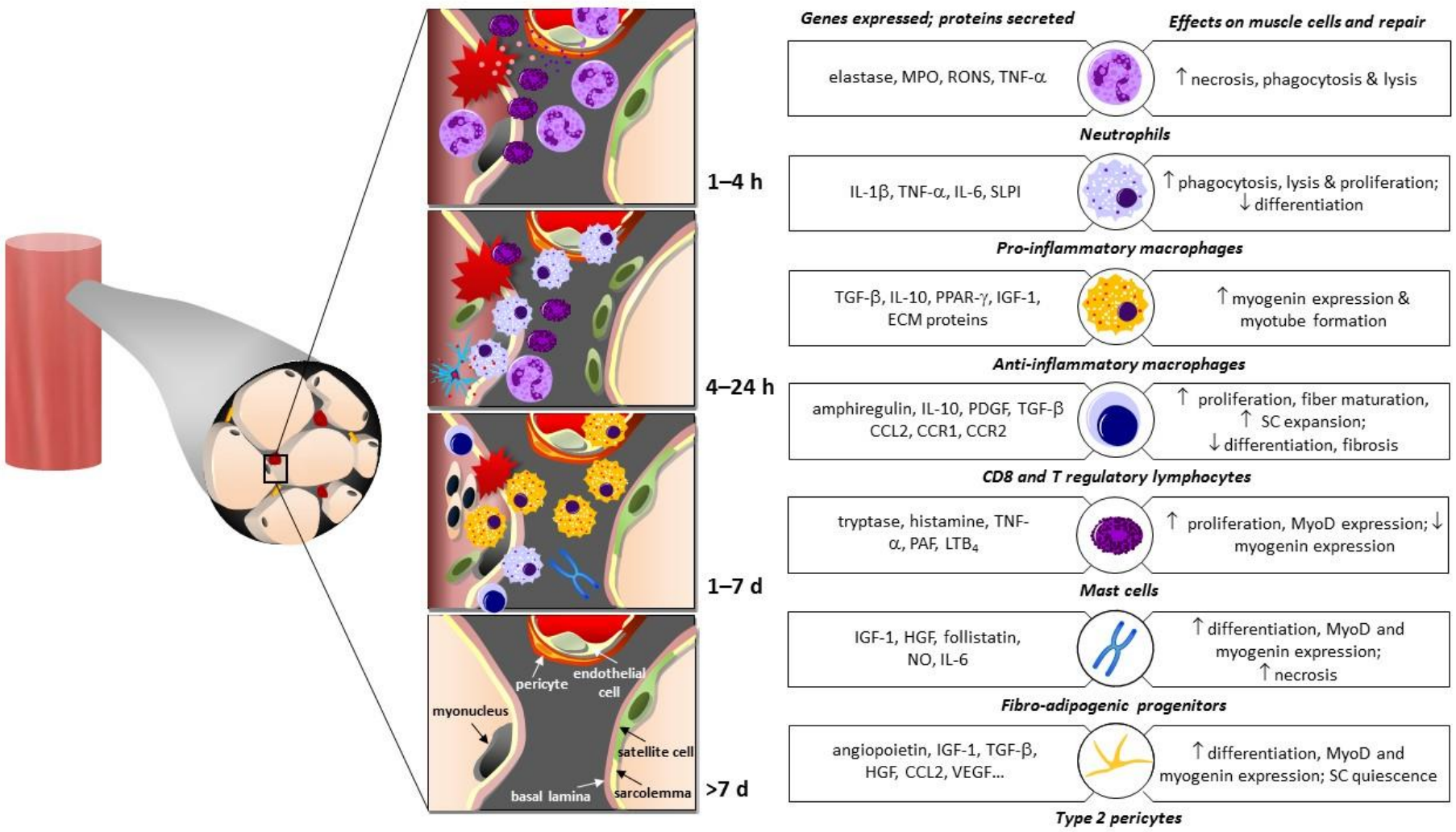

Genes expressed; proteins secreted
Effects on muscle cells and repair
elastase, MPO, RONS, TNF-α
↑ necrosis, phagocytosis & lysis
Neutrophils
1–4 h
IL-1β, TNF-α, IL-6, SLPI
↑ phagocytosis, lysis & proliferation; ↓ differentiation
Pro-inflammatory macrophages
TGF-β, IL-10, PPAR-γ, IGF-1, ECM proteins
↑ myogenin expression & myotube formation
Anti-inflammatory macrophages
4–24 h
amphiregulin, IL-10, PDGF, TGF-β CCL2, CCR1, CCR2
↑ proliferation, fiber maturation, ↑ SC expansion; ↓ differentiation, fibrosis
CD8 and T regulatory lymphocytes
tryptase, histamine, TNF-α, PAF, LTB4
↑ proliferation, MyoD expression; ↓ myogenin expression
Mast cells
1–7 d
IGF-1, HGF, follistatin, NO, IL-6
↑ differentiation, MyoD and myogenin expression; ↑ necrosis
Fibro-adipogenic progenitors
angiopoietin, IGF-1, TGF-β, HGF, CCL2, VEGF...
↑ differentiation, MyoD and myogenin expression; SC quiescence
Type 2 pericytes
>7 d
pericyte
endothelial cell
myonucleus
satellite cell
basal lamina
sarcolemma

Figure 2

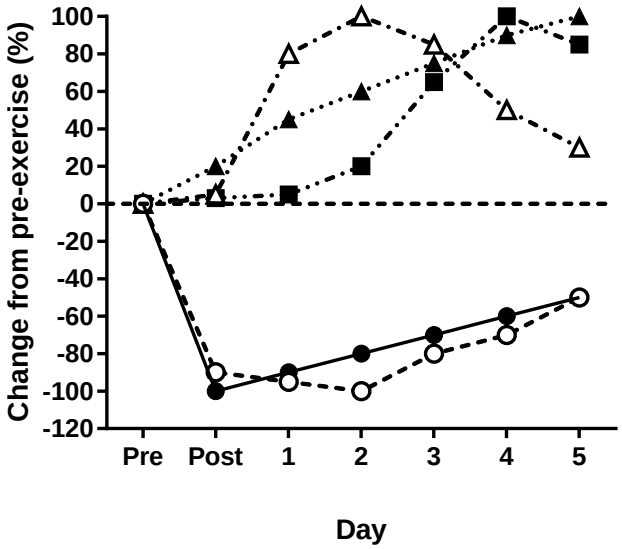
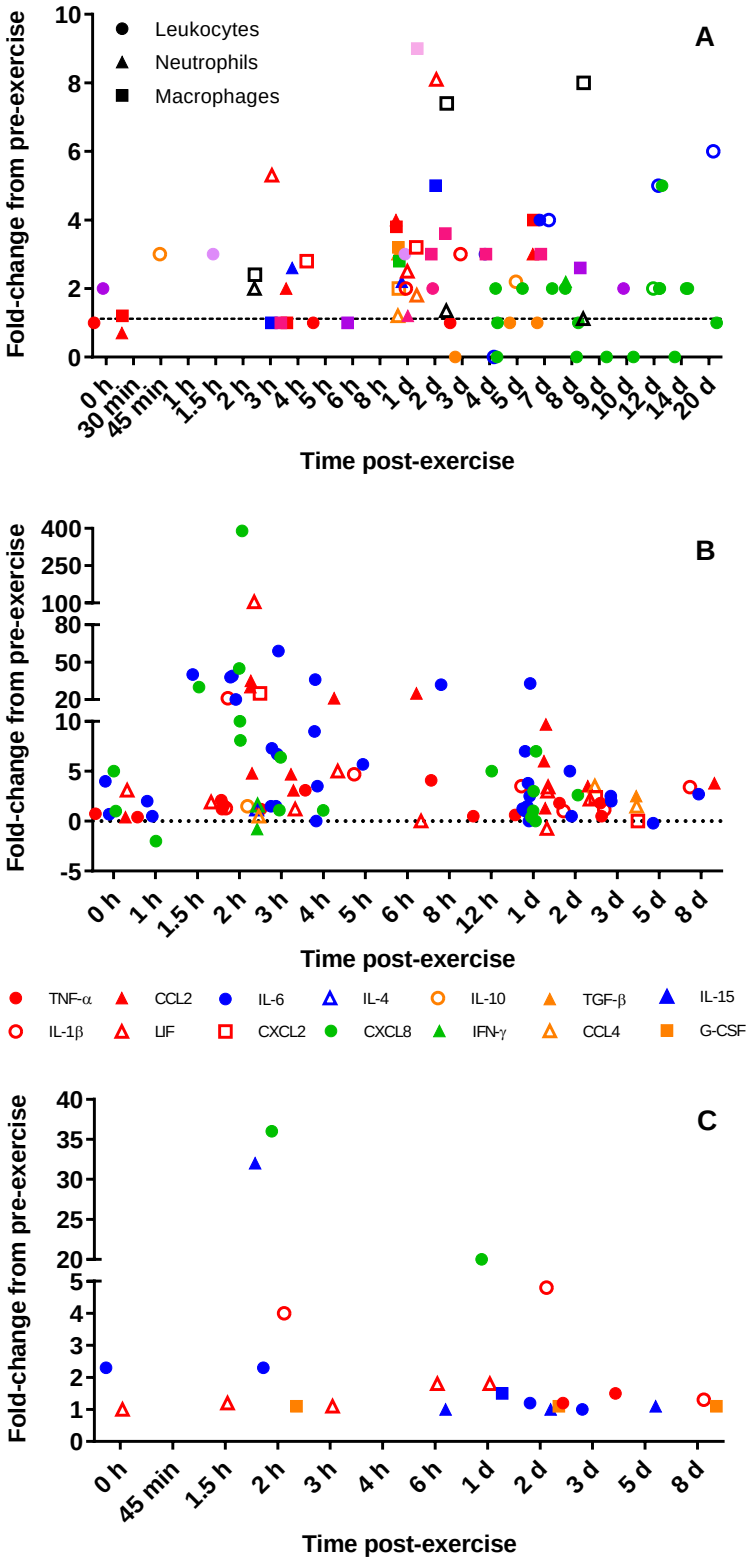

Change from pre-exercise (%)
100
80
60
40
20
0
-20
-40
-60
-80
-100
-120
Pre
Post
1
2
3
4
5
Day

Figure 3


A
Leukocytes
Neutrophils
Macrophages
Fold-change from pre-exercise
Time post-exercise
B
Fold-change from pre-exercise
Time post-exercise
TNF-α
CCL2
IL-6
IL-4
IL-10
TGF-β
IL-15
IL-1β
LIF
CXCL2
CXCL8
IFN-γ
CCL4
G-CSF
C
Fold-change from pre-exercise
Time post-exercise