FITNESS STATUS AND POST-EXERCISE INFLAMMATORY MARKERS IN 18-35 YEAR-OLD MALES

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIRMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Human Kinetics)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

June 2008

Abstract

Acute physical exercise results in transient systemic elevations of cytokines. The most significant elevation is seen in interleukin-6 (il-6). Elevated values of il-6 have been reported to enhance fatigue and diminish performance during endurance exercise. A delayed increase in C-reactive protein (CRP) has also been shown in response to il-6. Persistent elevations in systemic interleukin-6 and C-reactive protein values have been associated with increased risk of cardiovascular disease.

The purpose of this investigation was to determine whether differences exist in resting and post-exercise measures of il-6 and CRP between trained male endurance athletes and age matched untrained males.

Twenty-five eligible males were recruited; thirteen trained (T) mean (SD): age = 26.6(4.9) yrs, mass = 73.0(7.8) kg, height = 179.0(5.7) cm, BMI = 22.6(1.4) VO2 = 68.6(5.6) $ml \cdot kg^{-1} \cdot min^{-1}$and twelve untrained (U): age = 23.4(3.8) yrs, mass = 77.9(15.0) kg, height = 179.0(8.7) cm, BMI = 23.9(3.0) VO2 = 42.4(4.6) $ml \cdot kg^{-1} \cdot min^{-1}$. The two groups were matched for age and body mass index (BMI) and differed significantly in aerobic fitness and hours of exercise per week.

Days after an initial aerobic fitness assessment subjects were challenged with a 45 minute cycle ergometer exercise bout at an intensity corresponding to individual ventilatory threshold (VT). Serum il-6 was measured pre-exercise, 30 minutes post-, and 24 hours post-exercise. Serum CRP was measured pre-exercise and 24 hours post-exercise. Il-6 values were analyzed using a 2x3 mixed design ANOVA and CRP using a 2x2 mixed design ANOVA.

Il-6 values increased significantly in both groups 30 minutes post-exercise [T ($p<0.05$) and U ($p<0.05$)] and returned to baseline at 24 hours. Il-6 was not different between groups at any time point. CRP values did not increase significantly in either group between pre- and 24 hours post-exercise. CRP values were significantly higher in the untrained group pre- ($p<0.05$) and 24 hours post-exercise ($p<0.05$) compared to the trained group.

These results demonstrate no significant difference in il-6 between T and U at rest and or after exercise. This study also demonstrates a reduction in resting and post-exercise CRP in endurance trained males compared to untrained males matched for age and BMI.

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Acknowledgements

I would like to acknowledge and thank my supervisor, Dr. Jack Taunton, who gave me unconditional support throughout my graduate work. After each of our many meetings I left with a heightened interest in my project and medicine as a whole. You have inspired me to continue in this field. Thank you to my committee members, Dr. Ted Rhodes and Dr. Donna MacIntyre, who helped guide me through this project with their invaluable input. I would also like to recognize the generous financial support of the B.C Sports Medicine Research Foundation and the Faculty of Education for making this project possible. Last but not least, I would like to thank my family, Mom, Dad, Jamie, and Elizabeth, for your constant support of every kind.

Chapter 1: Introduction

Acute physical exercise of various modes has consistently been shown to result in transient systemic elevations in both pro- and anti-inflammatory cytokines. The most significant elevation is seen in interleukin-6 (il-6). Elevated values of circulating il-6 have recently been reported to enhance fatigue and diminish performance during endurance exercise (73). In addition, the post-exercise increase in circulating il-6 has been hypothesized by some researchers as an underlying cause of the unexplained overtraining syndrome (43, 73). A delayed increase in circulating acute phase proteins, most notably C-reactive protein (CRP), has also been demonstrated in response to elevations in circulating il-6 and other pro-inflammatory cytokines after a strenuous bout of exercise (48). Persistent elevations in systemic CRP values have been associated with an increased risk of cardiovascular disease when compared to adults maintaining lower levels of C-reactive protein (64).

Much research in past years has explored various methods of manipulating the post-exercise increase of inflammatory mediators. Of this research, only one study involving healthy human subjects has utilized prospective endurance exercise training to attenuate this response. The main finding of this prospective training study by Fischer et al, (23) was that a 10-week endurance exercise training program can reduce post-exercise il-6 mRNA expression in human skeletal muscle in response to a concentric exercise stimulus of the same relative workload. Although post-exercise plasma il-6 values in these subjects were similar before and after training, this study indicates that training status plays a role in skeletal muscle il-6 protein production. Cross-sectional analysis of

post-exercise plasma il-6 values between trained and untrained subjects in different studies indicate that trained subjects have an attenuated post-exercise il-6 elevation in comparison to lesser trained subjects. However, cross-sectional comparisons are difficult due to differing subject criteria, exercise modes and intensities, and differing sampling schedules. To date no research has directly compared the systemic post-exercise il-6 and CRP response to an identical relative exercise stimulus in trained and untrained humans matched for age, sex and body composition.

A comparison of post-exercise immunological markers between trained and untrained subjects will provide opportunity to analyze the relationship between these two variables. The main objective of this research is to determine whether post-exercise increases of systemic il-6 and CRP differ significantly between trained and untrained young males. Consequently, this research will contribute to the current understanding of the immunological and endocrine adaptations associated with exercise training.

1.1 Statement of Problem

1) Prior to this study, no human study has directly compared the systemic post-exercise il-6 and CRP in trained and untrained individuals in response to an exercise challenge of similar relative workload.

2) Although a cross-sectional analysis of current research studies indicates that a relationship exists between training status and post-exercise elevations of plasma il-6 and CRP, such a relationship has not yet been scientifically evaluated with a controlled study in men matched for age and body mass index.

3) Il-6 is a known stimulator of CRP; however, previous research has failed to evaluate a significant correlational relationship between the two inflammatory markers in response to physical exercise.

4) No available research has examined the differences in the ratio of peak post-exercise il-6 and CRP between trained and untrained individuals.

1.2 Purpose of the Investigation

The primary purpose of this study was to investigate the differences between aerobically trained and untrained young males in resting and post-exercise systemic concentrations of both il-6 and CRP in response to a predetermined concentric exercise stimulus. The secondary purpose of this study was to quantitatively evaluate the correlational relationship between circulating concentrations of il-6 30 minutes post-exercise and CRP 24 hours post-exercise.

1.3 Research Question/Hypotheses

The following research hypotheses were formulated prior to the investigation based upon available research literature:

1) Subjects in the trained study group will demonstrate a significantly lower ($\alpha \leq .05$) concentration of systemic il-6 post-exercise challenge (30-minutes post-exercise) compared the untrained subject group.

2) Males in the trained study group will demonstrate a significantly lower ($\alpha \leq .05$) concentration of systemic CRP post-exercise challenge (24-hours post-exercise) compared to the untrained subject group.

3) Following each exercise stimulus circulating concentrations of plasma il-6 in both groups will increase significantly ($\alpha \leq .05$) compared to pre-exercise values.

4) Systemic CRP concentrations will increase significantly ($\alpha \leq .05$) in response to the exercise challenge in the untrained group but will not increase significantly in the trained group.

5) Resting values of plasma il-6 and serum CRP will not be significantly different between training groups.

6) Differences in pre- and post-exercise concentrations of plasma il-6 will show a significant correlation ($\alpha \leq .05$) with differences in pre- and post-exercise concentrations of CRP in both study groups.

Chapter 2: Review of Literature

Il-6 is a ~27 kDa glycoprotein consisting of 212 amino acids, including a 28 amino acid signal peptide. Il-6 is in a sub-class of cytokines that share a similar helical protein structure and a similar signal transducer (glycoprotein 130). Cytokines are a class of polypeptide messenger proteins responsible for signal transmission amongst various immune system and other organ cells. Cytokines may act in autocrine, paracrine, or endocrine fashion. In response to trauma or infection pro-inflammatory cytokines tumor necrosis factor-α (TNF- α) and interleukin-1β (il-1β) are produced locally (65). The initial increase of Il-1β and TNF-α is followed by increases of il-6, and then anti-inflammatory cytokines il-1 receptor antagonist (Il-1ra), tumor necrosis factor receptors (TNF-R) and il-10. As such, il-6 has been termed inflammation-sensitive rather than pro-inflammatory (61). Il-1β, TNF-α, and bacterial endotoxin are the classic stimuli for il-6 release (2). Although synthesized by a variety of cells, il-6 is mainly secreted by activated monocytes, fibroblasts, and vascular endothelial cells in response to injury or infection (2). Il-6 individually, or in combination with inflammatory cytokines il-1β and TNF-α, stimulates a delayed hepatocyte production of acute-phase proteins termed an acute-phase response. CRP is an acute-phase protein that increases up to 1000-fold with trauma or infection (24). Il-6 has been identified as the chief stimulator of acute-phase protein production (26).

Systemic il-6 also has the ability to activate the hypothalamic-pituitary-adrenal axis (43). Prior research has indicated that il-6 can permeate the blood-brain barrier (4) and, when administered in doses equivalent to those during exercise, results in increased sensations of fatigue, depressed mood state, and decreased ability to concentrate (73). In a study by Robson-Ansley (73) subjects were injected with recombinant human il-6 in a

dosage equivalent to that following strenuous exercise significantly impaired performance in a 10km running time trial. The precise mechanism resulting in decreased performance following il-6 administration is unclear but Robson-Ansley indicates that serotonergic pathways are likely activated.

Marked post-exercise elevations of plasma il-6 concentration are a consistent finding throughout previous research (55, 60, 62, 63, 85, 52). The cytokine response to exercise differs from that of trauma or sepsis. Most notably, il-6 is the first cytokine to appear in circulation in response to exercise rather than il-1β or TNF-α (62). This pattern suggests that il-6 synthesis during exercise is stimulated by factors other than il-1β and TNF-α. Furthermore, il-6 increases greatly with exercise, while il-1β and TNF-α generally increase minimally or not at all (66).

Early research indicated that il-6 production during exercise was associated with, and likely a result of muscle damage (10). However, later research failed to report a similar association, suggesting that il-6 may be produced in response to muscle contractions without muscle damage (61, 17). Post-exercise plasma il-6 increases in response to exercise without muscle damage have been a common finding (12, 55, 58, 61, 62, 63). In order to elucidate the source of il-6 production Steensberg (83) designed a study that monitored the arterial-venous (a-v) difference of il-6 in both a working and resting leg. The exercise bout consisted of one-legged knee extensions for 5 hours at 40% of peak power output. The a-v difference in il-6 was equal between legs pre-exercise. Throughout the exercise bout the a-v difference in the active leg increased significantly and peaked at 9.77 ng/l, whereas the a-v difference in the resting leg remained similar to pre-exercise. Although a specific source could not be identified in this study, the relationship between a-v differences in il-6 from the active limb and systemic il-6

concentrations indicated that systemic post-exercise il-6 elevations could be accounted for by production in the active limb.

In both a human model and an animal model biopsies of the active muscle have demonstrated marked elevations in il-6 mRNA following strenuous concentric and eccentric exercise (62, 40). Furthermore, Penkowa (65) detected il-6 within muscle cells and displayed an increase following contraction. With the use of microdialysis catheters implanted in skeletal muscle and peritendinous tissue, Langberg (44) have suggested that peritendinous tissue also contributes to the post-exercise increase in systemic il-6. In all, the available research has established that il-6 production and release occurs in skeletal muscle cells in response to exercise and is likely the chief source of il-6 production during non-damaging exercise.

Other sources of il-6 release during exercise have been suggested; however, their relative contribution to post-exercise il-6 elevation appears to be minimal. Lyngso (49) examined the il-6 release from adipose tissue during exercise by comparing the a-v difference between a radial artery and a subcutaneous abdominal vein in nine healthy subjects. Systemic il-6 peaked 30 minutes after a 1 hour cycle ergometer exercise at 60% of VO_{2max}, increasing significantly above resting values. Elevations in adipose tissue il-6 production were unchanged until 1 hour post-exercise, at which point systemic il-6 concentrations were far below peak values. The demonstrated time-course of il-6 release from adipose tissue indicates that it does not contribute significantly to systemic peak post-exercise il-6 elevations.

Nybo (59) also used a-v difference between arterial and internal jugular venous concentrations of plasma il-6 to show that the brain produces il-6 during endurance exercise. Il-6 release and uptake by the brain at rest was nil but increased significantly

($p<.05$) after 1 hour on a cycle ergometer at 50% of VO_{2max}. Measurements of a-v differences in combination with absolute measures of cerebral blood flow indicated a net release of il-6 from the brain of .3 ng/min. Considering that peak il-6 output from an exercising leg reached approximately 30 ng/min (83), cerebral il-6 production likely accounts for a minute portion of elevated systemic il-6 concentration during exercise.

The time-course and degree of il-6 elevation after exercise is dependent upon exercise type, intensity, and duration. In response to damaging eccentric exercise circulating il-6 concentrations are elevated immediately after exercise, increase steadily and peak approximately 6 hours post-exercise (51, 17). Following concentric-based exercise, circulating il-6 concentrations demonstrate an exponential increase, setting on 10-30 minutes into exercise and reaching maximum concentrations upon cessation or within 30 minutes post-exercise (65, 62, 89). The intensity of exercise has shown a significant correlation with peak post-exercise circulating il-6 concentrations during running exercise (63). Concentrations of circulating il-6 have been reported to increase as much as 128-fold following a marathon run in healthy young males (61). Soon after cessation of activity in both cycling and running-based exercise models circulating il-6 levels decline quickly, reaching resting levels between 6 and 24 hours post-exercise (61, 62, 89).

No research to date has directly compared systemic il-6 concentrations during or following exercise in subjects with different fitness levels. A cross-sectional analysis between modes of exercise and fitness levels is difficult as systemic il-6 levels are dependent upon a variety of factors not controlled between studies including muscle mass involved and contraction type. However; in order to review the available literature a

cross-sectional analysis of comparable research incorporating similar exercise modes and intensities will follow.

In a study by Vassilakopoulos (89) healthy untrained males aged 28-44 years were subjected to a 45-minute exercise bout on an upright cycle ergometer at 70% of VO_{2max} in order to examine systemic cytokine responses. The men in this study had a mean VO_{2max} of 38.9 ml/min/kg and were not regularly participating in exercise. Resting pre-exercise plasma concentrations of il-6 had a mean concentration of ~1 pg/ml. Plasma il-6 reached 4 pg/ml upon cessation of exercise and peaked 30 minutes post-exercise at ~7.3 pg/ml. Contrary to similar studies, Vassilakopoulos (89) had subjects ingest a carbohydrate rich breakfast prior to the exercise bout. Based on research by Nehlsen-Cannarella (55) and Nieman (58) that indicate an attenuation of il-6 production with carbohydrate ingestion prior to exercise, post-exercise plasma il-6 concentrations likely would have increased to a greater extent had subjects completed the exercise bout after an overnight fast.

In a similar study Febbraio (20) recruited seven males aged 22.1+/-3.8 years with a mean VO_{2max} of 47.9 ml/kg/min. The exercise bout consisted of 120 minutes of cycling on a semi-recumbent ergometer at 65% of individual VO_{2max}. In this study subjects completed the predetermined exercise bout following an overnight fast. Systemic plasma il-6 was recorded at 0 (pre-exercise), 30, 60, 90, and 120 minutes into exercise with respective concentrations of 2, 4, 5, 7.5, and 12.5 pg/ml. No blood samples were recorded after cessation of exercise. Although exercise intensity was 5% less in this study compared to that of Vassilakopoulos et al. (89), the exercise bout was completed following an overnight fast and the exercise duration was more than 2-fold. Mean il-6 concentrations reached ~7.5 pg/ml at time points in both studies; at 75 minutes (30

minutes post-exercise) in Vassilakopoulos' study and 90 minutes in Febbraio's study. Considering subjects in Vassilakopoulos' study completed the exercise bout 30 minutes prior to this measure and subjects in Febbraio's study had been cycling continuously for 90 minutes prior to the measure, it would be expected that subject's in Febbraio's study would demonstrate higher concentrations of plasma il-6. Furthermore, subjects in Febbraio's study completed the exercise bout following an overnight fast, likely leading to greater post-exercise il-6 increases than if fed prior to exercise.

In another study incorporating a similar exercise bout MacDonald (50) examined post-exercise il-6 concentrations in elite athletes. These subjects were well trained and participated in physical training 5-8 times per week. Subjects included eight males aged 28 +/- 1 year with mean VO_{2max} values of 65 +/-1 ml/kg/min. The exercise stimulus consisted of 1 hour of upright cycle ergometer exercise at 70% of individual VO_{2max}; completed in the morning following a light breakfast consisting of 75% carbohydrates with a total energy intake of 713 KJ . Systemic plasma il-6 was recorded at 0 (pre-exercise), 10, 20, 30, 45, and 60 minutes of exercise. The corresponding il-6 concentrations were .8, 1, 1.2, 1.6, 2.7, and 4 pg/ml respectively. Considering the similar mode and relative exercise intensity in this study compared to Vassilakopoulos', it would be expected that both groups would demonstrate similar post-exercise il-6 concentrations. At similar time points of exercise (45 minutes into exercise) in Vassilakopoulos' and MacDonald's study, circulating plasma il-6 concentrations were 4 and 2.7 pg/ml respectively. This further suggests that training status has a large influence on plasma il-6 concentrations in response to exercise.

Another similar study by Starkie (83) examined circulating plasma il-6 during 2 hours of upright cycle ergometer exercise at 70% of individual VO_{2max}. The subjects in

this study were endurance trained males aged 25 +/-5 years with a mean VO_{2max} value of 60.2 ml/min/kg. Subjects completed the 2 hour trial in the morning following an overnight fast. Circulating plasma il-6 was sampled pre-exercise, after 60 minutes of exercise, and upon completion of exercise at 120 minutes. The corresponding plasma il-6 concentrations were .1, .2 and 1.5 pg/ml. These results are similar to another study by Starkie et al., (83) in which males with a mean VO_{2max} value of 61.03 ml/kg/min showed peak post-exercise plasma il-6 values of .8 pg/ml in response to 90 minutes of cycling exercise at 70% VO_{2max}. Unfortunately, in the latter study no pre-exercise nutritional measures were reported. These studies incorporated the same exercise mode and relative intensity as Vassilikapoulos et al., (89) with a markedly increased duration. As a result, it would be expected that mean post-exercise plasma il-6 concentrations would be much greater in these subjects. In contrast, the mean peak plasma il-6 concentrations for Starkie (82) and Starkie (83) after 120 and 90 minutes of cycle exercise corresponded to 20% and 11% of peak post-exercise plasma il-6 concentration in Vassilikapoulos' research.

In combination these studies display an important relationship between physical fitness, measured as maximal oxygen consumption, and circulating il-6 concentrations in response to concentric-based cycling exercise. The mean systemic plasma il-6 values throughout exercise in these studies are displayed in a table and plotted on one graph (Appendix A), which clearly demonstrates a relationship between training status and post-exercise plasma il-6 values at similar relative exercise intensities and durations. It is not possible to quantitatively compare values between research by Vassilakopoulos (89) and the others as blood sampling time points were not identical; however, the plasma il-6 increases in the more fit subjects appear markedly diminished when compared to the untrained subjects in Vassilakopoulos' work.

Two prospective training studies have been undertaken in order to investigate the effects of training on post-exercise plasma il-6 levels. Croisier (17) designed a study in which 5 moderately active males underwent an injury exercise protocol. The exercise protocol incorporated three stages of 30 maximal eccentric contractions of the knee flexors and extensors of both legs, before and after a submaximal eccentric training program. The training protocol consisted of 5 training sessions over three weeks; each training session involved 5 stages of 10 submaximal eccentric contractions similar to those of the injury exercise protocol. After training post-exercise values of serum myoglobin and delayed onset muscle soreness were significantly reduced. Croisier (17) attributed this reduction to a training-induced resistance of skeletal muscle to eccentric contraction. In contrast, post-exercise values of plasma il-6, measured immediately, 30 minutes, 48 hours, and 72 hours after exercise, were not different before and after the training regimen. This study demonstrated that short term eccentric training does not attenuate the eccentric exercise-induced plasma il-6 response.

In another prospective training study, Fischer (22) examined circulating skeletal muscle il-6 mRNA and plasma concentration of il-6 in 7 males following a 3 hour bout of isolated knee extensor contractions before and after a 10-week endurance training program. The contractions were performed at a rate of 60 contractions per minute and an intensity of 50% of the subject's maximum wattage sustainable for 1 minute. The training protocol consisted of 1 hour of isolated knee extensions 5 days per week at 75% of pre-exercise maximum wattage sustainable for 1 minute. The training workload was increased 5-10% every fortnight depending on subject progress. Upon completion of the 10-week training program subjects were retested and a post-training maximal wattage was determined. The workload corresponding to 50% of the subjects' maximum wattage

sustainable for 1 minute, and subsequent post-training exercise challenge, was increased by a mean of 44%, indicating that the training protocol improved functional performance. Resting il-6 mRNA was similar pre- and post-training in all subjects; however, post-exercise skeletal muscle mRNA increased only 8-fold post-training as opposed to 76-fold pre-training. Circulating plasma il-6 at rest, immediately post-exercise, and 2 hours post-exercise were similar before and after training. This is of great interest considering that the workload performed in the post-training exercise challenge was 44% higher than pre-exercise. Considering research by Ostrowski (63), that indicates post-exercise plasma il-6 concentration is highly correlated with exercise intensity ($r = .30$, $p < .05$), it would be expected that a 44% increase in exercise intensity would result in a markedly higher increase in post-exercise plasma il-6.

The systemic CRP response to exercise is lesser studied and is less sensitive than that of il-6. Resting CRP concentrations are generally <2 mg/l in healthy individuals. In response to moderate or prolonged endurance exercise systemic serum CRP concentrations may rise in similar fashion to that seen in trauma or infection. In response to an ultradistance 246 km foot race CRP values reached a mean of 97.3 +/- 57.6 mg/l Margeli (33). The post-exercise elevation in serum CRP tends to occur 16-24 hours after the exercise bout (80, 19, 12). In another study that examined inflammatory markers in two separate marathon groups the mean CRP concentrations 16 hours following a standard marathon were 15 mg/l and 11.8 mg/l respectively (12). Interestingly, 1 hour of cycle ergometer exercise at 60% of VO_{2max} stimulated a significant ($p=.04$) increase in serum CRP 24 hours post-exercise in untrained subjects (< 5 hrs/week of recreational activity) (80).

Although il-6 has been identified as the chief mediator of hepatocyte production of CRP, a significant correlation between the two inflammatory markers has not been demonstrated. Czarkowska-Paczek (18) designed a study examining the correlation between post-exercise values of il-6 and CRP. In the study 14 trained male cyclists performed a graded cycling test to exhaustion. Athletes began riding at 20 km/h and grade was increased every 3 minutes until exhaustion. Blood samples were drawn from an antecubital vein before, immediately after and 2 hours post-exercise. Serum CRP concentrations were 3.25, 2.39, and 2.70 mg/dl at the respective sampling times. Serum il-6 concentrations were 0.48, 1.21, and 10.07 pg/ml at the respective sampling times. No other sampling times were examined. As no correlation was evident between measures at cessation of exercise ($r = -.1588$, $p = .588$) or 2 hours post-exercise ($r = .358$, $p = .209$), the authors concluded that there is no significant correlation between post-exercise il-6 and CRP. Since the investigators only monitored CRP up to 2 hours post exercise it is not surprising there was no recorded increase in this marker. No data points were recorded 16-24 hours post-exercise. It is likely that an increase in CRP would be seen during this time since hepatocyte production of CRP in response to an inflammatory response has been shown to occur >6 hours following the inflammatory stimulus (92). A more appropriate measure of correlation between post-exercise il-6 and CRP would be to compare peak values of each marker; 0-1 hour post-exercise for il-6 and 16-24 hours post-exercise for CRP.

A mechanism for the apparent attenuated post-exercise circulating il-6 response displayed in trained individuals has yet to be elucidated. As a result, researchers have designed studies in order to manipulate variables that potentially have an effect on il-6 production. In unpublished research, Gleeson & Bishop (2000) reported that cycling

exercise in a glycogen-depleted state enhances the post-exercise plasma il-6 response. In this study cyclists performed 60 min of exercise at 75% VO_{2max} followed by a time trial (equivalent to 30 min work at 80% VO_{2max}). Peak post-exercise elevations of plasma il-6 were approximately 11-fold higher in the low CHO group (in which subjects were given < 1 g CHO/kg/day for three days prior to testing) in comparison to subjects in the high CHO group (receiving approximately 8 g CHO/kg/day for three days prior to testing). Although no data indicating a difference in skeletal muscle glycogen stores between groups was recorded, it is likely that the low CHO group began the exercise bout with lower levels of stored skeletal muscle glycogen. This research represents a crude relationship between pre-exercise glycogen stores and post-exercise systemic il-6.

Steensberg (84) further explored the relationship between skeletal muscle glycogen content and post-exercise muscular il-6 production by having young physically active males complete a two-legged concentric knee-extensor exercise, where one leg was depleted of glycogen prior to the exercise bout. The pre-exercise glycogen content of the glycogen depleted leg was ~40% lower ($P<.05$) than the control leg; 200 and 350 mmol glocosyl U (kg dry wt)$^{-1}$ respectively. The exercise stimulus consisted of concentric knee-extensions over a range of ~60 degrees (90-30 degree angle) at 40% of maximum power until exhaustion (4-5 hours). Net il-6 release from each leg was evaluated by measuring the arterial-venous difference in il-6 concentration in each leg before and during the exercise bout. Throughout the exercise bout the workload did not differ between legs. Prior to exercise no net il-6 release from either leg was detected. At 1 hour into the exercise protocol an arterial-venous difference was demonstrated in the glycogen depleted leg. In contrast, an arterial-venous difference in the control leg was not observed until subjects reached exhaustion. These findings suggest that pre-exercise

glycogen content in the active muscle influences il-6 release from that muscle regardless of circulating substrates and hormones. Considering this research in combination with previous research by Steensberg (85), demonstrating that exercise-induced elevations in circulating plasma il-6 can be accounted for by the active skeletal muscle; it appears that pre-exercise skeletal muscle glycogen stores affect the systemic plasma il-6 response to concentric exercise.

The multiple adaptations that occur in response to endurance training are well established. One of these adaptations is an increase in skeletal muscle glycogen storage. As evidenced by Putman (70), 7 or 8 consecutive days of cycle training for 2 hours per session at an intensity of 60% of individual VO_{2max} was enough to elevate skeletal muscle glycogen content both at rest and during exercise. The subjects in this study were healthy untrained males. In another training study, Fischer (23) had young healthy untrained males complete 1 hour of isolated knee extensions 5 days per week at 75% of pre-exercise maximum wattage sustainable for 1 minute. Training was performed on a modified ergometer and the duration of the training program was 10 weeks. The training workload was increased 5-10% every fortnight depending on subject progress. Mean values of resting skeletal muscle glycogen increased significantly ($p<.05$) from 400 to 750 mmol/kg dry wt. throughout the training program. Since endurance training elevates skeletal muscle glycogen, and pre-exercise glycogen stores dramatically affect post-exercise il-6 values, training-induced increases in skeletal muscle glycogen stores likely contribute to the attenuated il-6 elevation in trained subjects.

Oxidative stress has also been indicated as a stimulus for skeletal muscle il-6 production. Kosmidou (41) investigated the effect of reactive oxygen species on various skeletal muscle cells in response to work by Thannickal (88) that examined the cellular

signaling ability of free radicals. Results of this study demonstrated that skeletal myotubes, differentiated from C2C12 murine skeletal myoblasts, produced il-6 in response to pyrogallol (PYR), xanthine/xanthine oxidase (X/XO), or hydrogen peroxide (H_2O_2). In contrast, skeletal myoblasts and endothelial cells showed no il-6 response to similar exposure. It is also of interest that superoxide dismutase (SOD) and catalase (CAT) inhibited the reactive oxygen species-induced increase in il-6 from myotubes. Thus, it is suggested that oxidative stress plays a role in skeletal muscle il-6 production.

A number of researchers have administered exogenous antioxidants before exercise in order to attenuate the elevation of circulating il-6 post-exercise. The results of such research have demonstrated varying results. Vassilakopoulos (89) examined the cytokine response to 45 minutes of cycle ergometer exercise at 70% of VO_{2max} in the same subjects with and without antioxidant supplementation. Subjects were young, healthy, but untrained males with mean VO_{2max} values of 38.9 ml/kg/min. The two trials were completed in the same order for each subject and separated by one month. Antioxidant supplementation consisted of 200mg vitamin E, 50,000 IU vitamin A, and 1,000mg vitamin C daily for 60 days; allopurinol 600mg/day for 15days; and N-acetylcystein 2g/day for 3 days and 800mg the day before the second exercise bout. Without supplementation subjects displayed a peak post-exercise il-6 concentration of 7.3 pg/ml. With supplementation the peak il-6 concentration post-exercise was 2.5 pg/ml; significantly diminished ($p<.05$). Fischer et al. (22) also found a blunted post exercise il-6 elevation with antioxidant supplementation. Subjects in this study were physically active non-athletes. Subjects were assigned to either treatment, vitamin C 500mg/day and vitamin E 400 IU/day for 29 days, or placebo. The exercise bout consisted of 3 hours of concentric knee extensions at 50% of maximum power. Peak post-exercise plasma il-6

values for the placebo and treatment group were 21 pg/ml and 11 pg/ml respectively ($p<.05$). These studies indicate that antioxidant supplementation attenuates the post-exercise il-6 response to concentric exercise in untrained males.

In contrast, other research indicates that post-exercise il-6 elevations in highly trained athletes are unaffected by similar antioxidant supplementation (57, 54, 56). The exercise bouts incorporated in these studies included a 50km ultramarathon, a 60km ultramarathon, and an ironman triathlon respectively. Researchers have suggested that the discrepancy between the varied results may be due to enhanced muscular and systemic antioxidant capacity achieved from strenuous physical training (89). Much evidence has been reported suggesting that skeletal muscle antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) are enhanced in response to regular endurance training (67, 38, 45, 79). As such, enhanced skeletal muscle antioxidant status due to training may play a role in the attenuated il-6 response to concentric-based muscle activity.

Chapter 3: Methodology

3.1 Study Design

This study was cross-sectional mixed model design intended to monitor both resting and post-exercise measures of systemic inflammation at different time points prior to and in response to a sub maximal endurance cycle ergometer challenge in both aerobically trained and untrained males aged 19-35. The independent variables were training status and training history while the dependent variables, measuring systemic inflammation, were the systemic serum concentrations of il-6 and CRP pre-exercise and at assigned time points after a submaximal exercise bout. Blood glucose was also recorded before and immediately after the exercise bout to ensure similar blood glucose levels between groups. Subjects were assigned to either a trained or untrained group based upon measures of maximal oxygen consumption (VO_{2max}) and training history. Assignment to the trained group required a cycle VO_{2max} $\geq$65 ml/kg/min and a VO_{2max} $\leq$45 ml/kg/min for the untrained group. Experimental groups were matched for age and body mass index (BMI). Following assignment to experimental group, blood samples were analyzed for blood glucose, il-6 and CRP at rest (pre-exercise) and following a predetermined exercise challenge. For post-exercise measures il-6 samples were drawn 30 minutes and 24 hours post-exercise, and CRP samples were drawn 24 hours post-exercise. Blood glucose was recorded immediately pre- and immediately post-exercise. The exercise stimulus was a 45 minute bout on a cycle ergometer at an intensity corresponding to individual ventilatory threshold.

3.2 Participants

Healthy males aged 18-35 were selected from the University of British Columbia campus and the greater Vancouver area. Male participants only were selected in order to eliminate potential antioxidant effects of female sex hormone fluctuations. Estrogen has antioxidant properties potentially reducing oxidative stress while systemic estrogen is elevated (13). In addition, there seems to be a reduced il-6, il-1, and TNF-α response to typical stimuli in females compared to males (77). After providing informed consent participants were selectively enrolled into the study in accordance with the predetermined eligibility criteria for the respective experimental groups. After eligibility determination on the first day of testing subjects were assigned to groups based upon fitness status. A total sample size (N) of 26, 13 subjects per group, was determined via power analysis for a 2x3 mixed design analysis of variance (ANOVA) design. This sample size was designed to result in a power of .80 in detecting a difference between groups on the main factor of post-exercise plasma il-6 concentration, with a main effect size of (f^2=.32).

Of the 26 subjects that completed the study 13 trained and 12 untrained subjects were included in the data analysis. One subject from the untrained group was excluded from the study at a later date due to a breach of protocol.

3.3 Eligibility Criteria

(1) Males aged 18-35 years old,

(2) Normal BMI: 18.5-24.9 kg/m^2 [Weight (kg)/height $(m)^2$],

(3) Maximal Oxygen consumption $\leq$ 45 ml/kg/min (cycle ergometer) and not participating regularly in physical exercise $\leq$ 2 hours per week **OR** Maximal Oxygen consumption $\geq$ 65 ml/kg/min (cycle ergometer) and regularly training at an elite level,

(4) Current and expected Vancouver Resident for duration of study,

(5) No antioxidant/Vitamin supplementation $\leq$ 6 weeks prior to study,

(6) No use of steroidal anti-inflammatory medication $\leq$ 6 weeks prior to study,

(7) Healthy,

i. No history of chronic disease (CVD, endocrine, hepatic, inflammatory, etc…)

ii. No traumatic or chronic injuries present $\leq$ 6 months prior to study

iii. No febrile illness/infection $\leq$ 6 weeks prior to beginning of study

(8) Non-smoker

(9) No current or planned caloric intake restriction,

(10) No shift workers (potential for circadian rhythm il-6 abnormalities)

3.4 Procedures

After initial contact and voluntary agreement to participate, subjects provided informed consent and were immediately enrolled into the study. All procedures were approved by the Clinical Research Ethics Board of the University of British Columbia and Providence Healthcare. Prior to any physical testing subjects completed a physical activity readiness questionnaire (PAR-Q). Upon completion of a PAR-Q and medical clearance each subject underwent baseline aerobic fitness and body composition measures in the John M. Buchanan exercise physiology laboratory. Baseline fitness measures were completed following 24 hours without exercise and a ≥2 hour fast. Body composition was recorded as both BMI (mass (kg)/height $(m)^2$) and the sum of three skinfolds (axilla, abdominal, and mid-thigh). Also at this time subjects completed a questionnaire (Appendix B) in order to establish training history. Aerobic fitness was assessed using a maximal oxygen consumption (VO_{2max}) cycle ergometer protocol. Subjects were allowed a five minute self-selected warm up before the VO_2max protocol began. The VO_2max protocol began at 50 watts with workload increases of 25 watts every minute until volitional fatigue. A maximal test was confirmed based on pre-determined indicators; respiratory exchange ratio (RER) above 1.1, HR within ten beats of age predicted maximum HR (220-age), and a plateau in VO2max [either a decrease or an increase of <2 ml/kg/min]. VO_{2max} was determined by averaging the highest VO_2 values over two consecutive 20 s intervals.

In addition to measurement of VO_2max, ventilatory threshold was calculated using ventilatory equivalence (Ve/VO_2) in order to establish a subsequent individual exercise challenge workload for each subject. Ventilatory equivalence was assessed by trained exercise physiologists. The ergometer utilized in this protocol was an 800 series

Sensormedics electronically braked cycle ergometer. Expired gases were analyzed with a Sensormedics Vmax 29 metabolic cart.

At least 72 hours after baseline fitness measures were recorded subjects underwent the exercise challenge on a cycle ergometer at a predetermined workload. The work bout was a 45 minute cycle at a wattage corresponding to individual cycle ventilatory threshold. In preparation for the execise challenge subjects were asked to avoid strenuous physical activity 72 hours prior to and physical activity entirely for 48 hours prior to the exercise challenge. Also, subjects were asked to refrain from alcohol consumption within 48 hours of testing.

On the day of the exercise challenge subjects reported to the St. Paul's Hospital Healthy Heart fitness gym between 8am and 10am following an overnight fast (water only past 11pm the previous night). Subjects were encouraged to travel to the hospital using the least physically strenuous means available. At this time a technician drew one resting venous blood sample from an antecubital vein in the subjects' preferred arm; the sample was later divided for baseline serum CRP measurement and serum il-6 measurement. After this a finger prick blood sample was analyzed for blood glucose. Glucose was recorded to determine whether any subjects began the exercise bout with abnormal blood glucose concentrations or had a significant change throughout the bout. This was of great importance as blood glucose status has been shown to have a large affect upon skeletal muscle il-6 production. Upon completion of the blood sampling subjects completed a 5 minute self-selected warm up on an electronically braked cycle ergometer. After warming up subjects completed the predetermined exercise challenge (45 minutes at a wattage corresponding to individual cycle ventilatory threshold). Heart rate was recorded every minute and rating of perceived exertion (RPE), on a Borg 15

point scale, was recorded every five minutes during the exercise bout. If the assigned workload was not manageable individual workload was adjusted in order to maintain a heart rate within ten beats per minute of that corresponding to ventilatory threshold determined on day one. Immediately after the exercise bout another finger prick blood sample was analyzed for blood glucose. After this glucose analysis subjects remained seated in the laboratory for 15 minutes still fasting. Approximately 15 minutes post-exercise subjects were weighed in order to monitor fluid loss. Water was consumed accordingly to replenish fluid loss and achieve pre-exercise weight. Thirty minutes post-exercise another blood sample was drawn for measurement of post-exercise serum il-6. After this sample subjects were asked to return to the clinic in 24 hours and were free to leave. Subjects were asked to refrain from vigorous physical activity and alcohol consumption within this 24 hour period. Twenty-four hours following the cessation of the exercise bout subjects returned to the hospital lab for another blood sample to measure 24 hour post-exercise serum il-6 and CRP. A protocol timeline is included as Chart 4.1.

3.5 Outcome measures

Intereukin-6:

For il-6 analysis blood was collected in 5 ml samples into serum separator tubes containing silica and polymer gel for serum separation. Samples were drawn by venipuncture from an antecubital vein of the subjects arm at predetermined points in time. Serum separator tubes were then inverted 5 times and left to clot for 30 minutes. At that time samples were centrifuged at 1100-1300 x gravity for 10-15 minutes. Serum was then aliquoted and stored in a freezer at $\leq$ -80° C until assayed. Serum il-6 concentration

was determined using a commercially available quantikine high-sensitivity enzyme linked immunosorbent assay (ELISA) kit (R & D Systems, Minneapolis, Minn. USA). The ELISA kit used has a mean intra-assay coefficient of variation of 7.4 and a mean inter-assay coefficient of variance of 7.3. Samples were tested in duplicate.

C-reactive protein:

For CRP analysis blood was collected in 5 ml samples into serum separator tubes containing silica and polymer gel for serum separation. Samples were drawn by venipuncture from an antecubital vein of the subjects arm at predetermined points in time. Serum separator tubes were then inverted 5 times and left to clot for 30 minutes. At that time samples were centrifuged at 1100-1300 x gravity for 10-15 minutes. Serum was then aliquoted and stored in a freezer at $\leq$ -20° C until assay within 24 hours. Serum CRP was evaluated using an Immulite 2000 High Sensitivity solid-phase, chemiluminescent immunometric assay (Diagnostic Products Corporation, Los Angeles, California. USA). The selected assay has mean inter- and intra-assay coefficients of variation of < 5%.

Blood Glucose:

Low blood glucose concentrations during exercise have been shown to enhance the post-exercise increase in il-6. Blood glucose concentrations were recorded using a portable hand held glucose meter (Bayer Glucometer Elite with Bayer Ascensia ELITE test strips, Bayer Corporation, Elkhart, Indiana. USA). Finger prick capillary samples were drawn from a clean and dry self-selected finger for each analysis. In combination the Bayer Glucometer Elite and Bayer Ascensia ELITE test strips have an intra-assay coefficient of variation of 4.0% and an inter-assay coefficient of variation of 5.3% at normal blood glucose concentrations.

Rate of Perceived Exertion:

During the 45-minute cycle ergometer challenge subjects indicated ratings of perceived exertion every five minutes using a 15-point Borg scale. The mean of all RPE scores for each subject was taken as that subject's RPE score for the exercise challenge.

3.6 Data Analysis

Data analysis was completed using SPSS software (version 12.0). All data was first tested for normality of distribution and homogeneity of variance prior to further statistical analysis. All measurements subjected to analyses were reported as means. Demographic and physiological descriptive statistics compared between groups were analyzed by independent t-tests. Significance for each t-test was set at $\alpha = .05$. Systemic il-6 concentrations were analyzed using a 2 (Groups: trained and untrained) x 3 (Time: pre-, 30 minutes post-exercise, and 24 hours post-exercise) mixed design analysis of variance (ANOVA) with repeated measures on the second variable. If needed, violations for sphericity were corrected using the Greenhouse-Geisser correction. A 2 (Groups: trained and untrained) x 2 (Time: pre- and 24 hours post-exercise) mixed design ANOVA was used to analyze serum CRP concentrations. A 2 (Groups: trained and untrained) x 2 (Time: pre- and immediate post-exercise) mixed design ANOVA was also used to analyze blood glucose concentrations. Significant main or interaction effects for il-6, CRP, and blood glucose were further analyzed using post-hoc t-tests. Significance for each t-test was set at $\alpha = .05$.

Relative values of peak Il-6 per workload (wattage, relative oxygen consumption, and absolute oxygen consumption) at 30 minutes post-exercise were also compared between groups using independent t-tests. Significance was set at $\alpha = .05$.

The correlational relationship between pre- versus post-exercise differences in il-6 and CRP were analyzed in both fitness groups. The correlational relationship between fitness variables and baseline values of il-6 and CRP were also analyzed. Correlation was assessed using the Pearson product moment calculation. Significance was set at $\alpha = .05$.

Chapter 4: Results

4.1 Subject Demographic Characteristics

All subjects in the study were between the ages of 19 and 35, with an average age of (25.1 +/- 4.6 years), and prescreened for potential medical contraindications to participation. As planned, the trained and untrained groups in the study were matched with respect to non-performance related demographic characteristics (Table 4.1). No significant differences between the trained and untrained group were found in age, height, mass or BMI. However, the sum of skin folds SOS was greater in the untrained 36.2 +/- 8.5 mm compared to the trained group 22.8 +/- 4.7 mm $F(1,23) = 2.16$, $p = 0.000$.

In accordance with subject inclusion criteria the groups differed in training history. The trained group participated in 13.5 +/- 3.5 hours of general exercise per week compared to 2.8 +/-1.9 hours in the untrained group $F(1,23) = 2.5$, $p = 0.000$ and the trained group cycled for 9.46 +/- 3.9 hours per week compared to 0.2 +/- 0.4 hours in the untrained group $F(1,23) = 34.6$, $p = 0.000$.

4.2 Subject Physiological and Performance Characteristics

Due to the nature of the study, subjects were assigned to groups based upon training history and aerobic fitness on a cycle ergometer. As such the two groups differed significantly in most of these variables (Table 4.2).

The trained group had significantly higher values than the untrained group for both absolute maximal oxygen consumption (VO_{2max}); 5.01 +/- 0.61 L/min compared to 3.29 +/- 0.65 L/min O_2 $F(1, 23) = 0.067$, $p = 0.000$ and relative maximal oxygen

consumption (VO2max) 68.61 +/- 5.57 ml/kg/min compared to 42.42 +/- 4.62 ml/kg/min F (1, 23) = 0.233, p = 0.000 respectively. Absolute and relative oxygen consumptions at ventilatory threshold (VT) were also significantly higher in the trained group compared to the untrained group; 3.57 +/- 0.51 L/min compared to 2.00 +/- 0.36 L/min F (1, 23) = 3.18, p = 0.000 and 48.90 +/- 5.80 ml/kg/min compared to 25.80 +/- 2.68 ml/kg/min F (1, 17.20) = 5.08, p = 0.000 respectively. Although the workload assigned to subjects was an intensity corresponding to VT, VO2 at VT was a significantly greater percentage of maximal oxygen consumption [% VO2max (ml/kg/min) at VT] in the trained group compared to the untrained group; 71.1 +/- 4.6 % compared to 61.0 +/- 4.6 % F (1, 23) = 0.168, p = 0.000.

Maximal heart rate (HRmax) and average heart rate during the exercise bout (HRavg) were not significantly different between groups. However, heart rate at VT was significantly higher in the trained group 161.9 +/- 9.7 BPM than the untrained group 151.6 +/- 11.6 BPM F (1, 23) = 0.005, p = 0.024.

Performance characteristics differed greatly between groups. Peak aerobic power output and power output at VT were significantly higher in the trained group compared to the untrained group; 431 +/- 49 watts compared to 267 +/- 55 watts F (1, 23) = 0.042, p = 0.000 and 265 +/- 38 watts compared to 139 +/- 27 watts F (1, 23) = 0.751, p = 0.000 respectively.

4.3 Subject Compliance

4.3.1 Protocol

Of the initial 26 subjects enrolled in the study one failed to comply with protocol. This subject deviated from proposed protocol by consuming alcohol the night before day 2 of testing. This was not reported by the subject until after day 2 of testing. The data for this subject was excluded from data analysis resulting in an N of 25 instead of 26. Otherwise all subjects complied, to the best of our knowledge, with protocol including appropriate fasting, avoidance of exercise, and avoidance of alcohol consumption.

4.3.2 Performance

All subjects were able to complete the exercise bout on day 2. In Table 4.3 assigned and completed power outputs and heart rates are presented for each group demonstrating that both groups successfully completed their assigned exercise bouts. The power output (PO) achieved by each group was somewhat lower than that assigned but both groups achieved a PO not significantly different than that assigned from assessment on day 1 (Table 4.3). Both groups also achieved an average heart rate during the exercise bout on day 2 similar to that corresponding to VT.

4.4 Outcome Measures

4.4.1 Interleukin-6

It was hypothesized that peak post-exercise Il-6 concentrations would be significantly lower ($p < 0.05$) in the trained group compared to the untrained group. It was also hypothesized that baseline concentrations of Il-6 would not be significantly different between groups. At each of three time points, pre-exercise, 30 minutes post-exercise, and 24 hours post-exercise, mean Il-6 concentrations were somewhat lower in the trained group. However, this difference was not statistically significant (Table 4.4).

It was also hypothesized that Il-6 concentrations in both groups would increase significantly following the assigned 45-minute exercise challenge. When analyzed there was found to be a significant difference for the within-subjects effect over the three Il-6 samples $F(1.60, 21.31) = 30.49$, $p = 0.000$ after Greenhouse Geisser correction. Further post-hoc tests indicated Il-6 concentrations in both groups increased significantly between pre-exercise and 30 minutes post-exercise samples; trained 0.598 +/- 0.535 pg/ml to 1.826 +/- 0.741 pg/ml ($p < 0.01$) and untrained 0.914 +/- 0.896 pg/ml to 2.027 +/- 1.095 pg/ml ($p < 0.01$) (Table 4.5).

4.4.2 C-Reactive Protein

It was hypothesized that CRP concentrations would be similar between groups at rest and would increase significantly 24 hours post-exercise in the untrained group but not the trained group. When analyzed there were no significant within-subjects effects $F(1,23) = 0.604$, $p = 0.445$ indicating that CRP values remained similar over time within

each group (Table 4.3). A significant effect was found when between subjects effects were analyzed F (1,23) = 7.655, p = 0.011. Further post-hoc tests indicated that CRP concentrations were lower in the trained group for the pre-exercise and 24 hours post-exercise sample; pre-exercise 0.162 +/- 0.247 mg/L compared to 1.167 +/- 1.427 mg/L $p < 0.05$, 24 hours post-exercise 0.192 +/- 0.202 mg/L compared to 1.233 +/- 1.235 mg/L $p < 0.05$ (Table 4.4).

4.4.3 Correlations

It was hypothesized that differences in pre- and post-exercise concentrations of plasma il-6 would show a significant correlation ($\alpha \leq .05$) with differences in pre- and post-exercise concentrations of CRP in both study groups. Using the Pearson product moment correlation there was no significant correlation between pre- to 30 minutes post-exercise elevations of Il-6 and pre- to 24 hours post-exercise elevations of CRP in either group or both groups combined. However, when the two groups were analyzed separately, baseline concentrations of Il-6 and CRP were significantly correlated in the untrained group ($r=0.69$, $P=0.01$, $r^2=0.48$), representing a moderate relationship, but not in the trained group $r=-0.13$, $P=0.68$, $r^2=0.02$). Peak concentrations of Il-6 and CRP were not significantly correlated in either group.

4.4.4 Blood Glucose

Blood glucose concentrations were recorded immediately before and immediately after the exercise bout on day 2 for each subject to monitor potential differences between groups. There were no significant differences in blood glucose concentrations before or

after exercise and there were also no differences between pre- and post-exercise blood glucose concentrations in either group (Table 4.5). All blood glucose values were within healthy limits with a minimum of 4.6 mmol/L and a maximum of 6.3 mmol/L.

4.4.5 Ratings of Perceived Exertion

Average ratings of perceived exertion were recorder from each subject during the exercise bout on day 2 to compare subjective feelings of workload between groups. Ratings of perceived exertion were found to be similar between the trained and untrained groups (Table 4.4).

Figure 4.1 Subjects screened, consented, assigned, and completed

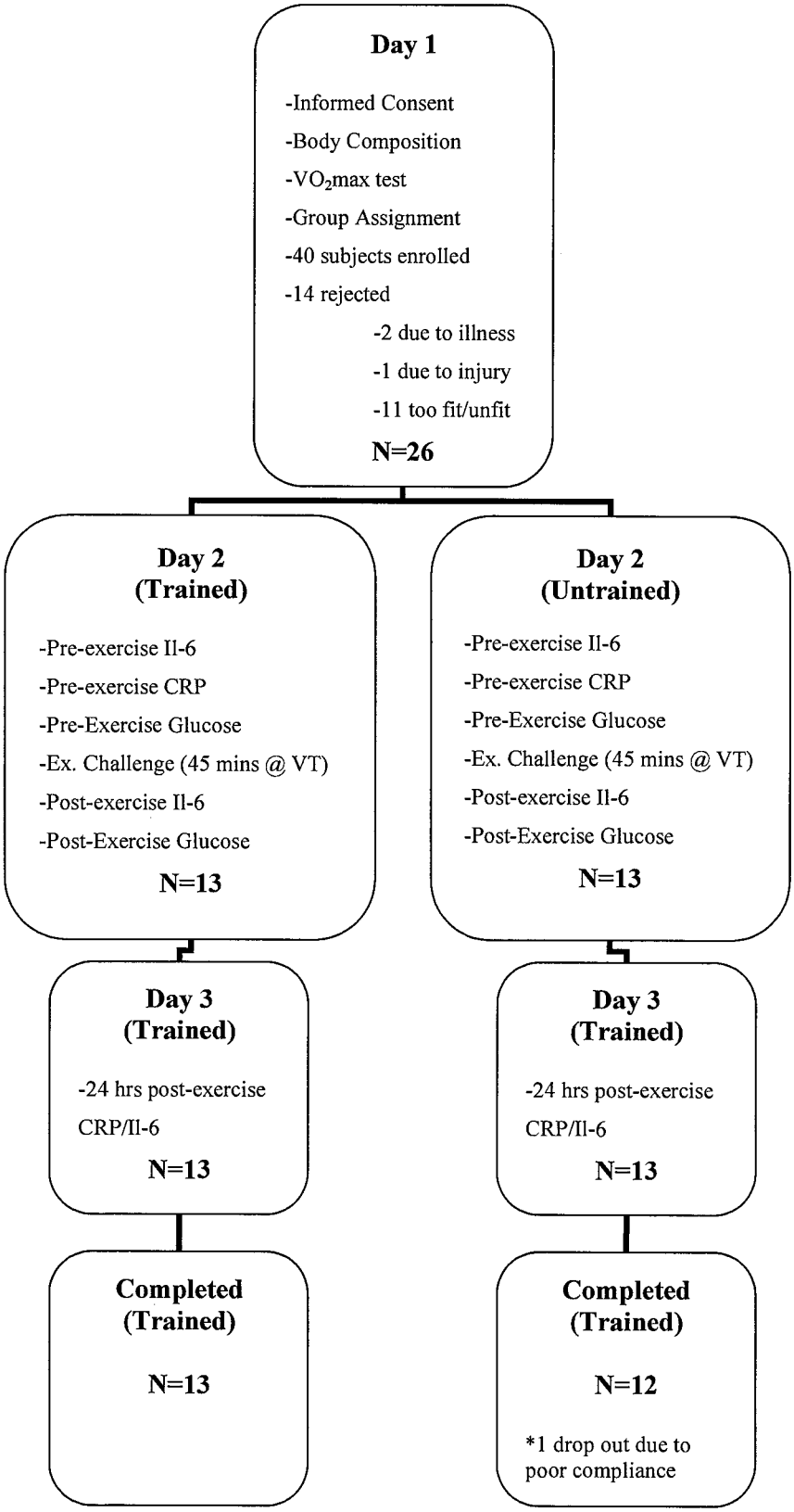


Table 4.1 Subject Demographic Characteristics

Variables	Trained (n=13)	Untrained (n=12)	*P*
Age (Years)	26.7 +/- 4.9	23.4 +/- 3.8	NS
Race			
Caucasian	12	10	NS
Asian	1	1	NS
	0	1	NS
Body Composition			
Height (cm)	179 +/- 5.7	179 +/- 8.7	NS
Mass (kg)	73.0 +/- 7.8	77.9 +/- 15.0	NS
BMI (kg/m^2)	22.6 +/- 1.4	23.9 +/- 3.0	NS
SOS	22.8 +/- 4.7	36.2 +/- 8.5*	0.000
Training Habits			
Exercise per week (hrs)	13.4 +/- 3.5	2.8 +/- 1.9*	0.000
Cycling per week (hrs)	9.5 +/- 3.9	0.2 +/- 0.4*	0.000
Pimary Event			
Cycling	10	0	NS
Triathlon	3	0	NS

Values are given as mean +/- SD, NS = Not Significant, * indicates significant difference ($p \leq 0.05$).

Table 4.2 Subject Physiological and Performance Characteristics

Variables	Trained (n=13)	Untrained (n=12)	*P*
Respiratory			
VO2max (L)	5.01 +/- 0.61	3.29 +/- 0.65*	0.000
VO2max (ml/kg/min)	68.61 +/- 5.57	42.42 +/- 4.62*	0.000
VO2 (L) at VT	3.57 +/- 0.57	2.00+/- 0.36*	0.000
VO2 (ml/kg/min) at VT	48.90 +/- 5.80	25.80 +/- 2.68*	0.000
% VO2max (ml/kg/min) at VT (%)	71.1 +/- 4.6	61.0 +/- 4.6*	0.000
Cardiovascular			
HRmax (BPM)	188.1 +/- 7.1	187.9 +/- 10.1	NS
HR at VT (BPM)	161.9 +/- 9.7	151.6 +/- 11.6*	0.024
HRavg during challenge (BPM)	162.7 +/- 7.1	153.0 +/- 18.5	NS
Performance			
Peak Aerobic PO (Watts)	430.8 +/- 49.1	266.7 +/- 54.7*	0.000
PO at VT (Watts)	265.4 +/- 37.5	138.75 +/- 26.9*	0.000
PO during challenge (Watts)	247.3 +/- 30.8	132.5 +/- 29.5*	0.000

Values are given as mean +/- SD, NS = Not Significant, * indicates significant difference ($p \leq 0.05$).

Table 4.3 Performance Outcome Measures Within Groups

Variable	Assigned	Completed	*P*
Heart Rate (bpm)			
Trained	161.9 +/- 9.7	162.7 +/- 7.1	NS
Untrained	151.6 +/- 11.6	153.0 +/- 18.5	NS
Power Output (Watts)			
Trained	265.4 +/- 37.5	247.3 +/- 30.8	NS
Untrained	138.75 +/- 26.9	132.5 +/- 29.5	NS

Values are given as mean +/- SD, NS = Not Significant, * indicates significant difference ($p \leq 0.05$).

Table 4.4 Outcome Measures Between Groups

Variables	Trained (n=13)	Untrained (n=12)	*P*
Il-6			
Pre-Exercise (pg/ml)	0.598 +/- 0.535	0.914 +/- 0.896	NS
30min Post-Exercise (pg/ml)	1.826 +/- 0.741	2.027 +/- 1.095	NS
24hrs Post-Exercise (pg/ml)	0.633 +/- 0.350	0.931 +/- 0.902	NS
CRP			
Pre-Exercise (mg/L)	0.16 +/- 0.25	1.17 +/- 1.43*	< 0.05
24hrs Post-Exercise (mg/L)	0.19 +/- 0.20	1.23 +/- 1.23*	< 0.05
Blood Glucose			
Pre-Exercise (mmol/L)	5.2 +/- 0.4	5.4 +/- 0.2	NS
Post-Exercise (mmol/L)	5.3 +/- 0.6	5.1 +/- 0.4	NS
Rating of Perceived Exertion	14.3 +/- 1.1	13.8 +/- 0.8	NS

Values are given as mean +/- SD, NS = Not Significant, * indicates significant difference ($p \leq 0.05$).

Table 4.5 Outcome Measures Il-6 and CRP Within Groups

Variable	Pre-Exercise	30min Post-Exercise	24hrs Post-Exercise	*P*
Il-6 (pg/ml)				
Trained	0.598 +/- 0.535	1.826 +/- 0.741*	X	< 0.01
Trained	0.598 +/- 0.535	X	0.633 +/- 0.350	NS
Trained	X	1.826 +/- 0.741	0.633 +/- 0.350*	< 0.01
Untrained	0.914 +/- 0.896	2.027 +/- 1.095*	X	< 0.01
Untrained	0.914 +/- 0.896	X	0.931 +/- 0.902	NS
Untrained	X	2.027 +/- 1.095	0.931 +/- 0.902*	< 0.01
CRP (mg/L)				
Trained	0.16 +/- 0.25	X	0.19 +/- 0.20	NS
Untrained	1.17 +/- 1.43	X	1.23 +/- 1.23	NS

Values are given as mean +/- SD, NS = Not Significant, * indicates significant difference ($p \leq 0.05$).

Table 4.6 Outcome Measure Blood Glucose Within Groups

Variable	Pre-Exercise	Post-Exercise	*P*
Blood Glucose (mmol/L)			
Trained	5.2 +/- 0.4	5.3 +/- 0.6	NS
Untrained	5.4 +/- 0.2	5.1 +/- 0.4	NS

Values are given as mean +/- SD, NS = Not Significant, * indicates significant difference ($p \leq 0.05$).

Figure 4.2 Interleukin-6 Concentrations

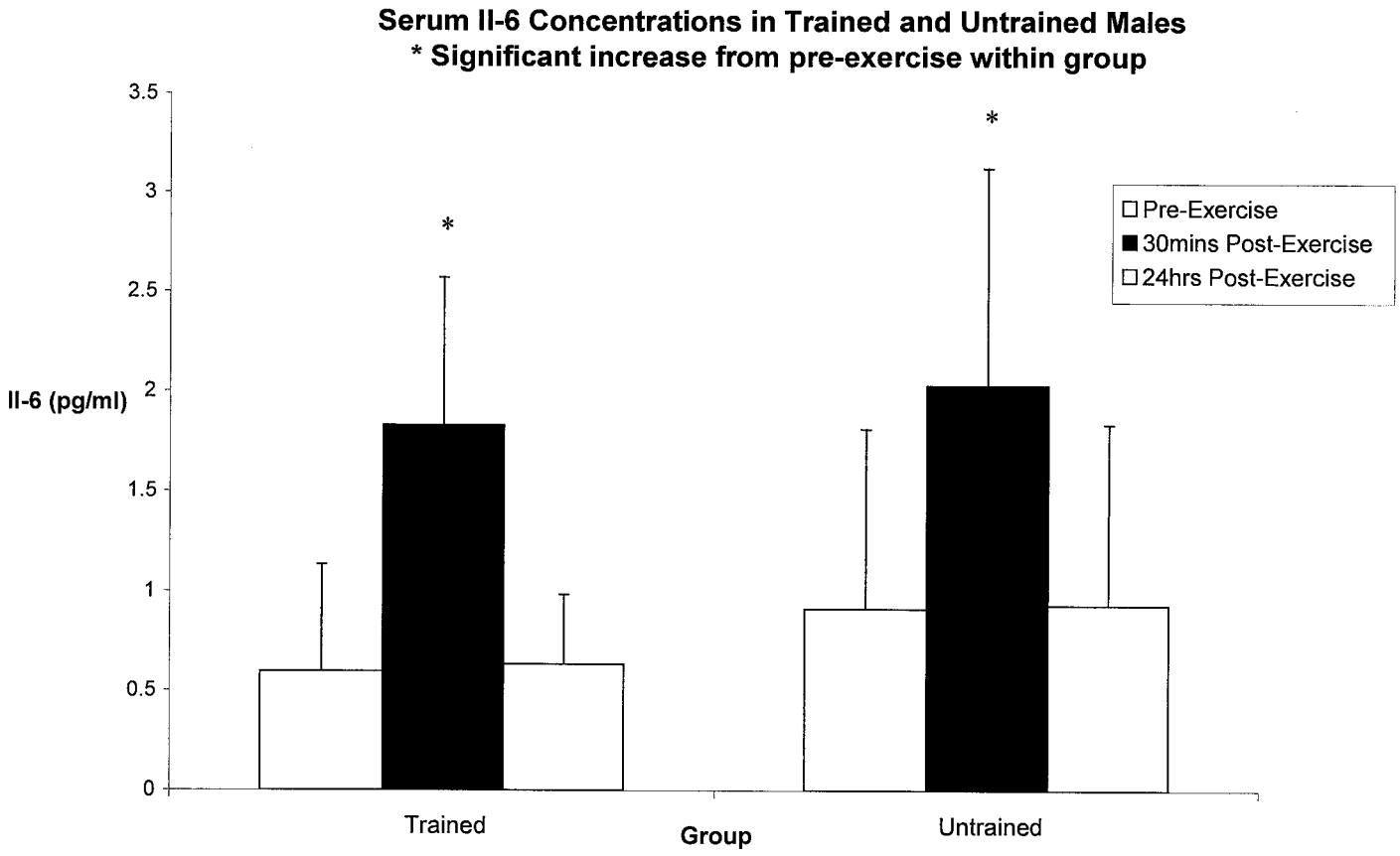


Figure 4.3 C-Reactive Protein Concentrations

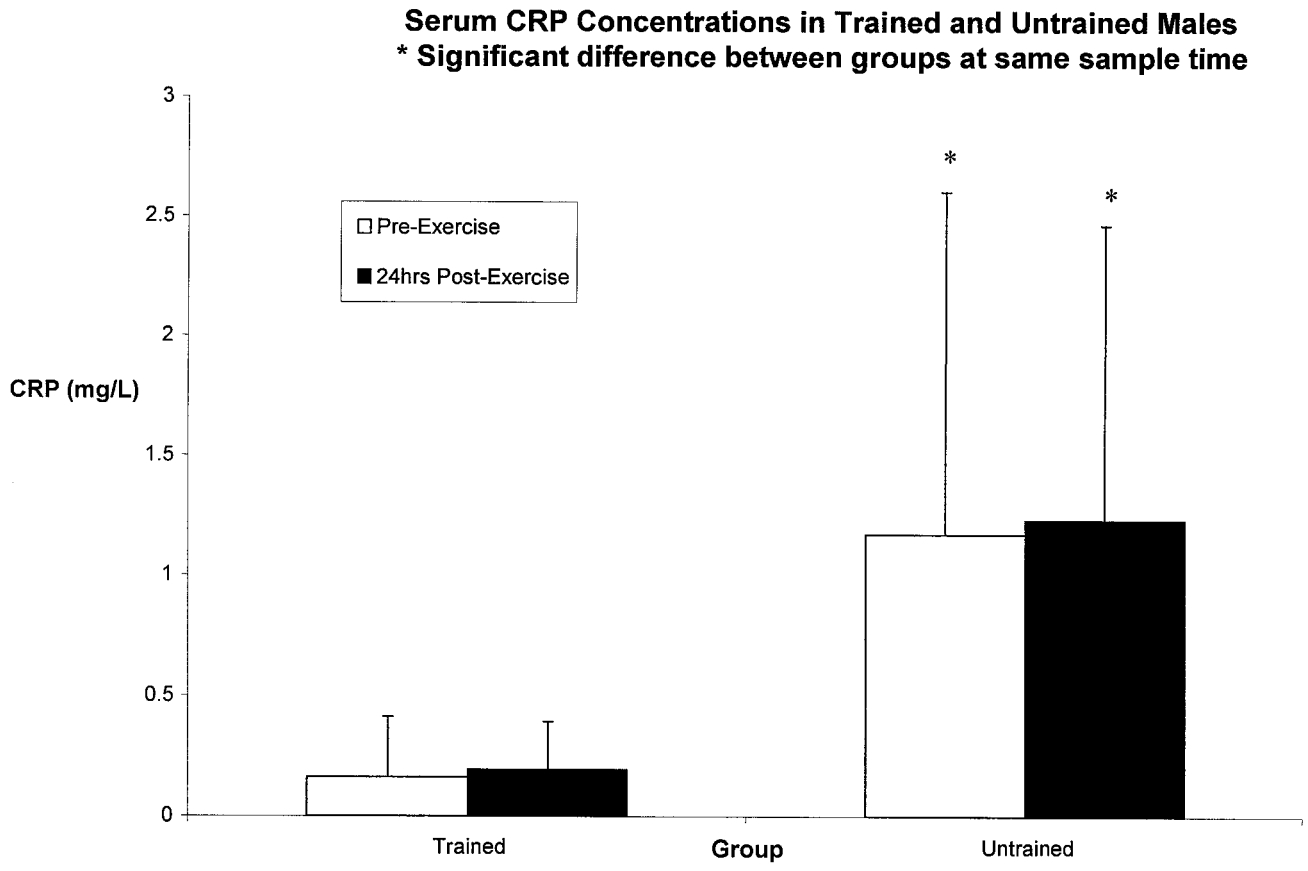
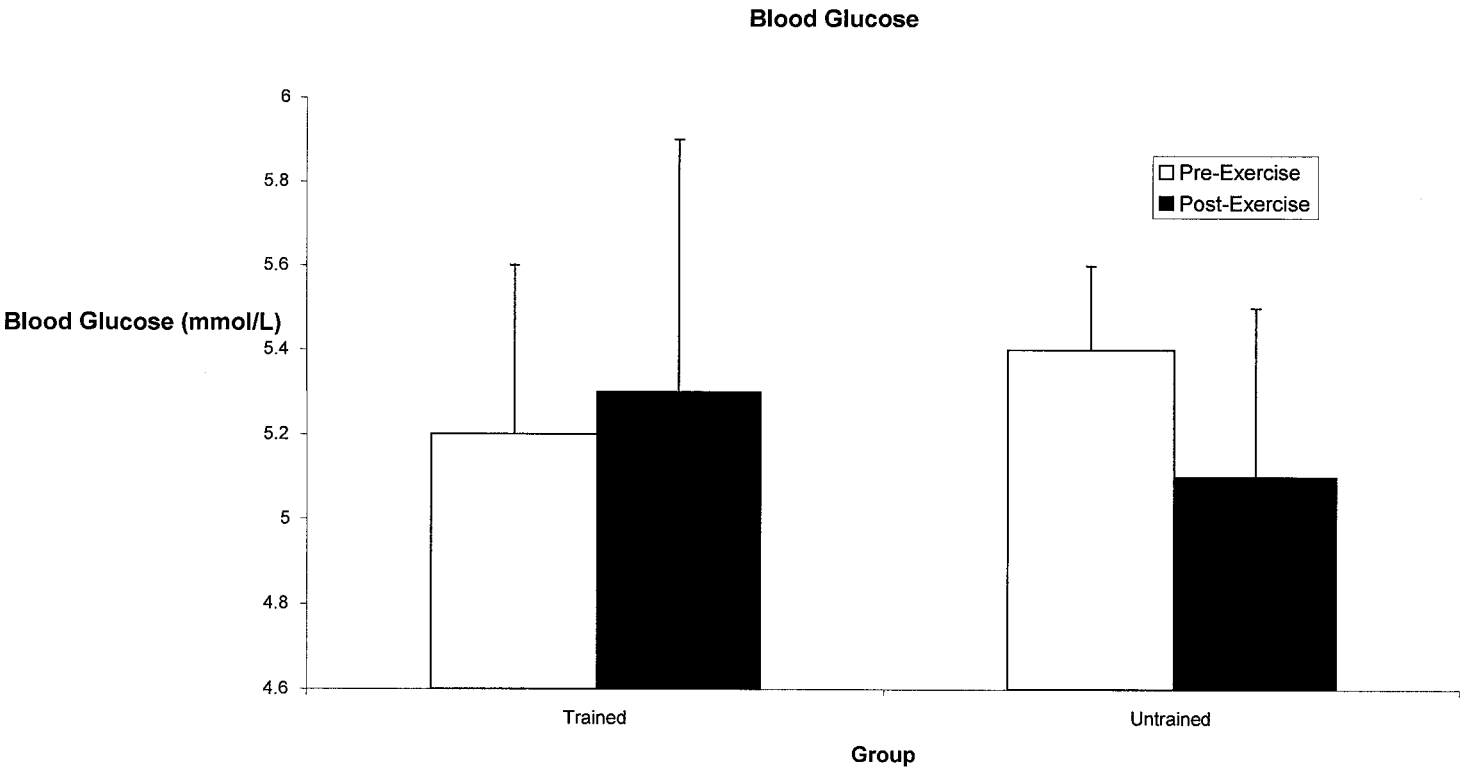


Figure 4.4 Blood Glucose Concentrations



Chapter 5: Discussion

5.1 General Findings

This cross-sectional study is, to the best of our knowledge, the first to compare resting and post-exercise measures of inflammatory proteins Il-6 and CRP between trained and untrained healthy males following a similar exercise challenge. This analysis provides new information on the effect of training on inflammation and the relationship between Il-6 and CRP. The main findings of this investigation are the following:
1) Systemic concentrations of Il-6 increased following exercise in both trained and untrained subject groups but concentrations between groups were similar at all testing points. 2) Neither the trained or untrained group showed a significant change in CRP concentrations in response to the exercise challenge. However, at rest and 24 hours post-exercise CRP concentrations were significantly lower in the trained compared to the untrained group. 3) Pre- to post-exercise changes in Il-6 and CRP concentrations were not significantly correlated. However, resting pre-exercise concentrations of Il-6 and CRP were significantly correlated in the untrained group but not in the trained group.

5.2 Il-6

The pattern, variability, and magnitude of Il-6 concentrations found in this study are in agreement with similar studies incorporating endurance cycle ergometer exercise with trained athletes; peaking at 1.826 +/- 0.741 pg/ml at 30 minutes post-exercise and returning to baseline values 24 hours post-exercise (60, 65, 89). The variability of il-6, measured as standard deviation, at peak values was 0.741 pg/ml in the trained group and 1.095 pg/ml in the untrained group. Although these standard deviation values are high

relative to the group means they are similar to or less than that found at peak values in similar previous studies: 8.0 +/- 2.0 pg/ml after 1 hour of treadmill running at 75% of VO2max (60) and 7.3 +/- 1.5 pg/ml after 45 minutes of cycle ergometry at 70% VO2max (89).

Only one other study, to the best of our knowledge, has examined resting and post-exercise Il-6 values in sedentary subjects using a similar exercise mode and blood sampling schedule (89) to that in our study. In this study sedentary males rode on a cycle ergometer at 70% of maximal oxygen consumption for 45 minutes with Il-6 values peaking 30 minute post-exercise with a mean of 7.3 +/- 1.5 pg/ml. Comparatively the Il-6 values demonstrated in our untrained group are not similar, reaching a mean peak concentration of only 2.027 +/- 1.095 pg/ml. When comparing the exercise protocols of these studies, our untrained group was assigned a workload at ventilatory threshold, corresponding to 61.0 +/- 4.6% of VO_{2max}, while subjects in the other study maintained a workload corresponding to 70% of VO_{2max} regardless of ventilatory threshold. This 9% difference in oxygen consumption may account for the peak Il-6 discrepancy between our untrained group and those of the other study (89) considering systemic peak post-exercise il-6 has been shown to increase linearly with exercise intensity (calculated as VO_2/VO_{2max}) (63).

Although the specific stimulus or stimuli for Il-6 production during exercise have not been elucidated, it is has been demonstrated that systemic lactate is not a significant stimulus (85). Furthermore, oxidative stress has been implicated as a potential stimulus for Il-6 production in skeletal muscle (41). Considering that free radical production increases as a function of oxygen consumption (68) it seems that exercise challenges may be better matched for relative oxygen consumption as opposed to ventilatory threshold or

lactate threshold when examining Il-6 production. From a practical perspective, it is appealing to assign workloads based upon a measure closely linked to performance such as ventilatory threshold. However, in order to understand the physiological effect of training upon skeletal muscle production of Il-6 it seems workload assignment should be based upon oxygen consumption.

As hypothesized, we found that Il-6 concentrations increased significantly in both trained and untrained groups, $p < 0.01$. Contrary to our hypothesis we found that there were no significant differences in Il-6 concentration between the two groups at any time point. This in itself is remarkable as the trained group was working at a significantly higher wattage (87% higher), relative oxygen consumption (90% higher), and absolute oxygen consumption (79% higher) than the untrained group. Furthermore, as was the instance comparing the results of Vassilakopoulos' study (89) to our untrained group, the workload assigned was not based upon relative oxygen consumption. When compared by relative oxygen consumption our trained group worked at an intensity corresponding to 71.1 +/- 4.6% of maximal oxygen consumption (VO_2/VO_{2max}) whereas our untrained group worked at an intensity corresponding to 61.0 +/- 4.6%; significantly different, $p < 0.01$. In a study by Ostrowski (63) it was demonstrated that peak Il-6 concentrations in similarly trained athletes formed a significantly correlated ($p < 0.01$) linear relationship with exercise intensity (calculated as VO_2/VO_{2max}). It can be extrapolated then that the peak post-exercise Il-6 concentration difference between groups would likely have been larger if the workloads for each group were assigned based upon relative oxygen consumption.

It should also be noted that our blood sampling times were assigned based on the best estimate of when peak il-6 concentrations would occur as found by previous research

(40, 41, 89). These studies indicated that il-6 concentrations in response to concentric exercise between 0 and 30 minutes post-exercise. As such we chose 30 minutes post-exercise to draw our peak il-6 blood sample. Some researchers have indicated that peak il-6 values may even occur before the end of the exercise bout; in which case the true peak il-6 values in our subjects may have been missed. Although this is possible, the majority of studies using a concentric exercise stimulus of similar duration to ours found peak il-6 values to occur within 30 minutes after exercise (40, 41, 89).

5.3 CRP

As hypothesized, CRP concentrations in the trained group remained similar between rest and 24 hours post-exercise. It was also hypothesized that CRP values would increase significantly in the untrained group from rest to 24 hours post-exercise. Surprisingly, our untrained group showed no such increase. This hypothesis was based upon an earlier study (80) that showed a significant increase ($p = 0.04$) in CRP 24 hours post-exercise in untrained subjects after riding on a cycle ergometer for 60 minutes at an intensity corresponding to 60% of VO_{2max}. The discrepancy in findings may be due to investigation methodology. In the earlier study (80) training status was not quantified leaving the possibility that these subjects were less active than our untrained group; 2.8 +/- 1.9 hours of exercise per week and a mean relative VO_{2max} of 42.42 +/- 4.62 ml/kg/min. Health factors such as recent injury, recent illness and chronic illness are all factors that may affect CRP values and were not accounted for (80). Also, the exercise intensity is difficult to compare between studies as Smith (80), indirectly estimated a workload of 60% VO_{2max} based upon maximal heart rate alone whereas our workload was

based on direct respiratory measures of ventilatory threshold. Although surprising our results are similar to those found in a study run concurrently to ours in which highly trained and moderately trained subjects both showed no significant increase in CRP in the days following a similar moderate endurance exercise bout (66). These results indicate that the exercise stimulus used was not great enough to examine an Il-6 induced increase in CRP. Sorichter (81) monitored the CRP response to endurance exercise in trained runners (VO2max 60.02 ml/kg/min) after a one hour run at 80% on VO2max. The results from this showed significantly increased CRP values at 6 and 24 hours post-exercise with values at 24 hours post-exercise being the greatest. Our untrained group worked at a VO2 corresponding to 61% of VO2max for 45 minutes and our trained group at a VO2 corresponding to 70% of VO2max for 45 minutes. Considering il-6 is the chief stimulus for CRP production and il-6 increases after exercise as a function of exercise duration and intensity our exercise bout should have lasted at least one hour and the intensity, measured as % VO2, should have been increased by 19% and 10% for our untrained and trained group respectively.

Other studies that have examined the CRP response to various forms of exercise have used different sampling timelines. In a study by Czarkowska-Paczek (18) trained cyclists with a relative VO2max of 65.7 ml/kg/min cycled to exhaustion. The total workload completed in this study was comparable to that in our study. CRP was recorded pre-exercise, immediately post-exercise, and two hours post-exercise. CRP values showed no significant change over time. CRP was not recorded 24 hours post-exercise. Researchers in this study assumed that if CRP was to increase following exercise it would do so within two hours of the exercise bout. To our knowledge few studies have monitored systemic CRP pre-exercise, immediately post-exercise, and at subsequent time

points up to 48 hours after a discrete endurance event. Taylor (87) recorded CRP before and after an ultradistance triathlon (160 km). Measures of CRP indicated no significant changes immediately after or within five hours of exercise. The next measure was taken 24 hours post-exercise and at that time was significantly elevated. CRP then returned to near baseline by 48 hours. This study suggests that CRP does not increase immediately after endurance exercise; even with exercise of duration found in an ultraendurance event. In a running study in which trained runners, VO2max 60.02 ml/kg/min, ran on flat ground for one hour at 80% of VO2max CRP concentrations were recorded pre-exercise, immediately post-exercise, and 1, 6, 24, and 48 hours post-exercise (81). CRP results showed a significant increase 6 hours after exercise (increase from 0.3 mg/L at baseline to 0.5 mg/L 6 hours post-exercise) and a greater increase 24 hours post-exercise (increase from 0.3 mg/L at baseline to 1.2 mg/L 24 hours post-exercise). At 48 hours after exercise CRP values returned to baseline. Although these studies outline the time course of CRP accumulation, likely peaking at approximately 24 hours post-exercise, there is a lack of research reporting CRP values between 6 and 24 hours post-exercise. From the available literature our sampling schedule seems appropriate to assess a significant elevation in CRP and the addition of a measure immediately post-exercise or up to 6 hours post-exercise would seem unnecessary. As mentioned, no research to our knowledge has recorded CRP values between 6 and 24 hours after endurance exercise possibly causing us and other researchers to miss the true peak post-exercise CRP elevation. This lack of blood sample collection is likely due to inconvenience as time points falling within the 6-24 hours pos-exercise often during sleep.

Another hypothesis we made a priori was that CRP concentrations would be similar between groups pre-exercise but significantly higher in the untrained group 24

hours post-exercise. We hypothesized that CRP concentrations at rest would be similar between groups since both groups were healthy, young, of similar BMI, and free of any medications. Also we felt that, although not highly active, our untrained group was not sedentary. We found that CRP was markedly higher in the untrained group pre-exercise [0.16 +/- 0.25 mg/L compared to 1.17 +/- 1.43 mg/L ($p < 0.05$)] and 24 hours post-exercise [0.19 +/- 0.20 mg/L compared to1.23 +/- 1.23 mg/L ($p < 0.05$)]. Our hypothesis that CRP values would be similar between groups was based upon values found by Czarkowska (18) in combination with large scale studies reporting normal CRP values in inactive individuals. Czarkowska recorded CRP before and after exercise in elite road cyclists of similar fitness to our study; mean VO2max 65.7 ml/kg/min. Resting CRP concentration in this group was reported as 3.25 mg/L indicating no reduction compared to inactive individuals. In retrospect, pre-sampling protocol in this study was not discussed indicating that samples could have been taken during a heavy training period. Considering CRP peaks 24 hours post-exercise, if participants had trained the day prior to sampling CRP may have been artificially elevated. Anecdotally it seems unlikely that athletes of this caliber would take 48 hours off of training without being prompted. This could account for the massive discrepancy in CRP values compared to our findings. In a study published during the course of our study a research team showed a non-significant difference in CRP concentration between physically active and inactive subjects (86). Although the difference was not significant the results are in line with our findings. In this study young physically active and inactive subjects aged 18-35 years were compared for resting CRP. The inactive group had a resting CRP of 1.2 mg/L and the physically active group had a resting CRP of 0.7 mg/L ($P > 0.05$). This shows a similar comparison to our results with the physically active group maintaining a reduced CRP concentration.

In Stewart's study physical fitness was assessed as a VO2max estimate from the Balke test; physically active subjects having a mean of 45 ml/kg/min and inactive subjects having a mean of 35 ml/kg/min. In our study relative VO2max had a mean difference of more than 20 ml/kg/min between groups; 42.4 ml/kg/min in the untrained group and 68.6 ml/kg/min in the untrained group. This larger difference in primary fitness measure likely accounts for our finding of such a vast difference in CRP in comparison to previous research.

In large scale population studies it has generally been assumed that reductions in CRP in more active individuals are due to reduced underlying CRP stimuli; mainly systemic il-6. Considering Il-6 concentrations were similar between groups at all time points it seems unlikely that the difference in CRP values can be attributed directly to Il-6. This was surprising as il-6 has been defined as the chief regulator and stimulus for CRP production (12).

One potential explanation for these findings is that secondary stimuli of hepatic CRP production, il-1 and TNF-α, were greater in the untrained group. This seems unlikely for two reasons. First, il-1 and TNF-α, when administered individually, showed a 1-fold increase in CRP production from human hepatocytes (12). In contrast, a similar addition of il-6 to human hepatocytes showed a 23-fold increase in CRP production. Second, it has been demonstrated that physically active and inactive groups of healthy males aged 18-35 years with similar BMI measures have shown no differences in resting concentrations of il-1 or TNF-α suggesting that training status should not play a role in resting il-1 or TNF-α values (86).

Another possible explanation is that leptin levels were higher in our untrained group than our trained group. Leptin is a hormone produced in adipocytes that is typically

associated with hunger and energy balance. In relation to our work, it is of note that systemic leptin concentrations are significantly correlated with systemic CRP (78). Previously it was assumed that this correlation was confounded by obesity and the subsequent increase in adipocytes and other inflammatory mediators. Later findings by Shamsuzzaman (78) showed a significant correlation between the two regardless of BMI, and other associated factors. The understanding of the relationship between leptin and CRP is not fully understood; however, it has been demonstrated in vitro that leptin stimulates CRP expression in human coronary artery endothelial cells (78). Interestingly leptin was found to be a more potent CRP stimulus in these cells than il-6, TNF-α, or il-1β. Also, the leptin receptor has been shown to have signaling capabilities similar to il-6 receptors indicating that leptin may act directly to induce CRP production in the absence of il-6, il-1 or TNF-α (6). Furthermore, a number of studies have found reduced leptin levels in trained individuals compared to untrained as well as within individuals after training for some weeks (9). The results typically were attributed to differences in fat mass but two groups that ran prospective endurance training studies found reductions in leptin with training without a concomitant reduction in fat mass (33, 37). With all of this in mind it seems reasonable that our trained group may have had diminished systemic leptin due to increased endurance training and a significantly lower sum of skinfolds reflecting reduced fat mass. Applying the mechanisms and information discussed this potential reduction in leptin may have influenced the significant reduction in CRP in our trained group.

It also seems possible that the il-6 cell surface receptor expression in the liver may be desensitized in trained individuals. Evidence of this comes from a study by Mackiewicz (52) that examined the acute phase response to il-6 in human hepatoma cells.

When repeatedly exposed to high levels of il-6 in vitro, the cell surface il-6 receptors became desensitized resulting in a limited acute phase response to an identical il-6 stimulus. By this same mechanism the constant peaks in systemic il-6 associated with strenuous exercise training may desensitize hepatocyte il-6 receptors in trained subjects. This may lead to lower CRP concentrations in trained subjects when compared to untrained subjects even while maintaining similar resting il-6 concentrations. Correlation results in our study lend some support to this mechanism. At baseline il-6 and CRP concentrations were significantly correlated in the untrained group ($r=0.69$, $p = 0.01$), representing a moderate relationship in which 48 percent of the change in CRP can be attributed to il-6, but not in the trained group ($r=-0.13$, $p=0.68$). This lack of correlation indicates that the il-6 to CRP relationship is dissimilar in the trained and untrained group. Furthermore, the mean post-exercise increase in CRP (mg/L) per increase in il-6 (pg/ml) was 0.0416 mg/L/pg/ml in the trained group and 1.2112 mg/L/pg/ml in the untrained group. This ratio measure objectively represents the il-6 to CRP relationship. In combination our results support this notion of a blunted CRP response to il-6 in trained subjects.

5.4 Training Adaptations

The results collected from our study indicate that trained subjects are able to do far more work and turn over far more oxygen than untrained subjects while showing a similar absolute elevation in il-6. Also, when corrected for work or oxygen consumption the trained group exhibited a blunted il-6 peak after exercise. With this information it seems as though training does likely affect skeletal muscle il-6 production. Upon

reviewing the research to date there are a number of skeletal muscle adaptations due to endurance training that may reduce il-6 production during exercise.

In exercise physiology it is well established that skeletal muscle undergoes various adaptations with endurance training. These adaptations include increased glycogen stores in both type 1 and 2 fibers, glycogen sparing due to preferential fatty-acid oxidation, and preferential type 1 fiber recruitment (1, 35, 28, 29). All of these adaptations may lead to a reduction in contraction-induced skeletal muscle il-6 production. It has been a consistent finding that intramuscular il-6 mRNA expression and protein release in response to concentric-based exercise are elevated with reduced pre-exercise intramuscular glycogen content (41, 84). Currently it is hypothesized that phosphorylation of nuclear p38 MAPK (mitogen activated protein kinase) activates il-6 transcription factors in skeletal muscle (14). It also appears that intramuscular glycogen binds upstream signaling molecules for p38 MAPK (MKK3, MKK6, AMPK) (14). Through this mechanism increased intramuscular glycogen storage inhibits p38 MAPK phosphorylation resulting in reduced il-6 transcription and subsequent protein production. Assuming this mechanism is correct training-induced increases in glycogen storage and glycogen sparing could reduce contraction-induced il-6 production.

Through other findings it also seems that training-induced preferential type 1 fiber recruitment during endurance exercise may reduce post-exercise il-6 production. In vitro work has demonstrated that mammalian muscle tissue shows an increase in il-6 mRNA expression and protein release when incubated with calcium inophore ionomycin identifying Ca^{2+} as a stimulus for il-6 production (36). The significance of this lies in the finding that type 2 fibers show a near 20-fold greater Ca^{2+} release during contraction compared to type 1 fibers. When fiber types were compared after a bout of concentric

contractions biopsy analyses showed greater il-6 mRNA content as well as protein production in the type 2 fibers compared to type 1 (34). Therefore, training-induced alterations in fiber type recruitment favoring type 1 fibers would likely also reduce contraction-induced il-6 production.

Another adaptation in muscle that may reduce contraction-induced il-6 production is enhanced antioxidant capacity. During heavy exercise oxygen consumption in the active muscle may increase as much as 100-fold (79). With this comes a subsequent increase in the production of free radicals; harmful molecules or ions containing reactive unpaired electrons. The chief source of free radical production during exercise is at complex 1 and 3 of the electron transport chain (15). Another source of free radical production during heavy exercise may be the xanthine oxidase pathway activated during tissue ischemia and reperfusion (32). Regardless of the source, free radical production has been directly identified as a potent stimulus for il-6 production in myocytes (41). Support of this finding comes from a subsequent study that demonstrated a reduction in post-exercise il-6 concentrations when subjects were administered antioxidant supplements (89). This research implicates oxidative stress as a primary stimulus for il-6 production. To quench free radicals and prevent tissue damage from oxidative stress, humans have innate antioxidant enzymes within skeletal muscle; primarily superoxide dismutase, glutathione peroxidase, and catalase. Much evidence has been reported suggesting that skeletal muscle antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) are enhanced in response to regular endurance training (69, 38, 45, 79). With this information it seems likely that training, leading to enhanced innate antioxidant capacity, could also likely reduce post-exercise il-6 elevations.

Although it has been demonstrated by many groups that antioxidants SOD and GPX are found in greater concentration in skeletal muscle (69, 38, 45, 79), athletes training heavily may be at increased risk of oxidative damage. The reason for this is that increases in skeletal muscle oxidative enzymes and antioxidant enzymes do not increase in parallel and in fact oxidative enzyme capacity is enhanced more so than systemic antioxidant enzyme capacity (16, 67). Such unparalleled improvements in oxidative and antioxidant capacity allow the body to overshoot its innate ability to quench free radicals produced during intense exercise. Intense endurance training then may serve to be protective against oxidative stress from ambient free radical attack (eg. diet, cigarettes, infection) and submaximal exercise but may be detrimental with respect to intense exercise.

The result may be that extreme endurance training can lead to oxidative stress related illness. Oxidative stress has been implicated as a cause in many illnesses; most notably cancer and heart disease. Free radicals are unstable products capable of reacting with lipids, proteins, and DNA in tissue. The cancer causing effects of oxidative stress are rooted in the ability of free radicals to alter DNA and cell replication. The role of free radicals in cardiovascular illness is due to low density lipoprotein oxidation which contributes to endothelial dysfunction and subsequent atherosclerosis (11, 3). It has also been hypothesized that oxidative stress may inhibit cardiac function acutely by causing intracellular calcium overload (40). In this study it was found that cardiac myocytes exposed to free radicals caused myocyte injury resulting in excess calcium influx and subsequent contractile abnormalities.

The results of a large scale epidemiological scale (71) potentially demonstrate the significance of this oxidative stress imbalance with ultraendurance training. In this study

it was found that ultraendurance athletes training heavily, defined as > 10500 kilojoules of energy expended per week through activity, were at greater risk of cardiovascular disease than any other group even the most sedentary group expending 0 kilojoules per week through activity (71). Also surprising was that subjects in the second most active group, expending 6300-10499 kilojoules per week through activity, were at greater risk of cardiovascular disease than those in less active groups, 1680-3779 kilojoules per week and 3780-6299 kilojoules per week. These two groups, expending 1680-3779 and 3780-6299 kilojoules per week were at the lowest risk for cardiovascular disease in this study. Although a causative relationship is not possible to analyze in such a study this report displays the potential harmful effects of excessive exercise and subsequent oxidative stress.

5.5 Overtraining Implications

In the past five years il-6 has received attention as a cause of overtraining or underperformance syndrome. Underperformance syndrome (UPS) is defined as a persistent decrement in athletic performance capacity despite 2 weeks of relative rest (72). UPS affects many athletes each year in a variety of sports with symptoms including fatigue, loss of appetite, irritability, and poor sleep quality (25). However, the defining symptom and most common reason for seeking treatment is poor performance. The potential causes of UPS have been studied for years but no theorized mechanism has been able to account for the onset or symptoms. It is clear that those affected by UPS not only have impaired performance but in severe cases also exhibit irregular hypothalamic-pituitary function (5). The challenge for past researchers has been linking peripheral damage and fatigue with the central nervous system. In 2000 Lakier-Smith (43)

hypothesized that cytokines, produced in response to constant musculoskeletal trauma, were likely candidates as a cause of UPS and coined the term "cytokine sickness". More recently, Robson (72) suggested a more specific and plausible mechanism by which il-6 may account for UPS and associated symptoms.

As demonstrated in our study and others (23, 60) endurance exercise leads to an increase in systemic il-6. Il-6 exerts its effects throughout the body including the central nervous system via passage through the blood brain barrier (4). When administered to healthy human subjects in dosages equivalent to those found during prolonged endurance exercise il-6 induced significant increases in systemic cortisol and adrenocorticotropic hormone (58). Subjects also reported increased overall fatigue, depressed mood, poor concentration, and sleep disturbances (measured as reduction in REM sleep) (58). Further, il-6 has been implicated as the chief source of debilitating fatigue in cancer patients (42). To our knowledge only one study has monitored exercise performance during il-6 administration (73). In this study athletes completed a 10 km running time trial with or without il-6 injections. When injected with il-6, in dosages designed to match plasma levels during prolonged exercise, subjects experienced more fatigue and performed significantly poorer than with placebo (73). These results are intriguing as the effects of systemic il-6 on the central nervous system can account for the majority of UPS.

The basis of the model proposed by Robson (72) is that excessive repetitive exercise bouts and/or concomitant increases in il-6 lead to a time-dependant sensitization (TDS). A TDS is a "progressive and persistent amplification of behavioural, endocrine and immunological responses to repeated intermittent stimuli over time"(72, 7). Robson draws similarities between UPS and TDS as athletes often end up in an overtrained state

following a period of probable repeated il-6 stimuli: infection, injury, heavy training or heavy competition (72). Also, UPS resembles TDS in that once UPS develops a worsening of symptoms is seen with subsequent exposure to training indicative of sensitization (72). Robson indicates that the sensitization may manifest as enhanced il-6 production, enhanced sensitivity to the il-6 protein or a combination of the two. There are a number of mechanisms which may result in elevated systemic il-6 in the heavily training athlete. Most commonly discussed is underlying musculoskeletal injury during which a recent or chronic injury may be causing an increase in il-6 regardless of exercise. A similar pattern may occur following a recent infection. For either reason il-6 in the system may be elevated leading to sensitization. As mentioned previously, low pre-exercise muscle glycogen content results in enhanced post-exercise il-6 values after a similar exercise bout (14, 21). With heavy training loads including multiple workouts each day, athletes may be unable to replenish glycogen stores between workouts again potentially resulting in greater il-6 concentrations. In addition, although antioxidant enzyme capacity is elevated in the skeletal muscle of endurance trained athletes, the oxidative capacity of muscle increases to a greater extent with training (67, 68, 69). The result is that an athlete may have a reduced ability to quench free radicals after high level endurance training; subsequently resulting in greater post-exercise il-6 elevations. Also, il-6 is found to be elevated in individuals after high stress situations (65) potentially similar to those during times of heavy competition. These potential causes of elevated il-6 seem likely to occur individually or in combination for the highly trained athlete.

A recent study showed that repetitive endurance training with insufficient recovery over three weeks resulted in elevated plasma il-6, worsened performance, and increased fatigue (74). In this study triathletes had extra run training sessions added to

their current schedules to induce overreaching; a state of training prior to UPS in which one has continued to increase training intensity but performance begins to decline. Results from this study support Robson's proposed il-6 hypothesis of UPS as resting il-6 production increased with repetitive training bouts. The elevation in il-6 in response to excessive training represented a TDS (74).

The results of our study suggest that endurance trained individuals can complete an exercise bout of much greater intensity than untrained individuals while maintaining similar il-6 values. This represents a training adaptation opposite to TDS in which the body successfully adapts to an enhanced stimulus. Considering that individuals in an overreached state develop a maladapted il-6 response to exercise training, and that this response coincides with early performance decrements, regular measures of systemic il-6 in heavily training athletes may serve as an early indicator of overtraining syndrome.

5.6 Health Implications

For years CRP has been seen as an indicator of underlying inflammation due to illness or injury. It was first linked to cardiovascular disease in 1985 when researchers extracted CRP from human atherosclerotic aortic intima (76). Until recently CRP had been thought of as a bystander in cardiovascular disease; not a cause. With further research it has become clear that CRP is causative at various stages of atherosclerosis. The following summary is not exhaustive but attempts to cover the primary mechanisms of CRP in atherosclerosis progression. First, CRP concentrations predictive of vascular events (> 3 mg/L) directly upregulate endothelial cell adhesion molecules (6-11) that assist in leukocyte-endothelial cell interaction. Once this interaction has occurred CRP aids in leukocyte transmigration through the endothelium by stimulating the release of

monocyte chemoattractant protein-1(46, 8). CRP further inhibits vascular function by stimulating endothelin from endothelial cells; a potent vasoconstrictor and a further stimulus for CRP upregulation of cell adhesion molecules (8). In addition, CRP inhibits the production of endothelial nitric oxide; a vasodilator maintaining vascular tone and function (29, 75). Further, nitric oxide inhibition leads to endothelial cell apoptosis serving to enhance the role of CRP in atherosclerosis (75). Although pro-inflammatory cytokines il-6, il-1, and TNF-α are the primary stimuli for CRP, CRP also provides a positive feedback mechanism by upregulating transcription factor-kappa B (NF-κB); a transcription pathway required by most endothelial pro-inflammatory cytokine genes (47). As a result, CRP itself elevates pro-inflammatory cytokine production. Finally, CRP also plays a role in accumulation of atherosclerotic plaque. Researchers have indicated that CRP encourages macrophage uptake of low density lipoproteins further enhancing the atherosclerosis process (8, 91).

As mentioned in chapter 5.3 il-6 is the chief stimulator of hepatic CRP production (12). Our results indicated that trained and untrained subjects had similar il-6 concentrations at rest and CRP values were significantly higher at rest in the untrained group. We also found that resting concentrations of CRP and il-6 were significantly correlated in the untrained but not the trained group. With this information it seems that the relationship between il-6 and CRP is different in trained than untrained individuals. Possibilities for this are described in chapter 5.3. Regardless of the mechanism for the lower concentrations of CRP pre- and post-exercise in the trained group this difference represents a reduction in cardiovascular risk in the trained group. These results are especially surprising as both groups were matched for age, BMI, health status, medication, and smoking status; all factors linked to systemic inflammation. Even more

surprising was that two subjects in the untrained group were considered to be in the high risk factor range for CRP (>3 mg/L), two were considered to be in the moderate risk range (2-3 mg/L), and the remaining 8 were considered low risk (<1 mg/L). In the trained group all subjects were considered low cardiovascular risk with respect to CRP (<1 mg/L). Although our study was a cross-sectional design our results indicate that endurance training aside from age, BMI, health status, medication, and smoking may reduce cardiovascular risk with respect to CRP.

5.7 Limitations

Due to the cross-sectional design of this study we are limited with respect to attributing differences in outcome measures to training alone. Although this study provides a relatively simple way to evaluate the effects of training on both Il-6 and CRP values, there are many variables between individuals that cannot be controlled for in a cross-sectional design. As such a longitudinal study, measuring the effects of training over time, must be done to eliminate such variables.

Another inherent limitation of this study came with attempting to match groups. Although groups were matched well for health, age, height, weight, and BMI, we were unable to match groups for sums of skinfolds. When recruiting both sedentary individuals and elite endurance cyclists, matching groups for sums of skinfolds was not possible with the resources available. This is a concern as it has been reported that obese individuals show elevated values of both il-6 and CRP (9, 24, 47). Even though the sums of skinfolds were significantly greater in the untrained group the values reported were still well within normal values; likely not affecting inflammatory status.

The main outcome measures reported in this study were systemic concentrations of Il-6 and CRP. From the available literature it is clear that the increases in systemic il-6 occurring soon after endurance exercise can be attributed to il-6 production and release from the active muscle (65). This has been found by comparing arterio-venous il-6 differences across the active muscle to systemic il-6 concentrations (85). For our study no arterio-venous measures were recorded leaving the possibility that the source of il-6 production, although unlikely, may not have been the active skeletal muscle. Also no studies, including ours, have monitored changes in il-6 degradation compared to production while exercising. It has been shown that a significant amount of il-6 is taken up in the hepatosplanchnic viscera during exercise but there may be other tissues that do as well (20). With this in mind, it may be that differences in post-exercise il-6 between individuals are due to il-6 uptake differences rather than production differences or a combination of both. Again, with a simple venous measure we cannot determine where il-6 was produced and removed; only the change in systemic concentrations. Similarly, the chief source of CRP is thought to be from hepatocytes and the main stimulus il-6 but CRP is also produced in endothelial cells in response to il-6. As is the case with determining the source of il-6, the location of production of CRP and the ratio of production to degradation cannot be determined with a systemic venous blood.

The goal of this study was to determine if there was a difference in resting and post-exercise inflammatory markers between aerobically trained and untrained healthy males. Generally the training effects that have been hypothesized to reduce the inflammatory response following exercise are enhanced glycogen storage in type I muscle fibers, resulting in increased endurance capacity for those fibers and reduced reliance on type II fibers and enhanced innate antioxidant defenses (23, 89). With subject

selection and protocol we tried to eliminate factors external to training that may have influenced these training effects by excluding subjects who had taken antioxidant supplements within six months and having subjects fast from the night before the exercise challenge. Without taking muscle biopsies to analyze antioxidant enzyme capacity and glycogen stores we were unable to evaluate these training adaptations in each group. As such we were unable to attribute any differences in the trained or untrained group directly to particular training adaptations.

In order to remove as many variables as possible in this study, subjects were all males aged 18-35 years. There is evidence of a blunted mononuyclear il-6 response to typical stimuli such as lypopolysaccharide exposure (77) which may affect both baseline and post-exercise il-6 concentrations in a study such as ours. As a result, the results of our study, including baseline systemic inflammatory values, cannot be transferred to females until a similar study is conducted in females. Similarly, there is little research comparing the inflammatory response to exercise in old and young individuals. One study to date (88) has evaluated the training effect of resting il-6 and CRP in both young (aged 18-35) and older (aged 65-85) previously sedentary individuals. Baseline values of both proteins show similar responses between groups in response to endurance exercise training; il-6 remained similar and CRP decreased with training.

5.8 Future Directions

This study was a cross-sectional study comparing two groups differing only in fitness. The results, in combination with previous findings, indicate that endurance training does alter the post-exercise increase in il-6. Our results also clearly demonstrate a

difference in CRP values at rest and post-exercise between trained and untrained subjects. With respect to study design the next step is to carry out a prospective study in which untrained subjects are trained in a concentric-based activity and challenged before and after training with an identical work bout to directly determine the effect of training on an individual. This would result in a statistically more powerful study that would have fewer confounding variables than a cross-sectional design.

There are also a number of other variables that would be useful to examine in the future if resources were available. With our results that trained individuals can work at a far greater wattage and oxygen consumption with a similar inflammatory response it would be useful to have objective evidence of what is different in the trained muscle and the untrained muscle. For example, low glycogen stores have been shown as a stimulus for il-6 production in active skeletal muscle (14) and it has also been found that trained muscle can store more glycogen (35). In future work it would be beneficial to take a muscle biopsy and analyze glycogen contact to determine if glycogen was in fact different between trained and untrained.

Considering oxidative stress is also a stimulus for skeletal muscle il-6 production (41) it would be beneficial to examine differences in antioxidant enzyme capacity and markers of oxidative stress concurrent to il-6 values in a trained and untrained state after exercise. By evaluating the relationship between the two, the role of oxidative stress and free radical production in the post-exercise il-6 response could be quantified.

Monitoring free radicals in tissue is challenging due to the particularly short half-life of free radical species. As a result, free radical production is usually expressed by indirect indicators of lipid, protein or DNA oxidation; oxidative damage. Most studies involving human subjects have employed byproducts of lipid peroxidation such as conjugated

dienes, lipid hydrocarbons, and thiobarbituric acid-reactive substances (TBARS) as measures of oxidative stress. Conjugated dienes are initial products of the peroxidation of unsaturated fatty acids, and are considered to be accurate and repeatable measures of lipid peroxidation measures (57, 67-69, 79). Unfortunately, conjugated dienes may be present in dieters, resulting in mis-represented whole-tissue oxidation. Other studies have used exhaled hydrocarbon products of lipid peroxide splitting, such as ethane and pentane, to indicate lipid peroxidation. Exhaled pentane is a particularly useful measure since both fatty acid types from which pentane is produced are found mostly within the cell membrane (57, 67-69, 79). The noninvasiveness of this measure is attractive; however, it should only be used supplementary to other oxidative stress measures.

More recently, electron spin resonance and paramagnetic resonance spectrometry have been used to directly measure superoxide radicals in animals. These techniques are the most powerful as they both directly measure transition states of the free radicals (79). To date, these measures have not been used as indicators of free radical production in humans.

Another technique, using chemiluminescence to detect lipid peroxidation, has been described by (57, 67-69, 79), in which biological samples assays are employed. In the sampled assay, the antioxidant capacity is estimated by a decrease in chemiluminescence when an oxyradical is coupled with the production of light. This method is reported to be rapid, sensitive, reproducible, and simple (57, 67-69, 79). It also allows for total antioxidant capacity measures from small volumes of fluid (57, 67-69, 79

Blood glutathione disulphide (GSSG) measures may also be a useful indicator of oxidative stress. In the presence of H_2O_2 and hydroperoxides, intracellular glutathione quickly oxidizes to GSSG, but is quickly reduced back to glutathione if the oxidative

stress is minimal. If the oxidative stress is beyond the cell's ability to reduce GSSG to glutathione, an accumulation of blood GSSG may serve as an indicator of oxidative stress (57, 67-69, 79).

The most commonly employed method to evaluate lipid peroxidation is the measure of TBARS, most commonly Malandialdehyde (MDA), which is created in peroxidizing systems (57, 67-69, 79). During oxidation of polyunsaturated fatty acids, MDA is generated as a secondary product. Altjough simple to measure, a variety of other factors such as side products of thromboxane or prostaglandin synthesis may also increase MDA levels in blood and tissues (57, 67-69, 79).

Measures of oxidative stress in conjunction with il-6 would aid in determining the effect of free radicals on post-exercise il-6. However, it should be noted that current lipid peroxidation and oxidative assessment methods should be used carefully due to lack of accuracy, validity, or both. It has been suggested that two or more techniques should be used to provide improved measures of oxidative stress.

It would also be of value to study the effects of different exercise workloads on il-6 production within individuals. This would provide a better understanding of the influence of both oxygen consumption and metabolic system use on il-6 production. Ostrowski (63) examined run intensity and il-6 production in marathoners during a marathon with results indicating that run intensity, measured as VO2max/run time, was significantly correlated with elevations in il-6. However, no researchers to our knowledge have designed a study to compare exercise bouts requiring different oxygen consumptions within the same individual. This could be done by having subjects go through a protocol identical to ours with initial aerobic fitness assessment and then an exercise challenge except with repeated exercise bouts days apart; each bout at an

intensity corresponding to a different percentage of maximal oxygen consumption. For example, each participant could complete three exercise bouts in random order at 50, 60, and 70 percent of maximal oxygen consumption. By analyzing il-6 peaks after each bout a powerful relationship between intensity, measured as percentage of maximal oxygen consumption, and il-6 production could be reported.

Another future direction from this research is in the area of overtraining. Although a longitudinal study is required to fully determine how an individual's inflammatory response will adapt to training, our work established a relationship for the post-exercise il-6 response in trained and untrained groups. With these results it seems that training would at least result in a similar post-exercise il-6 increase if not a blunted increase compared to pre-training. That said, an elevated post-exercise increase in il-6 with training may indicate a skeletal muscle maladaptation that could lead to overtraining. The mechanism by which il-6 may be linked to overtraining is discussed in chapter 5.5. In addition, elevated il-6 values at rest may be indicative of an underlying muscle maladaptation or unrecovered microtrauma. Regardless of the underlying cause elevated systemic il-6 leads to symptoms of and potentially full blown underperformance syndrome itself. To best study the link between training, overtraining, and il-6 subjects would take part in a training program aimed at inducing overreaching or even overtraining. This could be done in a similar fashion to a report by Robson-Ansley (74) in which triathletes had intense run interval training bouts added to an already taxing training schedule. By monitoring athletes, using an exercise challenge and blood sampling schedule similar to ours, at regular intervals throughout a strenuous training schedule it would be possible to evaluate each athlete's training adaptation with respect to resting and post-exercise il-6. Performance and symptoms of overtraining could then

be compared to changes in individual resting and post-exercise il-6 values to determine whether il-6 is a good indicator or predictor of overtraining.

Chapter 6: Conclusion

In summary, our results have shown that systemic il-6 concentrations are similar in trained and untrained subjects at rest and following a relatively similar exercise bout. However, when corrected for both work completed and oxygen consumed during the exercise bout, trained subjects demonstrated a blunted il-6 response compared to trained subjects. These findings bring to the forefront the need for a training study to monitor the inflammatory response to an identical exercise bout in the same individual before and after successful training to fully elucidate the effect of training. Our findings also indicated significantly lower concentrations of CRP in trained compared to untrained subjects. Further examination of the results has lead to proposal that the relationship between il-6 and CRP may be altered with regular exercise.

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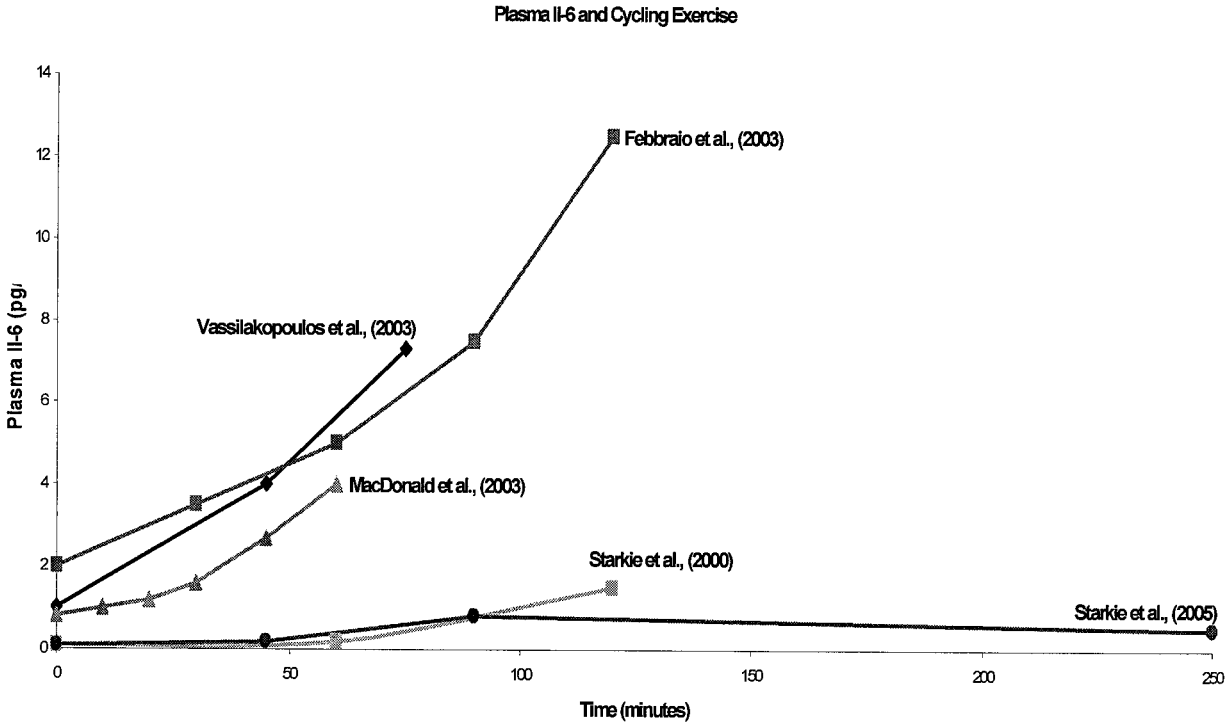
Appendices

Appendix A - Il-6 concentrations Following Cycle Exercise in Different Fitness Groups



Comparison of Fitness Status and Exercise Challenge Response

Study	Subject VO2max (ml/kg/min)	Exercise Challenge Intensity	Exercise Mode	Exercise Duration	Pre-Exercise Nutritional Status	Time at Peak Il-6 Concentration	Peak Il-6 Concentration
Vassilakopoulos et al., 2003	38.9	70% of VO2max	Cycle Ergometer	45 Minutes	Fed High-Carb Meal	30 Minutes Post-Exercise	7.5 pg/ml
Febbraio et al., 2003	47.9	65% of VO2max	Rucumbent Cycle Ergometer	120 Minutes	Overnight Fast	Immediate Post-Exercise	12.5 pg/ml
MacDponald et al., 2003	65	70% of VO2max	Cycle Ergometer	60 Minutes	713 KJ Meal (75%CHO)	Immediate Post-Exercise	4 pg/ml
Starkie et al., 2000	60.02	70% of VO2max	Cycle Ergometer	120 Minutes	Overnight Fast	Immediate Post-Exercise	1.5 pg/ml
Starkie et al., 2005	61.03	70% of VO2max	Cycle Ergometer	90 Minutes	N/A	Immediate Post-Exercise	.8 pg/ml

Appendix B - Participant Questionnaire

Aerobic Fitness Status and Pos-Exercise Inflammatory Markers in 18 to 35 year-old males

Participant Questionnaire

Participant name:_____

1.) Do you currently participate in any form of exercise training? **Yes/No** (*If no skip to question 5*)

2.) If you responded Yes to question 1, approximately how many hours per week do you train? and What type of training do you participate in? (*include a breakdown of time spent at each type of training)*

3.) Do you currently participate in cycle racing or training, **Yes/No**? If so, at what level do you participate at? (*ie. CAT 1, 2, 3, other?*)

4.) How long have you participated in this level of cycle training? (*weeks/months/years?)*

5.) Have you recently undergone an aerobic fitness assessment (VO2max test)? If so, do you recall the results of your assessment?

6.) To the best of your knowledge do all of the following inclusion criteria apply to you?

Yes/No

- Male aged 18-35 years old?

Yes/No

- Current and expected Vancouver Resident for duration of study?

Yes/No

- No antioxidant/Vitamin supplementation $\leq$ 6 weeks prior to study?

Yes/No

- No use of steroidal anti-inflammatory medication $\leq$ 6 weeks prior to study?

Yes/No

- Healthy,
    - No history of chronic disease (CVD, endocrine, hepatic, inflammatory, etc…)?

    Yes/No

    - No traumatic or chronic injuries present $\leq$ 6 months prior to study?

    Yes/No

    - No febrile illness/infection $\leq$ 6 weeks prior to beginning of study?

    Yes/No

- Non-smoker?

Yes/No

- No current or planned caloric intake restriction?

Yes/No

- Non shift worker?

Yes/No

Appendix C – Ethical Approval Certificate

The University of British Columbia
Office of Research Services,
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

Certificate of Full Board Approval

Clinical Research Ethics Board Official Notification

PRINCIPAL INVESTIGATOR	DEPARTMENT	NUMBER
Taunton, J.E.	Family Practice	C06-0088

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT

Providence Health Care, UBC Campus

CO-INVESTIGATORS:

MacIntyre, Donna, Rehabilitation Sciences; Rhodes, Edward, Human Kinetics

SPONSORING AGENCIES

British Columbia Sports Medicine Research Foundation

TITLE:

Aerobic Fitness Status and Post-Exercise Inflammatory Markers in 18 to 35 Year-Old Males

APPROVAL DATE	TERM (YEARS)	DOCUMENTS INCLUDED IN THIS APPROVAL:
28 March 2006	1	Protocol version 1 dated 02 February 2006; Subject Consent Form version 1 dated 02 February 2006; Advertisement

CERTIFICATION:

In respect of clinical trials:

1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.

2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.

3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The documentation included for the above-named project has been reviewed by the UBC CREB, and the research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved by the UBC CREB.

The CREB approval for this study expires one year from the approval date.

Approval of the Clinical Research Ethics Board by one of:
Dr. Gail Bellward, Chair
Dr. James McCormack, Associate Chair