The Effect of Glycogen Depletion on Responses of Interleukin-1β, Interleukin-6, and Interleukin-10 to Maximal Exercise

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ABSTRACT

KYLE LEPPERT: The Effect of Glycogen Depletion on Responses of Interleukin-1β, Interleukin-6, and Interleukin-10 to Maximal Exercise
(Under the direction of Robert G. McMurray, Ph.D.)

The purpose of this study was to investigate the influence of glycogen stores on the maximal responses of IL-1β, IL-6, and IL-10 to a discontinuous, progressive cycle ergometry test. Eight trained cyclists completed two progressive maximal cycle ergometry tests (5 min stages with 5 min recovery), one normally fed and one following glycogen-depletion (2-h at 60-65% VO_{2max}). Percent changes in circulating IL-1β, IL-6, and IL-10 were measured during exercise and recovery and peak changes were compared. IL-6 showed a trend towards significant interaction effect of exercise time and trial condition ($p = 0.054$) in both trials, but there were no significant differences among percent changes in IL-1β or IL-10. Glycogen-reduction appeared to cause all cytokines to peak earlier, but the trend was not significant.

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CHAPTER I

BASIS OF STUDY

Tissue damage may be caused by external battery, such as burns or contusions, or internal disruptions such as bacterial or viral infections. When damage occurs in tissue, there is a general response to protect and repair the injured area. This response is known as inflammation. Inflammation is commonly marked by local swelling, redness, heat, pain, and impaired function. Tissue damage can also develop in skeletal muscles during the stress of exercise. The inflammatory response to exercise can be similar to that caused by other forms of tissue damage (Smith & Miles, 2000).

This inflammatory response is very complex, with numerous mechanisms controlling and amplifying its own function. Cytokines are one set of molecules involved in the control of inflammation. When exercise persists for a sufficient duration and intensity, cytokines are synthesized by monocytes and macrophages near active muscle fibers. Cytokines coordinate signaling of other immune cells both locally and systemically. Cytokines are classified into one of several types, including interleukins (IL), tumor necrosis factors (TNF), interferon and growth factors (Smith & Miles, 2000).

The inflammation that develops in damaged tissue is the product of both pro- and anti- inflammatory signaling cytokines. Initially, the inflammation escalates as greater numbers of pro-inflammatory cytokines accumulate in the tissue. These cytokines coordinate the protection and repair of the injured area. As tissue begins to return to its healthy state, anti-inflammatory cytokines are released to downregulate inflammation.

Interleukin-6 (IL-6) and interleukin-1 beta (IL-1β) are two proinflammatory cytokines that most immediately trigger local proinflammatory events. Both of these cytokines are produced by monocytes and macrophages during strenuous exercise and beyond the end of the exercise bout (Smith & Miles, 2000). Anti-inflammatory cytokines limit, and eventually end, the inflammatory response; one anti-inflammatory cytokine is interleukin-10. Interleukin-10 (IL-10) suppresses monocytes' and macrophages' production of proinflammatory cytokines (Smith & Miles, 2000). While the plasma concentration changes in most cytokines across exercise bouts of many durations and intensities have been examined, there has been little evidence gathered on the rate of change within exercise.

In addition to their local control of inflammation, cytokines may exhibit endocrine influence. Interleukin-6 and interleukin-1 beta stimulate the hypothalamic-pituitary axis to produce adrenocorticotropic hormone (ACTH) and directly stimulate the adrenal cortex – both leading to the release of corticosteroids (Smith & Miles, 2000). These endocrine signals lead to effects on substrate selection during exercise and recovery. These signals favor increases in fat and protein metabolism, as well as glucose transport and glycogen synthesis (Pedersen, 2007).

The endocrine signal to make more fuel available is also stimulated by the demand to maintain blood glucose as glycogen stores become depleted. Maintenance of blood glucose to feed active muscles becomes a serious concern during intense exercise. However, the full set of mediators between decreased blood glucose and hypothalamic-pituitary activation is not known with certainty. Catecholamines have been shown to increase with exercise intensity and duration, and to be further increased during exercise when muscular glycogen is reduced ahead of activity (McAnulty et al., 2007). Since proinflammatory cytokines are

strong stimulators of the hypothalamic-pituitary axis – which is capable of triggering glucose-sparing actions – exercise beyond the point of glycogen depletion might stimulate greater release of pro-inflammatory cytokines and inhibit the release of anti-inflammatory cytokines. This heightened response would appear as higher elevation in pro-inflammatory cytokines such as IL-1β and IL-6, along with suppressed anti-inflammatory cytokine response, including IL-10. This exacerbated response could also cause inflammatory imbalance to persist longer into recovery. Thus far, there has been a lack of strong evidence to confirm or refute this glycogen-dependent alteration of inflammatory response.

Significance

The development and dissipation of inflammation are the result of changes in the relative proportions of free pro- and anti- inflammatory cytokines in circulation. This study was done in an effort to add to the base of knowledge in developing a model of the change in the balance between pro- and anti- inflammatory signals caused by maximal exercise. To date, the most comprehensive description of this response within an exercise bout has been theoretical (Febbraio & Pedersen, 2002). In that theoretical description, the concentration changes during exercise were assumed based on pre- and post- exercise measurements. In the current study, a measurement of cytokines prior to terminating exercise was made in order to better understand how the rate of accumulation of cytokines actually changes during exercise.

Most studies of inflammatory responses to exercise have chosen to investigate either pro- or anti- inflammatory cytokines. The action of either class of cytokines, however, may depend upon the relative plasma concentrations of both (Cox et al., 2007). This study investigated the responses of three cytokines, including one of proven anti-inflammatory

action (IL-10) and two of proven proinflammatory action (IL-1β and IL-6). By doing so, the relative concentrations of competing cytokines could be traced, showing which signal's effects are more dominant.

The perturbations of this inflammatory balance were investigated in two different conditions of glycogen storage: normally fed and rested versus low pre-exercise glycogen storage. With the increased risk of illness such as upper respiratory tract infection associated with inflammatory response dysregulation, nutritional factors that may affect the severity of inflammatory response to exercise could become an important consideration for athletes (Cox et al., 2007).

Purpose of Study

This study evaluated the changes in plasma concentrations of IL-1β, IL-6, and IL-10 in response to an acute bout of maximal exercise. This study also explored the effect of exercise-induced glycogen depletion on the responses of IL-1β, IL-6 and IL-10 to the same acute bout of maximal exercise. This effect was analyzed by comparing two bouts of progressive, maximal cycling; one with high glycogen supply (Control Trial) and one with glycogen notably depleted by a previous bout of intense exercise (Depletion Trial).

Research Hypotheses

1. Plasma concentrations of IL-1β and IL-6 will peak immediately after exercise in the Control Trial. Interleukin-10 response will peak during recovery in the Control Trial.
2. Plasma concentrations of IL-1β and IL-6 will peak immediately after exercise in the Depletion Trial. Interleukin-10 response will peak during recovery in the Depletion Trial.

3. The peak changes in plasma concentrations of IL-1β, IL-6, and IL-10 will be greater during the Depletion Trial compared to the Control Trial.
4. The time to peak plasma concentrations of IL-1β, IL-6, and IL-10 will differ between Control and Depletion trials.

Assumptions

1. The progressive exercise test was done to exhaustion.
2. Glycogen depletion protocol caused similar depletion in all participants.
3. Participants were infection-free for 3 weeks preceding participation.
4. Diet of the participants remained constant between trials.

Delimitations

1. Participants were healthy individuals with experience in cycling.
2. Participants owned their own cycling shoes and seat which were used for their testing.
3. Participants were highly trained – cycling/running 3-5 days per week, more than 60 minutes per day for 60 days prior to testing.
4. Participants were tested after ≥4 h fast and 24 h without exercise prior to the Control Trial and 12 h prior to the Depletion Trial.

Limitations

1. Results can only be generalized to healthy individuals experienced in cycling.
2. Results can only be generalized to lower body exercise modes.
3. Depletion trial may not have been sufficient to totally deplete glycogen in highly trained participants.
4. Diet of participants was not controlled for 24 hours prior to testing.

CHAPTER II

REVIEW OF LITERATURE

When injury occurs in tissue, a series of reactions occurs in local structures. The purposes of these reactions are to stave off further tissue damage and to instigate actions that repair the tissue to an extent that it can continue normal function. The encompassing term for these reactions and repair is inflammation. The earliest stages of inflammation are known as the acute phase response (APR). The events of the APR are predetermined and consistent for all scenarios of inflammation. The APR's main result is the release of mediator proteins capable of triggering a more extensive response throughout the organism (Baumann & Gauldie, 1994).

The acute phase response described by Baumann and Gauldie was first connected to exercise in a publication by Cannon and Kluger in 1983. In their study, the researchers presented that proteins were released from monocytes and macrophages in the acute phase response which appeared to trigger an increase in core temperature (Cannon & Kluger, 1983). These proteins are now understood to be pro-inflammatory cytokines, including certain interleukins. These interleukins are deemed pro-inflammatory because they exacerbate the development of inflammation and processes associated with that development.

In exercise, the APR commonly occurs as the direct result of macrophage or blood monocyte activation near the skeletal muscle tissue (Smith & Miles, 2000). These cells, when activated, release the mediators of inflammatory control – primarily cytokines. The first responders appear to be the pro-inflammatory cytokines, including interleukin-1 (IL-1) and

tumor necrosis factor-alpha (TNF-α). They act first locally and then spreading throughout the organism through blood delivery. Shortly after these cytokines appear as the first wave, a second wave of pro-inflammatory cytokines arrives at the damage site, including interleukin-6 (IL-6). The second wave is recognized as the force behind the continuation toward the full cytokine response characteristic of the APR (Baumann & Gauldie, 1994).

These three types of pro-inflammatory cytokines, IL-1, IL-6, and TNF-α, are known to possess two sets of effects. One set of effects target the hypothalamus, altering the temperature set-point and triggering the febrile response. The second set of alterations caused by these cytokines stimulates the adrenal pituitary axis to produce and release adrenocorticotropic hormone (ACTH). Adrenocorticotropic hormone then triggers production and release of cortisol. While cortisol does ultimately provide negative-feedback to inhibit further release of IL-1 and IL-6, cortisol's more immediate impact is at the liver, increasing hepatic glucose production (Baumann & Gauldie, 1994; Brooks et al., 1996). The connection between cytokines and glucoregulatory function in the adrenal pituitary axis is of particular interest in this investigation.

In addition to the cytokines involved in the escalation of inflammatory development, there are also those which act to limit and end the pro-inflammatory processes. These cytokines are termed anti-inflammatory and include interleukin-10 (IL-10). Interleukin-10 is released from monocytes, as well as from B cells and Type 2 T lymphocytes. Interleukin-10 is capable of inhibiting production of pro-inflammatory proteins. Steensberg et al. (2003) investigated the hypothesis that increased plasma IL-6 is the trigger of the anti-inflammatory response phase that includes IL-10. They analyzed the responses of several cytokines in healthy resting individuals caused by 3 hours of IL-6 injection of levels corresponding to the

response to strenuous exercise. Compared to saline placebo, plasma IL-10 concentrations were significantly higher within 1 hour of the beginning infusion and remained significantly elevated for the entire 3 hours following infusion. The accumulation of IL-6 from the acute phase response becomes the trigger for the increased release of IL-10, which in turn inhibits further release of IL-6 and other pro-inflammatory proteins (Gleeson, 2007).

Exercise Interleukin Responses

Developing a comprehensive model of the APR in exercise has not been a concise task since many factors contribute to how one's body responds to exercise. The responses of interleukins to various modes, durations, and intensities of exercise have been investigated, though few have studied more than one or two cytokines concurrently and fewer still have examined progressive maximal exercise responses. This section summarizes the exercise responses of IL-1β, IL-6, and IL-10 in healthy, variably trained subjects in varying exercise conditions demonstrated in previous studies. The key element of response for consideration here is timing; in terms of when a significant change occurs, when a peak is reached, and for how long the concentration remains significantly changed.

IL-1β

In a study to investigate IL-1 exercise response, Cannon et al. (1986) had healthy volunteers cycle for 1 hour at 60% VO_2max. Blood samples were taken immediately before and after exercise, and 3 hours post-exercise. These researchers did not analyze the blood directly for IL-1 concentration in plasma, but rather measured the samples' IL-1 activity augmenting lymphocyte proliferation. There was no significant change in IL-1 activity immediately post-exercise, but there was a significant IL-1 activity increase by 3-hours post-

exercise. Unfortunately, this does not show whether the amount of IL-1 in circulation changed.

Between 1986 and 1989, research showed that two distinct isoforms of IL-1 exist; these isoforms were labeled alpha and beta. Cannon et al. (1989) investigated the IL-1β specific response to a bout of eccentric exercise (45 minutes of downhill treadmill running at 70% VO_2max). Biopsies were taken from the vastus lateralis before exercise, and 45 minutes and 5 days after exercise. The biopsies at both post-exercise time points showed unquantified increase in the region's staining concentrations for IL-1β (Cannon, 1989). Importantly, this study demonstrated that the beta isoform of IL-1 was exercise-responsive, and that this response may not appear immediately post-exercise, but it may persist for several hours once it initiates. The same methods were repeated by Fielding et al. (1993), and again a significant increase in IL-1β was found by the 45-minute post-exercise time point. In addition, the IL-1β staining intensity was even greater by the 5-day post-exercise time point indicating an even greater duration of elevation.

Drenth et al. (1995) examined the response of interleukins in well-trained males in a bout of greater duration, 6-hour running. Similar to Cannon et al. (1986), Drenth et al. reported no significant difference in IL-1β between blood samples taken immediately post-exercise and those taken at rest the day before. No data were collected however to judge whether a change occurred in recovery.

Ostrowski et al. (1999) completed another study of cytokine response to long duration running, measuring the changes in plasma cytokines caused by a marathon. Ten males, with VO_2max values between 53 and 71 mL/kg/min, completed the marathon. Blood samples from the participants were taken 1 week prior, immediately after, and every 30 minutes for 4

hours after the race. The average plasma concentration of IL-1β in these men was elevated from rest immediately post-exercise, and continued to rise until peaking within the first hour of recovery. The mean peak concentration was more than double the average at rest.

Overall, IL-1β concentrations were elevated from resting levels by exercise in these studies. When exercise duration was less than an hour, the increase in IL-1β appeared during recovery. But, an increase was found immediately post-exercise in longer duration work (several hours), although one study (Drenth et al., 1995) failed to find a significant change in similar exercise.

IL-6

The 1995 study by Drenth et al. on marathon runners also analyzed the response of IL-6. While IL-1β in these participants did not change significantly, plasma IL-6 was significantly elevated immediately post-race. No samples were taken during recovery.

The study by Ostrowski et al. (1999) detailed in the previous section, also analyzed IL-6 response at the same intervals as IL-1β. And, like Drenth et al., Ostrowski et al. found the highest plasma IL-6 levels immediately post-exercise, approximately 128-fold over pre-race. Unlike Drenth et al., this group did take samples during recovery (every 30 minutes for 4 hours) and found that IL-6 declined from the immediate post-exercise peak but remained above resting values through 4 hours of recovery.

Steensberg et al. (2000) had participants complete 5 hours of single-leg exercise at 40% W_{max}. Blood draws taken near the working muscle every 60 minutes during the exercise showed a steady rise in IL-6 throughout, peaking nearly 19-fold over rest immediately post-exercise. Steensberg et al. (2002) used knee-extensor exercise once again, with both legs exercising concomitantly and for only 3 hours but at 55% W_{max}. Again, a significant 100-fold

increase in plasma IL-6 was found after 120 minutes of exercise. In both of these studies led by Steensberg, no blood sampling was done post-exercise; thus we do not know how long IL-6 remained elevated into recovery.

These studies, like most others with various exercise protocols, indicated an increase in IL-6 with exercise that was apparent immediately post-exercise. Those that continued to track IL-6 into recovery, Drenth et al. (1995) and Ostrowski et al. (1999) agreed that IL-6 peaked immediately post-exercise, but remained above resting levels for at least 4 hours.

IL-10

What may have been most notable about the study of Ostrowski et al. on marathon runners (1999) is that they analyzed the response, not only of pro-inflammatory cytokines, but also of anti-inflammatory cytokine IL-10. In these runners, mean IL-10 concentration peaked immediately post-race, approximately 27-fold over mean resting concentration. From the post-race time point onward, IL-10 fell toward resting levels throughout the 4-hour recovery period, but remained significantly above rest at all recovery points (Ostrowski, 1999).

Peake et al. (2005) had trained runners complete three different exercise protocols on separate days: treadmill running flat at 60% VO_2max for 60 minutes (Moderate Intensity), treadmill running flat at 85% VO_2max for 60 minutes (High Intensity), and downhill treadmill running at 60% VO_2max for 45 minutes (High Damage). Only the High Intensity trial significantly increased IL-10 over resting levels, where IL-10 was higher both immediately and 1-hour post-exercise. Fitting with its anti-inflammatory role, IL-10 was higher at 1-hour post-exercise (7-fold) than immediately after exercise (6.3-fold), but no blood samples were taken beyond 1 hour to see if this increase continued further. In addition

to exemplifying this anti-inflammatory role, this study showed greater IL-10 response with greater intensity and not with greater muscle damage.

Zaldivar et al. (2006) likewise found an IL-10 response to intense exercise when healthy men performed 30 minutes of heavy cycling above lactate threshold. Mean IL-10 plasma concentration 1 hour into recovery was significantly elevated over rest. Blood was also sampled immediately post-exercise; however, the increased IL-10 mean concentration found immediately post-exercise was not statistically significant.

Possible Causes and Roles of Cytokine Response to Exercise

Considering Muscle Damage

Knowing that these three interleukins are all responsive to exercise in varying degrees, we can begin to consider what stimulus within exercise causes the APR. Since inflammation is known to be a response to tissue damage (Baumann & Gauldie, 1994), the damage of muscle tissue due to forces in exercise has been given strong consideration as a stimulus for cytokine response. Creatine kinase is an enzyme known to leak out of damaged muscle tissue (Brooks et al., 1996). Bruunsgaard et al. (1997) compared creatine kinase release and IL-6 release between concentric and eccentric exercises. Creatine kinase rose, as expected, nearly 40-fold in the 4 days following eccentric exercise, but did not rise significantly following concentric exercise. In the eccentric condition, IL-6 levels increased five-fold and significantly correlated with creatine kinase in recovery; no IL-6 change was found in the concentric condition. Since IL-6 is intended to aid a process of tissue repair, it is fitting that it should respond to a signal of tissue damage such as creatine kinase. This study could not, however, establish a causal relationship between muscle damage and pro-inflammatory cytokine production.

Cannon et al. (1986) proposed that the increased IL-1 activity they saw following exercise was not due to muscle damage since the IL-1 activity increase did not correlate significantly with creatine kinase activity ($r = 0.55$). This pro-inflammatory response also occurred without using an eccentric exercise mode. This led the authors to suggest that pro-inflammatory response in exercise was not related to clean-up of local debris. Also, when blood samples from resting participants were treated with epinephrine, IL-1 release from monocytes was increased. But this effect was suppressed by the addition of hydrocortisone, which they said would reduce the likelihood that epinephrine was a physiological cause for IL-1 secretion in exercise (Cannon, 1986).

In the Fielding et al. (1993) study, the increase in IL-1β was significantly correlated with the increase of plasma creatine kinase. While Cannon et al. (1986) did not link muscle damage to IL-1β response, Fielding et al. (1993) did lend support to the connection. However, again this may not be a causal relationship in which muscle damage and the resultant creatine kinase trigger IL-1β release. Instead, it may be simply that the intensity of work in these bouts causes IL-1β release, as well as muscle tissue damage.

Possible Influence of Glycogen Status

Overall, the research detailed previously in this chapter specific to each cytokine's response demonstrates that, while results do vary with differences in the exercise administered and the conditioning levels of the participants, the results support the existence of an acute phase response to exercise related by some means to the intensity/duration of exercise. If the physical work of exercise, in and of itself, was the cause of cytokine response, the response would most likely recede immediately following exercise. But as this is not what the research has shown (Fielding et al., 1993; Steensberg et al., 2000, 2002), some

result of intensity/duration manipulation must linger into recovery. As mentioned in the opening of this chapter, IL-1β and IL-6 are known to stimulate the adrenal pituitary axis to ultimately cause cortisol release which increases hepatic glucose production. Since the rate of use of stored glycogen increases with intensity of exercise (Gollnick et al., 1973) and total use increases with duration, the results of Fielding et al. (1993) may lend support to a possible role of pro-inflammatory cytokines in responding to glycogen disappearance through their stimulus in the adrenal pituitary axis. Both the investigations lead by Cannon (1986, 1989) as well as that by Fielding et al. (1993) found an IL-1β response that appeared some time after the cessation of exercise unrelated to muscle damage. This could indicate that the signal for pro-inflammatory cytokine release is related to some element of recovery, such as restoration of muscle glycogen storage. When the results of the two studies led by Steensberg (2000, 2002) are compared, they lend additional support to an intensity/glycogen depletion effect on pro-inflammatory cytokine response as well: when more muscle mass was exercised at higher intensity (greater glycogen usage rate) despite shorter duration (one leg at 40% W_{max} for 5 h versus both legs at 55% W_{max} for 3 h), the IL-6 response was approximately 5 times greater.

Ostrowski et al. finding an IL-10 response initiation before exercise terminated suggested that anti-inflammatory cytokines begin to control the extent of inflammation before it peaks, just as Steensberg et al. (2003) showed to occur following IL-6 infusion. Still, if these pro-inflammatory cytokines influence hepatic glucose production and IL-10 inhibits the release of IL-1β and IL-6, then IL-10 should fully end pro-inflammatory actions only once glycogen has been sufficiently restored. The increases in IL-10 found by Peake et al. (2005) and Ostrowski et al. (1999) did begin within exercise but persisted into recovery,

significantly elevated for at least 4 hours post-exercise in the Peake et al. study. The more prolonged elevation noted by Peake et al. may suggest that, where more glycogen depletion requires more pro-inflammatory response, the greater pro-inflammatory response also demands greater anti-inflammatory response to return to normal function. More importantly, this may also suggest that anti-inflammatory inhibition may not rise enough to overpower pro-inflammatory signaling completely until sufficient time has been given to hepatic glucose production for glycogen replenishment. The following section will examine results from studies in which glycogen availability was manipulated either by supplementation or depletion prior to and/or during exercise.

Effects of Carbohydrate Manipulation on Interleukin Response

Studies investigating the possibility of a link between muscle glycogen status and exercise-induced APR have typically tried to change muscle glycogen before or during exercise and recovery by supplementing carbohydrates to boost glycogen storage. The hypothesis generally has been that, if falling glycogen availability in exercise causes the APR, this supplementation should dampen or eliminate cytokine response (Davison & Gleeson, 2005; Nehlsen-Cannarella et al., 1997).

Pre-exercise Carbohydrate Supplementation

Davison and Gleeson (2005) examined the effect of carbohydrate intake before and during exercise on immune and endocrine responses to 2.5 hours of cycling at 60% VO_2max. In both conditions (carbohydrate-supplemented and placebo), plasma glucose was significantly reduced by the exercise while ACTH, cortisol and IL-6 concentrations all significantly increased. When participants were supplemented with carbohydrate before and during the trial, plasma glucose loss was significantly reduced, as were the rises in ACTH

and cortisol. The authors noted a strong trend for IL-6 levels to be lower when carbohydrate was given (~40% lower) but attributed a lack of statistical significance to several large inter-individual variations.

Nehlsen-Cannarella et al. (1997) also discovered a difference in the exercise response of cytokines when carbohydrate supplementation was given immediately before and during 2.5 hours of running (~75-80% VO_2max). Plasma levels of IL-6 increased from rest during exercise in both groups and were in decline by 1.5 hours post-exercise in both groups, but were still significantly above rest. The supplemented group showed significantly higher plasma glucose and lower cortisol concentrations post-exercise. In these participants who received carbohydrate supplementation, total plasma IL-6 was significantly lower immediately and 1.5 hours post-exercise compared to placebo. Cortisol is known to provide negative feedback to inhibit IL-6 release (Baumann & Gauldie, 1994). The results of Nehlsen-Cannarella et al. (1997) may indicate that less production of cortisol to stimulate gluconeogenesis is the result of less IL-6 release. No significant correlations were found between IL-6 change and either cortisol or glucose change.

Supplemental Carbohydrate During Exercise

Febbraio et al. (2003) had participants cycle for 2 hours, with either carbohydrate supplement or placebo given throughout the bout, and compared biopsies from the vastus lateralis taken prior to and immediately after exercise to study the effect of carbohydrate supplementation on the muscular release of IL-6. When 250 mL of 6.4% carbohydrate drink was given during exercise, post-exercise arterial plasma IL-6 was significantly lower, as was IL-6 release measured at the working muscle. When carbohydrate was given, glucose uptake was higher and free fatty acid uptake lower. The finding that more IL-6 was released from

the muscle, and elsewhere, in control subjects (where supplement glucose was absent) points toward a cytokine role in demanding alternative fuel when less glucose is readily available.

When Nieman et al. (2003) gave participants carbohydrate during 3 hours of treadmill running at ~70% VO_2max, post-exercise plasma levels of both IL-6 and IL-10 were significantly lower compared to when the participants were given a placebo drink. These changes occurred despite no significant difference in muscle glycogen content in the vastus lateralis post-exercise compared to pre-exercise. Nieman et al. suggested that this could indicate that blood glucose concentration may control cytokine response, via catecholamine release, while muscle glycogen content is of little or no effect.

The common theme of these articles is that blood glucose concentration can be a major determinant of cytokine response. However, since these studies typically either did not induce or did not assess changes in muscle glycogen content before or during the exercise, the availability of this stored energy can not be judged as a factor (or non-factor) in cytokine responses. Also, by providing a glucose source so close to, and/or during, the exercise, these studies speak more to the effect of altered plasma glucose concentration than to that of stored fuel usage overall.

Pre-exercise Carbohydrate Reduction

Keller et al. (2001) studied the effect of lowered pre-exercise muscle glycogen levels on IL-6 response during 180 minutes of double-legged knee extension. In comparison to normal participants, participants whose muscle glycogen was previously reduced by ~40% (using exercise and fasting) had significantly greater increases in IL-6 release. Blood samples taken every 30 minutes within exercise revealed a significant difference between normal and depleted trials in IL-6 levels by 120 minutes – nearly 2-fold – which remained significant

through the end of exercise. No post-exercise measurements were taken (Keller et al., 2001). Since the pro-inflammatory response was increased when glycogen depletion was exacerbated beyond that proportionate to the intensity of exercise, glycogen availability becomes a strong candidate as the link between exercise intensity and cytokine response.

When MacDonald et al. (2003) depleted muscle glycogen in healthy men prior to 60 minutes of cycling at 70% VO_2max, IL-6 release from the working muscle was significant within 10 minutes, but no significant IL-6 release from the muscle occurred at all in the same bout without prior depletion. Despite differences in local muscular release of IL-6, the arterial plasma IL-6 content rose similarly over the bout in both conditions. The findings of MacDonald et al. suggest that while muscle glycogen status may impact IL-6 release near the muscle, the systemic rise of all-source IL-6 in the blood may not be greatly impacted by depletion.

The studies by Keller et al. and MacDonald et al. are possibly the only studies to date to directly investigate the effect of initially low muscle glycogen levels on cytokine response, and the results of these studies disagree on the ability of depleted glycogen storage to impact systemic circulating cytokine concentrations. Other studies, such as Steensberg et al. (2001), compared local release of cytokines between one glycogen-depleted and one glycogen-full leg within one subject and found an increase in local cytokine release at the depleted muscle but no change in circulating plasma cytokine concentrations. But these methods make it difficult to consider the impact of more extensive depletion on overall plasma circulation of cytokines.

Summary

The cytokines involved in the APR in exercise certainly can act within the adrenal pituitary axis to stimulate release of ACTH (Baumann & Gauldie, 1994). In various modes and intensities of exercise, the responses of IL-1β, IL-6, and IL-10 have been shown to vary in degree: including a delayed IL-1β increase following an hour of moderate cycling (Cannon et al., 1986) but no response to 6 hours running, increases in IL-6 of 4-fold after a 6-hour run and 128-fold after a marathon (Drenth et al., 1995; Ostrowski et al., 1999), and immediate IL-10 increases in both marathon running and 30 minutes heavy cycling (Ostrowski et al., 1999; Zaldivar et al., 2006). The timing of the onsets, peaks and endings for these responses has also varied. In some cases, the onset was noted before exercise ended (Steensberg et al., 2000), and in others the onset was delayed into recovery (Cannon et al., 1986). Peak concentrations of the pro-inflammatory IL-6 appeared immediately after exercise (Drenth et al., 1995; IL-6 in Ostrowski et al., 1999) but, the peak values for IL-1β were often not seen until later in recovery (Ostrowski et al., 1999; Fielding et al., 2003). Anti-inflammatory IL-10 typically peaked sometime into recovery and remained elevated up to 4 hours post-exercise (Ostrowski et al., 1999; Peake et al., 2005; Zaldivar et al., 2006).

When carbohydrate was supplemented before or during exercise, plasma IL-6 was consistently reduced (Davison & Gleeson, 2005; Nehlsen-Cannarella et al., 1997) but no causal relation could be made between cytokine response and glycogen status. When glycogen was significantly reduced prior to exercise, circulating IL-6 increased in one case (Keller et al., 2001) but not in another (MacDonald et al., 2003) despite increased muscular release of IL-6 in both. No such investigation of depletion or supplementation effects was available for IL-1β or IL-10.

In this study, we will investigate the impact of glycogen depletion (caused by previous exercise) on systemic circulation of both pro-inflammatory and anti-inflammatory cytokines. By examining both types of cytokines, we will also be able to assess the overall inflammatory balance between the two simultaneously, an element lacking in a large majority of the literature reviewed in this section. We will give special focus to the timing of increases and decreases of plasma concentrations of pro-inflammatory and anti-inflammatory cytokines, in an effort to more precisely identify the times of onset, peak, and return to resting concentration.

CHAPTER III

METHODOLOGY

The purposes of this study were to evaluate the changes in circulating levels of IL-1β, IL-6, and IL-10 in response to an acute bout of maximal exercise and to explore the effect of exercise-induced glycogen depletion on the responses of IL-1β, IL-6 and IL-10 to the same acute bout of maximal exercise. This effect was analyzed by comparing two bouts of progressive, maximal cycling; one with full glycogen supply (CTRL) and one with glycogen depleted approximately 40-60% (DEPL) by a previous bout of intense exercise. This chapter will explain the methodology used in this investigation – including criteria for subject inclusion, protocols for exercise trials, and data and statistical analyses.

Participants

Eight athletes concurrently training for competitive cycling (ages 18-45 years) completed the study protocol. The criteria for inclusion were as follows:

- Estimated VO_{2max} > 45 mL/kg/min
- Participant had been cycling 3-5 days per week, approximately 1 hour per day for 60 days prior to testing and had previously completed a cycling session of 2 hours duration or more
- Participant owned his/her own cycling shoes, pedal clips, and seat – which were used for maximal testing and glycogen depleting sessions

All possible risks and the purpose of the protocol to be administered were explained and any questions answered to the satisfaction of the participant. All participants completed a

medical history reviewed by the researchers prior to inclusion in the study. Participants subsequently signed their informed consent. Participants underwent a physical examination including a resting electrocardiogram; no abnormalities contraindicative of exercise were found.

Participants reported to the Applied Physiology Laboratory for all assessment and testing sessions. Participants' heights were measured at the start of the first visit; body masses were recorded before all sessions. Each participant's height was determined using a portable stadiometer (Perspectives Enterprises, Portage, MI). Body mass was measured using a mechanical scale (Detecto, Webb City, MO). The participants each brought the saddle, pedals, and cycling shoes they use for road cycling. Their personal saddles and pedals were affixed to a mechanically braked cycle ergometer (Monark, Varberg, Sweden). Heart rate (HR) was monitored during testing sessions by a Polar telemetry system (Polar Electro Inc., Lake Success, NY) fitted on the participant upon arrival to each session. All participants were requested to wear either a cycling or triathlon jersey during testing sessions.

Dietary Guidelines

Participants were requested to abstain from eating within 4 hours prior to the 2-hour glycogen depleting exercise session. After completion of the glycogen depleting session, they were requested to abstain from eating until 2 hours after the DEPL Trial session the following morning (which began 8 hours after completion of the glycogen depleting session). Participants were requested to abstain from eating within 4 hours prior to beginning the CTRL Trial session and for 2 hours after completion. Water intake was not restricted.

Exercise Protocols

On the participant's first visit to the laboratory, he/she completed a submaximal test to evaluate cardiovascular fitness following the YMCA cycle ergometer submaximal protocol. All participants completed the five-stage protocol. An estimation of maximal oxygen uptake per kilogram body mass (VO_2max) was then calculated using the equation designed for the protocol (Heyward, 2006). Participants were subsequently assigned randomly to an order of the two maximal cycling sessions (CTRL and DEPL) for a counterbalanced design (n = 4 for each order, 2 male and 2 female).

- CONTROL – One session of maximal exercise preceded by 24 hours without exercise.
- DEPLETION – One session 8 hours after completion of a 2-hour bout of cycling at 60-70% VO_2max designed to cause 40-60% depletion of stored glycogen (Gollnick, 1974).

The maximal cycling sessions in both trials consisted of a discontinuous maximal cycling protocol described here. After selecting a seat height and securing their feet to the pedals, participants completed three minutes of warm-up without resistance at 80 revolutions per minute (rpm). This pedal rate was used for the entire test. After the warm-up, the test began with five minutes of cycling at a workload of 240 kilogram-meters per minute (kgm/min). This stage and all subsequent stages completed were followed by a 2-minute collection period. This 2-minute window began with the collection of the blood sample, with the participant sitting still on the ergometer. Thereafter, a maximal voluntary contraction (duration < 10 seconds) of both legs downward against the pedals was completed as part of another study also using the data of these trials. After the contraction, three minutes were

spent cycling without resistance before the workload was increased and the next exercise stage began. The workload was increased by 240 kgm/min for each five-minute exercise stage. This progression continued until the participant was no longer able to maintain at least 70 rpm, desired to stop, or showed physical signs of severe fatigue (ACSM, 2006). Participants reported ratings of perceived exertion (RPE) during each stage according to Borg's original 6-20 RPE scale (Borg, 1970). Following termination and immediate blood draw, the participant was assisted in exiting the ergometer and moved to a chair to rest seated for two hours. The two maximal sessions were separated by 5 to 10 days.

During the maximal exercise bouts, metabolic rate was measured using a Parvo Medics TrueMax 2400 Metabolic System (Parvo Medics, Salt Lake City, UT). The system was calibrated before each trial. Use of the system followed standard operating procedures according to the manufacturer's instructions. Prior to each maximal cycling test, the participant was fitted with the headset holding the ventilation mouthpiece. The mouthpiece was attached to the Parvo Medics metabolic cart. The participant wore a nose clip for the duration of metabolic data collection.

Blood Sampling

In order to measure plasma levels of IL-1β, IL-6 and IL-10; blood samples were taken during the maximal exercise sessions. Samples of ~6 mL were taken via three syringe technique into heparinized vaccutainers. The antecubital area of the participant's dominant arm was sterilized with isopropyl alcohol, and a tourniquet was applied distal to the deltoid muscle of the involved arm. A catheter was then inserted in the antecubital vein of the dominant arm.

Samples were taken at rest, immediately following each stage of the maximal sessions – of these time points in exercise, only the sample taken prior to the second to last stage was assayed for interleukins. Samples were also taken immediately upon termination of the maximal exercise; and at 30, 60, and 120 minutes after termination. Blood samples at rest were taken with the participant seated in a chair or supine; all samples during exercise were taken with the participant remaining on the cycle ergometer and still.

Blood Analysis

Blood Sampling - Samples of whole blood were centrifuged using an Adams MHCT II microhematocrit centrifuge (Becton Dickinson, Franklin Lakes, NJ). Hematocrit (Hct) measurements were measured using the micro-hematocrit method, in duplicate. Hematocrit measures were read using an International Microcapillary Reader (International Equipment Company, Needham Heights, MA). Blood lactate concentration measurements were also taken from whole blood samples for each time point up to 1 hour post-exercise using an AccuTrend lactate analyzer. The remaining blood was cold centrifuged at 3000 rpm for 15 minutes at 3 ^{o}C, and separated plasma was immediately removed and stored in capped polypropylene tubes frozen at -80 ^{o}C until later analysis.

Cytokine Analyses - Plasma concentrations of each cytokine were made from whole blood samples taken 5 minutes prior to exercise; prior to the second to last stage of exercise; immediately upon termination of the last exercise stage; and 30, 60, and 120 minutes post-exercise. Concentrations of interleukins were determined using interleukin-specific Humakine ELISA kits (R&D Systems, Minneapolis, MN). A high sensitivity Humakine kit was selected for the analysis of IL-1β. Assays were completed according to manufacturer's instructions.

Data & Statistical Analysis

The ages and estimated VO_{2max} values of all subjects were summarized by means with standard deviations. The statistical analyses for each hypothesis were as follows:

Hypothesis 1 - Plasma concentrations of IL-1β and IL-6 will peak immediately after exercise in the Control Trial. Interleukin-10 response will peak during recovery in the Control Trial.

The CTRL Trial responses of all three cytokines were graphed in Microsoft Excel. The changes in plasma concentrations of each cytokine from rest to each subsequent time were compared by separate repeated measures analysis of variance (ANOVA). Significant ANOVA results were followed by paired t-tests with Bonferroni adjustment for multiple comparisons to determine which changes were significant.

Hypothesis 2 - Plasma concentrations of IL-1β and IL-6 will peak immediately after exercise in the Depletion Trial. Interleukin-10 response will peak during recovery in the Depletion Trial.

The DEPL Trial responses of all three cytokines were graphed in Microsoft Excel. The changes in plasma concentrations of each cytokine from rest to each subsequent time were compared by separate repeated measures ANOVA. Significant ANOVA results were followed by paired t-tests with Bonferroni adjustment for multiple comparisons to determine which changes were significant.

Hypothesis 3 - The peak changes in plasma concentrations of IL-1β, IL-6, and IL-10 will be greater during the Depletion Trial compared to the Control Trial.

The peak percent change values of each cytokine in the two conditions were compared using paired t-tests.

Hypothesis 4 – The time to peak plasma concentrations of IL-1β, IL-6, and IL-10 will differ between Control and Depletion trials.

Differences between trial conditions in the timing of peak changes in cytokines were assessed by comparing the time difference in the graphical peaks using Microsoft Excel.

Significance was set *a priori* at an α-level of 0.05 for all analyses (adjusted from 0.05 for Bonferroni adjustments). All statistical analyses were completed using SPSS statistical software, version 15.0 (SPSS, Chicago, IL).

CHAPTER IV

RESULTS

The purpose of this study was to investigate the influence of glycogen stores on the maximal exercise responses of IL-1β, IL-6, and IL-10 to a discontinuous, progressive cycle ergometry test. The first section of this chapter describes the physical characteristics of the participants and provides verification that the DEPL trial was completed by all subjects in a glycogen-reduced state. The chapter will thereafter detail the responses of the three cytokines as they pertain to the hypotheses set forth in the Introduction chapter.

Subject Characteristics

Eight participants completed all components of the study; the means and standard deviations, minimums and maximums of selected participant characteristics are provided in Table 1. The sample was even between genders (4 male, 4 female). The predicted VO_{2max} values of the population suggest that participants were highly trained. All participants were training for at least 6 months prior to participation in this study.

Table 1. Physical characteristics of participants

Characteristic	Mean ±std (n=8)	Minimum	Maximum
Body Mass (kg)	68.4±8.1	57.3	80.2
Height (cm)	172.8±10.0	159.0	186.0
Age (years)	28.0±6.4	21.0	38.0
Predicted VO_{2max} (mL/kg/min)	62.3±7.4	47.0	71.7

Glycogen Reduction Effectiveness

The DEPL trial was intended to be completed while participants' stored glycogen supplies were depleted. Glycogen depletion was defined as a significant decrease in respiratory exchange ratio (RER) and blood lactate concentration (La) during maximal exertion. Repeated measures t-tests were completed to compare the mean values of each measure in the two trial conditions for all participants. Significant differences were found between the trial conditions for both RER ($p = 0.002$) and La ($p < 0.001$) despite no significant difference in the number of stages completed between conditions ($p = 0.454$). There were no significant differences between trials in maximum heart rate ($p = 0.239$) or VO_{2peak} ($p = 0.627$).

Table 2. Means (± std) for maximum RER, maximum La, number of stages completed, maximum HR, and VO_{2peak} for the control (CTRL) and glycogen reduced (DEPL) trials (* $p<0.05$ CTRL vs. DEPL).

	Condition	Mean (± std)
Max RER*	CTRL	1.02 (± 0.04)
	DEPL	0.91 (± 0.07)
Maximum Lactate (mmol/L)*	CTRL	11.1 (± 2.4)
	DEPL	6.2 (± 1.8)
Number of Stages Completed	CTRL	7.5 (± 1.6)
	DEPL	6.9 (± 1.5)
Max HR (bpm)	CTRL	181.3 (± 10.8)
	DEPL	174.8 (± 10.2)
VO_{2peak} (mL/kg/min)	CTRL	53.2 (± 8.6)
	DEPL	51.0 (± 9.4)

Cytokine Response Comparisons

The mean resting plasma concentrations and standard deviations of the three cytokines are given in Table 3. Percent changes in plasma concentration were used for all analyses to minimize the influence of individual variation in resting values.

Table 3. Mean (± std) resting plasma concentrations of IL-1β, IL-6, and IL-10 (pg/mL) prior to CTRL and DEPL trials. Resting samples were taken 5 min prior to exercise.

	CTRL Plasma Concentration (± std)	DEPL Plasma Concentration (± std)
IL-1β (pg/mL)	0.53 (± 0.44)	1.03 (± 1.15)
IL-6 (pg/mL)	0.78 (± 0.62)	1.28 (± 0.76)
IL-10 (pg/mL)	27.81 (± 39.02)	15.01 (± 12.33)

The mean percent changes and standard errors of the means (SEM) for each cytokine to the two trial conditions at each selected time point after the start of exercise are depicted in Figures 1-3.

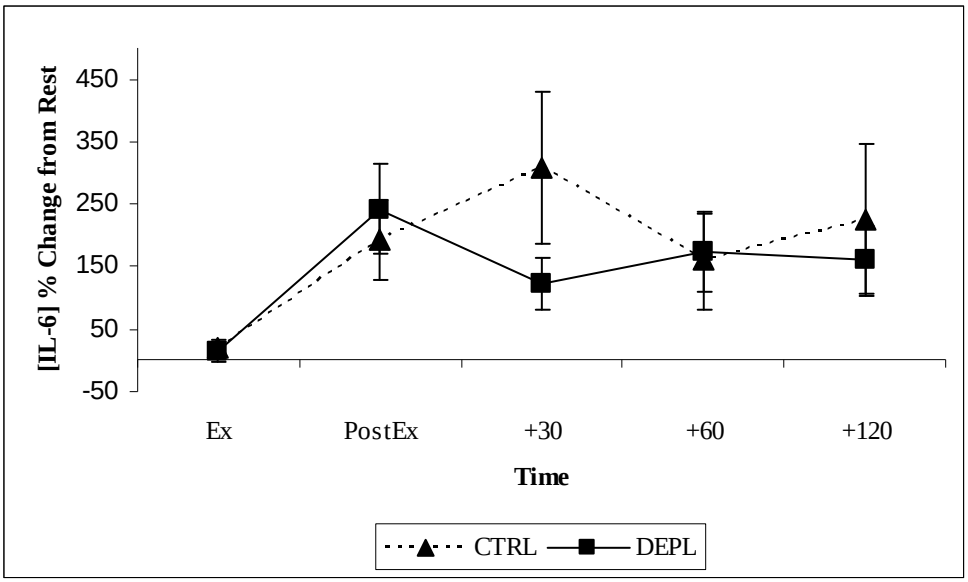

Fig.1 – Percent change (± SEM) in plasma concentration of IL-6 from rest prior to second to last exercise stage (Ex), immediately post-exercise (PostEx), and 30, 60, and 120 minutes post-exercise.

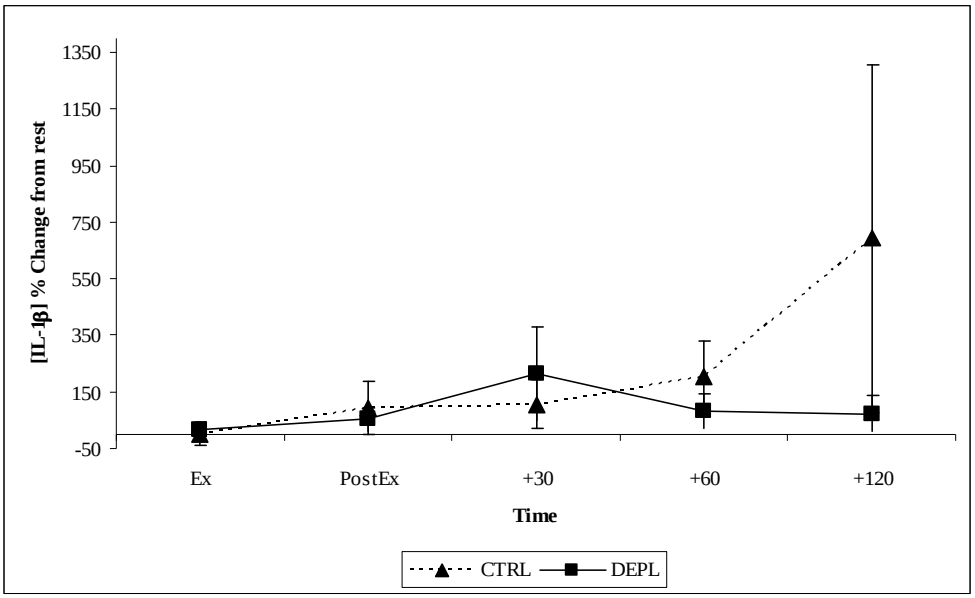

Fig. 2 – Percent change(± SEM) in plasma concentration of IL-1β from rest prior to second to last exercise stage (Ex), immediately post-exercise (PostEx), and 30, 60, and 120 minutes post-exercise.

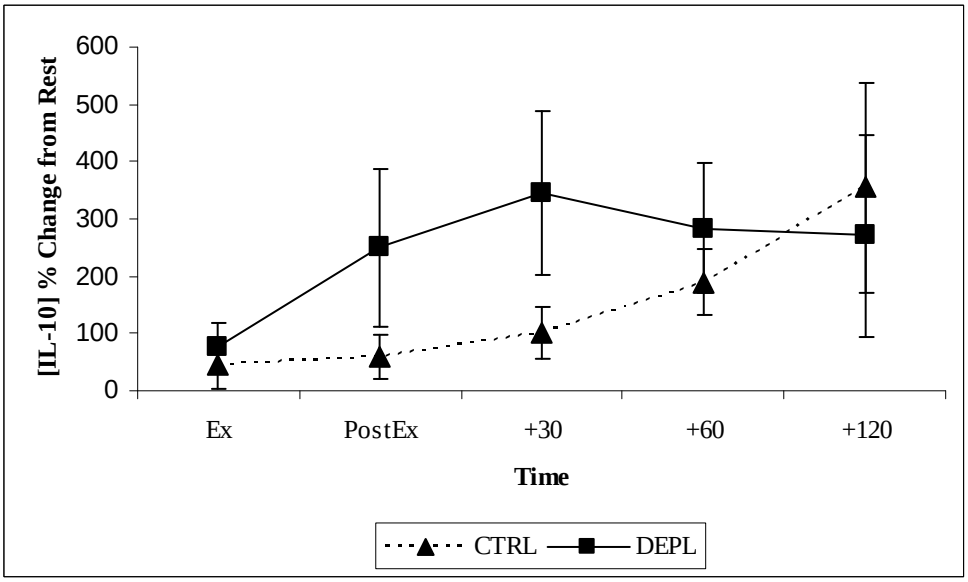

Fig. 3 – Percent change (± SEM) in plasma concentration of IL-10 from rest prior to second to last exercise stage (Ex), immediately post-exercise (PostEx), and 30, 60, and 120 minutes post-exercise.

Hypothesis 1 - Plasma concentrations of IL-1β and IL-6 will peak immediately after exercise in the Control Trial. Interleukin-10 response will peak during recovery in the Control Trial.

The responses of IL-1β, IL-6, and IL-10 to the CTRL trial condition are shown in Figures 1-3. In the CTRL trial, the peak in mean change of IL-6 occurred 30 minutes post-exercise. At that time, IL-6 was elevated 309 ± 122% over rest (Fig.1). This timing in response occurred in five out of eight of the participants. The peak mean change of IL-1β in the CTRL trial occurred 1-2 hours post-exercise in six of the eight participants, at which time it had risen by a mean of approximately 692 ± 613% (Fig. 2). The peak change of IL-10 in the CTRL trial occurred 1-2 hours post-exercise as well, at which time IL-10 was approximately 354 ± 184% over rest on average (Fig. 3); with 7 of the 8 participants responding similarly.

Hypothesis 2 - Plasma concentrations of IL-1β and IL-6 will peak immediately after exercise in the Depletion Trial. Interleukin-10 response will peak during recovery in the Depletion Trial.

The responses of IL-1β, IL-6, and IL-10 to the DEPL trial are shown in Figures 4-6. In this trial, the times of peak change in IL-6 were quite variable and appeared, for the majority of participants, to occur immediately post-exercise, up by approximately 242 ± 73% over rest (Fig. 1). In this trial, the IL-1β responses were also quite variable with the majority of the participants responding with the greatest in IL-1β at about the 30 minutes post-exercise or earlier (Fig. 2). The response of IL-10 was similar to IL-1β, with the greatest changes 30 minutes post-exercise or earlier; an approximate increase of 346 ± 144% (Fig. 3).

Hypothesis 3 - The peak changes in plasma concentrations of IL-1β, IL-6, and IL-10 will be greater during the Depletion Trial compared to the Control Trial.

There were no significant differences between the peak changes of any cytokine between the two trial conditions. Paired samples t-tests of the percent changes at peak were compared between conditions. For IL-6, the CTRL mean peak at 30 minutes post-exercise was compared to the DEPL mean peak immediately post-exercise ($p = 0.676$) (Fig. 1). For IL-1β, the CTRL mean peak at 120 minutes post-exercise was compared to the DEPL mean peak at 30 minutes post-exercise ($p = 0.504$) (Fig. 2). And for IL-10, the CTRL mean peak at 120 minutes post-exercise was compared to the DEPL mean peak at 30 minutes post-exercise ($p = 0.922$) (Fig. 3).

Hypothesis 4 – The time to peak plasma concentrations of IL-1β, IL-6, and IL-10 will differ between Control and Depletion trials.

There were differences in the timing of the peak change when comparing the two trials. The mean IL-6 peak appeared to occur 30 minutes post-exercise in the CTRL trial and immediately post-exercise in the DEPL trial. A repeated measures ANOVA revealed a trend towards interaction in the effects of time and trial condition ($p = 0.054$). Although there were no statistically evident peaks for IL-1β and IL-10, mean peaks in both appeared to occurred 2 hours post-exercise in the CTRL trial and 30 minutes post-exercise in the DEPL trial.

CHAPTER V

DISCUSSION

This study had two intended purposes. The first was to investigate the changes in IL-1β, IL-6, and IL-10 in response to a discontinuous, progressive cycle ergometry test. Evidence exists from previous literature that all three of these cytokines have some exercise responses (Fielding et al., 1993; Ostrowski et al., 1999). The second purpose was to assess the effects of exercise-induced glycogen depletion on the responses of IL-1β, IL-6, and IL-10. The lower RER and LA during the DEPL trials indicated that glycogen stores were significantly reduced (Table 2). In this chapter each of the cytokines will be discussed independently, focusing on the CTRL trial first followed by the DEPL trial and finally a comparison of the two trials.

IL-6 Response

In this study, a significant peak of IL-6 occurred 30 minutes into recovery following the CTRL trial ($p = 0.008$) (Fig.1). In previous studies, IL-6 was shown to increase steadily throughout exercise (Drenth et al., 1995; Ostrowski et al., 1999; Steensberg et al., 2000, 2002), and to decline throughout recovery (Ostrowski et al., 1999). However, all of these previous works involved steady-state exercise protocols, lasting at least 3 hours and only two measured IL-6 at 30 minutes post-exercise and beyond. The exercise protocol used in the present study was of shorter duration and progressing intensity (< 45 min active exercise time) and still resulted in an increase of IL-6 by the end of the exercise bout and a continued increase early in recovery period (Fig.1). One possible explanation for the continued rise of IL-6 in the present study could be that the stimulus responsible for IL-6 release may not have had sufficient time to maximally respond to the exercise duration, especially since the

greatest stimulus may have been in the last few stages when intensity was highest. This is similar to how catecholamines respond to progressive exercise, in comparison to steady-state exercise, as they work to make more fuel available to working muscles. Catecholamines are released more rapidly as intensity increases and continue to appear into early recovery because the demand for fuel delivery to muscles in progressive exercise is greatest in the late stages and can persist into recovery before being fully satisfied (Gollnick et al., 1973; Brooks et al., 1996). An IL-6 rise following the pattern of catecholamine response supports the theory that IL-6 release can be directly caused by catecholamines, as proposed by Keller et al. (2004).

Keller et al. (2001) and MacDonald et al. (2003) were able to find a greater IL-6 response with glycogen reduction. In the current study, no difference in the magnitude of peak changes in IL-6 was found, but glycogen reduction may have caused the peak change in IL-6 to arrive sooner (~30 min earlier than the CTRL trial) as seen in four of the eight subjects (Fig. 1). Since glycogen available in the muscle was already reduced, the need for mobilization of stored glycogen from elsewhere would develop more rapidly. Previous studies suggested that glycogen usage is the link between exercise intensity/duration and cytokine response (Steensberg et al., 2000, 2002). Since glycogen reduction appeared to have an impact on lactate, a posteriori, the maximal lactate values were correlated to the maximal changes in IL-6. This resulted in an $r = 0.797$ ($p = 0.018$) for the DEPL trial and an $r = 0.29$ for the CTRL trial, suggesting that a reduction in carbohydrate metabolism is linked to IL-6. This link is most likely catecholamines. Catecholamines respond to increases in intensity and duration (Brooks et al., 1996) and respond more rapidly when pre-exercise glycogen availability is reduced (Keller et al., 2004, McAnulty et al., 2007). The possibility of an

earlier IL-6 peak caused by reduced glycogen availability in the current study could fit this catecholamine response and lend support to a role of IL-6 in providing more fuel to working muscle.

IL-1β Response

Interleukin-1β did not increase significantly in the CTRL trials, but a small mean peak was noted 2 hours post-exercise. This response seems illogical if its pro-inflammatory role in exercise is similar to that of IL-6. Baumann and Gauldie (1994) described IL-1β as being in the first wave of pro-inflammatory response to antigens, even ahead of IL-6. It would therefore respond to the same stimulus with timing at least close to that of IL-6 in exercise if the stimulus were similar to that of an antigen. However, the exercise-induced response does differ from that caused by antigens, and a later response of IL-1β would agree with some previous results (Fielding et al., 1993; Drenth et al., 1995). Only one study of IL-1β during exercise noted any significant increase in IL-1β immediately post-exercise (Ostrowski et al., 1999); however, in that study, as well as those of Cannon et al. (1986, 1989) and Fielding et al. (1993), the greatest increase in IL-1β was consistently found later in recovery, up to 5 days post-exercise when followed so long (Fielding, 1993). If IL-1β measurements had continued further into recovery a significant change may have been evident.

The difference in the exercise response of IL-1β compared to its antigen response is likely due to a difference in purpose. In antigen response, IL-1β is known to increase body temperature set-point, but IL-1β can also function in coordinating muscular repair and protein synthesis (Cannon et al., 1986). The stimulus for IL-1β in antigen-response may be due to the need to increase body temperature in the fever process, a function not necessary in

exercise; while in exercise, its likely function is that of muscular repair coordination, an action that occurs almost entirely in recovery. The peak of IL-1β during recovery found in the present study is fitting for such differing roles.

Interleukin-1β did not significantly increase during the DEPL trial, but once again, a small peak was noted 30 min into recovery; 90 minutes earlier than in the CTRL trial. Since the role of IL-1β is to coordinate muscular repair and not to mobilize fuel for exercise, reducing muscular glycogen should have little or no effect on IL-1β release.

Comparing Pro-inflammatory Responses

The difference in stimuli for IL-6 and IL-1β may also relate to overall work intensity. Ostrowski et al. (1999) demonstrated that both IL-6 and IL-1β could increase in response to high-duration steady-state running. Only McMurray et al. (2007) examined the responses of these cytokines to an intense discontinuous exercise protocol, in a comparison of normal and overweight children. The children performed ten, 2-minute bouts of high intensity cycling separated by 1-minute rest intervals. In that study, IL-6 increased during exercise and recovery similar to the adults in the present study, and there was no significant increase in IL-1β. The intermittent and progressive exercise may be sufficient to cause immediate local release of IL-6 from muscle tissue in response to falling local muscle glycogen, but may not sustain the stimulus necessary for immediate systemic response of any of these cytokines (which includes all IL-1β sources). The stimulus (possibly diminishing stored glycogen) may instead need to accumulate before it can finally trigger systemic release.

IL-10 Response

As stated in the review of literature, IL-10 is an anti-inflammatory cytokine, released in response to the increase in pro-inflammatory mediators, which inhibits their

production/release (Gleeson, 2007). It seems fitting to this role that in both trials IL-10 should peak after IL-6. Though no significant change in IL-10 was found, the small IL-10 peaks did occur after IL-6 peaks in the majority of the subjects. Interleukin-10 peaked 90 minutes after IL-6 in the CTRL trial and 30 minutes after IL-6 in the DEPL trial (Fig.1, 3). In theory, glycogen reduction should prolong the pro-inflammatory period in recovery. However, IL-10 seemed to respond only to the increasing pro-inflammatory signals regardless of possible differences in glycogen status. The possible delay of IL-10 peak found in this study does not agree with the results of the Ostrowski et al. marathon study (1999) in which IL-10 peaked immediately post exercise, and declined over 4 hours of rest. The delayed IL-10 peak may, instead, be specific to exercise protocols. Zaldivar et al. (2006) had participants cycle for 30 minutes above lactate threshold. While there was no significant change in IL-10 immediately post-exercise, a significant increase was noted 1-hour into recovery. Furthermore, IL-6 was significantly elevated at both time points.

Unfortunately, the results of this study showed large inter-individual variability in IL-10 response, and the results of previous studies seem to suggest a variation in IL-10 response with any change in duration or intensity that may not follow a consistent pattern. The large variability in IL-10 responses was not related to VO_{2peak} ($r = -0.53$; $p>0.05$) nor lactate ($r = -0.14$; $p > 0.05$). Peake et al. (2005) found an immediate increase after an hour running at 85% VO_{2max} with a peak increase 1 hour post-exercise, but no change after an hour at 60% VO_{2max}. Brenner et al. (1999) found no change immediately after 2 hours of cycling at 60% VO_{2max} but found an immediate decrease following 5 minutes of cycling at 90% VO_{2max} that persisted for 3 hours. Therefore, the signal for IL-10 release in exercise may relate to factors other than IL-6 production.

Considering Impact of Peak Timing

As anticipated, the peak IL-10 response occurred after the peak IL-6 response, regardless of condition (Table 4). The small changes in the three cytokines appeared to peak sooner in the DEPL trial than in the CTRL trial (Table 4). The mean IL-6 peak occurred 30 minutes earlier in the DEPL trial (Table 4). The peaks in both IL-1β and IL-10 occurred 90 minutes earlier in the DEPL trial (Table 4). These results suggest that glycogen depletion does have a minor impact on the cytokine responses to exercise. Since the peak magnitudes of cytokine responses were not significantly changed by glycogen reduction, either glycogen stores were not effectively depleted (though RER and LA differences did exist between trials), or depletion simply modifies the rate and duration of cytokine changes instead of the magnitude.

Table 4. Timing of mean peaks in percent changes (max-baseline) of cytokines across trials

Cytokine	CTRL	DEPL
IL-6	30 min post exercise	Immediate Post-Exercise
IL-1β	120 min post exercise	30 min post exercise
IL-10	120 min post exercise	30 min post exercise

Conclusions

Despite lack of significant increases in the cytokines, these results did allow most of the hypotheses to be addressed to some degree. All of the hypotheses for this study concentrate on cytokine responses to this specific discontinuous, progressive exercise protocol. However, these hypotheses were based on previous research in which the exercise protocols were continuous and steady-state. The conclusions made regarding this protocol

may, therefore, provide a basis for future study of cytokine response to similar discontinuous and/or progressive exercise protocols.

Hypothesis #1 was that IL-1β and IL-6 would peak immediately post-exercise, and IL-10 would peak in the 2-hour recovery period in the CTRL trial. Both IL-6 and IL-1β peaked later than hypothesized. Based upon the results, the Hypothesis #1 regarding IL-1β and IL-6 was not accepted. Hypothesis #1 also stated that IL-10 would peak during recovery and, in fact, a small, but non-significant, change was noted 2 hours post-exercise (Fig. 3). Therefore, Hypothesis #1 regarding IL-10 was accepted.

Hypothesis #2 predicted the same timing of all peaks would occur in the DEPL trial as was hypothesized for the CTRL trial. This was not the case for IL-1β, but the trends for IL-6 and IL-10 peaks were in support. The peak for IL-6 response during the DEPL trial was immediately post-exercise; while small increases in IL-1β and IL-10 were noted 30 minutes post-exercise (Fig. 1-3). Therefore, Hypothesis #2 was accepted regarding IL-6 and IL-10, but not accepted regarding IL-1β.

Hypothesis #3 predicted that the peaks in changes of all three cytokines would be higher in the DEPL trial than in the CTRL trial. No difference between trial peaks of any statistical significance; therefore Hypothesis #3 was not accepted.

Hypothesis #4 stated that the time point at which peak changes of all three cytokines occurred would differ between trials. As previously stated, the results suggested possibly earlier peaks (though non-significant) for all three cytokines in the DEPL trial than in the CTRL trial. Therefore, Hypothesis #4 was accepted; although the peak responses were very small and not significant for IL-1β or IL-10.

Based upon these results, some conclusions can be made concerning cytokine responses to intermittent and progressive exercise. Similar to other modes and durations of exercise, IL-6 is elevated by this exercise protocol either during recovery or within exercise depending on glycogen status. This exercise protocol does not consistently increase IL-1β or IL-10, regardless of glycogen status. Lastly, progressive exercise, intermittent or not, may cause a cytokine response proportionate to the rise in intensity that may not reach peak until sometime in recovery.

Recommendations for Future Study and Application

This study featured a discontinuous, progressive exercise test. Rest intervals had to be included between each exercise stage to allow for collection blood samples along with other data relevant to a second research thesis project. In order to ease the transition into the start of the next stage, each rest interval ended with 2 minutes of active recovery (cycling at full speed without resistance). An advantage to using this discontinuous mode was that it allowed for a given number of exercise stages to be completed over more total time. This improved the chance that all participants would pass the estimated minimum time needed from initiation to cessation of exercise to detect an inflammatory response. A disadvantage, however, may have been that these recovery intervals allowed cytokine responses to recede after each stage and prevented reaching the highest responses possible for the given intensity of exercise. Future studies solely interested in cytokine response may want to reduce or remove rest intervals but also reduce the rate at which workload is increased to lessen time for recovery within exercise without cutting the total exercise time.

The glycogen-reduction protocol used in this study was based upon the work of Gollnick et al. (1973). It was a 2-hour continuous, steady-state exercise intended to be of the

highest intensity the participants could sustain for such duration. While all participants did show steady-state responses in VO_2, heart rate, and RER, they often struggled to maintain the assigned pace at the prescribed workload for the full duration. Future efforts may want to consider alternative protocols that may decrease the total exercise time, possibly using progressing workloads.

One limitation in the analysis of the cytokines was the choice of ELISA supplies and time points to be analyzed. These assay kits come in normal and high-sensitivity varieties. Since previous research showed smaller concentration changes in IL-1β (that could be below the detectable limit of a normal kit), we chose high-sensitivity kits for that cytokine, but used normal-sensitivity kits for the other two cytokines. Though there was still no significant differences detected with these kits for IL-1β, using high-sensitivity kits for the other two cytokines may have increased the accuracy of those results, possibly reducing the degree of inter-individual variability in concentrations throughout the trials. Adding additional time points would allow more precise determination of peak timing and overall patterns in concentration changes, but this also requires more kits and lengthens the analysis time, and could require more blood to be drawn from participants.

If reduced glycogen does consistently increase inflammatory response to exercise, and increased exercise response has implications on overall immune function, then nutritional status could become an important consideration in ensuring healthy training. Haight and Keatinge (1972) showed that long-duration exercise (9 hours) could cause a fever-like increase in core temperature. Increased IL-1β can persist 5 days following only 45 minutes of steady-state exercise (Cannon et al., 1989; Fielding et al., 1993). Cannon et al. (1986) suggested that a lasting change in IL-1 could cause the rise in temperature set-point

seen by Haight and Keatinge (1972). These long elevations in IL-1β could cause athletes to experience fever-like temperatures that could be dangerous when enduring subsequent exercise sessions which present additional spikes in body temperature. A significant increase in IL-1β was not found in the duration examined in this study, but may still have been possible later in recovery (as in Cannon et al. (1986)). Future research interested in long-term immune effects should track IL-1β changes for at least 5 days post-exercise and also track core temperature changes over that time.

Cox et al. (2007) stated that "post-exercise changes in cytokine kinetics are likely to play an important role in modulating some of the reported post-exercise changes in immune function," suggesting that disruptions in the inflammatory balance could lead to a loss of control over inflammation and overall immune function. In that study, well-trained illness-prone and healthy participants' cytokine responses were compared in three running protocols. Illness-prone athletes showed greater IL-6 response and a decline in IL-10, lasting 24 hours post-exercise while healthy subjects returned to pre-exercise values in that time. These differences could indicate altered inflammatory control and could be the cause of these athletes' increased susceptibility to upper respiratory infection. If the increased IL-6 response leads to greater infection risk, minimizing the duration of cytokine imbalance becomes important in sustaining illness-free training. This study provides evidence that glycogen supply may be a crucial consideration in determining the factors contributing to lasting effects of exercise on inflammatory balance. Additionally, this study shows possible differences in the cytokine responses to intermittent progressive exercise versus those to steady-state exercise that could help in understanding what exercise is best for maintaining healthy immune function. Future studies on nutritional influences should analyze the diets of

participants preceding both trials and may want additional verification of the effectiveness of the glycogen-reduction methods (such as biopsies of participants' vastus lateralis

References

Amano, Y., Lee, S.W., Allison, A.C. (1993) Inhibition by glucocorticoids of the formation of interleukin-1α, interleukin-1β, and interleukin-6: mediation by decreased mRNA stability. *Molecular Pharmacology*, 43: 176-182.

American College of Sports Medicine (2006) *ACSM's Guidelines for Exercise Testing and Prescription*, 7th ed. Philadelphia: Lippincott Williams and Wilkins.

Baumann, H., Gauldie, J. (1994) The acute phase response. *Immunology Today*, 15 (2): 74-80.

Borg, G. (1970) Perceived exertion as an indicator of somatic stress. *Scandinavian Journal of Rehabilitative Medicine*, 23: 92-98.

Brenner, I.K.M., Natale, V.M., Vasiliou, P., Moldoveanu, A.I., Shek, P.N., Shephard, R.J. (1999) Impact of three different types of exercise on components of the inflammatory response. *European Journal of Applied Physiology*, 80: 452-460

Brooks, G.A., Fahey, T.D., White, Baldwin, K.M. (1996) *Exercise Physiology: Human Bioenergetics and Its Applications*, 3rd ed. Mayfield Publishing Co.

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T.L., MacLean, D.A., Pedersen, B.K. (1997) Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *Journal of Physiology*, 449 (3): 833-841.

Cannon, J.G., Evans, W.J., Hughes, V.A., Meredith, C.N., Dinarello, C.A. (1986) Physiological mechanisms contributing to increased interleukin-1 secretion. *Journal of Applied Physiology*, 61 (5): 1869-1874.

Cannon, J.G., Fielding, R.A., Fiatrone, M.A., Orencole, S.F., Dinarello, C.A., Evans, W.J. (1989) Increased interleukin 1β in human skeletal muscle after exercise. *American Journal of Physiology*, 257 (Regulatory Integrative Comprehensive Physiology, 26): R451-R455.

Cannon, J.G., Kluger, M.J. (1983) Endogenous pyrogen activity in human plasma after exercise. *Science*, 220: 617-619.

Cox, A.J., Pyne, D.B., Saunders, P.U., et al. (2007) Cytokine responses to treadmill running in healthy and illness-prone athletes. *Medicine and Science in Sports and Exercise*, 39 (11): 1918-1926.

Davison, G., Gleeson, M. (2005) Influence of acute vitamin C and/or carbohydrate ingestion on hormonal, cytokine, and immune responses to prolonged exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, 15:465-479.

Drenth, J., Van Uum, S., Van Deuren, M., Pesman, G.J., Van Der Ven-Jongekrijg, J., Van Der Meer, J. (1995) Endurance run increases circulating IL-6 and IL-1ra but downregulates ex vivo TNF-α and IL-1β production. *Journal of Applied Physiology*, 79 (5): 1497-1503.

Febbraio, M.A., Pedersen, B.K. (2002) Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *FASEB Journal*. 16: 1335-47.

Fielding, R.A., Manfredi, T.J., Ding, W., Fiatarone, M.A., Evans, W.J., Cannon, J.G. (1993) Acute phase response in exercise III. Neutrophil and IL-1b accumulation in skeletal muscle. *American Journal of Physiology* 265 (Regulatory Integrative Comprehensive Physiology 34): R166-R172.

Gleeson, M. (2007) Immune function in sport and exercise. *Journal of Applied Physiology*, 103: 693-699.

Gollnick, P.D., Armstrong, R.B., Saubert, C.W. IV, Sembrowich, W.L., Shepherd, R.E., Saltin, B. (1973) Glycogen depletion patterns in human skeletal muscle fibers during prolonged work. *Pflugers Archives*, 344 (1): 1-12.

Haight, J.S.J. and Keatinge, W.R. (1973) Elevation in set point for body temperature regulation after prolonged exercise. *Journal of Physiology*, 229: 77-85.

Helge, J.W., Stallknecht, B., Pedersen, B.K., Galbo, H., Kiens, B., Richter, E.A. (2002) The effect of graded exercise on interleukin-6 release and glucose uptake in human skeletal muscle. *Journal of Physiology*, 546 (1): 299-305.

Heyward, V. (2006) *Advanced Fitness Assessment and Exercise Prescription*, 5th ed. Champaign, IL: Human Kinetics.

Keller, C., Steensberg, A., Pilegaard, H., Osada, T., Saltin, B., Pedersen, B.K., Neufer, P.D. (2001) Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *FASEB Journal*, 15 (14): 2748-2750.

Keller, P., Keller, C., Robinson, L.E., Pedersen, B.K. (2004) Epinephrine infusion increases adipose interleukin-6 gene expression and systemic levels in humans. *Journal of Applied Physiology*, 97: 1309-1312.

MacDonald, C., Wojtaszewski, J., Pedersen, B.K., Kiens, B., Richter, E. Interleukin-6 release from human skeletal muscle during exercise: relation to AMPK activity. *Journal of Applied Physiology*, 95: 2273-2277.

McAnulty, S.R., McAnulty, L.S., Morrow, J.D., Nieman, D.C., Owens, J.T., Carper, C.M. (2007) Influence of carbohydrate, intense exercise, and rest intervals on hormonal and oxidative changes. *International Journal of Sport Nutrition and Exercise Metabolism*, 17 (5): 478-490.

McMurray, R.G., Zaldivar, F., Galasetti, P., Larson, J., Eliakim, A., Nemet, D., Cooper, D.M. (2007) Cellular immunity and inflammatory mediator responses to intense exercise in overweight children and adolescents. *Journal of Investigative Medicine,* 55: 120-128.

Nehlsen-Cannarella, S.L., Fagoaga, O.R., Nieman, D.C., Henson, D.A., Butterworth, D.E., Schmitt, R.L., Bailey, E.M., Warren, B.J., Utter, A., Davis, J.M. (1997) Carbohydrate and the cytokine response to 2.5 h of running. *Journal of Applied Physiology*, 82 (5): 1662-1667.

Nieman, D.C., Davis, J.M., Henson, D.A., Walberg-Rankin, J., Shute, M., Dumke, C.L., Utter, A.C., Vinci, D.M., Carson, J.A., Brown, A., Lee, W.J., McAnulty, S.R., McAnulty, L.S. (2003) Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine level after a 3-h run. *Journal of Applied Physiology*, 94:1917-1925.

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., Pedersen, B.K. (1999) Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *Journal of Physiology*, 515 (1): 287-291.

Peake, J.M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K., Coombes, J.S. (2005) Plasma cytokine changes in relation to exercise intensity and muscle damage. *European Journal of Applied Physiology*, 95: 514-521.

Pedersen, B.K. (2007) IL-6 signalling in exercise and disease. *Biochemistry Society Transactions*, 35: 1295-97.

Smith, L.L., Miles, M.P. (2000) Exercise-induced muscle injury and inflammation. In: *Exercise and Sport Science*. Garrett, W.E. and Kirkendall, D.T. eds. Philadelphia: Lippincott Williams & Wilkins. pp. 401-409.

Steensberg, A., Febbraio, M.A., Osada, T., Schjerling, P., van Hall, G., Saltin, B., Pedersen, B.K. (2001) Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *Journal of Physiology*, 537 (2): 633-639.

Steensberg, A., Fischer, C.P, Keller, C., Moller, K., Pedersen, B.K. (2003) IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology: Endocrinology and Metabolism*, 285: E433-437.

Steensberg, A., Keller, C., Starkie, R.L., Osada, T., Febbraio, M.A., Pedersen, B.K. (2002) IL-6 and TNF-a expression in, and release from, contracting human skeletal muscle. *American Journal of Physiology: Endocrinology and Metabolism*, 283: E1272-E1278.

Steensberg, A., van Hall, G., Osada, T., Sacchetti, M., Saltin, B., Pedersen, B.K. (2000) Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *Journal of Physiology*, 529 (1): 237-242.

Vaisberg, M., de Mello, M.T., Seelaender, M., dos Santos, R., Rosa, L. (2008) Reduced maximal oxygen consumption and overproduction of proinflammatory cytokines in athletes. *Neuroimmunomodulation*, 14: 304-309.

Zaldivar, F., Wang-Rodriguez, J., Nemet, D., Schwindt, C., Galassetti, P., Mills, P.J., Wilson, L.D., Cooper, D.M. (2006) Constitutive pro- and anti-inflammatory cytokine and growth factor response to exercise in leukocytes. *Journal of Applied Physiology*, 100 (4): 1124-1133.