ABSTRACT

Effects of G-trainer, Cycle Ergometry, and Stretching on Physiological and Performance Markers of Recovery from Endurance Exercise

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The purpose of this study was to compare the effectiveness of 3 recovery treatments (AlterG Anti-Gravity Treadmill, stationary cycling, and static stretching) on physiological and psychological markers of muscle fatigue and recovery following an acute bout of exhaustive exercise. In a cross-over design, twelve aerobically-trained males (21.25 ± 2.3 yrs, 72.05 ± 8.09 kg, 178.42 ± 6.27 cm) completed an 18.25 mile time trial performed by stationary cycling. Immediately following the endurance bout, participants completed 30 minutes of AlterG G-trainer or cycle ergometry (at ~40% VO2max) or static stretching exercises. A significant main effect for time was detected for lactate ($p = 0.010$) and cortisol (0.039) post-exercise. No other main effects for time, treatment or treatment by time interaction were identified. When compared to stationary cycling and static stretching, exercise performed on the AlterG G-trainer treadmill was unable to further reduce systemic markers of stress and inflammation, blood lactate, or improve anaerobic performance and/or psychological mood states following endurance exercise.

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by

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A Thesis

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ACTH: adrenocorticotrophan

ADP: adenosine monophosphate

Cr: creatine

DOMS: delayed onset muscle soreness

IL-6: interleukin 6

POMS: profile of mood states

SIRS: systemic inflammatory response syndrome

TNFα: tumor necrosis factor alpha

VO_{2max}: maximal oxygen consumption

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CHAPTER ONE

Introduction

Athletes continually strive to push their capacity to exercise harder and longer during training and/or competition. Endurance exercise presents a challenge to the body with such exercise demands as elevated heart rate, blood pressure, energy expenditure, fluid loss, and thermal stress (Kimber et al., 2002; Padilla et al., 2000; Sharwood et al., 2004). Moreover, these demands may create strains on the body that exist in the form of increased systemic stress and inflammation and disruption to the skeletal muscle architecture (Suzuki, Peake, Nosaka, Okutsu, Abbiss, Surriano, et al., 2006). Over time, these demands will likely lead to muscular soreness and fatigue and possible muscle injury (Neubauer et al., 2008). Therefore, given that demanding training schedules of an endurance athlete may occur multiple times a day or on consecutive days, adequate rest is essential to aid in tissue repair, function restoration, and both muscle and psychological recovery for subsequent optimal performance.

It is evident that exhaustive endurance exercise provokes oxidative, metabolic, and hormonal stresses, all of which can lead to the release of cytokines and the activation of numerous cell subpopulations within the immune system (Neubauer et al., 2008). In response to homeostasis disruption during exercise, a stereotypical inflammatory response ensues which consists of neutrophil, macrophage, free radical, growth factor, and chemokine invasion of the affected musculature (Tidball, 2005; Warren et al., 2004; Zhang et al., 2009). The systemic inflammatory and stress response may lead to increased elevations in cortisol (Gleeson, 2002; Neubauer et al., 2008; Hill et al., 2008), interleukin-

6 (IL-6) (Suzuki et al., 2006; Neubauer et al., 2008; Ostrowski et al., 2008; Robson-Ansley et al., 2007), and tumor necrosis factor alpha (TNFα) (Neubauer et al., 2008; Ostrowski et al., 2008). Increased elevations in stress and inflammation hormones can lead to modulation of immune function and possible subsequent damage to the muscle architecture, and thus decrements in muscle strength and function (Enoka & Duchateau, 2008). In addition, blood lactate is also anticipated to rise after exhaustive endurance exercise for several possible reasons: the stimulation of glycolysis through muscle contraction or sympathetic nervous system activation, an increase in lactate dehydrogenase enzyme activity, and insufficient oxygen to meet metabolic demands (Plowman & Smith, 2007). Power output is expected to decrease after intensive endurance activity due to an increase in lactate accumulation, depletion of muscle glycogen, failure of the neuromuscular system (Plowman & Smith, 2007), calcium and potassium losses, local ischemia, the depletion of ATP, Cr, and/or glycogen stores, and/or the interference of the muscle excitation-contraction coupling cycle (Mika et al., 2007).

After a training session and/or following competition, it is the athlete's goal to recover from the demands of exercise and/or possible injury as quickly as possible in order to reduce the impact on subsequent exercise endeavors. Because such demands may have an effect on subsequent performance in both physiological and psychological manners, finding ways to reduce such an impact on the body by enhancing post-exercise recovery is essential for subsequent athletic performance. Post-exercise recovery promotes the clearance of lactate and other systemic stress and inflammation markers and improves subsequent performance through restoring muscular, inflammatory, and immune parameters. Recovery also allows positive adaptation to the stress of exercise,

replenishment of energy stores, and the repair of damaged and/or fatigued tissues (Mika, 2007; Neubauer et al., 2008).

A wide array of recovery modalities is utilized by athletes to create a balance between the stresses of training and competition as well as to enhance between-training session recovery in athletes. Some of these recovery techniques include cryotherapy, hyperbaric oxygen therapy, massage, compression garments, electromyostimulation, rehydration, active recovery, stretching, and non-steroidal anti-inflammatory drugs (Barnett, 2006). Two dominant forms of recovery exist: active and passive. Passive recovery is defined as inactive rest, such as performing no physical activity. Active recovery generally involves performing aerobic exercise at a low intensity. Stretching can also be considered a form of active recovery. Low intensity aerobic exercise is defined as exercise at approximately 40% of VO_{2max} (Martin et al., 1998). Active recovery at 40% maximal oxygen uptake potentiates the enhanced ability to remove blood lactate induced by endurance training as compared to passive recovery (Taoutaou, Granier, Mercier, Mercie, Ahmaidi, & Prefaut, 1996). Active recovery refers to performing low intensity exercise in the immediate post-exercise window as well as the days following the workout. Active recovery, to a greater extent than passive recovery (Mika et al., 2007), plays a major role in lactate removal; this form of recovery aids lactate clearance by increasing blood velocity and blood flow to the affected musculature thereby increasing lactate metabolism by augmenting the transport of lactate from the active muscle to the removal sites (Martin et al., 1998; Choi et al., 1994). Cycle ergometry and stretching have been documented as methods of active recovery (Choi et al., 1994; Mika et al., 2007; Herbert & Gabriel, 2002). However, there is little research available on the use of

the G-trainer, a relatively new anti-gravity treadmill, as a recovery aid. Alter-G, Inc. claims that the G-trainer enables users to enhance performance (Alter-G, Inc., 2009). A particular aspect of performance enhancement is the ability to recover from exercise. Futhermore, one of the health applications of the G-trainer is to aid aerobic conditioning. The G-trainer claims to do this by reducing force of impact on the body and protecting tissues during recovery while maintaining an effective cardiovascular workout. There is little evidence available as to the efficacy of the G-trainer as a recovery modality in enhancing recovery in the immediate post-exercise window as well as during the time between training sessions.

Statement of the Problem

Studying markers of recovery following an exhaustive bout of endurance exercise will allow for the most effective recovery pattern to be determined (Peterson, Hansen, Aagaard, & Madsen, 2007). The training, competition, and recovery schedule of an athlete must be fine-tuned in order for progression to occur and a high level of performance to be maintained. Recovery must be implemented in order to restore muscular, inflammatory, and immune parameters (Neubauer et al., 2008). Researchers have reported that it is an ordinary practice for athletes to "warm-down", or actively recover, in order to reduce the accumulation of blood and muscle lactate and other physiological products after competition or training (Choi, Cole, Goodpaster, Fink, & Costill, 1994). Cycle ergometry and stretching have been documented as methods of active recovery (Choi et al., 1994; Mika et al., 2007; Herbert & Gabriel, 2002), and were therefore selected as recovery modalities in the present study. However, there is little research available on the use of the G-trainer as a recovery aid and specifically on the

effectiveness of the anti-gravity treadmill compared to other well known recovery modalities. To the author's knowledge, there is no research available that is specific to the effectiveness of the G-trainer as a recovery modality following an acute bout of cycling endurance exercise in aerobically trained males.

Purpose of the Study

The overall purpose of the proposed study was to examine the specific differences between the G-trainer, cycling, and stretching on physiological, performance, and psychological markers of recovery from endurance exercise. The aims of the study were 3-fold. First, the study aimed to compare the effects of three active recovery methods (G-trainer, cycle ergometry, and stretching) on the physiological markers cortisol, TNFα, and IL-6 following an exhaustive bout of endurance exercise. Second, the study aimed to compare the effects of the recovery methods on the performance markers blood lactate and power output following an exhaustive bout of endurance exercise. Third, the study aimed to compare the effects of the recovery methods on psychological parameters following an exhaustive bout of endurance exercise.

Hypotheses

Ho_1: There will be no significant difference in blood lactate concentrations between the G-trainer, Computrainer, and stretching in response to active recovery.

Ho_2: There will be no significant difference in Tumor Necrosis Factor alpha (TNFα) levels between the G-trainer, Computrainer, and stretching in response to active recovery.

Ho_3: There will be no significant difference in cortisol levels between the G-trainer, Computrainer, and stretching in response to active recovery.

Ho_4: There will be no significant difference in Interleukin-6 (IL-6) levels between the G-trainer, Computrainer, and stretching in response to active recovery.

Ho_5: There will be no significant difference in power output between the G-trainer, Computrainer, and stretching in response to active recovery.

Ho_6: There will be no significant difference in profile of mood states assessments between the G-trainer, Computrainer, and stretching in response to active recovery.

Delimitations

- Twelve aerobically trained male individuals aged 18-26 participated in this study. Aerobically trained individuals were defined as those that had been training a minimum of one year for an average of 5 hours/week of aerobic exercise (i.e., running, cycling, rowing, and/or swimming).
- Diet and hydration were monitored.
- Intra and extracellular water was monitored via Bioelectrical Impedance Analysis (BIA).

Limitations

- Subjects were asked to volunteer for the study. Subjects were recruited through fliers posted around Baylor University, through HHPR activity classes, through the Baylor Cycling Club, Triathlon Club, and Crew Team.
- Each subject used his personal bicycle.
- The daily schedules of each subject and inherent circadian rhythms that exist for all humans may have been different as a result of slightly different testing times, stresses, etc.

Assumptions

- All subjects completed the health risk assessment honestly and understood the protocol and directions given to them.
- The equipment and instruments used for measuring and recording VO_{2max}, power, heart rate, blood lactate, and biochemical markers were valid and reliable.
- The calibration of the equipment and instruments used during data collection was performed accurately.
- Subjects adhered to primary investigator's requests of refraining from exercise at least 48 hours pre-test.
- The testing protocol utilized in this study provided an accurate measurement of each subject's true maximal effort.
- Subjects gave their maximum effort during all testing and training sessions.

Definition of Terms

- *Active Recovery:* low intensity aerobic exercise at approximately 40% VO_{2max}
- *Aerobically trained:* those that have been training a minimum of one year for an average of 5 hours/week of aerobic exercise (i.e., running, cycling, rowing, and/or swimming).
- *Blood Lactate:* the concentration of lactates (a product of anaerobic respiration) dissolved in the blood
- *Computrainer Lab:* electronic bicycle ergometer that allows the user to ride his/her own bicycle. A trainer consists of a frame, a clamp to hold the bicycle securely, a roller that presses up against the rear wheel, and a mechanism that provides resistance when the pedals are turned.

- *Cortisol:* a catabolic hormone that reduces the utilization of amino acids for protein formation in muscle cells
- *G-trainer:* a computer-controlled integrated body-weight-supported treadmill system which uses differential air pressure to support the user while walking or running on the treadmill
- *Immune function indicators:* white blood cells; eosinophils, basophils, monocytes, granulocytes, lymphocytes, macrophages
- *Interleukin-6:* a cytokine derived from activated T lymphocytes that has many functions, including induction of B-cell growth; induction of B-cell differentiation and antibody production; induction of differentiation and proliferation of T cells; synergistic induction with IL-3 of hematopoietic cell growth; and induction of hepatocyte secretion of acute-phase inflammatory proteins
- *Muscle fatigue:* an exercise-induced reduction in the ability of muscle to produce force or power whether or not the task can be sustained
- *Standard stretching protocol:* 3 sets of 30 seconds of static stretching per bilateral muscle group including hip, knee, and ankle flexors and extensors.
- *Tumor Necrosis Factor α:* a cytokine involved in the inflammatory process and regulation of immune cells
- VO_{2max}*:* the maximum rate at which oxygen can be taken up and used by the body during exercise

CHAPTER TWO

Review of Literature

Introduction

Demanding training schedules are a normal part of an athlete's life. Training for competition may occur multiple times a day or on consecutive days. After a training session and/or following competition, it is the athlete's goal to recover from the demands of exercise and/or possible injury as quickly as possible and thus, reduce the impact on subsequent exercise endeavors. Depending on the nature of exercise, athletes who specifically train/compete in predominantly endurance exercise experience such exercise demands as elevated heart rate, blood pressure, energy expenditure, fluid loss, and thermal stress (Kimber et al., 2002; Padilla et al., 2000; Sharwood et al., 2004). Over time, these demands will likely lead to fatigue, which is commonly depicted as a decrease in the ability to perform physical actions (i.e. decreased muscle power) (Enoka & Duchateau, 2008) and normally coincides with elevations is blood lactate (Rowlands et al., 2008; Gleeson, 2002). Furthermore, such demands can at times lead to a systemic inflammatory and stress response with increased elevations in cortisol (Gleeson, 2002; Neubauer et al., 2008; Hill et al., 2008), interleukin-6 (IL-6) (Suzuki et al., 2006; Neubauer et al., 2008; Ostrowski et al., 2008; Robson-Ansley et al., 2007), and tumor necrosis factor alpha (TNFα) (Neubauer et al., 2008; Ostrowski et al., 2008). Because such demands normally may have an effect on subsequent performance in both physiological and psychological manners, finding ways to reduce such an impact on the

body by enhancing recovery immediately following or within the days following exercise is essential for subsequent athletic performance.

The following chapter will review the physiological demands of endurance exercise on an athlete's body, specifically focusing on muscle fatigue, systemic inflammatory response, and muscle damage and subsequent repair. Additionally, the importance of recovery from endurance exercise will be presented, emphasizing the effects of active and passive recovery, including the new AlterG Anti-Gravity Treadmill on muscle recovery.

The Demands of Endurance Exercise

Endurance exercise inflicts tremendous strain on the bodies of athletes. This strain exists in the form of skeletal muscle damage, systemic stress and inflammation, and normally presents itself as muscular soreness and fatigue (Suzuki, Peake, Nosaka, Okutsu, Abbiss, Surriano, et al., 2006). Such physical strain can challenge the body's internal milieu (i.e. homeostasis). The autonomic nervous system and the hypothalamic-pituitary-adrenal axis (HPA axis) react to and play a role in maintaining this homeostasis. However, during high demands of stress such as endurance exercise, increased cytokine secretion (i.e. IL-6 and TNFα) can lead to modulation of immune function; increased cortisol (which is catabolic in nature) and prolonged elevations in lactate can all lead to subsequent damage to the muscle architecture, and thus decrements in muscle strength and function (Enoka & Duchateau, 2008).

Skeletal Muscle Fatigue

The term muscle fatigue denotes a transient decrease in the capacity to perform physical actions. Muscle fatigue can be defined as 1) an exercise-induced reduction in the

ability of muscle to produce force or power whether or not the task can be sustained or 2) a decrease in the maximal force or power that the involved muscles can produce that develops gradually soon after the onset of sustained physical activity (Enoka & Duchateau, 2008). There are many causes of fatigue. Peripheral fatigue in endurance athletes is likely to due to an increase in lactate accumulation, depletion of muscle glycogen, and/or failure of the neuromuscular system (Plowman & Smith, 2007). Muscle homeostasis during exercise may be altered due to hydrogen ion accumulation, calcium and potassium losses, local ischemia, and depletion of ATP, Cr, and/or glycogen stores. The disruptions to homeostasis may be contributing factors to the interference of the muscle excitation-contraction coupling cycle during intense exercise and post-exercise, leading to muscular fatigue (Mika et al., 2007). Excitation-contraction coupling is hindered due to the reduction in Ca^{2+} release and uptake from the sarcoplasmic reticulum. This may negatively influence the rate of force development of the muscle. The neuromuscular system may also influence rate of force development through the alteration of nerve signal conduction between the nerve ending and the motor endplate. These factors directly impact peripheral fatigue (Peterson et al., 2007). A decrease in the rate of force development can be quantified as the decline in the maximal power or force measured immediately after fatigue (Enoka & Duchateau, 2008). Fatigue may also be manifested as a more gradual loss of force (Gibson & Edwards, 1985). For example, Halson and colleagues (2002) examined the effects of exercise stress on performance and fatigue indicators in endurance cyclists. After a period of intensified cycling, a significant decline in maximal power output and significant increase in time to complete a simulated

time trial were observed, suggesting that exercise stress does play a role in subsequent performance.

Lactate metabolism and its rate of elimination from the muscle and blood are important contributors to muscle fatigue (Martin, Zoeller, Robertson, & Lephart, 1998). The accumulation of hydrogen ions in the muscle is detrimental for numerous reasons. As the concentration of metabolites increases, force and tension development declines. This is a leading indicator of fatigue (Messonnier, Kristensen, Juel, & Denis, 2007). One of the numerous proposed causes to muscular fatigue includes the build-up of metabolites in the active muscle (Mika et al., 2007). Additionally, the decline of cellular pH is among the probable contenders for muscle fatigue. The hydrogen ion's involvement in muscle function disturbances has been recognized. Both H^+ and lactate impair excitation-contraction coupling and relegate calcium-activated force (Messonnier et al., 2007). Changes in intracellular pH may also alter channel properties, modify protein correspondence (Messonnier et al., 2007), and diminish the activity of crucial enzymes in glycolysis (Messonnier et al., 2007; Chasiotis et al., 1983), reducing the rate of ATP resynthesis (Messonnier et al., 2007; McCartney et al, 1986; Sahlin, 1992). Therefore, increasing clearance of lactate post-exercise is important for reducing the impact that lactate has on the muscle architecture.

Systemic Stress and Inflammation

Endurance exercise presents a challenge to the body of an athlete. Typically, the body reacts through a systemic inflammatory response (Neubauer et al., 2008). Pathophysiological mechanisms that disturb homeostasis after acute stress-related incidences may be involved in the systemic inflammatory response syndrome (SIRS) on

the basis of systemic cytokine release, also known as hypercytokinemia. The primary suspects linked to SIRS are circulating neutrophils because these cells are activated by cytokines and unfavorably affect microcirculation and destroy tissue. SIRS indicates the presence of two or more of the following: 1) body temperature exceeding 38˚C; 2) heart rate exceeding 90 beats/min; 3) respiration exceeding 20 breaths/min; 4) leukocyte count exceeding 12,000 cells/µl or less than 4,000 cells/µl; and 5) immature cells exceeding 10%. Exhaustive endurance exercise is likely to meet the majority of the above requirements. Because of the impact exercise has on disturbing skeletal muscle, neutrophil infiltration is a common occurrence after endurance exercise, specifically following activity at greater than 60% VO_{2max} and for longer than 60 minutes. In addition to immediately after prolonged endurance exercise, delayed onset of cytokines has been reported in the recovery period up to several hours following muscle-damaging exercise. Moreover, stress hormones such as cortisol, have been associated with mediating exercise-induced cytokine secretion and modulating neutrophil count and function. This suggests a multifaceted interaction among the fundamental mechanisms of exercise-induced muscle damage and the systemic inflammatory response (Suzuki, Manabu, Shigeyuki, Mutsuo, Satoru, Qiang, et al., 1999).

Immune function indicators (white blood cells; eosinophils, basophils, monocytes, granulocytes, lymphocytes, macrophages) have been studied as markers of systemic stress and/or inflammation and are anticipated to increase following exhaustive endurance exercise (Neubauer et al, 2008; Tzai & Cheng, 2007; Li & Gleeson, 2004; Suzuki et al, 2002). Leukocyte regulation and distribution is a multifaceted process that involves a number of factors. Exhaustive endurance exercise provokes oxidative,

metabolic, and hormonal stresses, all of which can lead to the release of cytokines and the activation of numerous cell subpopulations within the immune system (Neubauer et al., 2008). Specific immune parameters, such as neutrophil function and leukocyte distribution, are impacted by exercise. Tzai and Cheng (2007) noted an increase in leukocyte, neutrophil, and monocyte counts 9 hours after a 2 hour prolonged cycling bout that was performed at 55% peak aerobic power. This acute immune response results in a mobilization of neutrophils, lymphocytes, and monocytes in response to the need for muscle regeneration and repair. In a study examining the inflammatory responses and muscular stress following an Ironman triathlon, Neubauer et al. (2008) reported an increase in total leukocyte counts immediately post-race (237%) and 1 day post-race (56%). In addition, mean changes in leukocyte subpopulations (granulocytes, monocytes, and lymphocytes) were also found. Furthermore, Li and Gleeson (2004) observed an immunoendocrine response, specifically neutrophilia and monocytosis, after a 2 hour prolonged cycling bout performed at 60% VO_{2max}. The exercise-induced mobilization of these cell subpopulations within the immune system demonstrates the physiological stress that endurance exercise places on immunological parameters.

During prolonged, high intensity exercise, adrenocorticotrophan (ACTH) is secreted in response to stress and decreasing blood glucose levels. ACTH stimulates cortisol release which in turn promotes muscle proteolysis. Cortisol is a catabolic steroid hormone that reduces the utilization of amino acids for protein formation in muscle cells and aids in the maintenance of blood glucose homeostasis (Gleeson, 2002). Cortisol, through protein mobilization, gluconeogenesis, and free fatty acid mobilization, is thought to have an effect on glucose and glycogen replenishment during recovery from

exercise (Gaesser & Brooks, 1980; Fell et al, 1980). Blood cortisol levels increase at a rate proportional to exercise intensity and reach a final concentration dependent on the duration of exercise (Brandenberger & Follenius, 1975). Neubauer et al. (2008) found an increase in cortisol levels immediately following an Ironman triathlon. The Ironman triathlon consists of a 3.8 km swim, a 180 km bike, and a 42.2 km run. Ironman triathletes are extensive in their level of training and perform at an extremely high intensity and extended duration. Therefore, they are a notable group to investigate due to both the physiological demands of performance and the possibility of subsequent harmful effects of the ultra-endurance sport (Neubauer et al., 2008). Hill and colleagues (2008) examined the influence of exercise intensity on the cortisol response of the HPA-axis. Cortisol levels in moderately active trained men were investigated before and after 30 minutes of exercise at 40, 60, and 80% of their VO_{2max}. Results indicated that the 60% and 80% intensity magnitude of change (39.9% and 83.1%, respectively) was significantly greater than the 40% intensity session (5.7%) as well as between one another. Researchers concluded that moderate to high intensity exercise provokes increases in circulating cortisol levels, likely due to a combination of hemoconcentration and HPA axis stimulus. Conversely, low intensity exercise at 40% VO_{2max} actually resulted in a reduction in circulating cortisol levels (Hill, 2008). The lack of elevation during the low intensity exercise may be due to an increased metabolic clearance rate that is triggered by target tissue uptake of cortisol (Hill, 2008; Davies & Few, 1973).

Cytokines

Several studies have examined the cytokine responses to endurance exercise. Certain proinflammatory and immunomodulatory cytokines are known to increase

markedly in the circulation following endurance exercise (Suzuki, Nakaji, Yamada, Totsuka, Sato, & Sugawara, 2002). Suzuki et al. (2006) noted that endurance exercise, such as an Ironman triathlon, cause considerable muscle damage and inflammation which produces a cytokine response. Cytokine production and release is induced due to damage to muscle tissue, oxidative stress (Suzuki et al., 2006), and other metabolic and hormonal factors (Neubauer et al., 2008). The immune and cytokine responses work successfully in a collective manner. Cytokine release at the site of inflammation facilitates an influx of monocytes, lymphocytes, neutrophils, and other cells that aid in the clearance of metabolites and promote recovery (Petersen et al., 2001). Cytokine release may also be attributable to an increased epinephrine concentration and oxidative stress during exercise (Suzuki et al., 2006). The complex cytokine response associated with muscle damage includes the simultaneous presence of naturally occurring cytokine inhibitors and anti-inflammatory cytokines. Cytokine production and subsequent release facilitate the inflammation response and aid in the healing of tissue (Ostrowski, K., Rohde, T., Asp, Sven., Schjerling, P., & Pederson, B.K., 1999).

Both TNFα and IL-6 are involved in most aspects of the acute phase response. These cytokines are therefore typically referred to as inflammatory or pro-inflammatory cytokines. Because IL-6 does not itself induce inflammation, this cytokine is generally identified as an "inflammation-responsive" cytokine (Ostrowski, Rohde, T., Asp, S., Schjerling, & Pederson, 1999). TNFα promotes the release of IL-6. However, systemic IL-6 increases are more likely due to catecholamine stimulation through B_2-adrenergic stimulation rather than through inflammation itself (Mastorakos et al., 2005). Marked increases in IL-6 levels after exercise is consistent in the literature (Ostrowski, 1999;

Bruunsgard, 1997; Ullum, 1994). However, reported findings of post-exercise TNFα concentrations are inconsistent. Plasma TNFα reportedly increased 2 hours and 1 hour after completing both a 2.5 hour run and following a 5km race (Dufaux & Order, 1989; Espersen et al., 1990), respectively, while other studies have failed to detect TNFα post-exercise (Ostrowski et al., 1999; Rivier et al., 1994; Ullum et al., 1994). Many studies reported a lack of or minor elevations in TNFα following heavy exercise except for a few studies that examined extreme endurance exercise such as marathon running (Pederson, 1997; Northoff & Berg, 1991). Typcially, IL-6 is the first cytokine present in the circulation following exercise and may increase by 100-fold (ACSM, 2006; Petersen, 2001). This is likely attributable to the fact that IL-6 is produced locally in contracting skeletal muscle and that larger amounts of IL-6 are produced in response to exercise than any other cytokine (Pedersen, 2001).

Ostrowski and colleagues (1999) examined the pro and anti-inflammatory cytokine balance in male marathon runners before, immediately after, and every 30 minutes in a 4 hour post-exercise recovery period. Plasma concentrations of IL-6 and TNFα were observed, among other cytokines. The highest concentration of IL-6 was found immediately post-race, with a 128-fold increase compared to pre-race levels. IL-6 levels decreased during the 4 hour post-exercise period, but remained significantly elevated above pre-race values. Plasma TNFα levels peaked within the first hour after the marathon, with a 2.3-fold elevation compared to pre-race.

Elevated plasma IL-6 levels were reported in trained male triathletes following an acute period of intense interval training. This acute, intensified training is postulated to induce a suppression of the immune system and an elevation in IL-6. It has been

proposed that cytokines are linked to fatigue and that cytokine release during and following exercise may cause a chronic inflammatory response. Specifically, significant physical stress is likely linked to an excess production and/or a heightened sensitivity to IL-6 during exercise. IL-6 levels have been shown to increase greater than 100-fold during prolonged exercise with levels typically returning to pre-exercise levels within a few hours of rest (Robson-Ansley, Blannin, & Gleeson, 2007).

Rowlands et al. (2008) and Suzuki et al. (2006) examined TNFα as a marker of stress, inflammation, and muscle damage during recovery from high-intensity cycling and an Ironman triathlon race, respectively. Suzuki et al. (2006) reported that, following an Ironman triathlon race, plasma IL-6 concentrations were significantly elevated above pre-race values within 30 minutes after the race. Additionally, IL-6 remained significantly elevated above pre-race values 1 day after the race. Similarly, Neubauer et al. (2008) found an increase in IL-6 both immediately (10408%) and one day (345%) following an Ironman triathlon. Release of IL-6 from the skeletal muscle into systemic circulation is attributed to the decreased availability of blood glucose and muscle glycogen. Strenuous exercise induces an increase in the pro-inflammatory cytokine TNFα and a dramatic increase in the inflammation responsive cytokine IL-6 (Ostrowski et al., 1999). In addition to legitimate increases in these cytokines in blood, there have been significant associations between perceived stress and both IL-6 and TNFα as observed in elite rowers who participated in endurance training (Main et al., 2009). Bruunsgaard and colleagues (1997) examined the hypothesis that the exercise-induced increase in circulating cytokine levels is associated with muscle damage. Two 30 minute high-intensity trials were performed; the first consisted of normal concentric cycling and the

second consisted of braking with eccentric reversed revolution exercise. Researchers found that the eccentric exercise caused a more pronounced increase in plasma IL-6 levels 2 hours post-exercise, suggesting that the post-exercise cytokine response is related to skeletal muscle damage. This damage may specifically be due to increased levels of myofiber enzymes in plasma, ultrastructural damage to the muscle fiber, and the infiltration of inflammatory cells into the affected musculature. Petersen and colleagues (2001) point out a noteworthy detail regarding IL-6 kinetics: it is probable that the kinetics of IL-6 differ between exercise inducing early muscle damage and prolonged muscle damage. For example, when an eccentric exercise model was used, IL-6 levels did not peak until 1 to 1.5 hours after exercise. In contrast, when IL-6 concentrations were measured during and following a prolonged treadmill run or marathon, levels peaked 2.5 to 3.5 hours after exercise.

Research has demonstrated that many factors determine the degree to which serum enzyme activities increase during and following exercise. Two of the main factors include the intensity and duration of preceding exercise (Noakes, 1987; Petersen, 2001). Regarding duration, the most substantial post-exercise elevations of these enzymes are found after prolonged competitive exercise such as triathlons or ultra-endurance events and in activities which include eccentric muscle contractions such as downhill running or bench stepping (Noakes, 1987). Petersen and colleagues (2001) concur that the magnitude of increase in circulating cytokines is closely related to the duration of exercise. Another factor determining serum enzyme activity is individual variability. For example, a 50-fold difference in post-race enzymes was found in equally trained athletes completing the same 90 km ultra-marathon race. It is likely that the noted elevations that

occur following prolonged exercise is attributable to myofibrillar damage (Noakes, 1987). The damage that the muscle fibers incur as a result of exercise is an important issue to address. Damaged muscle must be restored in order for recovery and subsequent performance to be optimized. While the present study focuses on markers of system stress and inflammation, a parallel can be drawn between increases in enzyme activity that is due to changes in muscle parameters, specifically muscle damage and inflammation.

Muscle Damage

Inflammatory cell populations are likely to appear at the site of both muscle use and injury in a rapid and sequential manner and may continue to persist for days to weeks while muscle repair, regeneration, and growth occur (Tidball, 2005). In response to homeostasis disruption during exercise, a stereotypical inflammatory response ensues which consists of neutrophil, macrophage, free radical, growth factor, and chemokine invasion of the affected musculature (Tidball, 2005; Warren et al., 2004; Zhang et al., 2009). This occurrence is a complex process in which inflammatory cells may promote both injury *and* repair. For example, macrophages have been shown to promote muscle damage through the release of free radicals but have also been shown to play a role in muscle restoration through growth factors and cytokine-mediated signaling (Tidball, 2005; Suzuki et al, 1996). Neutrophils are responsible in eliciting muscle damage soon after exhaustive use but there has been no conclusive evidence yet to endorse the role neutrophils may play in muscle repair or regeneration. The underlying consensus demonstrates that whether the inflammatory response has an overall positive or negative effect on muscle function depends on the magnitude of the response, the previous history

of muscle use, and possibly injury-specific interactions between muscle and the invading inflammatory cells (Tidball, 2005; Tidball et al, 1998; Jungersten et al., 1997).

In addition to research concerning inflammatory parameters initiated by muscle damage or injury, recovery modalities have largely been investigated with regard to their ability to reduce the severity and duration of exercise-induced muscle injury and delayed onset muscle soreness (DOMS) (Barnett, 2006). Both unaccustomed exercise and eccentrically-biased exercise may result in significant damage to skeletal muscle and cause delayed onset muscle soreness. Both metabolic and mechanical mechanisms have been suggested to elucidate how exercise instigates this skeletal muscle damage (Pyne, 1994). When examining the mechanical mechanism of delayed onset muscle soreness, DOMS is generally implicated as a result of eccentric movement and typically occurs 48-72 hours following demanding exercise. Symptoms can range from muscle tenderness to severe debilitating pain. The intensity and duration of exercise are important factors in the onset of DOMS. Several theories have been proposed for the mechanism of DOMS, some of which include lactic acid, muscle spasm, connective tissue damage, muscle damage, inflammation, and the enzyme efflux theories (Cheung et al, 2003). Although not well substantiated, post-exercise free radical production has been linked to exercise/contraction-induced oxidative muscle damage as well (Close et al, 2005 & Pyne, 1994). It is likely that a combination of these factors contribute to DOMS (Cheung, Hume, Maxwell, 2003). Fortunately, skeletal muscle has the ability to complete a rather rapid and extensive regeneration in response to severe damage, as incurred by demanding physical activity. Muscle regeneration is characterized by two phases: a degenerative phase and a regenerative phase. The former is distinguished by the activation of an

inflammatory response which leads to the loss of muscle architecture. This phase of muscle injury is typically accompanied by the activation of mononucleated cells, primarily inflammatory cells and myogenic cells. Inflammatory cells are activated by certain factors that reside within and are released by the injured muscle that may provide the chemotactic signals to circulating inflammatory cells. Both neutrophils and macrophages are involved in this inflammatory cell cascasde **(**Chargé & Rudnicki, 2004; Petersen et al., 2001). Neutrophils are the first inflammatory cells to invade the injured muscle, with a significant increase in their number being observed as early as 1–6 hours after exercise-induced muscle damage. Macrophages become the predominant inflammatory cell type within the site of injury after neutrophil infiltration, usually 48 hours post-injury. The regenerative phase is characterized by the activation of myogenic cells to proliferate, differentiate, and fuse to necrotic fibers for repair or to each other for new fiber formation **(**Chargé & Rudnicki, 2004; Armstrong et al., 1991). Repair of the muscle fibers appears to be a comprehensive process, with the fibers adapting during this process so that future bouts of exercise of similar type, intensity, and duration cause less injury to the muscle (Armstrong et al., 1991).

Endurance exercises are typically not muscle damaging, meaning they cause no overt structural damage to the muscle. However, a major stress response is still noticed in relation to exercise-induced reactive oxygen species (ROS) production. ROS production during endurance exercise can be contributed to increased oxygen uptake which may lead to an overflow of electrons through the electron transport chain and an increased activity of immune and metabolic processes. The stimulus for free radical production is likely to occur due to proteolysis, with protein then used as a fuel source. The subsequent muscle

damage is then likely to lead to oxidative stress. In addition to substrate degradation eliciting ROS production, immune system cells such as macrophages, neutrophils, and monocytes form ROS to initiate the inflammatory response to exercise-induced muscle injury (Knez, Coombes, & Jenkins, 2006).

As demonstrated by the aforementioned research, the many demands of endurance exercise coupled with the subsequent inflammatory response and issues related to muscle damage, the aspect of recovery from exercise becomes increasingly important.

Muscle Repair and Recovery

An appropriate balance between training and competition stresses and recovery is important in maximizing athletic performance. In addition to serum variables and performance data, psychological indicators of fatigue and recovery are also important to an athlete's performance. These psychological indicators have been monitored by administering the Profile of Mood States Assessment. Kentta and colleagues (2006) monitored the mood state of elite kayakers during training and recovery to assess both training-induced mood disturbances and the extent of recovery following a training load. Researchers concluded that utilizing the POMS fatigue, vigor, and depression scores during periods of intensified training may help prevent athletes from becoming severely overreached. Likewise, the POMS was utilized by Halson and colleagues (2002) to monitor the effects of exercise stress and subsequent recovery on performance changes and fatigue indicators in endurance cyclists. Global mood state scores on the POMS were significantly increased from the normal training period to the intensified training period. Specifically, the subscales of tension, fatigue, and confusion were elevated, whereas vigor declined. On completion of recovery training, scores returned to normal training

levels. Researchers concluded that the POMS, when used in conjunction with other performance decrement measures, can be a useful tool in determining early overreaching.

A wide array of recovery modalities is utilized by athletes to create a balance between the stresses of training and competition as well as to enhance between-training session recovery in athletes. Some of these recovery techniques include cryotherapy, hyperbaric oxygen therapy, massage, compression garments, electromyostimulation, rehydration, active recovery, stretching, and non-steroidal anti-inflammatory drugs (Barnett, 2006). Two dominant forms of recovery exist: active and passive. Passive recovery is defined as inactive rest, such as performing no physical activity. Active recovery generally involves performing aerobic exercise at a low intensity. Stretching can also be considered a form of active recovery. Low intensity aerobic exercise is defined as exercise at approximately 40% of VO_{2max} (Martin et al., 1998). Active recovery at 40% maximal oxygen uptake potentiates the enhanced ability to remove blood lactate induced by endurance training as compared to passive recovery (Taoutaou, Granier, Mercier, Mercie, Ahmaidi, & Prefaut, 1996). Active recovery refers to performing low intensity exercise in the immediate post-exercise window as well as the days following the workout. Active recovery plays a major role in lactate removal; this form of recovery aids lactate clearance by increasing blood velocity and blood flow to the affected musculature thereby increasing lactate metabolism by augmenting the transport of lactate from the active muscle to the removal sites (Martin et al., 1998; Choi et al., 1994). The removal of these metabolites is higher during active recovery than passive recovery (Mika et al, 2007).

Studying indirect markers of recovery following an exhaustive bout of exercise will allow for the most effective recovery pattern to be determined (Peterson, Hansen, Aagaard, & Madsen, 2007). The training and recovery schedule of an athlete must be fine-tuned in order for progression to occur. In order for a high level of performance to be maintained, there must be a balance between training, competition, and recovery. Recovery must be implemented in order to restore muscular, inflammatory, and immune parameters (Neubauer et al., 2008). Researchers have reported that it is an ordinary practice for athletes to "warm-down", or actively recover, in order to reduce the accumulation of blood and muscle lactate and other physiological products after competition or training (Choi, Cole, Goodpaster, Fink, & Costill, 1994). Cycle ergometry (Choi et al., 1994); Mika et al., 2007), stretching (Herbert & Gabriel, 2002; Mika et al., 2007), and aqua exercise (Takahashi, Ishihara, & Aoki, 2006; Coffey, 2004; Cortis, 2010) have been utilized in recovery. Cycling, stretching, and the G-trainer have been chosen as recovery methods in the present study. The following information explains why these recovery procedures were implemented.

Stretching

Many athletes stretch before and/or after engaging in athletic activity. The purpose of stretching is to reduce risk of injury, reduce soreness after exercise, and/or enhance athletic performance (Herbert & de Noronha, 2007). Kinugasa and Kilding (2009) examined the effect of three post-match recovery modalities on vertical jump height after a 90 minute soccer match. The three recovery modalities included cold water immersion with hot shower, cold water immersion with active recovery on a cycle ergometer, and passive recovery with stretching. Researchers discovered that neither

recovery modality had a significant substantial effect on vertical jump height performance 24 hours post-match. Similarly, Dalrymple and colleagues (2010) determined the effects of stretching on peak jump height. Female volleyball players performed a light warm-up and then completed eight minutes of one of three stretching protocols: static stretching, dynamic stretching, or no stretching. One minute later, five maximal countermovement jumps were performed on a force platform with one minute passive rest between jumps. Results indicated that there were no significant differences between the three stretching protocols. Another study (Miladi et al., 2010) examined the effect of passive, active, or dynamic stretching on exercise time to exhaustion and blood lactate responses during supramaximal cycling exercise. Soccer players participated in two series of four repeated, intermittent supramaximal cycling exercises interspersed in random order with passive, active, or dynamic stretching. Results indicated lower blood lactate concentrations were observed with both dynamic and active stretching as compared to passive stretching. Furthermore, a greater exercise time to exhaustion was observed for dynamic stretching compared to active or passive stretching. Robey and colleagues (2009) compared the effects of hot/cold water immersion, static stretching, and no recovery interventions on leg strength, rowing performance, and indicators of muscle damage/soreness in the 72 hours following strenuous stair-climb running. In a crossover manner, rowers performed the run on three separate occasions followed by 15 minutes of one of the recovery procedures, which were repeated at 24 and 48 hours post-run. Researchers found that no significant strength or performance differences existed between the three recovery treatments. The results concluded that neither water immersion nor static stretching accelerated recovery at the 72 hour time point beyond that

achieved by the control group. Researchers site that the lack of differentiation between treatment groups may have been due to the type of hydrostatic pressure applied, the need for more dynamic performance measures, and the need for measurements on multiple occasions. Nevertheless, the return to baseline values for all variables except muscle soreness by the 48-72 hour time point suggests that time is an important factor in the post-exercise recovery process.

Cycling

In addition to stretching, cycling has also been utilized as a recovery aid. Gleeson et al. (1995) examined the hematological responses to submaximal cycling two days after either eccentric or concentric exercise bouts in order to determine if DOMS-inducing exercise affects physiological responses to subsequent submaximal exercise. The eccentric bout consisted of 30 minutes of bench stepping while the concentric bout consisted of 30 minutes of uphill walking. Results indicated that venous blood lactate concentration and plasma cortisol concentration were higher during cycling after eccentric exercise as compared to concentric exercise. Because eccentric exercise produced a larger exercise stress, this type of exercise is likely to limit the intensity and duration of subsequent training or competition. In a similar study conducted by Gleeson and colleagues (1998), DOMS-inducing exercise was explored in regard to its effects on blood lactate responses to subsequent incremental exercise. An eccentric exercise bout consisting of 40 minutes of bench stepping and a control condition of no prior exercise were utilized. Participants returned 2 days later and performed an incremental cycling protocol to exhaustion. Blood lactate concentration was higher during cycling after eccentric exercise as compared to the control condition of no prior exercise. Blegen and

colleagues (2008) explored the effects of varying intensities of cycle ergometry on select immune regulators and metabolic responders. One hour exercise bouts were performed at both 40% and 60% VO_{2max} in both normoxic and hypoxic environments. No significant differences were detected between condition or intensity for TNFα, likely because the level of hypoxia was not great enough and the exercise intensity was not strenuous enough to elicit changes in this immune parameter. Researchers reported that a modest increase in cortisol was identified with respect to intensity. This elevation likely coincides with the glucose elevations noted during hypoxia, suggesting that cortisol contributes to glucose maintenance.

Weight Supported Exercise and the G-trainer

Along with the use of stretching and cycling as recovery aids, aqua exercise has also been implemented in the recovery process. Takahashi and colleagues (2006) reported that aqua exercise promoted physiological functioning (muscle soreness, serum creatine kinase activity, muscle power, flexibility, whole-body reaction time and muscle stiffness) of the leg muscles following high-intensity downhill running for a period until the damaged muscles had recovered almost completely. Coffey and colleagues (2004) compared the effectiveness of active recovery, passive recovery, and contrast temperature water immersion on the performance of repeated treadmill exercise. Participants performed two pairs of treadmill runs at 120% and 90% of peak running speed over a four hour period and then engaged in one of the three recovery methods for 15 minutes. The recovery modalities were active running at 40% peak running speed, passive recovery while standing stationary, or contrast temperature water immersion (CTW) which alternated between 60 seconds cold and 120 seconds hot water immersion. Results

indicated that post-exercise blood lactate concentration was lower with active recovery and CTW as compared to passive recovery. Although a reduction in blood lactate was identified through active and CTW recovery after high intensity running, running performance returned to baseline 4 hours after the initial exercise bout regardless of the recovery strategy implemented (Coffey et al., 2004). Cortis and colleagues (2010) examined the effects of three post-exercise recovery interventions (low intensity water exercise, supine electrostimulation, and passive recovery) on physiological and performance parameters on subsequent daily submaximal running tests. No significant differences were reported between the morning and afternoon physiological parameters (blood lactate, oxygen consumption, percent hemoglobin saturation) and performance parameters (countermovement, bouncing jumps). This data suggests that post-exercise recovery interventions may not differ over a limited time period. The G-trainer, a relatively new anti-gravity treadmill, has been compared to aqua exercise in that the device is used for rehabilitation and performance enhancement purposes. For example, Coffey and colleagues (2004) report that active treadmill and active water recovery are equally effective in enhancing blood lactate removal.

Cycle ergometry and stretching have been documented as methods of active recovery (Choi et al., 1994; Mika et al., 2007; Herbert & Gabriel, 2002), and have therefore been selected as recovery modalities in the present study. However, there is little research available on the use of the G-trainer as a recovery aid. Alter-G, Inc. claims that the G-trainer enables users to enhance performance (Alter-G, Inc., 2009). A particular aspect of performance enhancement is the ability to recover from exercise. Furthermore, one of the health applications of the G-trainer is to aid aerobic conditioning.

The G-trainer claims to do this by reducing force of impact on the body and protecting tissues during recovery while maintaining an effective cardiovascular workout. There is little evidence available as to the efficacy of the G-trainer as a recovery modality in enhancing recovery in the immediate post-exercise window as well as during the time between training sessions. To the author's knowledge, there is no research available that is specific to the effectiveness of the G-trainer as a recovery modality following an acute bout of cycling endurance exercise in aerobically trained males.

Summary of Findings

The need for adequate recovery during and after exercise is vital for an athlete to return to normal training and competition. Recovery allows the body to adapt to the stress of exercise, to replenish energy stores, repair damaged tissues, and clear lactate. There are two primary forms of recovery: active and passive. Passive recovery is defined as inactive rest. Active recovery generally involves performing aerobic exercise at a low intensity. Active recovery refers to performing low intensity exercise in the immediate post-exercise window as well as the days following the workout. Cycle ergometry and stretching have been documented as methods of active recovery. However, research regarding the use of the G-trainer as an effective recovery method is limited. Studying the fatigue recovery process of endurance athletes through the examination of markers of skeletal muscle membrane damage, systemic stress and inflammation, peripheral fatigue, and psychological indicators will allow for the most effective recovery pattern to be determined.

CHAPTER THREE

Methodology

Experimental Design

Subject Demographics

Twelve aerobically trained male individuals between the ages of 18 and 26 participated in this study. Aerobically trained individuals had been training an average of 11.4 ± 4.7 months for an average of 8.5 ± 3 hours/week and 4.5 ± 1 days/week of aerobic exercise (i.e., running, cycling, rowing, and/or swimming). All subjects signed an informed consent document and the study was approved by the Baylor University Institutional Review Board prior to any data collection. Subjects were excluded from participation in this study if they reported any of the following: 1) current or past history of anabolic steroid use; 2) any metabolic disorders or taking any thyroid, hyperlipidmeic, hypoglycemic, anti-hypertensive, or androgenic medications; 3) ingested any ergogenic levels of creatine, HMB, thermogenics, ribose, pro-hormones (i.e., DHEA, androstendione, etc.) or other purported anabolic or ergogenic nutritional supplements within 2 months prior to beginning the study and to not take any additional nutritional supplement or contraindicated prescription medication during the protocol.

Study Site

All testing and training was conducted in the Exercise & Nutrition Sport Nutrition Laboratory (ESNL) in the Department of Health, Human Performance, and Recreation at Baylor University.

Independent and Dependent Variables

Table 1 shows the general research design and time course of assessments administered in this study.

The independent variables were the recovery treatments (Stretching, Cycle Ergometry and G-Trainer), and the sampling times during the course of this study. Dependent variables included the information collected on food records, blood lactate, inflammation and systemic stress-responsive serum variables (cortisol, TNF-α, IL-6), skeletal muscle power output, and profile of mood states assessment.

Entry/Familiarization Session

Subjects expressing interest in participating in this study were interviewed on the phone to determine whether they appeared to qualify to participate in this study. Subjects believed to meet eligibility criteria were then be invited to attend an entry/familiarization session. Once reporting to the lab, subjects completed a medical history questionnaire and underwent a general physical examination to determine whether they met eligibility criteria. Once cleared, subjects were familiarized to the study protocol via a verbal and written explanation outlining the study design. Eligible subjects who agreed to participate in the study read and signed university-approved informed consent documents and were given a dietary recall sheet that was completed prior to the baseline testing session. Further, subjects were instructed to refrain from any form of physical exercise for 48 hours and fast 8 hours prior to the baseline session.

Baseline Assessment

Prior to the endurance exercise bout, each subject had their body weight, height, heart rate, blood pressure and total body water determined. Subjects also completed a POMS questionnaire to determine their physiological state.

Each subject was assessed for aerobic fitness [maximal oxygen uptake ($VO_{2\ max}$)] by performing a cardiopulmonary graded exercise test on a LODE cycle ergometer (Amsterdam, Netherlands). The cycle ergometry test began at a power output of 100 watts. The workload was increased by 50 watts every two minutes until volitional fatigue. Table 2 illustrates the Astrand cycle ergometry protocol that was used.

Table 1

Maximal Graded Exercise Test Protocol

Warm-up	10 minutes	----------
Stage 1	Minutes 0-2	100 watts
Stage 2	Minutes 2-4	150 watts
Stage 3	Minutes 4-6	200 watts
Stage 4	Minutes 6-8	250 watts
Stage 5	Minutes 8-10	300 watts

Oxygen uptake (VO_2) was measured every 30 seconds via an open-circuit sampling system (Parvo Medics, Sandy, UT). VO_{2max} was determined if two of the following criteria were met: respiratory exchange ratio (RER) $\geq$ 1.15 and/or RPE $\geq$ 19 on the RPE scale and/or maximum heart rate within $\pm$ 10 beats of age-predicted maximum (HR_{max}). If such criteria were met, the highest level of VO_2 will be defined as $VO_{2\ max.}$ If such criteria were not met, then the highest VO_2 reached will be termed VO_{2peak} rather than VO_{2max}. Heart rate was determined from a continuously monitored heart rate monitor with chest strap and wristwatch (Polar Electro, Lake Success, NY).

Participants were randomly assigned through block randomization to one of three treatment groups: G-trainer, cycling, and/or stretching. The subjects participated in a crossover design and performed each recovery treatment on three separate occasions separated by 14 days.

Dietary Records and Psychological Assessment (POMS)

The participants' diets were not standardized and participants were asked not to change their dietary habits during the course of the study. In an attempt to assess the average daily macronutrient consumption of fat, carbohydrate, and protein, each participant was asked to keep dietary records the day prior to, the day of, and they day after the testing session. Each participant was instructed how to fill out the record prior to use. The dietary records were analyzed with the Food Processor Dietary Assessment Software program (ESHA Research Inc., Salem, OR). A psychological assessment was conducted using the current POMS (profile of mood states) questionnaire. The POMS is a validated, standardized self-rating scale consisting of 65 items that measures six identifiable mood states; Tension-Anxiety; Depression-Dejection; Anger-Hostility; Vigor-Activity; Fatigue-Inertia; Confusion-Bewilderment. A 5-point scale is used from 0 = not at all to 5 = extremely. The POMS was conducted at the entry/familiarization session and immediately after, three hours after, and 24 hours after the recovery treatment.

Nutritional Bar Intake

Participants were provided with a Kashi TLC Chewy Granola Bar to ingest 15 minutes prior to the endurance exercise bout. Each bar contained 140 calories, 5 grams of

fat, 105 milligrams of sodium, 100 milligrams of potassium, 20 grams of carbohydrate, and 6 grams of protein.

Endurance Exercise Bout

One week following baseline testing, each participant reported back to the lab for the endurance exercise bout. Participants were instructed to refrain from aerobic exercise for 48 hours and fast for 8 hours prior to the exercise session. The Computrainer is a stationary electronic bicycle ergometer that allows the user to ride their own bicycle. A trainer consists of a frame, a clamp to hold the bicycle securely, a roller that presses up against the rear wheel, and a mechanism that provides resistance when the pedals are turned. The protocol was selected from the Computrainer 3D Version 3 Software (Copyright 2006 Racermate) and was programmed into and used in conjunction with the bicycle ergometer to simulate a race course and race effort. The USAT Nationals St. Joe course, an 18.25 mile course with an average grade of 0.3%, a maximum grade of 9.7%, and 1702 total feet of climbing was used. Participants were provided with a viewing screen that depicted the race course. The participants were instructed to ride as hard as possible for the duration of the protocol. A fan providing constant wind speed was placed directly in front of the participants and positioned so that the airflow was directed towards the head and torso when in a normal cycling position. Water was available to the participants ad libitum. After the exercise session, participants donated blood and performed one of the three recovery treatments.

Recovery Treatment

The G-trainer (Alter-G Inc., Fremont, CA), is a computer-controlled integrated body-weight-supported treadmill system which uses differential air pressure to support

the user while walking or running on the treadmill. The G-trainer enables its users to reduce their effective body weight in as few as 1% increments from 100% of body weight to 20% of body weight.

The Computrainer Lab is an electronic bicycle ergometer that allows the user to ride his/her own bicycle. A trainer consists of a frame, a clamp to hold the bicycle securely, a roller that presses up against the rear wheel, and a mechanism that provides resistance when the pedals are turned.

The standard stretching protocol consisted of eight stretches that were performed for 3 sets of 30 seconds of static stretching per bilateral muscle group including hip, knee, and ankle flexors and extensors (Appendix F).

If recovering on the G-trainer, the participant ran at 40% VO_{2max} and 75% of body weight for 30 minutes. If recovering on the Computrainer, participants cycled at 40% VO_{2max} for 30 minutes. If recovering with stretching, participants performed standard static stretching exercises for approximately 30 minutes. The American College of Sports Medicine metabolic guidelines were used to determine the initial workload that elicited an oxygen uptake of an approximate equivalent intensity of 40% VO_{2max} for each participant. However, during the recovery protocol, 40% of maximal oxygen uptake was maintained by measuring oxygen uptake every 5 minutes and adjusting workload accordingly.

Blood Collection Procedure

Venous blood samples were obtained from the antecubital vein into a 10 ml collection tube using a standard vacutainer apparatus. Blood samples were allowed to stand at room temperature for 10 minutes and then centrifuged. A total of 13 blood

samples were obtained. For each sample, the serum was removed and frozen at -80°C for later analysis. Blood samples were collected at baseline, prior to and immediately post-endurance exercise bout and at three and 24 hours after the endurance exercise bout. Except for the three post-exercise samples, all blood samples were obtained after an 8-hour fast and standardized to the same time of day for each sample.

Blood Lactate Analysis

Blood was analyzed for lactate levels (mm) using an Accusport™ Blood Lactate Analyzer. Blood samples were collected at 0, 5, 10, 15, and 30 minutes after the endurance exercise bout, and at 3 and 24 hours post recovery.

Anaerobic Muscle Assessment

At baseline and 24 hours following the endurance exercise bout, participants performed a 30 second Wingate anaerobic capacity test. Participants warmed up for 2 min at 70-80 rpm on a stationary bicycle ergometer before performing the Wingate test. This warm-up continued into the start of the sprinting portion of the Wingate test, which allows for a flying start. The technician gave the participant a verbal 5 second countdown in order to ensure that the participant was pedaling at maximal speed by the end of the countdown. This countdown period was employed so that the participant was able to increase pedaling speed against a low resistance in order to overcome both the inertial and frictional resistance of the flywheel and shorten the time encompassed in the acceleration phase when full flywheel resistance was applied. After the countdown period, the workload was applied. Each participant was given verbal encouragement to perform to the best of their ability during each 30 second sprint test. The Wingate

anaerobic capacity test was performed on the LODE cycle ergometer (Amsterdam, Netherlands) with a resistance of 0.7 Nm/kg. Peak power was determined in watts. The product of distance traveled and average power produced during the endurance bout was reported as total work done.

Assessment of Serum Markers of Stress and Inflammation

Cortisol, IL-6, and TNFα levels were determined using enzyme-linked immunoabsorbent assay (ELISA) commercially available kits. The serum levels of each marker were determined with a Wallac Victor-1420 microplate reader (Perkin-Elmer Life Sciences, Boston, MA).

Cortisol

The quantitative measurement of serum cortisol was determined by using a competitive enzyme immunoassay (EIA) kit (Catalog #582121 Caymen Chemicals Company, Ann Arbor, MI). This ELISA has a minimum detectable sensitivity for human cortisol of 12pg/ml. 100 µL and 50 µL of EIA buffer was added to non-specific binding wells and maximum binding wells, respectively. 50 µL standards and unknown serum samples were added to the appropriate wells of the microplate that was pre-coated with a cortisol monoclonal antibody. 50 µL of Cortisol-acetylcholinesterase (ACh-E) conjugate (cortisol tracer) and cortisol EIA antiserum were added to appropriate labeled wells and incubated overnight at ~4˚C. Following overnight incubation, the microplate was emptied and rinsed five times with buffered saline solution containing a nonionic detergent (wash solution). 200 µL of Ellman's reagent (5, 5'-dithiobis-(2-nitrobenzoic acid) was added to all wells and 5 µL of tracer was added to total activity well. The microplate was then incubated for 90 min [shaken at 400 rpm at room temperature (~25˚C)] and absorbances

were read immediately at 405 nm. Absorbances are directly proportional to the concentration of cortisol present in serum. The cortisol standard (prepared using recombinant human cortisol lypholized) that was included in the kit was reconstituted to a concentration of 10ng/mL. Serial dilutions of this high standard were made six times not including a zero concentration standard resulting in eight total standards (ranging from 7.8 to 1000 pg/mL). These standards were used to plot a standard curve of absorbances utilizing linear regression analysis.

IL-6

The quantitative measurement of serum IL-6 was determined by using a competitive enzyme immunoassay (EIA) kit (Catalog #583361, Caymen Chemicals Company, Ann Arbor, MI). This ELISA has a minimum detectable sensitivity for human IL-6 of 7.8pg/ml. The IL-6 standard (prepared using recombinant human IL-6 lypholized) that was included in the kit was reconstituted to a concentration of 5ng/mL. Serial dilutions of this high standard were made seven times including a zero concentration standard resulting in eight total standards (ranging from 0 to 250 pg/mL). These standards were used to plot a standard curve of absorbances utilizing linear regression analysis. 25 µL aliquot of non-specific mouse serum was added to each 500 µL aliquot of sample and standard prior to addition to the well. 100 µL standards and unknown serum samples were added to the appropriate wells of the microplate that was pre-coated with an IL-6 monoclonal antibody. 100 µL of IL-6 acetylcholinesterase (ACh-E) Fab' conjugate was added to each well except the Blank wells and incubated overnight at ~4˚C. Following overnight incubation, the microplate was emptied and rinsed five times with buffered saline solution containing a nonionic detergent (wash solution). 200 µL of

Ellman's reagent (5, 5'-dithiobis-(2-nitrobenzoic acid) was added to all wells. The microplate was then incubated for 90 min [shaken at 400 rpm at room temperature (~25˚C)] and absorbances was read immediately at 405 nm. Absorbances are directly proportional to the concentration of IL-6 present in serum.

TNFα

The quantitative measurement of serum TNFα was determined by using a competitive enzyme immunoassay (EIA) kit (Catalog #589201, Caymen Chemicals Company, Ann Arbor, MI). This ELISA has a minimum detectable sensitivity for human TNFα of 3.9pg/ml. The TNFα standard (prepared using recombinant human TNFα lypholized) that was included in the kit was reconstituted to a concentration of 5ng/mL. Serial dilutions of this high standard were made seven times including a zero concentration standard resulting in eight total standards (ranging from 0 to 250 pg/mL). These standards were used to plot a standard curve of absorbances utilizing linear regression analysis. 25 µL aliquot of non-specific mouse serum was added to each 500 µL aliquot of sample and standard prior to addition to the well. 100 µL standards and unknown serum samples were added to the appropriate wells of the microplate that was pre-coated with a TNFα monoclonal antibody. 100 µL of TNFα acetylcholinesterase (AChE) Fab' conjugate were added to each well except the Blank wells and incubated overnight at ~4˚C. Following overnight incubation, the microplate was emptied and rinsed five times with buffered saline solution containing a nonionic detergent (wash solution). 200 µL of Ellman's reagent (5, 5'-dithiobis-(2-nitrobenzoic acid) was added to all wells. The microplate was then incubated for 90 min [shaken at 400 rpm at room

temperature (~25˚C)] and absorbances were read immediately at 405 nm. Absorbances are directly proportional to the concentration of TNFα present in serum.

Statistical Analysis

The dependent variables (cortisol, lactate, IL-6, TNFα, anaerobic power, and macronutrient intake) were analyzed for significance by utilizing an analysis of variance (ANOVA) for repeated measures for time, group, and group by time. Bonferroni post-hoc test (pairwise comparison) was utilized as a follow-up test if a significant interaction was observed. Post-hoc tests of any interaction effects demonstrated in the ANOVA were investigated via an independent samples t-test. The alpha level was set at $p \leq .05$.

CHAPTER FOUR

Results

Participants

Fourteen aerobically trained male participants began the study. Two participants were excluded from the study due to medical concerns. Baseline demographic data for the 12 participants who completed the study are presented in Table 2.

Table 2

Participant Demographics

Age (years)	21.25 ± 2.26
Weight (kg)	72.05 ± 8.09
Height (cm)	178.42 ± 6.27
BMI (kg/m^2)	22.62 ± 1.80
VO_{2max} (ml/kg/min)	53.71 ± 6.28
Heart Rate (bpm)	55.00 ± 9.00
Systolic Blood Pressure (mmHg)	115 ± 13
Diastolic Blood Pressure (mmHg)	80 ± 7

Note. Data are presented as means ± standard deviations.

Confounding Variables

Prior to data collection, certain variables were identified as potential confounding variables that may affect exercise performance and/or recovery. These variables included dietary intake, participants' hydration status, and total work performed during the endurance exercise session. To account for dietary intake, participants were asked to

complete 2 day dietary food record prior to and following the endurance exercise session. Hydration status [total body water (including both intracellular and extracellular fluid)] was determined through bioelectrical impedance. Finally, total work (KJ) performed by each participant during their endurance exercise session was analyzed.

Nutritional Intake Analysis

Data for macronutrient intake (total calories, carbohydrate, protein, and fat) are presented in Table 3. All participants were instructed to consume their usual diet during the 6 week course of the study. Participants were asked to complete a 2 day dietary food record prior to and following the endurance exercise session. A two-way [treatment (3) x time point (2)] repeated measures ANOVA to control for the within-individual variation was conducted to evaluate the participants' nutritional intake and its possible effect on performance and recovery. The within-subjects factor was time with two levels (2 days before exercise = Pre and 2 days post-exercise = Post) and treatment with three levels (G-trainer, cycling, and stretching). Prior to the endurance exercise session, no significant differences were observed between treatments for daily caloric intake ($p = 0.96$) or macronutrient intake of carbohydrate ($p = 0.95$), protein ($p = 0.82$), and fat ($p = 0.72$). Following the endurance exercise session, no significant differences were observed between treatments for total daily caloric intake ($p = 0.62$) or macronutrient intake of carbohydrate ($p = 0.54$), protein ($p = 0.40$), and fat ($p = 0.55$).

Table 3

Average Macronutrient Intake

Variable	G-trainer	Cycling	Stretching
Total Calories (Kcal)			
Pre	2715.80 ± 1144.08	2657.70 ± 1273.64	2658.40 ± 1411.85
Post	2925.50 ± 1281.84	2561.75 ± 1652.53	2731.25 ± 1485.91
Carbohydrate (g)			
Pre	362.95 ± 172.19	362.47 ± 198.33	349.52 ± 195.99
Post	381.47 ± 172.53	357.85 ± 246.72	330.59 ± 168.13
Protein (g)			
Pre	94.18 ± 49.01	103.15 ± 51.72	100.59 ± 53.58
Post	113.12 ± 48.91	92.06 ± 54.55	103.87 ± 50.38
Fat (g)			
Pre	101.62 ± 41.80	91.63 ± 49.59	100.21 ± 58.80
Post	112.62 ± 60.36	92.25 ± 64.91	108.36 ± 68. 45

Note. Data are presented as means ± standard deviations.

Hydration Status

Data for markers of hydration status (total body water, extracellular, and intracellular fluid) are presented in Table 4. Participants' intracellular, extracellular, and total body water content was determined via bioelectrical impedance analysis before the endurance bout and 24 hours after the endurance bout in order to monitor the consistency of the participants' hydration status over the course of the study. A two-way [treatment (3) x time point (2)] repeated measures ANOVA to control for the within-individual variation was conducted to determine any differences in participants' hydration status between the three recovery treatments. The within-subjects factor was time with two levels (immediately before exercise = T2Pre and 24 hours post-exercise = T4) and treatment with three levels (G-trainer, cycling, and stretching). Univariate analysis for intracellular fluid revealed no significant main effect for time ($p = 0.13$), treatment ($p = 0.33$), or treatment by time interaction ($p = 0.29$). Similarly, no significant main effect

for time (p = 0.098), treatment (p = 0.91), or treatment by time interaction (p = 0.68) was detected for extracellular fluid. In the same way, there were no significant main effect for time (p = 0.93), treatment (p = 0.48), or treatment by time interaction (p = 0.68) for total body water content.

Table 4

Hydration Analysis Data

Variable	G-trainer	Cycling	Stretching
Intracellular (L)			
T2Pre	22.98 ± 2.70	23.04 ± 2.65	23.15 ± 2.44
T4	23.39 ± 2.65	22.96 ± 2.58	23.36 ± 2.34
Extracellular (L)			
T2Pre	17.410 ± 2.12	17.40 ± 1.98	17.46 ± 1.77
T4	17.24 ± 2.04	17.34 ± 1.78	17.32 ± 1.71
Total Body Water (L)			
T2Pre	40.70 ± 4.46	40.69 ± 4.27	40.83 ± 3.87
T4	40.74 ± 4.27	40.53 ± 4.04	40.91 ± 3.82

Note. Data are presented as means ± standard deviations.

Endurance Exercise Session

Data for endurance performance variables (total work, duration, average power and average speed) are presented in Table 5. The endurance protocol was selected from the Computrainer 3D Version 3 Software (Copyright 2006 Racermate) and was programmed into and used in conjunction with a bicycle ergometer and the participants' personal bicycles to simulate race conditions and race effort. Duration, average power, average speed, and total work were recorded for each of the three endurance bouts in order to monitor the consistency of the participants' efforts over the course of the study. A one-way repeated measures ANOVA to control for the within-individual variation was conducted to evaluate variations in indices of endurance performance under each

treatment (G-trainer, cycling, stretching) condition. No significant differences were observed between endurance exercise bouts for duration ($p = 0.24$), average power ($p = 0.13$), average speed ($p = 0.14$), or total work ($p = 0.56$).

Table 5

Endurance Bout Performance Data

Variable	Gtrainer	Cycling	Stretching
Duration (mins)	69.99 ± 13.38	67.36 ± 10.93	70.53 ± 13.25
Average Power (watts)	172.83 ± 51.04	180.83 ± 49.53	168.17 ± 42.95
Average Speed (mph)	16.15 ± 2.92	16.63 ± 2.67	15.97 ± 2.59
Total Work (KJ)	689.48 ± 83.00	695.58 ± 82.02	681.41 ± 73.70

Note. Data are presented as means ± standard deviations.

Blood Lactate Analysis

Data for blood lactate are presented in Figure 1. A two-way [treatment (3) x time point (8)] repeated measures ANOVA to control for the within-individual variation was conducted to evaluate the effects of the different recovery treatments (G-trainer, cycling, stretching) on the dependent variable blood lactate following an intense endurance exercise bout. The within-subjects factor was time with eight levels (immediately before exercise = T2Pre; immediately post exercise = 0; 5, 10, 15, and 30 minutes post exercise = 5, 10, 15, 30); three hours post-exercise = T3; 24 hours post-exercise = T4) and treatment with three levels (G-trainer, cycling, and stretching). Except for the three post-exercise samples, all blood samples were obtained after an 8-hour fast and standardized to the same time of day for each sample. Univariate analysis revealed a significant main effect for time for lactate ($p = 0.010$). Subsequent pairwise analysis revealed a significant decrease in lactate from immediately post exercise (0) to 5 minutes after the

endurance bout (p=0.008). However, no treatment (p = 0.14) or treatment by time interaction (p = 0.28) was detected.

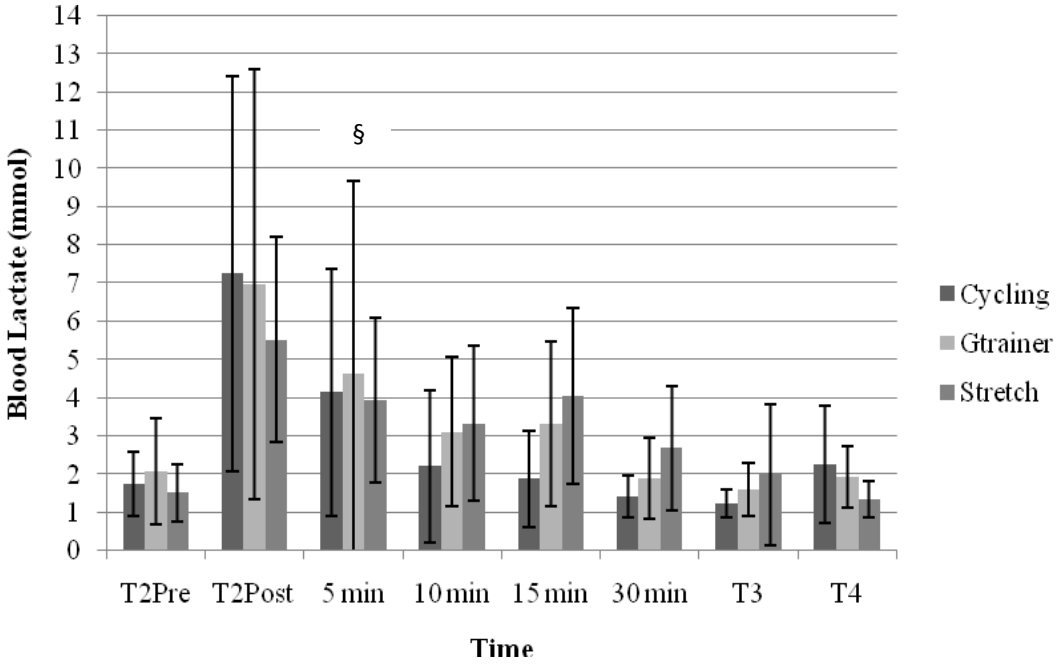

Figure 1. Time Course of Blood Lactate Concentration (mmol). Data (mean ± SD) represents blood lactate concentration (mmol) prior to and following an acute bout of endurance exercise in the three recovery groups (G-Trainer, cycling and stretching). §Significantly different from T2Post (p=0.008).

Serum Variable Analysis

Data for serum variables (IL-6, TNFα, and cortisol) are presented in Table 6 and Figure 2. At baseline, there were no significant differences between participants with regards to cortisol (p = 0.097), IL-6 (p = 0.57), or TNFα levels (p = 0.22). A two-way [treatment (3) x time point (4)] repeated measures ANOVA to control for the within-individual variation was conducted to evaluate the effects of the different recovery treatments (G-trainer, cycling, stretching) on various blood markers that may be involved in systemic stress and inflammation. The dependent variables that were analyzed in this univariate analysis were: cortisol, TNFα, and IL-6. The within-subjects

factor was time with four levels (immediately before exercise = T2Pre, immediately post exercise = T2Post, three hours post-exercise = T3, and 24 hours post-exercise = T4) and treatment with three levels (G-trainer, cycling, or stretching). Blood samples were collected and analyzed for cortisol, TNFα, and IL-6 prior to and immediately post endurance exercise bout and 3 and 24 hours after the endurance exercise bout. Except for the three post-exercise samples, all blood samples were obtained after an 8-hour fast and standardized to the same time of day for each sample. Univariate analysis revealed a significant main effect for time for cortisol ($p = 0.039$) with subsequent pairwise analysis revealing a significant decrease in cortisol from T2Pre to T3 ($p = 0.030$) and a significant increase from T3 to T4 ($p = 0.024$). However, no treatment ($p = 0.47$) or treatment by time interaction ($p = 0.42$) was detected. Additionally, no main effect for time ($p = 0.37$), treatment ($p = 0.37$), or treatment by time ($p = 0.43$) interactions were detected for IL-6. Similarly, no main effect for time ($p = 0.17$), treatment ($p = 0.54$), or treatment by time ($p = 0.63$) interactions were detected for TNFα.

Hypothesis one states that there will be no significant difference in blood lactate concentrations between the three recovery treatments in response to active recovery, therefore, we failed to reject the null hypothesis. Hypothesis two states that there will be no significant difference in Tumor Necrosis Factor alpha (TNFα) levels between the three recovery treatments in response to active recovery, therefore we failed to reject the null hypothesis. Hypothesis three states that there will be no significant difference in cortisol levels between the three recovery treatments in response to active recovery, therefore we failed to reject the null hypothesis. Finally, hypothesis four states that there will be no

significant difference in Interleukin-6 (IL-6) levels between the three recovery treatments in response to active recovery, therefore we failed to reject the null hypothesis.

Table 6

Serum Variables

Blood Parameter	G-trainer	Cycling	Stretching
IL-6 (pg/mL)			
T2Pre	7.89 ± 6.51	7.77 ± 8.13	9.41 ± 6.79
T2Post	10.99 ± 5.67	10.43 ± 7.50	8.75 ± 7.01
T3	11.90 ± 8.13	14.27 ± 12.29	8.13 ± 8.17
T4	10.26 ± 10.21	10.68 ± 7.91	10.68 ± 7.91
TNFα (pg/mL)			
T2Pre	14.42 ± 5.08	12.74 ± 5.06	14.84 ± 5.86
T2Post	15.32 ± 4.36	15.32 ± 5.85	13.43 ± 4.47
T3	14.71 ± 5.55	15.07 ± 3.85	14.53 ± 6.16
T4	18.25 ± 15.55	15.65 ± 5.77	16.94 ± 6.67

Note. Data are presented as means ± standard deviations.

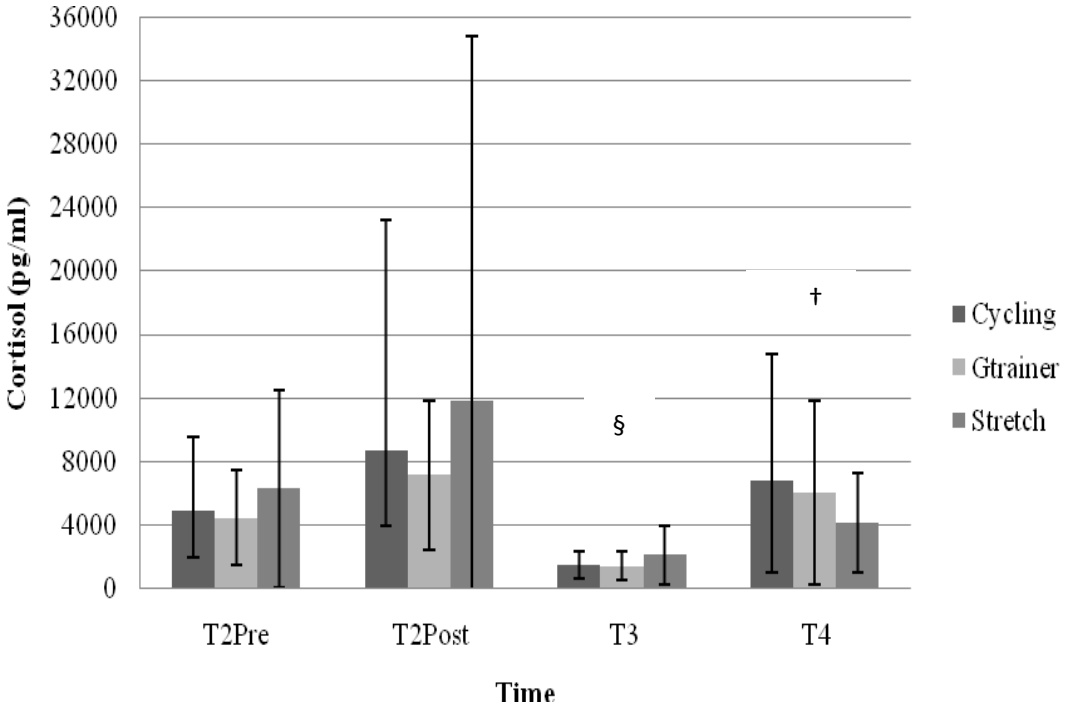

Figure 2. Time Course of Cortisol. Data (mean ± SD) represents cortisol concentration (pg/ml) prior to and following an acute bout of endurance exercise in the three recovery groups (G-Trainer, cycling and stretching). §Significantly different from T2Pre to T3 (p=0.030).
† Significantly different from T3 to T4 (p=0.024).

Indices of Anaerobic Capacity Assessment

Data for anaerobic capacity variables (peak power, mean power, time to peak power, and rate to fatigue) are presented in Table 7. A 30 second Wingate anaerobic capacity test was performed at baseline and subsequent performance/measurement was conducted 24 hours after each of the three endurance bouts to assess indices of recovery and anaerobic performance. A one-way repeated measures ANOVA to control for the within-individual variation was conducted to evaluate the effects of the different recovery treatments on indices of anaerobic performance The within-subjects factor was treatment with four levels (Baseline, G-trainer, cycling, stretching). No statistically significant differences were observed among treatments at the 24 hour time point in skeletal muscle peak power output ($p = 0.23$), mean power output ($p = 0.41$), time to peak power ($p = 0.42$), or rate to fatigue ($p = 0.35$). In the same way, no significant differences between baseline and 24 hour anaerobic capacity data were detected in mean power output ($p = 0.50$), time to peak power ($p = 0.40$), or peak power ($p = 0.05$). However, a significant main effect for time between baseline and the 24 hour time point was observed for rate to fatigue ($p = 0.04$), but subsequent pairwise analysis did not reveal the specific time point that was different. Hypothesis five states that there will be no significant difference in power output between the three recovery treatments in response to active recovery, therefore, we failed to reject the null hypothesis.

Table 7

Anaerobic Capacity Variables

Variable	Baseline	G-trainer	Cycling	Stretching
Peak Power (watts)	1323 ± 322.89	1371.83 ± 364.08	1411.08 ± 355.36	1430.83 ± 428.64
Mean Power (watts)	713 ± 114.19	707.67 ± 115.82	705.33 ± 127.55	718.50 ± 125.71
Time to Peak (sec)	4.68 ± 0.83	4.59 ± 0.70	4.38 ± 0.53	4.43 ± 0.49
Rate to Fatigue§ *(watt/sec)*	36.17 ± 12.54	38.72 ± 12.80	40.15 ± 12.31	40.71 ± 15.48

Note. Data (mean ± SD) represents anaerobic capacity variables at baseline and 24 hours following an acute bout of endurance exercise in the three recovery groups (G-Trainer, cycling and stretching). §Significantly different from baseline (p=0.04).

Profile of Mood States Assessment

Data for the profile of mood states assessment are presented in Table 8. A psychological assessment was conducted using the current POMS (profile of mood states) questionnaire in order to determine possible psychological indicators of fatigue and recovery from exercise. The POMS is a validated, standardized self-rating scale consisting of 65 items that measures six identifiable mood states: tension, depression, anger, vigor, fatigue, and confusion. A 5-point scale is used from 0 = not at all to 5 = extremely. The global mood disturbance score is reported. The POMS was conducted at baseline, immediately after (T2Post) the recovery bout, and 24 hours (T4) after the recovery bout. A two-way [treatment (4) x time point (2)] repeated measures ANOVA was conducted to evaluate the effects of an acute bout of endurance exercise and subsequent recovery treatment on psychological parameters. The within-subjects factor was time with two levels (immediately post exercise = T2Post and 24 hours post-exercise = T4) and treatment with three levels (G-trainer, cycling, and stretching). No statistically significant differences were observed among treatments with the psychological

assessment either immediately after ($p = 0.80$) or 24 hours after ($p = 0.06$) the endurance exercise bout. Similarly, no significant differences between the baseline and T2Post data ($p = 0.18$) or the baseline and the 24 hour psychological assessment data were observed ($p = 0.09$). Hypothesis six states that there will be no significant difference in profile of mood states assessments between the three recovery treatments in response to active recovery, therefore, we failed to reject the null hypothesis.

Table 8

Profile of Mood States Data

Time Point	Baseline	G-trainer	Cycling	Stretching
	13.82 ± 15.66			
T2Post		6.82 ± 20.41	5.91 ± 14.82	6.36 ± 17.79
T4		10.82 ± 27.30	0.82 ± 13.72	5.73 ± 18.50

Note. Data are presented as means ± standard deviations.

CHAPTER FIVE

Discussion

The overall purpose of the present study was to determine if weight–supported exercise utilizing the AlterG G-trainer treadmill would enhance muscle recovery following an exhaustive bout of endurance exercise in aerobically trained males. Specifically, the study sought to compare efficacy of the AlterG G-trainer to commonly used recovery modalities, stationary cycle and static stretching, on indices of anaerobic performance, systemic markers of stress (cortisol) and inflammation (TNFα and IL-6), blood lactate and psychological indicators of mood state (POMS) following endurance exercise. The effects of stationary cycling and static stretching on recovery from exercise have been well documented over the past 15 years (Choi et al., 1994; Mika et al., 2007; Herbert & Gabriel, 2002). However, to date, no controlled scientific studies have examined the efficacy of the AlterG G-trainer on various aspects of muscle recovery. The major finding of the current study is that 30 minutes of weight-supported exercise on the AlterG G-trainer was unable to enhance muscle recovery following an intense bout of endurance exercise when compared to stationary cycling and static stretching following an acute bout of exhaustive endurance exercise. Despite minimal differences between select recovery methods, further research is necessary to validate these observations, and thus, determine individualized recovery modalities to create balances between the stresses of training and competition.

Systemic Markers of Stress and Inflammation

The cytokines IL-6 and TNFα are pleiotropic molecules that play important roles in the inflammatory process (Suzuki et al., 2006; Neubauer et al., 2008; Ostrowski et al., 2008; Robson-Ansley et al., 2007). Studies have shown that as little as 30 minutes of exercise can lead to significant elevations in biomarkers of stress and inflammation in the blood, thereby eliciting an acute phase response (Robson-Ansley et al., 2007). Post-exercise increases in IL-6 are consistent in the literature (Ostrowski et al., 1999; Bruunsgard, 1997; Ullum, 1994). IL-6 is the first cytokine present in the circulation following exercise and may increase by 100-fold during prolonged exercise (ACSM, 2006; Petersen, 2001; Robson-Ansley, 2007). Levels typically peak between 30 minutes (Suzuki et al., 2006) and 3.5 hours post-exercise (Peterson, 2001) and return to pre-exercise levels within a few hours of rest (Robson-Ansley, 2007). Levels may remain significantly elevated 1 day post-exercise (Suzuki et al., 2006) depending on the demands of the exercise performed. In contrast to the marked post-exercise increases in IL-6 levels that are consistent in the literature, reported findings of post-exercise TNFα concentrations are inconsistent (Ostrowski et al., 1999). Plasma TNFα reportedly increased 2 hours and 1 hour after completing both a 2.5 hour run and following a 5km race (Dufaux & Order, 1989; Espersen et al., 1990), respectively, while other studies have failed to detect TNFα post-exercise (Ostrowski et al., 1999; Rivier et al., 1994; Ullum et al., 1994). Many studies reported a lack of or minor elevations in TNFα following heavy exercise except for a few studies that examined extreme endurance exercise such as marathon running (Pederson, 1997; Northoff & Berg, 1991). Plasma TNFα levels typically peak within the first hour after exercise and slowly decline and

return to baseline levels around 2.5 hours post-exercise (Ostrowski et al., 1999). It has been proposed that cytokines are linked to fatigue and that cytokine release during and following exercise may cause a chronic inflammatory response. Despite a ~16 and ~5% post-exercise increase in IL-6 and TNFα, respectively; the acute bout of endurance exercise utilized in the current study failed to increase both significantly. Since increases in the magnitude of 100-fold and 3-fold for IL-6 and TNFα, respectively, are normally observed following more demanding exercises such as a marathon (Ostrowski et al., 1999); the likely explanation for current results is that the endurance exercise chosen was not intense enough. Moreover, increases in such inflammation biomarkers, especially IL-6, appear to be activated in response to eccentric exercise, as it is a key activator of proteolysis. Therefore running, especially prolonged bouts, are associated with repeated eccentric contractions and thus could explain the normally large increases following such an exercise. In the current study, since cycling exercise is predominantly concentric, it could explain why non-significant increases were observed. In addition, since only 3 and 24 hour time points were sampled, it could be speculated that peak expression of these biomarker may have been missed.

Similarly to inflammation biomarkers IL-6 and TNFα, serum cortisol was also increased immediately post exercise (43%), but returned to baseline levels with a significant decrease following recovery treatment. Interestingly, serum cortisol levels significantly increased 24 hours post exercise. Cortisol, through protein mobilization, gluconeogenesis, and free fatty acid mobilization, is thought to have an effect on glucose and glycogen replenishment during recovery from exercise. Additionally, blood cortisol levels increase at a rate proportional to exercise intensity and reach a final concentration

dependent on the duration of exercise (Brandenberger & Follenius, 1975). Therefore, the post-exercise elevation observed in this stress hormone was anticipated. The findings of the present study coincide with the results presented by Hill and colleagues (2008), who examined the influence of exercise intensity on cortisol concentrations. Cortisol levels in moderately active trained men were investigated before and after 30 minutes of exercise at varying intensities (40, 60, and 80% of their VO_{2max}). Results indicated that at higher intensities (i.e. between 60% and 80% of VO_{2max}), the magnitude of change was in the order of ~39.9-83.1%. This was compared to a ~5.7 change at 40%. Authors concluded that moderate to high intensity exercise provokes increases in circulating cortisol levels due to a combination of hemoconcentration and HPA axis stimulus. In the current study, the large increase in serum cortisol post-exercise was most likely due to a stress response to the prolonged, high intensity endurance exercise bout that resulted in decreasing blood glucose levels and muscle proteolysis. The significant decrease in cortisol levels observed following each recovery treatment was most likely due to an increase in metabolic clearance rate that is normally triggered by target tissue uptake of cortisol (Hill, 2008; Davies & Few, 1973). Indeed, Hill and colleagues (2008) demonstrated a significant reduction in circulating cortisol levels following low intensity exercise.

Notwithstanding, it is clear that in the current study, 30 minutes of weight-supported exercise utilizing the AlterG G-trainer was unable to have any significant effects on systemic markers of stress and inflammation. The premise behind the G-trainer in reducing these markers is to aid in aerobic conditioning by reducing force of impact on the body and protecting tissues during recovery (Alter-G, Inc., 2009). Given no research has examined the effects weight-supported exercise on systemic markers of stress and

inflammation, it is not readily apparent as to why no significant benefits were seen. It could be suggested that since only moderate increases in IL-6 and TNFα were observed, the ability to detect any differences between the 3 recovery modalities was limited. It is interesting to note that a significant decrease in cortisol was observed following each recovery modality and since no true control (i.e. no recovery) was utilized in the current study; it could be speculated that each recovery treatment was effective. Clearly, more research is needed to validate such observations

Blood Lactate

Elevations in blood lactate following incremental or near maximal exercise is a common occurrence (Rowlands et al., 2008; Gleeson, 2002; Messonnier et al., 2007). Gleeson et al. (1998) reported an average blood lactate level of 11.6mM following an incremental cycling bout. In the current study, blood lactate was increased immediately post-exercise (74%, ~ 6.6 mM) and subsequently returned to baseline during the recovery period. However, no statistical significance in blood lactate between recovery treatments were reported following the endurance exercise bout. Active recovery at 40% maximal oxygen uptake potentiates the enhanced ability to remove blood lactate that is induced by endurance exercise (Taoutaou, Granier, Mercier, Mercie, Ahmaidi, & Prefaut, 1996). This form of recovery aids lactate clearance by increasing blood velocity and blood flow to the affected musculature thereby increasing lactate metabolism by augmenting the transport of lactate from the active muscle to the removal sites (Martin et al., 1998; Choi et al., 1994). Lactate metabolism and its rate of elimination from the muscle and blood are important contributors to muscle fatigue (Martin et al, 1998; Messonnier et al, 2007; Mika et al., 2007). Since post-exercise blood lactate kinetics

were similar for all three recovery treatments, the current study suggests that static stretching was able to increase blood lactate clearance to a similar extent as both active recovery treatments (AlterG G-trainer and stationary cycling). It is most likely that given the large variation in blood lactate response following endurance exercise (83% cycling, 74% G-trainer, 67% stretching), and more importantly, the ability to clear lactate from the blood, it could be speculated that the ability to detect small significant differences between treatments was reduced. In theory, active recovery should increase lactate clearance, which at 10, 15, 30 minutes and 3 hours post endurance exercise this was evident in the cycling group compared to AlterG G-trainer and static stretching (albeit not significant). At 5 minutes post-exercise, lactate had declined by 25, 36, and 28% for the cycling, G-trainer, and stretching treatments, respectively. When compared to immediately post-exercise, lactate at 10 minutes post-exercise had declined by 55, 58, and 37% for the cycling, G-trainer, and stretching treatments, respectively. When compared to immediately post-exercise, lactate at 15 minutes post-exercise had declined by 68, 53, and 30% for the cycling, G-trainer, and stretching treatments, respectively. When compared to immediately post-exercise, lactate at 30 minutes post-exercise had declined by 77, 73, and 51% for the cycling, G-trainer, and stretching treatments, respectively. When compared to immediately post-exercise, lactate at 3 hours post-exercise had declined by 74, 73, and 53% for the cycling, G-trainer, and stretching treatments, respectively. The passive nature of static stretching can explain the slow clearance rate of lactate from the blood. In contrast, the AlterG G-trainer is a form of active recovery, and thus should also increase lactate clearance rate. It is possible that the air pressure produced by the AlterG G-trainer on the lower limbs to reduce

participants' weight on the treadmill may have hindered blood lactate clearance within the muscle. This is highly speculative and further research is clearly warranted.

Indices of Anaerobic Performance

The current study compared 24 hour post-exercise anaerobic power output to baseline power output as an indicator of functional recovery from systemic stress, inflammation and peripheral fatigue. No significant changes in skeletal muscle peak power output, mean power output, time to peak power, or rate to fatigue were observed at 24 hours following the acute endurance bout. Rate to fatigue was significantly higher at the 24 hour post endurance exercise; however, this is likely due to a familiarization effect in which the participants became more accustomed to the anaerobic power test at each successive testing bout.

With regards to the effectiveness of the recovery modalities on indices of anaerobic performance, no significant differences between recovery treatments were evident. Because the present study examined anaerobic performance only at the 24 hour post-endurance bout, it is likely that the window of opportunity to identify any differences in power output between the three recovery treatments was missed. Indeed, most indices of anaerobic performance had returned to pre-exercise levels. Robey and colleagues (2009) suggested possibly using several performance measures on multiple occasions in order to identify potential differences that may exist between recovery treatments. Despite the possibility of overlooking a more favorable time point to examine post-exercise anaerobic power, the present study corresponds with other research in utilizing power output as an indicator of recovery from peripheral fatigue (Breen et al., 2009; Pritchett et al., 2009). Typically, the demands of endurance exercise

will likely lead to fatigue, which is commonly depicted as a decrease in muscle power (Enoka & Duchateau, 2008), normally coincides with elevations is blood lactate (Rowlands et al., 2008; Gleeson, 2002), and can at times lead to a systemic inflammatory and stress response with increased elevations in cortisol (Gleeson, 2002; Neubauer et al., 2008; Hill et al., 2008), interleukin-6 (IL-6) (Suzuki et al., 2006; Neubauer et al., 2008; Ostrowski et al., 2008; Robson-Ansley et al., 2007), and tumor necrosis factor alpha (TNFα) (Neubauer et al., 2008; Ostrowski et al., 2008). The lack of differences between treatments in regard to power variables may possibly coincide with an absence of differences between treatments in relation to blood lactate, cortisol, IL-6, and TNFα. It should be noted that all 24 hour testing sessions for each recovery treatment were only compared to one "pre-endurance performance baseline value" and thus not a "true" baseline value obtained prior to each recovery treatment. This is a limitation to the current study; however, given that a 2 week washout period was taken between recovery treatments, and most markers of systemic stress, inflammation and peripheral fatigue returned to baseline within 24 hours, it is likely that any effect was minimal.

Psychological Mood States (POMS)

In the current study, no statistical significance was observed between treatments with the psychological assessment obtained at either immediately after or 24 hours after the endurance exercise bout. In addition, no statistical significance was observed between baseline and 24 hour post-exercise POMS data. Similar to the present study, the POMS was utilized by Halson and colleagues (2002) to monitor the effects of exercise stress and subsequent recovery on performance changes and fatigue indicators in endurance

cyclists. Data from the current study does not coincide with Halson and colleagues observation of significantly increased global mood state scores from the normal training period to the intensified training period. This is likely due to the lack of prolonged stress accumulated in the present study, as an acute endurance protocol was used. Halson (2002) reported that on completion of recovery training, POMS scores returned to normal training levels. Data from the current study also demonstrates that following the recovery treatments, global mood state scores corresponded to baseline scores.

Conclusions and Future Recommendations

Overall, the present study does not support the notion that weight-supported exercise utilizing the AlterG G-trainer treadmill can enhance muscle recovery following an acute exhaustive bout of endurance exercise. When compared to stationary cycling and static stretching, exercise performed on the AlterG G-trainer treadmill was unable to further reduce systemic markers of stress (cortisol) and inflammation (IL-6 and TNF-α), blood lactate, or improve anaerobic performance or psychological mood states following endurance exercise. Despite limited findings, the current study contributes to the literature, as it is one of a few studies to assess the effect of an acute bout of cycling endurance exercise on physiological and biochemical markers of stress and inflammation, but moreover, compare weight-supported exercise utilizing the AlterG G-trainer treadmill to the common recovery modalities stationary cycling and static stretching.

As demonstrated by the research presented above, the current study, in certain aspects, employed a differing methodology or protocol and at times a dissimilar participant demographic. For example, the present study examined submaximal exercise as a recovery modality instead of nutritional intervention, massage, cryotherapy, etc. The

present study investigated aerobically trained males during an acute endurance bout that consisted of primarily concentric muscle actions as opposed to supramaximal exercise, eccentric exercise, or ultra-endurance exercise. Additionally, the current study examined metabolic damage/inflammation as opposed to mechanical damage caused by exercise. Therefore, these dissimilarities may be the cause of differences in study outcomes such as more robust changes or lack of change in physiological and/or performance markers. Although the aforementioned studies may have utilized different recovery methods, subject populations, and sports and may have examined different physiological and performance parameters, there are parallels that can be drawn between the research presented and the present study. First, recovery from exercise is a necessity; post-exercise recovery strategies and procedures are commonly implemented. Second, no one recovery method has been documented as the most effective technique. Third, certain physiological and performance variables are known to be effected by exercise and subsequent recovery. The present study examined the effects of different recovery methods and their effects on hematological markers and anaerobic power.

The limitations inherent in this study were a lack of further biochemical testing which would provide more information as to the effects of an acute bout of endurance exercise on additional cytokines as well as immune function indicators such as macrophages, neutrophils, and eosinophils. Future studies may want to investigate these biomarkers. Additionally, a more homogeneous population may be more conducive to investigation. For example, participants who are considered to be elite athletes (higher, more selective VO_{2max}) and who only participate in one discipline (only runners, only cyclists, etc.) may be favorable to examine. Future studies should address potential

confounding factors such as the mode of exercise used to induce systemic stress and inflammation, the recovery intervention, and subject characteristics such as training history. Moreover, prospective studies should consider monitoring hematological data past the 24 hour post-exercise time point, as data from the present study suggests that complete recovery from hypercytokinemia and systemic stress following an acute bout of cycling endurance exercise was not achieved by 24 hours post-exercise. Furthermore, because a control treatment was not used alongside the G-trainer, cycling, and stretching treatments, it is not possible to infer the exact magnitudes of the physiological, biochemical, and performance effects that were observed. However, despite the absence of a control treatment (i.e. no exercise), as many extraneous variables as possible were accounted for through the monitoring of hydration status, macronutrient intake, exercise prior to the endurance bout, and total work performed during the endurance bout. Regardless, future studies may want to implement a control treatment for comparison purposes.

Despite the subdued findings in this investigation, it is well known that athletes who train for competitive sports are exposed to demanding training. This training may occur multiple times a day or on consecutive days. After a training session or following competition, it is the goal of an athlete to be able to train or compete at full capacity during subsequent exercise endeavors. For this reason, recovery is regarded as an essential element of training and performance. As a result, conclusions from this study warrant inclusion of recovery modalities to create a balance between the stresses of training and competition.

APPENDICES

APPENDIX A

BAYLOR UNIVERSITY

Department of Health, Human Performance, and Recreation
Informed Consent Form

Title of Investigation: Effects of G-trainer, Cycle Ergometry, and Stretching on Physiological and Performance Markers in Active Recovery from Exercise

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Rationale:
Cycle ergometry and stretching have been documented as methods of active recovery. However, there is little research available on the use of the G-trainer as a recovery aid. Alter-G, Inc. claims that the G-trainer enables users to enhance performance. One aspect of performance enhancement is the ability to recover from exercise. One of the health applications of the G-trainer is to aid aerobic conditioning. The G-trainer claims to do this by reducing force of impact on the body and protecting tissues during recovery. Information regarding the use of the G-trainer as an effective recovery method is limited. This study aims to compare two well known recovery treatments (stretching and cycle ergometry) to active non-weight bearing exercise (G-trainer). In addition, this study aims to verify the alterations between recovery methods after demanding endurance exercise.

Description of the Study
Baseline Testing
I will be one of approximately 15 apparently healthy recreationally active male subjects between the ages 18 and 30 who will participate in this study. During an initial familiarization session, I will be informed of the requirements of the study and sign an informed consent statement in compliance with the Human Subjects Guidelines of Baylor University. A trained individual will examine me to determine if I am qualified to participate in this study. If I am cleared to participate in the study, I will be familiarized

to the testing procedures. This session will take approximately 30 minutes to complete. I also understand that the study is a cross-over design, therefore, I will repeat the same battery of tests listed below (with the exception of recovery session) separated by 14 days. Once I complete the familiarization/baseline session (testing session one), I will be scheduled for testing session two, one week later.

Following the familiarization session, I will be instructed to refrain from exercise for 48 hours prior to baseline testing. I will be provided with two 4-day dietary analysis forms that I am to complete the 4 days prior to familiarization/baseline testing, and testing session three. Once I report to the lab for the testing session, I will turn in my dietary analysis form.

I understand that I will then donate about 10 milliliters of blood from a vein in my arm. Blood samples will be obtained using standard/sterile procedures using a needle inserted into a vein in my arm. I understand that personnel who will be taking my blood are experienced in phlebotomy (procedures to take blood samples) and are qualified to do so under guidelines established by the Texas Department of Health and Human Services. This will take about 5 minutes and I understand that I will be asked to donate the same volume of blood on five separate occasions throughout the study. I understand that I will have my blood pressure, resting heart rate, height, weight, body composition, and total body water measured and recorded. I understand that I will be tested for VO_{2max}, heart rate max, and lactate threshold during an incremental ride to exhaustion on a cycle ergometer (GXT). I will be told that I should continue to exercise until I can exercise no more. I understand that no type of encouragement will be offered. I will perform my own warm-up routine and will then be instructed to produce a power output of 100 watts. Upon achieving 100 watts, the test will begin. Each stage will be three minutes in duration. The following stages require an increase of 50 watts to be sustained per stage until 250 watts is reached, wherein power output will be increased by 50 watts every stage until volitional fatigue. Test termination will occur 1) when I am no longer able to maintain the required power output, 2) when I have reached self-reported volitional fatigue, or 3) when the investigator or supervisors consider me at risk for physical harm. 30 minutes following the GXT, I understand that I will perform a maximal power test using the Wingate Protocol. I will warm up for 2 min at 50-60 rpm on a stationary bicycle ergometer before performing the Wingate test. This warm-up will be continued into the start of the sprinting portion of the Wingate test, which allows for a flying start.

Following familiarization/baseline testing, I will return to the lab for testing session two. I understand that I will again have my weight, total body water, blood pressure and heart rate measured and recorded. I will also rate my level of perceived effort and psychological state using the Borg Scale and POMS questionnaire, respectively. I understand that I will also donate about 10 milliliters of blood from my vein in my arm prior to and immediately following the exercise bout and recovery session. I also understand that I will be tested to blood lactate concentrations via finger prick prior to and directly after the exercise bout and 5, 10 and 15 minutes following the recovery exercise session. I understand that I will perform a fatiguing bout of exercise on a Computrainer which is approximately 25 miles with an average grade of 0.3%, a

maximum grade of 9.7%, and total climbing of 1702 total feet. Following the exercise bout, I will perform one of three recovery sessions for approximately 30 minutes. Three hours following the recovery session, I understand that I will undergo blood sampling and also rate my level of perceived effort and psychological state using the Borg Scale and POMS questionnaire. I will then be instructed to refrain from exercise and return to the lab 24 hours later.

Twenty-four (24) hours following the recovery bout, I will again be tested for blood lactate concentrations through a finger stick. Additionally, approximately 10 ml of blood will be drawn from a vein in my arm. I will again be asked to rate my level of perceived effort and psychological state using the Borg Scale and POMS questionnaire. I understand that I will perform a maximal power test using the Wingate Protocol. I will warm up for 2 min at 50-60 rpm on a stationary bicycle ergometer before performing the Wingate test. This warm-up will be continued into the start of the sprinting portion of the Wingate test, which allows for a flying start. Following all testing, I understand that I will continue with my normal activities and return to the lab 14 days later to repeat the same battery of tests, with the exception of a different recovery session.

Exercise Fatigue Protocol

I will perform the fatiguing bout of exercise on a Computrainer. The Computrainer is a stationary electronic bicycle ergometer that allows the user to ride their own bicycle. A trainer consists of a frame, a clamp to hold the bicycle securely, a roller that presses up against the rear wheel, and a mechanism that provides resistance when the pedals are turned. The protocol will be selected from the Computrainer 3D Version 3 Software (Copyright 2006 Racermate) and will be programmed into and used in conjunction with the bicycle ergometer to simulate a race course and effort. The USAT Nationals St. Joe course, a 24.46 mile course with an average grade of 0.3%, a maximum grade of 9.7%, and 1702 total feet of climbing will be used. I will be provided with a viewing screen that depicts the race course. I will be instructed to ride as hard as possible for the duration of the protocol. A fan providing constant wind speed will be placed directly in front of me and positioned so that the airflow will be directed towards the head and torso when in a normal cycling position. Water will be available to me ad libitum (as desired).

Recovery Session

Immediately following the exercise bout, I will perform one of three recovery bouts (G-trainer, Computrainer, or stretching) for 30 minutes. I will be randomly assigned to one of three treatment groups: G-trainer, Computrainer, or stretching. I will participate in a crossover design and will perform each recovery session on three separate occasions separated by 14 days. On each occasion, I will perform the same battery of tests as mentioned previously; however, the recovery modality will be different. If recovering on the G-trainer, I will run at 40% VO_{2max} and 75% of body weight for 30 minutes. If recovering on the Computrainer, I will cycle at 40% VO_{2max} for 30 minutes. If recovering with stretching, I will perform standard static stretching exercises for approximately 30 minutes.

Anaerobic Muscle Assessment
I will perform a maximal power test using the Wingate Protocol 24 hours following the exhaustive exercise bout. I will warm up for 2 min at 50-60 rpm on a stationary bicycle ergometer before performing the Wingate test. This warm-up will be continued into the start of the sprinting portion of the Wingate test, which allows for a flying start. Peak power will be determine in watts.

I agree to do my best to follow the instructions outlined by the investigators and show up to all scheduled testing times. I agree not to take any other nutritional supplements or performance enhancing aids during this study (i.e. vitamins/minerals, creatine, protein, etc). In addition, I agree not to take any non-medically prescribed medications and to report any medication that is prescribed for me to take during this study. I understand that if I take any other nutritional supplements or medications during the course of the study that I may be removed from the study.

Exclusionary Criteria
I understand that in order to participate in the study, a trained individual will examine me to determine whether I qualify to participate. I understand I will not be allowed to participate in the study if: 1) I have any known metabolic, pulmonary, or cardiovascular disease; 2) I am taking any medications to treat metabolic, pulmonary, or cardiovascular diseases; 3) I have any absolute or relative contraindication for exercise testing or prescription as outlined by the American College of Sports Medicine; 4) I have taken any ergogenic supplements within the past month or are currently taking any supplements other than a multi-vitamin. I have reported all nutritional supplements, medically prescribed drugs, and non-medically prescribed drugs that I am presently taking. I have completed a medical history questionnaire and am not aware of any additional medical problems that would prevent me from participating in this study. I agree to report all changes in medical status, nutritional and/or pharmacological agents (drugs) that I take during the course of the investigation to Amy West (573-986-7903) or Dr. Mike Greenwood (254-710-7687).

Potential Risks & Methods to Minimize Risks
I understand that I will have approximately 10 milliliters of blood drawn by an experienced researcher from a vein in my forearm using a sterile needle and blood tubes five (5) times on different occasions during this study. This procedure may cause a small amount of pain when the needle is inserted into my vein as well as some minor bleeding and bruising. I may also experience some dizziness, nausea, and/or faint if I am unaccustomed to having blood drawn.

I understand that the exercise tests that will be performed may cause symptoms of fatigue, shortness of breath, and/or muscular fatigue/discomfort. The exercise tests may also cause short-term muscle soreness and moderate fatigue for several days following the tests. I understand that I may also experience muscle strains/pulls during the exercise testing and/or training program. However, these risks will be similar to the risk of participating in my normal training program. I also understand that trained, non-physician exercise specialists certified in CPR/AED will supervise exercise assessments. I

understand that a telephone and an automated electronic defibrillator are in the laboratory in case of any emergencies and that there will be no less than two researchers working with me during each testing session. I understand that emergency procedures are posted in the lab in the unlikely of an emergency.

The greatest risk associated with participating in this study will likely be from the muscle fatigue I may experience from participating in the training protocol. However, the intensity of the training protocol will be no more than when I engage heavily in a new or different form of physical activity. Therefore, the potential benefits of participating in this study outweigh the potential risks. If clinically significant side effects are reported, the participants will be referred to discuss the problem with the laboratory nurse, and if deemed necessary Dr. Mike Greenwood or Dr. Cooke will refer the participant to Ronald Wilson, MD for medical follow-up. Dr. Wilson is one of the Sports Medicine physicians for Baylor University and is an adjunct Professor in the Department of HHPR.

Benefits
Participants may gain insight about their health and fitness status from the assessments to be performed. Participants will be provided with their VO_{2max}, HR_{max}, $power_{max}$ and lactate threshold following testing. Participants may also gain valuable information regarding the most effective form of active recovery from endurance exercise.

Alternative Treatments
This is not a medical treatment. Therefore, if medical treatment is needed, I must obtain treatment for any medical problem I might have from my personal physician.

Costs and Payments
Participants completing all familiarization and testing sessions as well as turning in all required materials (i.e., dietary logs, testing sessions, etc) in the study will be paid $75.00. Subjects may receive information regarding results of these tests if they desire. If subjects are Baylor students, they will not receive any academic credit for participating in this study.

New Information
Any new information obtained during the course of this research that may affect my willingness to continue participation in this study will be provided to me. In addition, I will be informed of any unusual/abnormal clinical findings in which medical referral to my personal physician may be warranted. If I desire, I may request that this information be provided to my physician.

Confidentiality
I understand that any data collected about me throughout the course of this study (questionnaires, medical history, lab findings, or physical examinations) will be kept confidential to the extent permitted by law. I understand that my information will be assigned a number that will be known to the principle investigator only. However, I understand that my records of this research may be subpoenaed by court order or may be inspected by federal regulatory authorities. I understand that my data may be used in reports, presentations, and publications. I understand that I will not be individually

identified unless I have given written consent. I understand that once blood samples are analyzed, they will be discarded.

Right to Withdrawal
I understand that I am not required to participate in this study and I am free to refuse to participate or to withdraw from the study at any time. Further, that my decision to withdraw from the study will not affect my care at this institution or cause a loss of benefits to which I might be otherwise entitled. If there is concern about my medical safety, I may be referred to seek medical attention.

Compensation for Illness or Injury
I understand that if I am injured as a direct result of taking part in this study, I should consult my personal physician to obtain treatment. I understand that the cost associated with the care and treatment of such injury will be the responsibility of me or my insurance carrier. In some cases, insurers may not reimburse claims submitted for a research-related injury resulting from medical procedures or treatments performed as part of a research study. I understand that neither Baylor University nor the investigators have budgeted funds to compensate me for injury or illness that may result from my participation in this study and thus will not be accountable for illness or injury acquired during the course of this study. However, I may be referred to my personal physician if any clinically significant medical/psychological findings are observed during the course of this study.

I agree to indemnify and hold harmless Baylor University, its officers, directors, faculty, employees, and students for any and all claims for any injury, damage or loss I suffer as a result of my participation in this study regardless of the cause of my injury, damage or loss.

Statement of Conflict of Interest
I understand that the researchers involved in collecting data in this study have no financial or personal interest in the outcome of results or sponsors.

Voluntary Consent
I certify that I have read this consent form or it has been read to me and that I understand the contents and that any questions that I have pertaining to the research have been or will be answered by Dr. Mike Greenwood (254-710-7687), Dr. Matthew Cooke (254-710-4025) or Amy West (573-986-7903).

My signature below means that I am at least 18 years of age and that I freely agree to participate in this investigation. I understand that I will be given a copy of this consent form for my records. If I have any questions regarding my rights as a research subject in this study, I may contact Baylor's University Committee for Protection of Human Subjects in Research. The IRB Chairman is Dr. Matt Stanford, Professor, Department of Psychology and Neuroscience, P.O. Box 97334, Waco, TX 76798-7334, phone number 254-710-2236.

Date _____ Subject's Signature_____

I certify that I have explained to the above individual the nature and purpose of the potential benefits and possible risks associated with participation in this study. I have answered any questions that have been raised and have witnessed the above signature. I have explained the above to the volunteer on the date stated on this consent form.

Date _____ Investigator's Signature_____

APPENDIX B

Application to the Baylor IRB
For Review of Research/Activity Proposal

Part 1: Signature Page

1. Name _____**Amy West**_____

2. Email Address _____**Amy_West@baylor.edu**_____

3. Complete Mailing Address ____**2620 S. 3rd St., Waco, TX 76706**_____

4. Position _____**Graduate Student**_____

5. Faculty Advisor (if researcher is Graduate Student)____**Dr. Mike Greenwood**_____

6. Department/School_____**HHPR**_____

7. Telephone # ____**573-986-7903**_____ FAX #_____

8. Are you using subjects in research (Y or N) Y or in teaching exercises (Y or N) N

9. Title of the research project/teaching exercise:

Effects of G-trainer, cycle ergometry, and stretching on physiological and performance markers in active recovery from exercise

10. Please return this signed form along with all other parts of the application and other documentation to the University Committee for Protection of Human Subjects in Research; Dr. Matt Stanford, P.O. Box 97334, Waco, TX 76798-7334. If you have questions, or if you would like to see a copy of the OHRP Report on protection of human subjects in research, contact Dr. Stanford at 254-710-2236.

Signature of Principal Investigator Date

Signature of Faculty Advisor (required if researcher is a Graduate Student)

Departmental Review:

Department Chair or the Chair's Designate

Part 2: Introduction & Rationale

Endurance exercise inflicts tremendous strain on the bodies of athletes. This strain exists in the form of skeletal muscle damage, systemic stress and inflammation, and muscular fatigue, soreness, and function (Suzuki et al., 2006). Muscle fatigue can be defined as any exercise-induced reduction in the ability to exert muscle force or power, regardless of whether the task can be sustained or whether it has peripheral or central causes. One of the numerous proposed causes to muscular fatigue includes the build-up of metabolites in the active muscle (Mika A, Mika P, Fernhall, & Unnithan, 2007).

Recovery from exercise is important in order for an athlete to return to normal training. Active recovery has been suggested to improve muscle blood flow and remove exercise metabolites such as ADP, carbon dioxide, free radicals, and/or hydrogen ions (Mika et al., 2007). The effectiveness of recovery can be measured in the body's ability to overcome trauma and to repair and adapt to a new level. The need for proper recovery during and after training is vital. Recovery allows the body to adapt to the stress of exercise, to replenish energy stores, repair damaged tissues, and clear lactate. Consequently, inadequate rest following prolonged endurance events may lead to chronic systemic stress, inflammation, and fatigue that may impair subsequent performance (Neubauer, Konig, & Wagner, 2008). Inadequate rest could be characterized by progressive fatigue that would accompany a high volume and high intensity training schedule, not allowing for recovery from previous performances. Lactate metabolism and its rate of elimination from the muscle and blood are important components of recovery (Martin, Zoeller, Robertson, & Lephart , 1998). Recovery contributes to an increase in blood and lymph circulation to the traumatized muscle which may improve the regenerative process by reducing metabolite accumulation and local edema (Mika et al., 2007). There are two primary forms of recovery: active and passive. Passive recovery is defined as inactive rest, such as performing no physical activity. Active recovery generally involves performing aerobic exercise at a low intensity. Low intensity aerobic exercise is defined as approximately 40% of VO_{2max} (Martin et al., 1998). Active recovery refers to performing low intensity exercise in the immediate postexercise window as well as the days following the workout. Active recovery aids lactate clearance by increasing blood flow to the affected musculature thereby increasing lactate metabolism (Martin et al., 1998). Low intensity recovery exercise may also increase blood velocity and aid in the removal of metabolites following exercise. The removal of these metabolites is higher during active recovery than passive recovery (Mika et al, 2007). Choi et al. (1994) have reported that it is an ordinary practice for athletes to "warm-down", or actively recover, in order to reduce the accumulation of blood and muscle lactate after competition or training. It has been established that lactate disappears at a higher rate during active recovery than during passive recovery. This occurs due to an increase in blood flow which augments the transport of lactate from the active muscle to the removal sites (Choi, Cole, Goodpaster, Fink, & Costill, 1994).

There are numerous biochemical markers that are important to examine in order to determine recovery from exercise. Markers of systemic stress and inflammation include coritsol (Gleeson, 2002; Neubauer et al., 2008), C-Reactive protein, IL-6 (Suzuki et al., 2006; Neubauer et al., 2008), TNFα (Neubauer et al., 2008), and immune function indicators (Neubauer et al, 2008; Tzai & Cheng, 2007; Li & Gleeson, 2004; Suzuki et al,

2002). Markers of peripheral fatigue include concentrations of blood lactate (Rowlands et al., 2008; Gleeson, 2002) and power output (Enoka & Duchateau, 2008).
Studying the fatigue recovery process of endurance athletes through the examination of markers of systemic stress and inflammation and peripheral fatigue will allow for the most effective recovery pattern to be determined (Peterson, Hansen, Aagaard, & Madsen, 2007). The training and recovery schedule of an athlete must be fine-tuned in order for progression to occur. In order for a high level of performance to be maintained, there must be a balance between training, competition, and recovery. Recovery must be implemented in order to restore muscular, inflammatory, and immune parameters (Neubauer et el., 2008).
Cycle ergometry and stretching have been documented as methods of active recovery. However, there is little research available on the use of the G-trainer as a recovery aid. Alter-G, Inc. claims that the G-trainer enables users to enhance performance. One aspect of performance enhancement is the ability to recover from exercise. One of the health applications of the G-trainer is to aid aerobic conditioning. The G-trainer claims to do this by reducing force of impact on the body and protecting tissues during recovery. Information regarding the use of the G-trainer as an effective recovery method is limited. This study aims to compare two well known recovery treatments (stretching and cycle ergometry) to active non-weight bearing exercise (G-trainer). In addition, this study aims to verify the alterations between recovery methods after demanding endurance exercise.

Part 3: Methodology

Methods

Subjects

Fifteen recreationally trained male athletes ages 18-30 will participate in this study. Subjects must be recreationally trained endurance athletes (i.e. cyclists, runners, or triathletes). Subjects must have participated in at least 1 continuous hour, 3x/week of endurance activity (i.e. cycling or running) for at least 6 months.

Study Site

All testing and training will be conducted in the Exercise & Nutrition Sport Nutrition Laboratory (ESNL) in the Department of Health, Human Performance, and Recreation at Baylor University.

Independent and Dependent Variables

Independent Variables

G-trainer

The G-trainer is comparable to aqua exercise. Aqua exercise has been shown to enhance recovery through the benefits of water resistance and buoyancy. The recovery of muscle power, stiffness, and soreness has been documented with the use of aquatics (Takahashi, Ishihara, & Aoki, 2006). Body weight support on the legs is believed to become 25-30% of body mass when immersed in water up to the xiphisternum and 10% when immersed up the shoulder. Similar to aqua exercise, the G-trainer also provides weight support on the legs. The G-trainer enables its users to reduce their effective body weight in as few as 1% increments from 100% of body weight to 20% of body weight.

Cycle ergometry

Mika et al. (2007) have reported that, following dynamic muscle fatigue, the most appropriate and effective recovery method is light, active exercise, such as cycling with minimal resistance. Choi et al. (1994) used an active recovery bout on a cycle ergometer at 42% VO_{2max}. They reported that the intensity was nearly identical to the optimal recovery intensity of between 32% and 43% VO_{2max} for lactate removal for cycling exercise. A Computrainer will be used for the cycling modality. A Computrainer is an electronic bicycle ergometer that allows the user to ride his/her own bicycle. A trainer consists of a frame, a clamp to hold the bicycle securely, a roller that presses up against the rear wheel, and a mechanism that provides resistance when the pedals are turned.

Stretching

Stretching has been utilized as a recovery method. This technique has been a popular strategy for recovery after muscle fatigue in the realms of both rehabilitation and sports (Mika et al., 2007). The purpose of stretching is usually to reduce muscle soreness following exercise, to reduce the risk of injury, or to improve athletic performance (Herbert & Gabriel, 2002). Rodenburg et al. have reported positive recovery effects from a stretching and massage combination (Rodenburg, Steenbeek, Schiereck, & Bar, 1994). However, the benefits could be more accredited to stretching than massage (Mika et al., 2007). The stretching protocol will consist of 3 sets of 30 seconds of non-assisted static stretching per bilateral muscle group including hip, knee, and ankle flexors and extensors (Please see Appendix G).

Dependent Variables

Markers of systemic stress and inflammation will include coritsol, C-Reactive protein, TNFα, interleukin 6 (IL-6), and immune function indicators. Endurance exercise presents a challenge to the body of an athlete. Typically, the body reacts through a systemic inflammatory response (Neubauer et al., 2008). Several studies have examined the cytokine responses to endurance exercise. Certain proinflammatory and immunomodulatory cytokines are known to increase markedly in the circulation following endurance exercise (Suzuki, Nakaji, Yamada, Totsuka, Sato, & Sugawara, 2002). Suzuki et al. (2006) noted that endurance exercise, such as an Ironman triathlon, cause considerable muscle damage and inflammation which produces a cytokine response. Cytokine production and release is induced due to damage to muscle tissue, oxidative stress (Suzuki et al., 2006), and other metabolic and hormonal factors (Neubauer et al., 2008). Cytokine release may also be attributable to an increased epinephrine concentration and oxidative stress during exercise (Suzuki et al., 2006). Neubauer et al. (2008) found an increase in cortisol levels immediately following an Ironman triathlon. Cortisol is a catabolic hormone that reduces the utilization of amino acids for protein formation in muscle cells (Gleeson, 2002). In a study examining markers of muscle damage and inflammation after an Ironman triathlon race, C-reactive protein was measured as an indicator of the acute inflammatory response (Suzuki et al., 2006). Neubauer et al. (2008) found an increase in high-sensitive CRP both immediately (543%) and one day (7702%) after an Ironman triathlon compared to baseline values. This data suggests that major systemic stress was caused by the competition. Rowlands et al. (2008) and Suzuki et al. (2006) examined TNFα as a marker of stress, inflammation, and muscle damage during recovery from high-intensity cycling and an Ironman triathlon

race, respectively. Suzuki et al. (2006) reported that, following Ironman triathlon races, plasma IL-6 concentrations were significantly elevated above pre-race values within 30 minutes after the race. Additionally, IL-6 remained significantly elevated above pre-race values 1 day after the race. Similarly, Neubauer et al. (2008) found an increase in IL-6 both immediately (10408%) and one day (345%) following an Ironman triathlon. Release of IL-6 from the skeletal muscle into systemic circulation is attributed to the decreased availability of blood glucose and muscle glycogen. Leukocyte regulation and distribution is a multifaceted process that involves a number of factors. Exhaustive endurance exercise provokes oxidative, metabolic, and hormonal stresses, all of which can lead to the release of cytokines and the activation of numerous cell subpopulations within the immune system (Neubauer et al., 2008). Specific immune parameters, such as neutrophil function and leukocyte distribution, are impacted by exercise. Tzai and Cheng (2007) report that the acute immune response results in a mobilization of neutrophils, lymphocytes, and monocytes. In a study examining the inflammatory responses and muscular stress following an Ironman triathlon, Neubauer et al. (2008) reported an increase in total leukocyte counts immediately post-race (237%) and 1 day post-race (56%). In addition, mean changes in leukocyte subpopulations (granulocytes, monocytes, and lymphocytes) were also found. Similarly, Tzai and Cheng (2007) noted an increase in leukocyte, neutrophil, and monocyte counts following a prolonged cycling bout. Furthermore, Li and Gleeson (2004) observed an immunoendocrine response, specifically neutrophilia and monocytosis, after prolonged cycling.

Markers of peripheral fatigue will include a decrease in power output and accumulation of blood lactate. The term muscle fatigue denotes a transient decrease in the capacity to perform physical actions. Muscle fatigue can be defined as 1) an exercise-induced reduction in the ability of muscle to produce force or power whether or not the task can be sustained or 2) a decrease in the maximal force or power that the involved muscles can produce that develops gradually soon after the onset of sustained physical activity (Enoka & Duchateau, 2008). Peripheral fatigue in endurance athletes is likely to due to an increase in lactate accumulation, depletion of muscle glycogen, and/or failure of the neuromuscular system (Plowman & Smith, 2007). Muscle homeostasis during exercise may be altered due to hydrogen ion accumulation, calcium and potassium losses, local ischemia, and depletion of ATP, Cr, and/or glycogen stores. The disruptions to homeostasis may be contributing factors to the interference of the muscle excitation-contraction coupling cycle during intense exercise and postexercise, leading to muscular fatigue (Mika et al., 2007). Excitation contraction coupling is hindered due to the reduction in Ca^{2+} release and uptake from the sarcoplasmic reticulum. This may negatively influence the rate of force development of the muscle. The neuromuscular system may also influence rate of force development through the alteration of nerve signal conduction between the nerve ending and the motor endplate. These factors directly impact peripheral fatigue (Peterson et al., 2007). A decrease in the rate of force development can be quantified as the decline in the maximal power or force measured immediately after fatigue (Enoka & Duchateau, 2008).

Procedures

Medical Monitoring

Participation is voluntary and subjects may cease to participate at any time. Subjects must read, understand, and sign an informed consent form. Subjects must read and sign a medical history questionnaire. In the event of an unlikely emergency, an emergency phone will be available to call Baylor Campus Police at extension 2222. Sanitary procedures (i.e. sterilized lancets, alcohol prep pads, surgical gloves) will be followed during blood analysis. All invasive materials will be disposed of and contained in a biohazard container. The skin surface broken during each finger stick or blood draw will be sanitized with an alcohol swab. At no time will there be less than two researchers tending to each subject. The primary investigator and research team are CPR/AED certified.

Baseline Testing

Approximately 15 apparently healthy recreationally active male subjects between the ages 18 and 30 will participate in this study. During an initial familiarization session, subjects will be informed of the requirements of the study and sign an informed consent statement in compliance with the Human Subjects Guidelines of Baylor University. A trained individual will examine the subjects to determine if they are qualified to participate in this study. If the subjects are cleared to participate in the study, they will be familiarized to the testing procedures. This session will take approximately 30 minutes to complete. The study is a cross-over design, therefore, subjects will repeat the same battery of tests listed below (with the exception of recovery session) separated by 14 days. Once subjects complete the familiarization/baseline session (testing session one), they will be scheduled for testing session two, one week later.

Following the familiarization session, subjects will be instructed to refrain from exercise for 48 hours prior to baseline testing. Subjects will be provided with two 4-day dietary analysis forms that they are to complete the 4 days prior to familiarization/baseline testing, and testing session three. Once subjects report to the lab for the testing session, they will turn in their dietary analysis form.

Subjects will then donate about 10 milliliters of blood from a vein in the arm. Blood samples will be obtained using standard/sterile procedures using a needle inserted into a vein in the arm. The personnel who will be taking blood are experienced in phlebotomy (procedures to take blood samples) and are qualified to do so under guidelines established by the Texas Department of Health and Human Services. This will take about 5 minutes and subjects will be asked to donate the same volume of blood on five separate occasions throughout the study. Blood pressure, resting heart rate, height, weight, body composition, and total body water measured and recorded. Subjects will be tested for VO_{2max}, heart rate max, and lactate threshold during an incremental ride to exhaustion on a cycle ergometer (GXT). Subjects will be told that they should continue to exercise until they can exercise no more. Subjects will perform a 10 minute warm-up routine and will then be instructed to produce a power output of 100 watts. Upon achieving 100 watts, the test will begin. Each stage will be three minutes in duration. The following stages require an increase of 50 watts to be sustained per stage until 250 watts is reached, wherein

power output will be increased by 50 watts every stage until volitional fatigue. Test termination will occur 1) when the subject is no longer able to maintain the required power output, 2) when the subject has reached self-reported volitional fatigue, or 3) when the investigator or supervisors consider the subject at risk for physical harm. 30 minutes following the GXT, subjects will perform a maximal power test using the Wingate Protocol. Subjects will warm up for 2 min at 50-60 rpm on a stationary bicycle ergometer before performing the Wingate test. This warm-up will be continued into the start of the sprinting portion of the Wingate test, which allows for a flying start.

Following familiarization/baseline testing, subjects will return to the lab for testing session two. Subjects will again have weight, total body water, blood pressure and heart rate measured and recorded. Subjects will also rate their level of perceived effort and psychological state using the Borg Scale and POMS questionnaire, respectively. Subjects will also donate about 10 milliliters of blood from a vein in the arm prior to and immediately following the exercise bout and recovery session. Subjects will be tested for blood lactate concentrations via finger prick prior to and directly after the exercise bout and 5, 10 and 15 minutes following the recovery exercise session. Subjects will perform a fatiguing bout of exercise on a Computrainer which is approximately 25 miles with an average grade of 0.3%, a maximum grade of 9.7%, and total climbing of 1702 total feet. Following the exercise bout, subjects will perform one of three recovery sessions for approximately 30 minutes. Three hours following the recovery session, subjects will undergo blood sampling and also rate their level of perceived effort and psychological state using the Borg Scale and POMS questionnaire. Subjects will then be instructed to refrain from exercise and return to the lab 24 hours later.

Twenty-four (24) hours following the recovery bout, subjects will again be tested for blood lactate concentrations through a finger stick. Additionally, approximately 10 ml of blood will be drawn from a vein in the arm. Subjects will again be asked to rate their level of perceived effort and psychological state using the Borg Scale and POMS questionnaire. Subjects will perform a maximal power test using the Wingate Protocol. Subjects will warm up for 2 min at 50-60 rpm on a stationary bicycle ergometer before performing the Wingate test. This warm-up will be continued into the start of the sprinting portion of the Wingate test, which allows for a flying start. Following all testing, subjects will continue with their normal activities and return to the lab 14 days later to repeat the same battery of tests, with the exception of a different recovery session.

Exercise Fatigue Protocol

Subjects will perform the fatiguing bout of exercise on a Computrainer. The Computrainer is a stationary electronic bicycle ergometer that allows the user to ride their own bicycle. A trainer consists of a frame, a clamp to hold the bicycle securely, a roller that presses up against the rear wheel, and a mechanism that provides resistance when the pedals are turned. The protocol will be selected from the Computrainer 3D Version 3 Software (Copyright 2006 Racermate) and will be programmed into and used in conjunction with the bicycle ergometer to simulate a race course and effort. The USAT Nationals St. Joe course, a 24.46 mile course with an average grade of 0.3%, a maximum grade of 9.7%, and 1702 total feet of climbing will be used. Subjects will be provided

with a viewing screen that depicts the race course. Subjects will be instructed to ride as hard as possible for the duration of the protocol. A fan providing constant wind speed will be placed directly in front of the subject and positioned so that the airflow will be directed towards the head and torso when in a normal cycling position. Water will be available to the subject ad libitum (as desired).

Recovery Session

Immediately following the exercise bout, subjects will perform one of three recovery bouts (G-trainer, Computrainer, or stretching) for 30 minutes. Subjects will be randomly assigned to one of three treatment groups: G-trainer, Computrainer, or stretching. Subjects will participate in a crossover design and will perform each recovery session on three separate occasions separated by 14 days. On each occasion, subjects will perform the same battery of tests as mentioned previously; however, the recovery modality will be different. If recovering on the G-trainer, I subjects will run at 40% VO_{2max} and 75% of body weight for 30 minutes. If recovering on the Computrainer, subjects will cycle at 40% VO_{2max} for 30 minutes. If recovering with stretching, subjects will perform standard static stretching exercises for approximately 30 minutes.

Anaerobic Muscle Assessment

Subjects will perform a 30 second Wingate anaerobic capacity test at baseline and 24 hours following the exhaustive exercise bout. Subjects will warm up for 2 min at 50-60 rpm on a stationary bicycle ergometer before performing the Wingate test. This warm-up will be continued into the start of the sprinting portion of the Wingate test, which allows for a flying start. The Wingate anaerobic capacity test will be performed on the LODE cycle ergometer (Amsterdam, Netherlands) with a resistance of 0.7 Nm/kg. Test-to-test variability in performing repeated Wingate tests in our laboratory yielded correlation coefficients of $r = 0.98$ ±15% for mean power. Peak power will be determine in watts.

Exercise Fatigue Protocol

The subject will perform the fatiguing bout of exercise on a Computrainer. The Computrainer is a stationary electronic bicycle ergometer that allows the user to ride their own bicycle. A trainer consists of a frame, a clamp to hold the bicycle securely, a roller that presses up against the rear wheel, and a mechanism that provides resistance when the pedals are turned. The protocol will be selected from the Computrainer 3D Version 3 Software (Copyright 2006 Racermate) and will be programmed into and used in conjunction with the bicycle ergometer to simulate a race course and effort. The USAT Nationals St. Joe course, a 24.46 mile course with an average grade of 0.3%, a maximum grade of 9.7%, and 1702 total feet of climbing will be used. Subjects will be provided with a viewing screen that depicts the race course. The subjects will be instructed to ride as hard as possible for the duration of the protocol. A fan providing constant wind speed will be placed directly in front of the subjects and positioned so that the airflow will be directed towards the head and torso when in a normal cycling position. Water will be available to the participants ad libitum.

Research Team

Amy West, BS. Ms. West is a graduate teaching assistant in the Department of HHPR and will serve as the primary investigator in this study.

Mike Greenwood, PhD, FNSCA, FACSM, FISSN, CSCS*D. Dr. Greenwood is Professor and Graduate Exercise Physiology Director in the Department of HHPR at Baylor University.

Matt Cooke, PhD. Dr. Cooke is Assistant Professor in the Department of HHPR at Baylor University.

Matthew Stanford, PhD. Dr. Stanford is Professor in the Department of Psychology and Neuroscience at Baylor University.

Equipment

Metabolic cart (aerobic capacity testing)

Maximal oxygen consumption will be measured via the Parvo Medics TrueMax 2400 Metabolic Measurement System (Parvo Medics, Provo, UT). Oxygen uptake will be measured every 15 seconds via an open-circuit sampling system and the highest level VO_2 will be defined as VO_{2max}.

Cycle Ergometer

Maximal power testing will be performed on a LODE cycle ergometer (Amsterdam, Netherlands).

Heart Rate Monitor

Heart rate will be determined from a continuously monitored heart rate monitor with chest strap and wristwatch (Polar Electro, Lake Success, NY).

Blood lactate analyzer

Blood will be analyzed for lactate levels (mm) using an Accusport™ Blood Lactate Analyzer.

Biochemical analyses

Using an enzyme-linked immunoabsorbent assay (ELISA) commercially available kit, serum pro-inflammatory markers (coritsol, C-reactive protein, IL-6, TNFα) will be determined. The serum levels of each marker will be determined with a Wallac Victor-1420 microplate reader (Perkin-Elmer Life Sciences, Boston, MA). The assay will be performed at a 450 nm wavelength against a known standard curve.

Computrainer

The fatigue protocol, maximal aerobic capacity test, and peak power test will be performed on an electronic bicycle ergometer that allows the user to ride their own bicycle. A trainer consists of a frame, a clamp to hold the bicycle securely, a roller that presses up against the rear wheel, and a mechanism that provides resistance when the pedals are turned (Racermate, Seattle, WA).

G-trainer
The G-trainer is a computer-controlled integrated body-weight-supported treadmill system which uses differential air pressure to support the user while walking or running on the treadmill. Alter-G, Inc. claims that the G-trainer enables users to enhance performance. One of the health applications of the G-trainer is to aid aerobic conditioning. The G-trainer claims to do this by reducing force of impact on the body and protecting tissues during recovery. In addition, the G-trainer enables its users to reduce their effective body weight in as few as 1% increments from 100% of body weight to 20% of body weight. The device accomplishes this unweighting through an air pressure regulation system that used positive air pressure around the lower body to support body weight (Grabowski & Kram, 2008; Alter-G). The G-trainer provides the same benefits of weight supported recovery such as a rise in muscle temperature and an increase in muscle blood flood without the impact of ground reaction forces.

Anthropometric and Body Composition Testing Procedures
Total body mass (kg) will be determined on a standard dual beam balance scale (Detecto).Total body water (total, intracellular, and extracellular) will then be estimated using a Xitron 4200 Bioelectrical Impedance Analyzer (San Diego, CA).

Dietary Analysis
The participants' diets will not be standardized and subjects will be asked not to change their dietary habits during the course of the study. The dietary records will be evaluated with the Food Processor IV Nutrition Software dietary assessment program (ESHA Research Inc., Salem, OR).

Hemodynamic Assessments
Heart rate will be determined by palpitation of the radial artery using standard procedures. Blood pressure will be assessed after resting for 5 minutes using a mercurial sphygmomanometer using standard procedures.

Blood Collection Procedure
Venous blood samples will be obtained from the anticubital fossa into 10 milliliter collection tube using a standard vacutainer apparatus for both plasma and serum separation. Blood samples will be allowed to stand at room temperature for 10 minutes and then centrifuged. For each sample, the serum will be removed and frozen at -80°C for later analysis.

Subjects
Recruitment
Participants will be recruited from the metropolitan area of Waco, TX. Subjects will be recruited through fliers posted around Baylor University, through HHPR activity classes, through two local cycling clubs, the Waco Bicycle Club and the Baylor Cycling Club, and through a local running and triathlon club, the Waco Striders.

Selection Criteria
Subjects will not be allowed to participate in the study if they:
1. Have any known metabolic, pulmonary, or cardiovascular disease;
2. Are taking any medications to treat metabolic, pulmonary, or cardiovascular disease
3. Have any absolute or relative contraindication for exercise testing or prescription as outlined by the American College of Sports Medicine;
4. Have taken any ergogenic supplements within the past month or are currently taking any ergogenic supplements other than a multi-vitamin

Compensation or Incentives
Subjects will receive $75 at the completion of the study. If a Baylor University student, the subject will not receive any academic credit for participating in this study.

Possible Risks & Methods to Minimize Risks
The venipuncture procedure may cause a small amount of pain when the needle is inserted into the vein as well as some minor bleeding and bruising. Subjects may experience some dizziness, nausea, and/or faint if unaccustomed to having blood drawn. The exercise tests may cause symptoms of fatigue, shortness of breath, and/or muscular fatigue/discomfort. The exercise tests may also cause short-term muscle soreness and moderate fatigue for several days following the tests. The investigators of this study are well qualified. Ms. West is a graduate teaching assistant in the Department of HHPR, is CPR/AED, blood borne pathogen, and radiation safety certified and is a certified personal trainer through the American College of Sports Medicine. Mike Greenwood, PhD, FNSCA, FACSM, FISSN, CSCS*D, is Professor and Graduate Exercise Physiology Director in the Department of HHPR at Baylor University. Matt Cooke, PhD**,** is Assistant Professor in the Department of HHPR at Baylor University. In the event that any unexpected problems or adverse events occur, subjects will be referred to discuss the matter with the laboratory nurse, and if deemed necessary Dr. Mike Greenwood or Dr. Matt Cooke will refer the participant to Ronald Wilson, MD for medical follow-up. Dr. Wilson is one of the Sports Medicine physicians for Baylor University and is an adjunct Professor in the Department of HHPR.

Potential Benefits
Participants may gain insight about their health and fitness status from the assessments to be performed. Participants will be provided with their VO_{2max}, HR_{max}, $power_{max}$ and lactate threshold following testing. Participants may also gain valuable information regarding the most effective form of active recovery from endurance exercise.

Assessment of Risk
The potential benefits of active recovery from exercise have been well studied. The results of this study will help determine the effects of the G-trainer, cycle ergometry, and stretching on biochemical marker and blood lactate response in active recovery from exercise. The greatest risk associated with participating in this study will be to participate in the testing protocol. However, since the subjects to be used in this study will be recreationally trained endurance athletes, these risks would be no different than the

athletes participating in their own training program. It is the view of the researchers that the potential benefits of subjects participating in this study outweigh the potential risks.

Confidentiality

Information obtained from this research (including questionnaires, medical history, laboratory findings, or physical examination) will be kept confidential to the extent permitted by law.

Data Analysis, Presentation, and Publication

Data will be presented at an appropriate scientific conference (e.g., American College of Sports Medicine, American Society of Exercise Physiologists, National Strength and Conditioning Association, etc) and published in a peer reviewed scientific journal (e.g. Medicine and Science in Sports and Exercise, Journal of Exercise Physiology, Journal of Strength and Conditioning, etc).

Statement of Conflict of Interest

Researchers involved in collecting data in this study have no financial or personal interest in the outcome of results or sponsors.

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APPENDIX C

BAYLOR UNIVERSITY
ESNL
Medical History Inventory

Directions. The purpose of this questionnaire is to enable the staff of the Exercise and Sport Sciences Laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **CONFIDENTIAL** as described in the **Informed Consent Statement.**

Name:_____ Age _____Date of Birth_____

Name and Address of Your Physician:_____

MEDICAL HISTORY
Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in blank).

____	Heart murmur, clicks, or other cardiac findings?	____	Asthma/breathing difficulty?
____	Frequent extra, skipped, or rapid heartbeats?	____	Bronchitis/Chest Cold?
____	Chest Pain of Angina (with or without exertion)?	____	Melanoma/Suspected skin Lesions?
____	High cholesterol?	____	Stroke or Blood Clots?
____	Diagnosed high blood pressure?	____	Emphysema/lung disease?
____	Heart attack or any cardiac surgery?	____	Epilepsy/seizures?
____	Leg cramps (during exercise)?	____	Rheumatic fever?
____	Chronic swollen ankles?	____	Scarlet fever?
____	Varicose veins?	____	Ulcers?
____	Frequent dizziness/fainting?	____	Pneumonia?
____	Muscle or joint problems?	____	Anemias?
____	High blood sugar/diabetes?	____	Liver or kidney disease?
____	Thyroid Disease?	____	Autoimmune disease?
____	Low testosterone/hypogonadism?	____	Nerve disease?
____	Glaucoma?	____	Psychological Disorders?

Do you have or have you been diagnosed with any other medical condition not listed?
_____

Please provide any additional comments/explanations of your current or past medical history.
_____

Please list any recent surgery (i.e., type, dates etc.).
_____

List all prescribed/non-prescription medications and nutritional supplements you have taken in the last month.

What was the date of your last complete medical exam?

Do you know of any medical problem that might make it dangerous or unwise for you to participate in this study?
(Including maximal exercise tests) ____ If yes, please explain:

Recommendation for Participation

____ No exclusion criteria presented. Participant is ***cleared*** to participate in the study.
____ Exclusion criteria is/are present. Participant is ***not cleared*** to participate in the study.

Signed: _____ Date: _____

APPENDIX D

Baylor University
Exercise and Sport Nutrition Laboratory

Personal Information
Name: _____

Address: _____

City: _____ State: _____ Zip Code _____

Home Phone: (___) _____ Work Phone: (___)_____

Cell Phones: (___) _____ Fax: (___)_____

Email address:_____

Birth date: _____ / _____ / _____ Age: _____ Height: _____ Weight: _____

Exercise & Supplement History/Activity Questionnaire

1. Describe your typical occupational activities.

2. Describe your typical recreational activities.

3. Describe any exercise training that you routinely participate.

4. How many days per week do you exercise/participate in these activities?

5. How many hours per week do you train?

6. How long (years/months) have you been consistently training?

7. When was the last time you ingested a performance enhancing aid or supplement (excluding multi-vitamin)?

APPENDIX E

Baylor University
Exercise & Sport Nutrition Laboratory

NAME _____ Date _____

INSTRUCTIONS

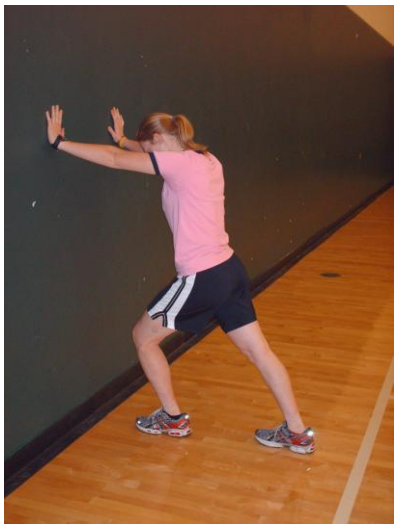
1. Record everything you eat for 4 days prior to the testing session. If you eat pretzels, record how many. If you eat a bag of chips, record the number of ounces. For drinks, record the number of cups or ounces. Record everything you drink except water.
2. Record the Food, Amount, Brand Name, and Preparation Methods. For example: baked vs. fried chicken; 1 cup of rice; 2 teaspoons of margarine; 1 cup of 2% milk; McDonald's, Healthy Choice, or Frosted Flakes.
3. Record immediately after eating. Waiting until that night may make it difficult to remember all foods and quantities. **PLEASE BE AS SPECIFIC AS POSSIBLE.**

Food (include brand)	**Method of Preparation**	**Quantity (cups, oz., no.)**
BREAKFAST:		
LUNCH:		
DINNER:		
SNACKS:		

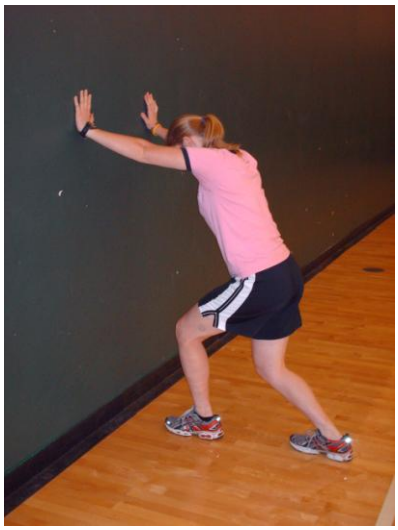
APPENDIX F

Passive Stretching Protocol

Straight knee ankle extensor wall stretch



Bent knee ankle extensor wall stretch



Standing quad stretch

Hip flexor stretch (lunge position, pushing hips forward)

Seated hamstring stretch

Lying piriformis stretch (on back, knee to chest)

Lying glut/low back stretch (on back, knee across body)

Bathing Beauty stretch

**Complete 3 sets of 30 seconds of static stretching per bilateral muscle group.

**Complete each stretch prior to continuing to the next.

**Hold to the point of mild discomfort, but not pain.

APPENDIX G

Kashi TLC Chewy Granola Bar

Ingredients: **Kashi Seven Whole Grains & Sesame®** Blend (Whole: Hard Red Winter Wheat, Oats, Rye, Barley, Triticale, Long Grain Brown Rice, Buckwheat, Sesame Seeds), Whole Almonds, Brown Rice Syrup, Soy Protein Isolate, Evaporated Cane Juice Crystals, Soy Grits, Chicory Root Fiber, Raisins, Sunflower Seeds, Evaporated Cane Juice Syrup, Cranberries, Vegetable Glycerin, Corn Flour, Honey, Rice Starch, Expeller Pressed Canola Oil, Oat Fiber, Evaporated Salt, Natural Flavors, Molasses, Soy Lecithin, Peanut Flour, Whey, Annatto Color.

Nutrition Facts

Serving Size: 1 Bar (35g)	
Amount per Serving	% Daily Value
Calories 140 Calories from Fat 45	
Total Fat 5g	8%
Saturated Fat 0.5g	3%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 105mg	4%
Potassium 100mg	3%
Total Carbohydrate 20g	7%
Dietary Fiber 4g	14%
Soluble Fiber 1g	
Insoluble Fiber 3g	
Sugars 5g	
Protein 6g	
Vitamin A	0%
Vitamin C	0%
Calcium	0%
Iron	6%

APPENDIX H

OVERVIEW OF RESEARCH DESIGN

Testing Session 1: Familiarization/ Baseline	Testing Session 2: Fatigue Protocol (1 week after baseline)	Recovery Method (immediately following fatigue protocol)	Testing Session 3: 3 hours post-recovery	Testing Session 4: 24 hours post-recovery
Crossover Design: Repeated for trials 2 & 3; separated by 14 days each				

○ Collect food log
○ Consent form
○ Medical history questionnaire
○ Demographic information
○ POMS
○ Anthropometric measures
○ Resting HR
○ Resting BP
○ BIA
○ Blood draw #1
○ Aerobic Capacity Test
○ 30 second Wingate Test
○ Familiarization session
  ○ G-trainer
  ○ Computrainer
  ○ Stretching
○ Distribute food log
	○ BIA
	○ Blood draw #2
	○ La^+
	○ Kashi Bar
	
	After fatigue protocol:
	○ Blood draw #3
	○ La^+ @ 0,5,10,15, 30 min
	○ Borg Scale
	○ POMS
		○ G-trainer
		or
		○ Computrainer
		or
		○ Stretching
			○ Borg Scale
			○ POMS
			○ Blood draw #4
			○ La^+
				○ Collect food log
				○ Borg Scale
				○ POMS
				○ BIA
				○ Blood draw #5
				○ La^+
				○ 30 second Wingate Test

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