Characterization of inflammatory responses to eccentric exercise in humans

Running title: Inflammation and eccentric exercise

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Abstract

Eccentric exercise commonly results in muscle damage. The primary sequence of events leading to exercise-induced muscle damage is believed to involve initial mechanical disruption of sarcomeres, followed by impaired excitation-contraction coupling and calcium signaling, and finally, activation of calcium–sensitive degradation pathways. Muscle damage is characterized by ultrastructural changes to muscle architecture, increased muscle proteins and enzymes in the bloodstream, loss of muscular strength and range of motion and muscle soreness. The inflammatory response to exercise-induced muscle damage is characterized by leukocyte infiltration and production of pro-inflammatory cytokines within damaged muscle tissue, systemic release of leukocytes and cytokines, in addition to alterations in leukocyte receptor expression and functional activity. Current evidence suggests that inflammatory responses to muscle damage are dependent on the type of eccentric exercise, previous eccentric loading (repeated bouts), age and gender. Circulating neutrophil counts and systemic cytokine responses are greater after eccentric exercise using a large muscle mass (e.g. downhill running, eccentric cycling) than after other types of eccentric exercise involving a smaller muscle mass. After an initial bout of eccentric exercise, circulating leukocyte counts and cell surface receptor expression are attenuated. Leukocyte and cytokine responses to eccentric exercise are impaired in elderly individuals, while cellular infiltration into skeletal muscle is greater in human females than males after eccentric exercise. Whether alterations in intracellular calcium homeostasis influence inflammatory responses to muscle damage is uncertain. Furthermore, the effects of antioxidant supplements are variable, and the limited data available indicates that anti-inflammatory drugs largely have no influence on inflammatory responses to eccentric exercise. In this review, we compare local versus systemic

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inflammatory responses, and discuss some of the possible mechanisms regulating the inflammatory responses to exercise-induced muscle damage in humans.

Keywords: muscle damage, inflammation, leukocytes, cytokines, cellular infiltration, repeated bout effect

Contents

Introduction

The following theory describes the current understanding of the events in the process of exercise-induced muscle damage (63). When myofibrils within a muscle fiber are stretched during contraction, some sarcomeres are more resistant to stretching than others. Consequently, the weaker sarcomeres absorb more of the stretch, and depending on the length-tension ratio, these sarcomeres become weaker until there is little or no overlap between the myofilaments. During repeated eccentric contractions, first the weak and then the stronger sarcomeres are progressively overstretched. During the muscle relaxation phase, the myofilaments of overstretched sarcomeres may fail to reconnect, resulting in disrupted sarcomeres. This structural disruption can spread to adjacent areas of the muscle, and can ultimately lead to damage to the membranes of the sarcoplasmic reticulum, transverse tubules or the sarcolemma. At the same time, excitation–contraction coupling is disrupted, and Ca^{2+} moves freely into the sarcoplasm where it activates proteolytic pathways related to muscle fiber degradation and repair (63). This process appears to produce some of the symptoms associated with muscle damage, including loss of muscle function, delayed-onset muscle soreness (DOMS), and increases in muscle proteins in the circulation, which represent plasma membrane damage. Skeletal muscle adapts to exercise-induced damage, such that there is less muscle damage and soreness when eccentric exercise using the same muscles is repeated up to 6 months after an initial bout. However, the precise mechanisms contributing to this repeated bout effect remain unclear (37).

The immune system plays a role in the degeneration and regeneration process of muscle and surrounding connective tissue after exercise-induced muscle damage. The inflammatory response to exercise-induced muscle damage is illustrated in Figure 1. Briefly, neutrophils are rapidly mobilized into the circula-

tion after exercise, and soon invade the damaged muscle tissue. Natural killer cells and lymphocytes are also mobilized, and anti-inflammatory cytokines are released into the systemic circulation during and immediately after eccentric exercise. Within 1 day after exercise, neutrophils are replaced in damaged muscle tissue by macrophages, and pro-inflammatory cytokines are produced in muscle. These inflammatory responses are important for the regulation of the acute-phase response and removal of fragments of damaged muscle after eccentric exercise.
The purpose of this review is to compare local and systemic inflammatory responses to eccentric exercise, and to discuss the factors affecting these responses. Although the focus of the review is on human inflammatory responses, we have cited data from some animal studies to explain some possible mechanisms.

1. Local inflammatory responses

Muscle damage resulting from lengthening contractions attracts leukocytes to the site of injury. Neutrophils invade skeletal muscle within several hours (4), and remain present up to 24 h after exercise (4, 30, 31, 34, 64, 77). Macrophages are present in muscle from 24 h to 14 days after exercise (3, 4, 23, 27, 34, 55, 69). Neutrophils and macrophages contribute to the degradation of damaged muscle tissue by release reactive oxygen and nitrogen species (38, 39), and may also produce pro-inflammatory cytokines (13). The pro-inflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α are expressed within skeletal muscle up to five days after exercise (9, 21, 23). IL-1β and TNF-α play a role in initiating the breakdown of damaged muscle tissue (13). Other cytokines such as IL-6 and transforming growth factor (TGF)-β1, and inflammatory antigens such as leukemia inhibitory factor (LIF) and hypoxia inducible factor (HIF)-1β are also expressed in skeletal muscle in the days following eccentric exercise (23, 35). Therefore, the local inflammatory response within skeletal muscle after eccentric exercise is predominantly pro-inflammatory. Less is known about the expression of anti-inflammatory cytokines such as IL-1 receptor antagonist (IL-1ra), IL-4 and IL-10 in muscle after exercise (34, 41). These cytokines may be produced by immune cells rather than muscle cells.

One limitation of assessing local inflammatory responses is whether the muscle biopsy procedure itself actually contributes to local inflammation. Malm *et al*. have reported that in one of their studies, there were no significant differences in the expression of inflammatory antigens within muscle in response to eccentric exercise compared to the control condition that involved a muscle biopsy without exercise (34). Therefore, it is important to consider this issue when interpreting data on local inflammatory responses to eccentric exercise.

2. Systemic inflammatory responses

In comparison to the number of studies that have investigated local inflammatory responses in muscle after eccentric exercise, there is a greater body of knowledge relating to systemic inflammatory responses after exercise. This likely reflects the fact that blood sampling is less invasive than the muscle biopsy procedure. Furthermore, the measurement of leukocyte numbers and cytokines in blood samples is easier to perform than immunohistochemical staining and gene expression studies in muscle samples. The greater number of studies examining systemic inflammation allows for the comparison of responses to different types of eccentric exercise.

A large volume of data is available relating to changes in neutrophils, monocytes, natural killer cells, T and B lymphocytes after eccentric exercise. These data are summarized in Tables 1 to 3. Evidence suggests that changes in circulating leukocyte counts after eccentric exercise are dependent on the muscle groups used, or the amount of muscle mass recruited during eccentric exercise. Circulating leukocyte numbers are generally highest after exercise such as downhill running and eccentric cycling (11, 12, 34, 50, 54, 61). In contrast, there are smaller changes after stepping exercise (22, 33) and eccentric contractions of the quadriceps and elbow flexors (58-60, 71). These differences may reflect the greater influence of stress hormones on leukocyte mobilization following dynamic systemic exercise versus local muscle contractions.

In addition to the mobilization of leukocytes, muscle damage has the potential to modify leukocyte receptor expression and functional activity. Alterations in receptor expression may mediate infiltration of neutrophils and monocytes into damaged muscle tissue (2), where these cells release proteolytic enzymes and reactive oxygen species that assist in the breakdown of tissue fragments (80). A number of studies have investigated changes in leukocyte receptor expression after different types of eccentric exercise, and the data from these studies are summarized in Table 4. Other studies have examined alterations in neutrophil degranulation and respiratory burst activity after eccentric exercise (for review, see ref. 48). There is no clear effect of different types of eccentric exercise on leukocyte receptor expression or functional activity. This disparity might be due to differences in the type of eccentric exercise, exposure to previous eccentric exercise, as well as the methods used to isolate neutrophils and measure receptor expression and functional activity.

The data from studies examining changes in systemic cytokine concentrations after eccentric exercise have been extensively reviewed elsewhere (25, 78). Table 5 provides data from a selection of studies to highlight differences in the systemic cytokine response to various types of eccentric exercise. Downhill running and eccentric cycling at high intensities (≥75% VO_{2max}) (54, 82) stimulate a greater increase in the plasma concentrations of IL-6 and IL-1 and IL-1ra than other forms of eccentric exercise such as moderate-intensity downhill running (49, 50, 79), and local eccentric contractions of the elbow flexors (15, 25, 56) or quadriceps (17, 32). This difference is more likely related to the intensity and duration of exercise (8, 49, 78, 82), and possibly increased core temperature (66) rather than muscle damage. While systemic IL-6 concentration generally returns to baseline within 24 h following downhill running and eccentric cycling (50, 54, 79, 82), it may remain elevated for several days following eccentric contractions of the elbow flexors, depending on the contraction workload (15, 56). This delayed response may occur because this muscle group is exposed to eccentric muscle strain less often than leg muscles (26), or because subjects might restrict the use of their arm following this type of eccentric exercise.

3. Pro- versus anti-inflammatory responses to eccentric exercise

In contrast to the local production of pro-inflammatory cytokines within skeletal muscle after eccentric exercise, the systemic levels of IL-1β and TNF-α increase only slightly, if at all, following eccentric exercise (8, 25, 74, 82). Conversely, a stronger systemic anti-inflammatory response occurs following eccentric exer-

cise, as indicated by elevated plasma levels of IL-1ra, IL-10 and soluble TNF-α receptors (49, 54, 74, 82). Other anti-inflammatory cytokines such as IL-4 and IL-13 either remain unchanged or below detection limits (25, 49). Evidence is lacking to suggest that muscle is the source of these anti-inflammatory cytokines. Instead, they may be produced by mononuclear cells of the immune system (75).

Therefore, although pro-inflammatory responses occur within skeletal muscle after eccentric exercise, the release of pro-inflammatory cytokines into the circulation appears to be inhibited. The mechanism of this inhibition is not immediately obvious at present; however, IL-6 may be involved. IL-6 has been termed an „inflammation-responsive" myokine, because it does not directly reduce inflammation and is produced within skeletal muscle (51, 53). Evidence suggests that IL-6 acts indirectly to restrict inflammation by stimulating the production of anti-inflammatory cytokines including IL-1ra, IL-10, cortisol and soluble TNF-α receptors (53). Following marathon running, the plasma concentrations of TNF-α (2.3×), IL-1β (2.1×), IL-6 (128×) and IL-10 (27×) are elevated immediately after running, while soluble TNF-α receptors (~2×) and IL-1ra (39×) peak 1–1.5 h later (44). IL-1ra, IL-10 and soluble TNF-α receptors exert their respective anti-inflammatory effects by inhibiting signal transduction via the IL-1 receptor (20), inhibiting cytokine gene expression and production in mononuclear cells (6, 86), binding and neutralizing circulating and membrane-bound TNF-α (73). Hence, following eccentric exercise, circulating anti-inflammatory cytokines respond to local production of pro-inflammatory cytokines and restrict systemic inflammation.

4. Factors influencing inflammatory responses

Research has focused on a variety of factors that may potentially influence inflammatory responses to muscle damage after eccentric exercise. These factors include age, gender, repeated bouts of eccentric exercise, antioxidant supplements, intracellular calcium homeostasis, and anti-inflammatory drugs. The following sections address each of the factors in detail.

4.1 Aging

The aging process is associated with a decline in skeletal muscle mass and strength. Aging muscle exhibits reduced contraction speeds, rate of force development and the ability to accelerate limb movement, and together, these factors contribute to muscle weakness and impaired stability in elderly individuals (16). Therefore, these responses have generated interest in whether aging muscle is more vulnerable to exercise-induced damage. Ultrastructural damage after eccentric exercise is greater in elderly individuals (36), and the rate of recovery after eccentric exercise and strenuous resistance training is slower (19, 62, 68).

Recently, Hamada *et al*. (23) reported increased mRNA expression for the leukocyte antigen CD18 *in vastus lateralis* muscle samples after downhill running. CD18 mRNA was significantly lower in elderly than young individuals. This finding might have been due to the lower mRNA expression of TGF-β1 that was also found in the elderly (23). However, other recent experimental data does not necessarily support a role for TGF-b1 as a neutrophil chemoattractant in injured skeletal myotubes *in vitro* (85).

Several studies have also investigated the influence of aging on systemic inflammatory responses to eccentric exercise. After downhill running, circulating

Table 1: Summary of changes in neutrophil counts after eccentric exercise

Type of eccentric exercise	Amount of muscle mass involved	Immediately after exercise	1–4 h after exercise	4–12 h after exercise	≥ 24 h after exercise	References
Downhill running	Large					
Low intensity (≤ 60% VO_{2max})		20 to 30% ↑	30 to 80% ↑	30% ↑	No change	Smith *et al*. (1989) Peake *et al*. (2005b)
High intensity (70–75% VO_{2max})		50% to 1.2× ↑	30% to 3× ↑	20 to 100% ↑	No change	Cannon *et al*.. (1990, 1994) Pizza *et al*. (1995) Petersen *et al*. (2001)
Young individuals (< 33 yrs old)		10% ↑	50 to 80% ↑	60 to 100% ↑	0-10% ↑	Cannon *et al*. (1990, 1994) Toft *et al*. (2002)
Old individuals (> 60 yrs old)		30% ↑	30% to 2× ↑	20 to 70% ↑	0-50% ↑	
Effect of antioxidant		100% ↑	1.6 to 2.8× ↑			Petersen *et al*. (2001)
Placebo		1.2-fold ↑	1.9 to 2.8× ↑			
Eccentric contractions of the quadriceps	Moderate		75% ↑	≤ 20% ↑		Saxton *et al*. (2003)
Eccentric cycling	Large	10 to 40% ↑	70% ↑	120% ↑	≤ 20% ↑	Bruunsgaard *et al*. (1996) Malm *et al*. (2000)
Eccentric stepping	Small	20% ↑	60 to 75% ↑	75% ↑	0-20% ↓	Gleeson *et al*. (1995) Malm *et al*. (1999) Saxton *et al*. (2003)
Eccentric contractions of the elbow flexors	Small					Pizza et al. (1996, 1999)
First bout			10 to 40% ↑	25 to 75% ↑	≤ 20% ↑	
Second bout			15 to 20% ↑	10 to 30%	0%	
Effect of anti-inflammatory drugs			0%	20% ↑	≤ 20% ↑	Pizza et al. (1999)
Placebo			15% ↑	30% ↑	≤ 20% ↑	

Table 2: Summary of changes in natural killer cell counts after eccentric exercise

Type of eccentric exercise	Amount of muscle mass involved	Immediately after exercise	1–4 h after exercise	4–12 h after exercise	≥ 24 h after exercise	References
Downhill running	Large					Pizza et al. (1995)
$CD16^+$ cells		3.2×↑	60%↓	15%↓	25%↓	
$CD56^+CD3^-$ cells		3.4×↑	55%↓	30%↓	65%↑	
Effect of antioxidant						Petersen et al. (2001)
$CD56^-CD16^+CD3^-$		2.5×↑	50%↓			
$CD56^+CD16^+CD3^-$		3.5×↑	75%↓			
$CD56^+CD16^-CD3^-$		1.5×↑	0%			
Placebo						
$CD56^-CD16^+CD3^-$		5×↑	0%			
$CD56^+CD16^+CD3^-$		3.6×↑	60%↓			
$CD56^+CD16^-CD3^-$		4×↑	0%			
Eccentric cycling	Large					Bruunsgaard et al. (1996)
$CD16^+CD3^-$		5×↑	20%↓			
$CD56^+CD3^-$		5×↑	25%↓			
$CD56^+CD16^+$		5×↑	35%↓			
$CDRA^+CD16^+$		5.4×↑	25%↓			
$CD56^+CD16^+CD57^+CD3^-$		10%↑		20%↓	≤ 20%↑	Malm et al. (2000)
$CD56^+CD16^+CD57^-CD3^-$		15%↑		0%	≤ 20%↑	
Eccentric stepping	Small					Malm et al. (1999)
$CD56^+CD16^+CD57^-CD3^-$				30%↓	20%↓	

Table 3: Summary of changes in T and B lymphocyte counts after eccentric exercise

Type of eccentric exercise	Amount of muscle mass involved	Immediately after exercise	1–4 h after exercise	4–12 h after exercise	≥ 24 h after exercise	References
Downhill running	Large					Petersen et al. (2001)
Effect of antioxidant						
$CD3^+$		60%↑	15-20%↓			
$CD4^+$		50%↑	15-20%↓			
$CD8^+$		80%↑	15-25%↓			
Placebo						
$CD3^+$		60%↑	0-30%↓			
$CD4^+$		50%↑	0-30%↓			
$CD8^+$		60%↑	5-30%↓			
$CD3^+$		0-50%↑	40%↓	0-5%↓	0-20%↓	Pizza et al. (1995)
$CD4^+$		10%↓ to 30%↑	50%↓	≤ 5%↓	0-30%↓	Malm et al. (2004)
$CD8^+$		100%↑	40%↓	5%↓	20%↓	
$CD20^+$	Large	10%↑	20%↓	40%≠	10-20%↓	
Eccentric cycling						Malm et al. (2000)
$CD3^+$		10%↓		5%↑	10%↓ to 5%↑	Bruunsgaard et al. (1996)
$CD8^+$		60%↑	20%↓			
$CD8^+CD11b^+$		50%↑		10%↑	20%↓ to 100%↑	
$CD8^+CD62^+$		5%↓		5%↓	10%↓ to 25%↓	
$CD20^+$						
$CD20^+CD23^+$						

Table 4: Summary of changes in neutrophil and monocyte cell surface receptor expression after eccentric exercise

Type of eccentric exercise	Amount of muscle mass involved	Immediately after exercise	1–4 h after exercise	4–12 h after exercise	≥ 24 h after exercise	References
Eccentric cycling	Large					
Monocyte CD11b+62L+		No change		5%↑	10%↓ to 5%↑	Malm et al. (2000)
Monocyte CD4/62L+		No change		10%↓	0-15%↓	
Monocyte CD4/62L-		No change		10%↓	0-10%↓	
Downhill running	Large					
Neutrophil CD11b		No change	10%↓		5%↓	Peake et al. (2005b)
Neutrophil CD16		No change	20%↓		No change	
Neutrophil CD35		10%↓	25%↓		25%↓	
Eccentric contractions of the quadriceps	Moderate					
Neutrophil CD11b			30%↑		10%↓ to 1.4-fold↑	Saxton et al. (2003)
Neutrophil CD64			No change		10%↓ to 15%↑	
Monocyte CD11b			30%↑		10-40%↓	
Monocyte CD64			10%↑		10%↓ to 10%↑	
Eccentric contractions of the elbow flexors	Small					
First bout						Pizza et al. (1996)
Neutrophil CD11b			30%↑	3 to 6×↑	3- to 7-fold↑	
Neutrophil CD18			5%↓	10-20%↓	15%↓ to 45%↑	
Neutrophil CD64			15%↓	80-100%↑	40-80%↑	
Monocyte CD11b			15%↑	0-50%↓	50%↓ to 2.5×↑	)
Monocyte CD18			No change	No change	10-50%↑	
Monocyte CD64			10%↑	50-70%↑	30-90%↑	

Table 4: Summary of changes in neutrophil and monocyte cell surface receptor expression after eccentric exercise

Type of eccentric exercise	Amount of muscle mass involved	Immediately after exercise	1–4 h after exercise	4–12 h after exercise	≥ 24 h after exercise	References
Second bout						
Neutrophil CD11b			No change	3- to 5-fold ↑	50% to 4× ↑	
Neutrophil CD18			5% ↓	5-15% ↓	5% ↓ to 30% ↑	
Neutrophil CD64			5% ↓	70% ↑	5% ↓ to 30% ↑	
Monocyte CD11b			15% ↑	30% ↓ to 30% ↑	50% to 3× ↑	
Monocyte CD18			No change	No change	10% ↓ to 10% ↑	
Monocyte CD64			No change	20% ↑	5-25% ↑	
Eccentric stepping exercise	Small					
Neutrophil CD11b			30% ↑		10-20% ↓	Saxton et al. (2003)
Neutrophil CD64			No change		0-20% ↓	Malm et al. (1999)
Monocyte CD11b			30% ↑		10% ↓ to 40% ↑	
Monocyte CD64			10% ↑		10% ↓ to 10% ↑	

neutrophil numbers were around 20% lower in elderly males (61–72 yr) than young males (20–32 yr) (11, 12) (see Table 1). The same group reported that the capacity of neutrophils to generate reactive oxygen species in vitro increased significantly five and 12 days after downhill running in elderly males (> 55 yr), whereas there was no significant change in young males (< 30 yr) (11). Consistent with the findings of Hamada et al., Toft et al. (82) reported that after eccentric cycling, plasma IL-6 concentration increased by around 9× in young males (20–27 yr), whereas the increase was much smaller (~90%) in elderly males (65–75 yr) (see Table 5). Plasma IL-1ra concentration also tended to be lower in the elderly males.

Therefore, aging impairs leukocyte mobilization and migration into skeletal muscle after eccentric exercise. This impairment may be related to weaker local and systemic cytokine responses in the elderly. This situation may be limited to eccentric exercise, however, because the IL-6 response to concentric exercise is similar in elderly and young individuals (52). Taken together, these findings suggest that inflammatory response to exercise in the elderly may depend on the extent of muscle damage.

4.2 Gender

There are no differences between males and females with respect to muscle damage after eccentric exercise (30, 67, 76, 77). However, several studies have noted gender differences in leukocyte infiltration into skeletal muscle. In particular, neutrophil infiltration is significantly greater in females than males 24

Table 5: Summary of changes in systemic cytokines after eccentric exercise.

Type of eccentric exercise	Amount of muscle mass involved	Immediately after exercise	1–4 h after exercise	4–12 h after exercise	≥ 24 h after exercise	References
Downhill running	Large	IL-1ra 100%↑ IL-6 4.6×↑ IL-8 30%↑ IL-10 No change IL-12p40 No change G-CSF 25%↑ MCP-1 55%↑	IL-1ra 2.4×↑ IL-6 4.6×↑ IL-8 15%↓ IL-10 No change IL-12p40 15%↑ G-CSF 20% MCP-1 70%↑		IL-6 0% IL-8 25%↓ G-CSF 0%	Peake et al. (2005a,b)
Effect of antioxidant		IL-6 13×↑ IL-1ra 100%↑	IL-6 4 to 7×↑ IL-1ra 100%↑		IL-6 0% IL-1ra 20%↑	Petersen et al. (2001)
Placebo		IL-6 14×↑ IL-1ra 1.3×↑	IL-6 5 to 7×↑ IL-1ra 3 to 4×↑		IL-6 0% IL-1ra 20%↑	
Eccentric cycling	Large					Toft et al. (2002)
Young individuals (< 33 yrs old)		IL-6 2 to 4×↑ IL-1ra 40%↑ TNF-α 0% sTNF-αR1 45%↑	IL-6 3 to 9×↑ IL-1ra 40-60%↑ TNF-α 0% sTNF-αR1 40-50%↑		IL-6 ≤ 100%↑ IL-1ra 25-100%↑ TNF-α 0% sTNF-αR1 10-20%↑	
Old individuals (> 60 yrs old)		IL-6 20%↑ IL-1ra 20%↑ TNF-α 0% sTNF-αR1 10%↑	IL-6 50 to 100%↑ IL-1ra 20 to 50%↑ TNF-α 0% sTNF-αR1 ≤ 20%↑		IL-6 ≤ 20%↑ IL-1ra 5 to 20%↑ TNF-α 0% sTNF-αR1 10-20%↑	
Eccentric contractions of the quadriceps	Moderate	IL-6 4×↑	IL-6 6×↑		0%	Croisier et al. (1999)
First bout		IL-6 7×↑	IL-6 8×↑		0%	

Abbreviations: IL, interleukin. ra, receptor antagonist. G-CSF, granulocyte-colony stimulating factor. MCP-1, monocyte chemotactic protein 1. TNF, tumor necrosis factor. sTNF-αR1, soluble TNF-α receptor 1.

Table 5: Summary of changes in systemic cytokines after eccentric exercise.

Type of eccentric exercise	Amount of muscle mass involved	Immediately after exercise	1–4 h after exercise	4–12 h after exercise	≥ 24 h after exercise	References
Second bout						
Eccentric contractions of the elbow flexors	Small					Hirose et al. (2004)
First bout		G-CSF 0%	G-CSF 40-50% ↑	G-CSF 70% ↑	G-CSF 10-60% ↑	
		TNF-α 0%	TNF-α 0%	TNF-α 0%	TNF-α 10-15% ↓	
		IL-8 20% ↓	IL-8 10-40% ↓	IL-8 80% ↓	IL-8 70-80% ↓	
		IL-10 0%	IL-10 0%	IL-10 0%	IL-10 0%	
Second bout		G-CSF 30% ↑	G-CSF 10-30% ↑	G-CSF 30% ↑	IL-10 0 to 60% ↑	
		TNF-α 0%	TNF-α 0%	TNF-α 0%	TNF-α 0%	
		IL-8 10% ↓	IL-8 30-60% ↓	IL-8 70% ↓	IL-8 40-70% ↓	
		IL-10 10% ↓	IL-10 0-100% ↑	IL-10 130% ↑	IL-10 10 to 80% ↑	

Abbreviations: IL, interleukin. ra, receptor antagonist. G-CSF, granulocyte-colony stimulating factor. MCP-1, monocyte chemotactic protein 1. TNF, tumor necrosis factor. sTNF-αR1, soluble TNF-α receptor 1.

h after eccentric contractions of the quadriceps (30, 77). Macrophage infiltration also increased significantly in females after a second bout of eccentric exercise, whereas there was no significant change in males (77). Conversely, another study observed a strong trend (P = 0.052) toward greater infiltration of leukocytes expressing the leukocyte common antigen within *vastus lateralis* after exercise in males versus females 48 h after exercise (76). This result was supported by higher circulating neutrophil numbers in males than females at the same time point. The conflicting findings in this latter study with respect to leukocyte infiltration may be due to the fact that a different antigen was used to assess cellular infiltration. Alternatively, because the muscle biopsy was taken at 48 h, rather than 24 h after exercise, there may have been more macrophages than neutrophils present in the muscle (69).

Animal studies suggest that estrogen protects against leukocyte infiltration after eccentric exercise by inhibiting calpain-activated protease activity in muscle (81). However, data from the human studies described above conflict with this particular model. One possible mechanism to explain the differences in leukocyte infiltration between males and females in humans is gender differences in muscle membrane perme-

ability after contraction-induced injury. Further work is needed to examine this concept in more detail.

4.3 Repeated bouts of eccentric exercise

The effects of repeated bouts of eccentric exercise are well established. Following an initial bout of eccentric exercise, skeletal muscle adapts and is thereafter less susceptible to damage following subsequent bouts of the same exercise (42). Experimental data is lacking to establish the precise role of neutrophils and macrophages in the processes of repair and adaptation of skeletal muscle following injury (80). One study has examined differences in leukocyte infiltration after repeated bouts of eccentric exercise (77). In male subjects, neutrophil and macrophage infiltration into skeletal muscle was not affected by repeated bouts. In contrast, female subjects showed evidence of significantly greater neutrophil and macrophage infiltration after the second bout of exercise. The force deficit was smaller and plasma CK activity was lower in both groups after exercise in the second bout. Therefore, it is uncertain whether leukocytes invading skeletal muscle after exercise enhance repair and adaptation. The role of leukocytes in this process may be gender-dependent.

Circulating neutrophil numbers are significantly attenuated by 10–45% in response to repeated bouts of eccentric exercise (58, 60) (see Table 1). There is also a moderate decrease ($P < 0.05$) in the expression of CD11b and CD64 on neutrophils, and CD18 and CD64 on monocytes after a second bout of eccentric exercise (60) (see Table 4). Importantly, this decrease in leukocyte receptor expression corresponded with substantially smaller changes in serum myoglobin concentration and creatine kinase activity after the second bout. Therefore, these findings provide evidence that leukocyte receptor expression is decreased after repeat bouts of eccentric exercise, and this adaptation appears to be a secondary response to the reduced degree of exercise-induced muscle damage.

4.4 Antioxidant supplementation

Evidence suggests that reactive oxygen species (ROS) such as superoxide produced by neutrophils and nitric oxide (NO) generated by macrophages contribute to muscle damage (38, 39). Therefore, by neutralizing ROS and NO, antioxidant supplements may help to reduce muscle damage. A large number of studies have investigated the effects of antioxidant supplementation on neutrophil activation and muscle damage after exercise, reporting variable findings (3, 14, 16, 29, 54, 70, 79; for review see ref. 47). Other studies have examined the effects of antioxidant supplements on local and systemic inflammatory responses.

Beaton *et al*. (3) observed that infiltration of CD68+ macrophages in *vastus lateralis* increased after eccentric contractions of the quadriceps muscles. However, vitamin E supplementation did not influence this response. These findings suggest that either (a) vitamin E was ineffective at preventing oxidative stress, or (b) muscle damage and cellular infiltration after exercise was not related to oxidative stress. It should be noted, however, that only one muscle biopsy was taken at 24 h after exercise (3), and macrophage infiltration may have been altered by vitamin E supplementation at a later time point.

In the first of two studies by Cannon *et al*., vitamin E supplementation increased circulating neutrophil numbers after downhill running in elderly males, such that

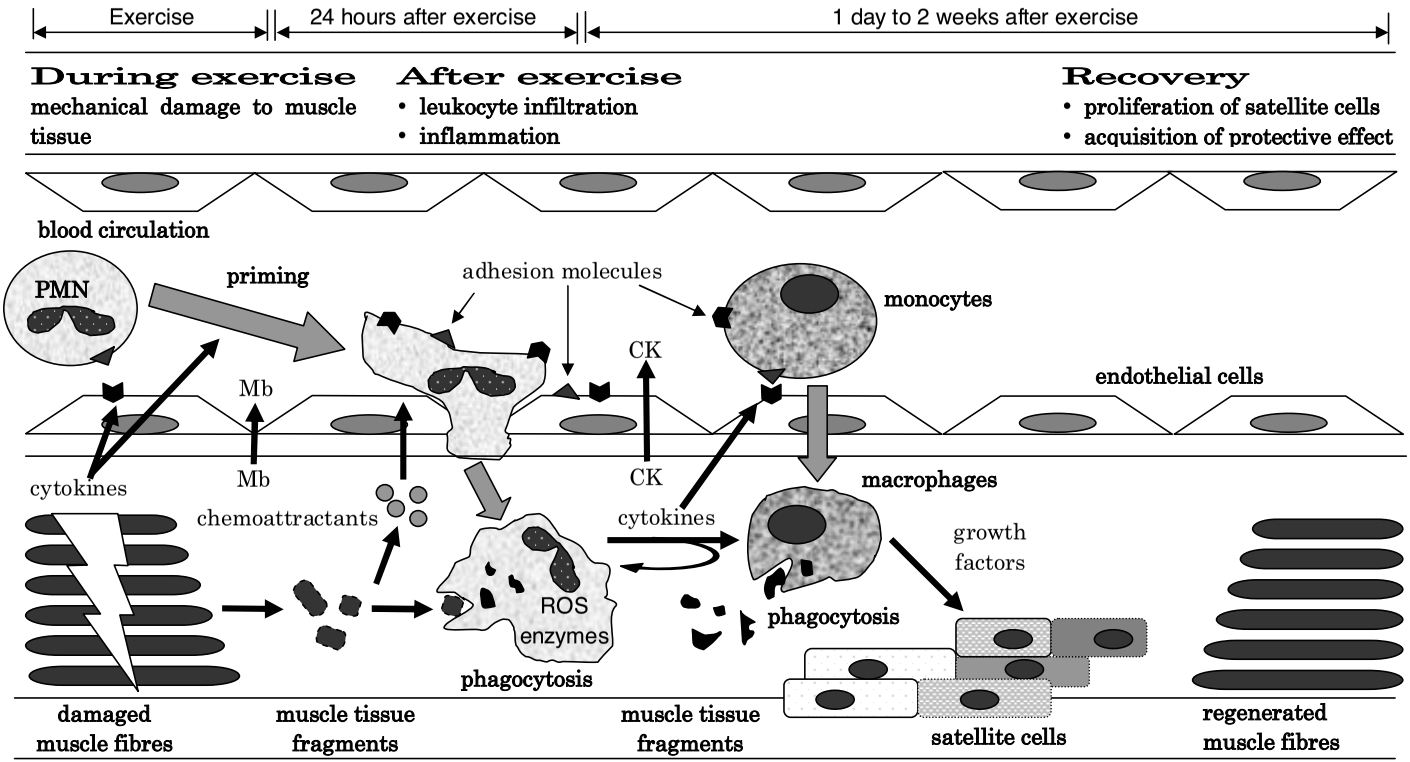

Figure 1. Exercise-induced muscle damage and subsequent muscle inflammation and regeneration process (PMN, polymorphonuclear leucocyte; Mb, myoglobin; CK, creatine kinase; ROS, reactive oxygen species)

the neutrophil count after exercise was similar to that of younger males (11). One explanation for this response is that vitamin E improved neutrophil mobilization in the elderly males by counteracting dysregulation of intracellular calcium homeostasis and calpain activity that accompanies the aging process (18). In the second of their studies, vitamin E supplementation suppressed plasma IL-1β concentration and in vitro monocyte production of IL-1β and IL-6 after downhill running (10). Vitamin E may have acted by neutralizing ROS produced during exercise, which are believed to stimulate cytokine production by skeletal muscle cells via activation of nuclear transcription factor-κ B (1, 28). However, not all studies support the idea that antioxidant supplementation attenuates systemic cytokine responses to eccentric exercise (15, 54, 56, 79). Possible reasons for this variation in experimental findings include differences in the dose and biological activity of individual antioxidants. In addition, the endogenous antioxidant defenses of study participants may affect their responsiveness to supplementation.

4.5 Intracellular calcium homeostasis

As described in the introduction, disruption of the muscle cell membrane after repeated contractions leads to increased influx of Ca^{2+} into the cell (45, 46). Elevated $[Ca^{2+}]i$ activates calpain-mediated proteases, which can attract neutrophils to the site of tissue injury (65) and contribute to further degradation of muscle tissue (5). Blocking of calcium channels may theoretically reduce leukocyte infiltration and muscle damage after eccentric exercise. Beaton et al. (4) reported that eccentric contractions of the knee extensors significantly increased neutrophil infiltration into vastus lateralis 4 h and 24 h after exercise. However, at 24 h neutrophil infiltration was unexpectedly greater in those individuals who received the calcium channel blocker. In explanation of these findings, the calcium channel blocker may have promoted neutrophil migration into the muscle tissue via its effects on vascular and smooth muscle tone (4). Macrophage infiltration also increased significantly 24 h after exercise; however this occurred independently of the calcium channel blocker (4). Therefore, although $[Ca^{2+}]i$ is implicated in muscle damage (63), it is not clear whether dysregulation of calcium homeostasis affects local inflammatory responses in skeletal muscle after eccentric exercise.

4.6 Anti-inflammatory drugs

The effects of anti-inflammatory drugs on markers of muscle damage are equivocal (7, 24, 43, 59, 72, 84). The variable research findings likely reflect differences in a range of factors including the extent of muscle damage after exercise, sampling times, and training status of subjects, in addition to the type, dose and timing of drug administration (7). Anti-inflammatory drugs exert their effect on inflammatory reactions through a number of pathways, including the inhibition of prostaglandin synthesis (83), neutrophil adhesion and activation (40, 57). Three studies have investigated the effects of anti-inflammatory drugs on local and systemic leukocyte responses to eccentric exercise.

In a study by Peterson *et al*., untrained males received 1200 mg ibuprofen before and after eccentric contractions of the quadriceps (55). Exercise did not alter the number of CD15+ neutrophils in the *vastus lateralis* muscle. The number of CD68+ macrophages increased markedly after exercise, however there was no significant effect of ibuprofen. Peterson *et al*. (55) suggested that ibuprofen may have

been ineffective in this study because it does not reduce pro-inflammatory mediators such as prostaglandin E_2, but does reduce mediators such as prostaglandin F_{2a} that facilitate the resolution of inflammation (83). Others have also reported no effect of naproxen sodium on the expression of leukocyte common antigen (CD45) after eccentric contractions of the quadriceps (7). However, there was no main effect of exercise on leukocyte common antigen expression either, so it was difficult to interpret the true significance of these findings.

Administration of 2400 mg ibuprofen per day for five days before and 10 days after eccentric elbow flexion increased neutrophil numbers, but did not significantly alter the capacity of neutrophils to generate reactive oxygen species *in vitro* (59). In explanation of these findings, ibuprofen treatment may have permitted an increase in leukotriene B_4 synthesis which can act as a chemoattractant (59). Alternatively, exercise may have counteracted the inhibitory effect of ibuprofen on neutrophil production of reactive oxygen species by reducing intracellular cyclic AMP, promoting chemoattractant binding and inhibiting changes in membrane fluidity (59).

5.Inflammatory responses, muscle function and delayed-onset muscle soreness

The issue of whether inflammation is a cause or consequence of muscle damage after eccentric exercise has been a point of debate for some time. A number of studies have attempted to address this issue by comparing the time course of changes in local inflammatory responses with loss of muscular strength and the development of delayed-onset muscle soreness after eccentric exercise. Malm *et al*. (34) used multiple regression analysis to assess the relationships between inflammatory responses and delayed-onset muscle soreness after eccentric exercise. They found no correlation between these variables. Furthermore, as stated previously, the inflammatory response to exercise was similar to the muscle biopsy procedure on its own. On the basis of these findings, they concluded that delayed-onset muscle soreness is not related to inflammation, but may be more closely related to muscle adaptation (34). In their second study, which involved downhill running, Malm *et al*. reported similar findings insomuch that downhill running did not cause greater expression of inflammatory antigens in skeletal muscle than the muscle biopsy procedure itself (35). However, one interesting finding was that those subjects who experienced delayed-onset muscle soreness showed evidence of greater staining for inflammatory antigens (CD3, CD11b, LIF and HIF-1β) in the fibrous connective tissue surrounding skeletal muscle (known as the 'epimysium') than those subjects who did not experience delayed-onset muscle soreness. They therefore suggested that delayed-onset muscle soreness may only develop in response to activation of leukocytes that are already present in the epimysium before exercise (35).

In contrast to the invasiveness of taking muscle biopsies, several other studies have used a less invasive technique that involves radio-labeling to assess neutrophil infiltration into damaged tissue after eccentric contractions of the quadriceps (30, 31, 64). This technique has the advantage that large areas of muscle can be assessed, whereas muscle biopsies are restricted to a much smaller section of muscle (31). Conversely, a disadvantage of this technique is that the process of extracting neutrophils for radio-labeling and subsequent re-injection into the experimental subjects may alter the expression and avidity of adhesion molecules

on the surface of the neutrophils. This effect may in turn modify the manner in which neutrophils migrate into the muscle tissue. Neutrophil infiltration increased up to 24 h post-exercise, and this increase coincided with the highest degree of muscle soreness and a secondary decrease in eccentric torque (31, 64). These findings suggest firstly that delayed-onset muscle soreness could be associated with an acute inflammatory response, and secondly that inflammatory events may contribute to a decrease in muscle force production after eccentric exercise.

Summary

Eccentric exercise generates a local pro-inflammatory response, but the systemic response is tightly regulated by anti-inflammatory cytokines. Few studies have systematically compared inflammatory responses to different types of eccentric exercise. Circulating leukocyte counts and systemic cytokine concentrations appear to increase to a greater extent after eccentric exercise involving a large muscle mass (e.g., downhill running, eccentric cycling) than after eccentric exercise using isolated muscle groups (e.g., elbow flexors, stepping exercise). However, it is uncertain whether these responses reflect a greater degree of muscle damage in larger muscle groups. Circulating leukocyte numbers and cytokine concentrations increase and return to baseline more rapidly after downhill running and eccentric cycling than after local eccentric exercise. These responses suggest that during downhill running and eccentric cycling, systemic factors (e.g., stress hormones, metabolism, oxidative stress) may be stronger stimuli for the leukocytosis and systemic cytokine production than during local eccentric exercise. Following repeated bouts of eccentric exercise, neutrophil counts are lower, and the expression of leukocyte cell surface receptors is attenuated, whereas there are no major differences in the cytokine responses. These adaptations probably reflect the lower degree of muscle damage that occurs after repeated bouts of eccentric exercise. Aging reduces circulating neutrophil counts, leukocyte infiltration and cytokine mRNA expression in skeletal muscle and plasma IL-6 responses after eccentric exercise. Therefore, aging seems to be associated with an impaired inflammatory response to eccentric exercise. This impairment may be mediated by currently unknown inhibitory factors within skeletal muscle that increase with age. Leukocyte infiltration into skeletal muscle after eccentric exercise seems to be greater in females versus males, at least in humans. However, the reasons for this gender difference are unknown at present. Finally, the small amount of data available indicates that anti-inflammatory drugs may increase circulating neutrophil counts, whereas they do not affect leukocyte infiltration into skeletal muscle after eccentric exercise.

In terms of directions for future research, the following ideas seem worthy of further investigation: (1) a systematic comparison of inflammatory responses to different types of eccentric exercise; (2) an investigation into whether cytokines respond to repeat bouts of other types of eccentric exercise, such as downhill running; (3) identification of the factors within skeletal muscle that inhibit inflammatory responses to exercise-induced muscle damage in elderly individuals; (4) the factors contributing to gender differences with respect to leukocyte infiltration into skeletal muscle after eccentric exercise in humans, and (5) the effects of anti-inflammatory drugs on cytokine responses.

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