The Effect of Carbohydrate Consumption during Intensive Exercise Training on the Free Testosterone to Cortisol Ratio

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ABSTRACT

AMY LANE: The Effect of Carbohydrate Consumption during Intensive Exercise Training on the Free Testosterone to Cortisol Ratio
(Under the direction of Anthony C. Hackney, Ph.D., D.Sc.)

This study examined the effect of dietary carbohydrate (CHO) consumption on the free testosterone to cortisol (fTC) ratio during an intense microcycle of exercise training. The ratio is a proposed biomarker for overreaching-overtraining in athletes. Two groups, control-CHO (~60% of daily intake, n=12) and low-CHO (~30% of daily intake, n=8), of male subjects performed three consecutive days of intensive training (~70-75% maximal capacity) with a dietary intervention (day before and days of training). The fTC ratio decreased ($p<0.01$) from pre-study resting measurement to the final post-study resting measurement in the low-CHO group. No significant change occurred in the control group. Findings suggest if the fTC ratio is utilized as a marker of training stress a diet of ~60% CHO needs to be consumed to maintain validity of the ratio value.

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To the casual observer, a thesis project may appear to be solitary work. However, to complete a project of this magnitude requires a network of support. I would like to thank my family for their endless support. I am appreciative to my advisor, Dr. Anthony C. Hackney for his guidance, motivation, and support. I would also like to acknowledge the rest of my committee, Dr. Kristin Ondrak and Mr. Alain Aguilar, M.A., A.T.C., for their willingness to assist me and their suggestions. I would like to finally acknowledge my fellow colleagues, without their early morning sacrifice of time and energy I would never have completed the process of data collection.

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CHAPTER I

BASIS OF STUDY

Introduction

Athletes must continually challenge themselves in their training in order to maintain the high level of physical fitness necessary to be competitive and optimize their sporting performance. The typical training approach consists of repeated exposures to strenuous activities. In order to maximize the adaptive response these activities must become increasingly more strenuous over time; i.e., progress to more difficult levels and continue to overload the body. However, there is a fine, unidentified line where beneficial training becomes detrimental. Pushing too hard without proper recovery time can result in a loss of performance (Rowbottom, 2000). Kuipers (1998) has depicted this as an inverse U-shaped relationship between performance and training overload (Figure 1).

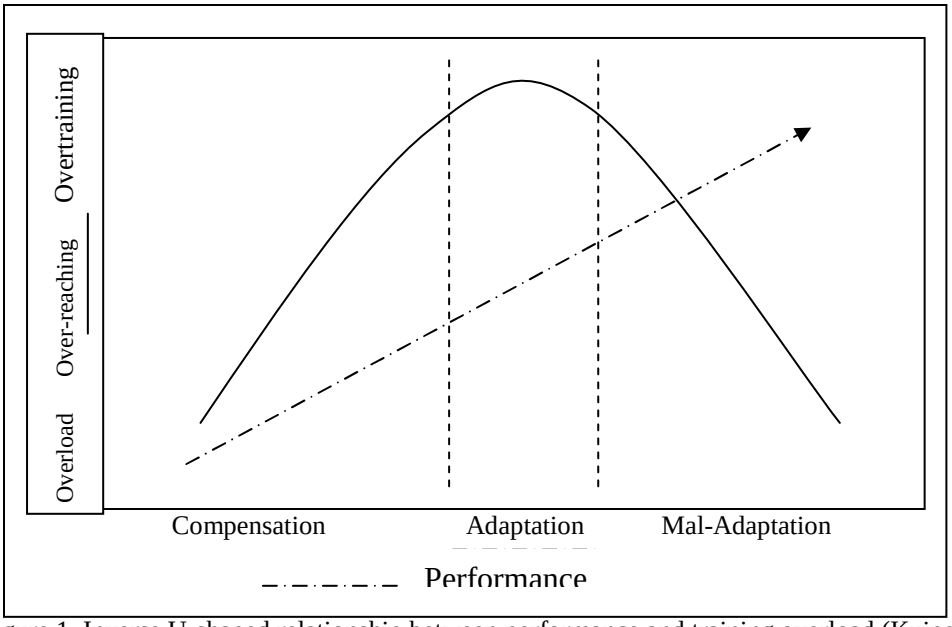

Figure 1. Inverse U-shaped relationship between performance and training overload (Kuipers, 1998).

Continued training overload without proper rest and recovery time leads to overreaching. At this point positive adaptations cease and continued efforts could lead to development of the Overtraining Syndrome (OTS) which decreases performance capacity. Regrettably, once OTS is reached it can take weeks or months for athletes to recover (Hackney, 2006). Because declining performance is undesirable for athletes, a method of monitoring training progress to prevent or avoid the OTS is desirable. Since many factors are involved in the progression, and individuals respond differently to stress, there is no standard measure (or known point) that identifies negative progress at this time (Angeli, Minetto, Dovio, & Pacotti 2004).

A suggested measure is to compare the recovery, or building up (anabolic) activity to the breaking down (catabolic) activity of proteins, which is regulated through endocrine factors. Protein status dramatically controls the plasticity of skeletal muscle adaptation. Therefore, by comparing the anabolic and catabolic hormonal activity, a state of positive or negative protein activity may be (Brooks, Fahey, & Baldwin, 2005). For these reasons, the analysis of the resting levels of blood free testosterone to cortisol (fTC) ratio has been proposed as a potential endocrine marker to measure training adaptation (Adlercreutz et al., 1986; Hoogeven & Zonderland, 1996; Urhausen, Gabriel, & Kindermann, 1995). A measured decrease of 30% or an absolute ratio value below $0.35x10^{-3}$ at rest (as measured over several days) is said to suggest an overtraining effect (Lehmann, Foster, & Keul, 1993). This marker has been used with varying degrees of success, some very promising, although many researchers continue to investigate its usefulness.

Testosterone, an anabolic steroid, has been shown to increase protein synthesis as well as red blood cell formation. Conversely, cortisol stimulates muscle proteolysis, and

gluconeogenesis. Exercise of a specific intensity and or duration is known to produce significant changes in testosterone and cortisol (McMurray & Hackney, 2000). Testosterone tends to increase with short-term bouts of exercise relative to intensity. These increases can mainly be attributed to a decrease in metabolic clearance rate, an increase in production and secretion, or the result of hemoconcentration from the exercise. A minimum intensity of 50-60% of VO_{2max} must be met for cortisol to show a significant increase (McMurray & Hackney, 2000). The increase results in the breakdown of triglycerides, hepatic gluconeogenesis, and an increase in muscle proteolysis in an effort to maintain blood glucose homeostasis (Brooks et al., 2005).

Hormones are also affected by energy substrate availability in the body, in particular the amount of carbohydrate (CHO). For example, diminished glucose concentrations in the blood will stimulate an exaggerated response by fuel mobilizing hormones such as epinephrine, glucagon, growth hormone and cortisol. Specifically, Galbo, Holst, and Christensen, (1979) found that subjects on a low CHO diet (daily caloric intake = 11.5% CHO) for several days had elevated levels of these fuel mobilizing hormones compared to subjects on a high CHO diet (daily caloric intake = 77% CHO). A consequence of ingesting a low CHO diet is the effect that it will have on cortisol and testosterone levels. Without abundant CHO to replenish both glycogen stores and maintain sufficient blood glucose levels, cortisol will be secreted in an effort to maintain blood glucose through muscle proteolysis and amino acid oxidation (Brooks et al., 2005). Testosterone also experiences alterations in bioavailability with differing diets. Anderson et al. (1987) found that testosterone levels decreased in a high protein diet, compared to a high CHO diet, while cortisol showed the opposite response. The binding globulins of both hormones experienced

parallel changes. The increase in sex hormone binding globulin provided more sites for free testosterone to bind, therefore decreasing the free testosterone concentration in the blood for anabolic purposes. Cortisol binding protein levels were increased with the high protein diet and suppressed in the high CHO, comparatively.

The recommended intake of CHO for athletes is approximately 60-70% of their diet, especially for endurance-based activities (Snyder, Kuipers, Cheng, Servais, & Fransen, 1995). By consuming the appropriate macronutrient balance, the body is able to replenish glycogen stores and maintain the intense level of training necessary. Due to training schedules, personal habits, and inappropriate nutritional education, most athletes overestimate their CHO intake by 10-25% (Snyder et al., 1995). This suggests that many athletes are not consuming an adequate amount of CHO. Therefore, in many situations it is possible that athletes are not physiologically prepared to handle the stressors of their intense training programs.

The above evidence suggest that to consider the fTC ratio as a training adaptation marker without taking into account the influence of prior daily diet may invalidate the measurement. That is, variations in daily CHO consumption potentially hinder the reliability of the markers results, leaving the conclusions suspect and inconsistent (Jeukendrup, 2003; Evans & Hughes, 1985; Galbo et al., 1979; Tsai et al., 1993; Kavouras, Troup, & Berning, 2004). The influence of daily CHO intake on cortisol and free testosterone levels in combination with acute exercise have been examined in the research literature. However, the influence CHO intake on the fTC ratio over repeated days of training has not.

Therefore, the purpose of this study was to investigate the effect of CHO consumption on the fTC ratio over repeated days of intense exercise. The subjects were

randomly assigned to a control (normal) or low carbohydrate group, and exercised (cycling) for three consecutive days. Blood was drawn at 24 hr intervals and analyzed for free testosterone and cortisol levels. The research question was: Does the quantity of CHO consumed affect the fTC response to intensive training? It was hypothesized that a low CHO consumption diet would produce a greater reduction in the fTC ratio than a control CHO diet.

Delimitations

1. The subjects consisted of males ages 18 to 45 who were recruited from The University of North Carolina at Chapel Hill and surrounding areas.
2. The subjects were healthy, moderately to highly trained individuals involved in competitive endurance sport.
3. The subjects began each trial after a minimum 8 hour fast and refrained from physical activity in addition to the training protocol during each trial.

Limitations

1. The results can only be generalized across the population studied: males 18 to 45 who were moderately to highly trained in endurance sports.
2. Although subjects were asked to refrain from normal physical activity during the training sessions and follow a control or low CHO diet, it is possible an occasional deviation occurred.

Definition of Terms

1. Overreaching: An increase in training meant to provide positive adaptations with appropriate rest. This can result in overtraining and/or OTS (Halson & Jeukendrup, 2003).

2. Overtraining: A condition identified through decreased performance due to an imbalance of exercise intensity and volume to recovery time (Angeli et al., 2004).
3. Overtraining Syndrome (OTS): A stress related condition resulting from too little rest and recovery, a primary symptom is decreased performance (Angeli et al., 2004).
4. Cortisol: A glucocorticoid hormone produced in the zona fasciculata layer of the adrenal cortex and is stimulated by the adrenocorticotropic hormone (ACTH) of the anterior pituitary (Neal, 2000).
5. Testosterone: A hormone synthesized and secreted from the Leydig cells of the testis, it is stimulated by the luteinizing hormone (LH) of the anterior pituitary and has both androgenic and anabolic effects (Neal, 2000).
6. Free Testosterone to Cortisol Ratio (fTC): The molar ratio of free testosterone to cortisol.

Significance

In the ever increasingly competitive world of athletics, the need to push oneself in training is greater than ever. The training load athletes endure is extremely intense. Currently, there is no identifier to warn coaches or athletes that they need to reduce the training load or increase resting periods to prevent maladaptations from occurring. The suggestion of a hormonal marker to identify such a situation, the fTC ratio, has been studied for the last 20 years. Research has been conducted in an effort to validate the use of the fTC ratio as a marker of potential overtraining. Results have been positive, except for a number of inconsistencies. However, the diets of the subjects in these studies have rarely been controlled for. The results of this study may be able to contribute to the validity of this

measure by providing guidelines concerning the influence of CHO during training on the fTC ratio. The results could direct future studies about dietary needs to be more complete and accurate. It could also be utilized as an educational tool for coaches and athletes alike, who may not know the extent to which a low CHO affects one's performance. Perhaps these results can help individuals avoid unnecessary setbacks.

CHAPTER II

REVIEW OF LITERATURE

I. Introduction

This study examines how the amount of CHO one consumes may impact the free testosterone to cortisol (fTC) ratio. This ratio has been suggested as a marker for identifying individuals with overtraining. However, it may be that the lack of proper nutrition is the driving force behind shifts in the ratio rather than only the intense exercise training. This literature review will examine the information available regarding cortisol and free testosterone and the potential dietary impact on them. In addition, it will review why the fTC ratio is considered a potential marker for overtraining. The research concerning the effect of exercise on the fTC ratio and the work that has been done in both short-term and long-term training studies will be evaluated, and whether dietary influences were considered.

II. Hormones

A. Cortisol

1. Function

The hormone cortisol is classified as a glucocorticoid, produced in the zona fasciculata of the adrenal cortex. Cortisol is the most abundant of the glucocorticoids, and is controlled by negative feedback loop. The release of cortisol is initiated from a disturbance in homeostasis (Tortora & Derrickson, 2006). An increase in stress or decrease in blood glucose levels will cause the hypothalamus to release corticotrophin releasing factor (CRF),

which results in the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary. ACTH will stimulate the adrenal cortex to release cortisol (Brooks et al., 2005).

The release of cortisol will stimulate lipolysis, breakdown proteins, and cause glucose formation. Cortisol will specifically cause a breakdown of triglycerides, releasing free fatty acids into the blood from adipose tissue. In the muscle, cortisol increases the rate of protein breakdown resulting in more amino acids in the blood available for remodeling or energy production. In the liver, amino acids, or lactate can be converted into glucose. This latter process, gluconeogenesis, increases the blood glucose levels (Tortora & Derrikson, 2006).

2. Dietary Impact

The nutritional status of an individual will have an impact on how cortisol responds. Galbo (1983) explains that with a decrease in CHO consumption, there is an increased response in sympathoadrenal activity. This will lead to elevated stimulation of cortisol in an effort to enhance lipolysis and maintain blood glucose homeostasis. He illustrated how following a ~ 60 hour fast, cortisol (nmol/l) levels were elevated from a resting basal state prior to the fast. Additionally, glucose (mmol/l) levels were decreased following the ~ 60 hour fast.

Tegelman, Lindeskog, Carlstrom, Pousette, and Blomstrand (1986) had ten women and six men eat a decreased caloric intake for 3 days, then 7 days with only 50g CHO (fasting), followed by 3 days back at their normal diet. Venous blood was drawn immediately before and after the fasting period, as well as one week after fasting. They found that a decrease in consumption of CHO could result in changes in peripheral hormones levels due to decreased metabolic clearance of some steroids (i.e., glucocorticoids).

Anderson et al. (1987) found that low-CHO diet influenced cortisol and its binding globulins. They looked at two isocaloric diets, high-protein (44%, 35% CHO) and high-CHO (70% CHO, 10% protein) over a 10 day period each in seven healthy 22-43 year old men. Cortisol and cortisol binding globulin (CBG) decreased during the high-CHO diet. During the high-protein diet 6% to 43% less CBG were available for binding resulting in more cortisol in the blood. Cortisol binding globulin is synthesized in the liver. According to Anderson et al. (1987) this suggests that synthesis and degradation may be influenced by changes in dietary composition, however, they could not identify a specific mechanism.

3. Exercise Response

Intensity and duration are two important factors in how cortisol will respond to exercise. With low intensity exercise ($\leq$ 40% of VO_{2max}), cortisol tends to show a decrease in concentration in the blood. This can be misleading. Cortisol is still being released in response to the exercise, but the metabolic clearance rate (MCR) is greater than the secretion/production rate, therefore the concentration appears to be decreasing (Galbo, 1983). There seems to be a threshold at which cortisol is produced and secreted at a greater rate than clearance. As exercise continues to an extended duration, cortisol will continue to increase in response to the stress (Brooks et al., 2005).

Hill et al. (2008) confirmed that a workload of at least 60% of an individual's maximal oxygen consumption will induce an increased cortisol response. They had twelve moderately trained males exercise at 40%, 60%, and 80% of VO_{2max} to determine what intensity would elicit a significant increase in circulating cortisol. Both the 60 and 80% sessions were significantly greater than the resting and 40% session. At 40%, a decrease in cortisol was found, which agrees with Galbo (1983).

The main role of cortisol during exercise is maintaining blood glucose levels. In an effort to do so, cortisol stimulates lipolysis in adipose tissue, accelerates the breakdown of protein in the muscle, and increases gluconeogenesis in the liver. These functions occur during both the actual exercise and continue on for up to 2 hours after completion (Brooks et al., 2005) (see Figure 2).

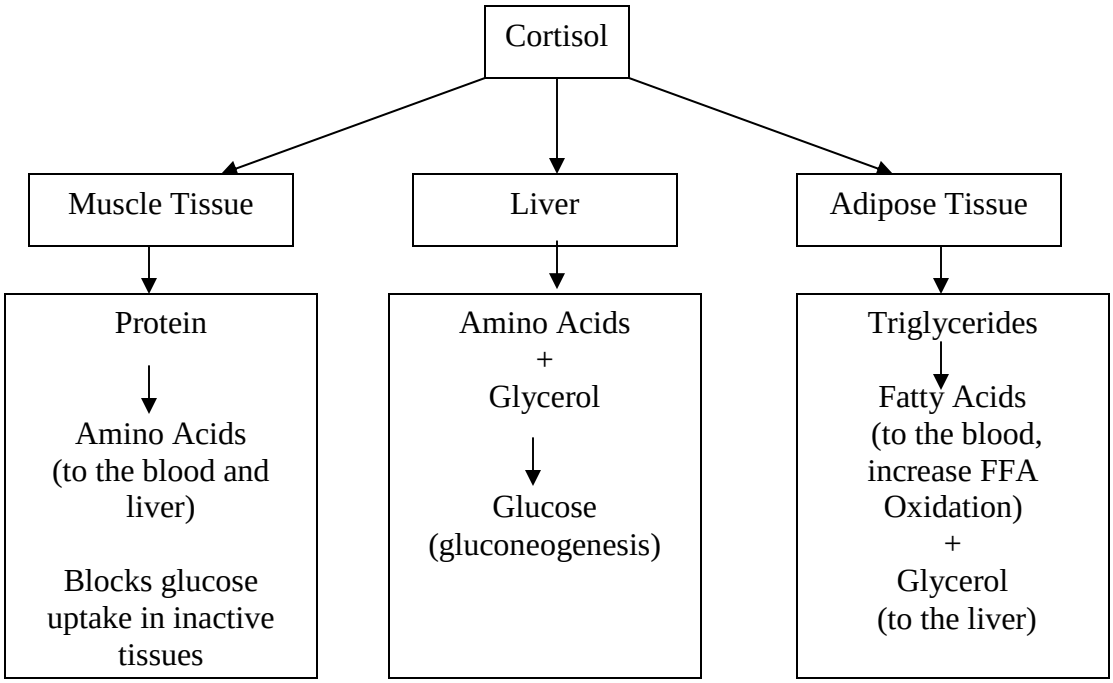

Figure 2. The effect of cortisol on muscle tissues, liver, and adipose tissue during exercise and recovery.

The lipolysis stimulation is done in an effort to decrease the use of glycogen and make lipids a greater source of energy for the muscles. At the same time that is happening, cortisol blocks the muscles uptake of glucose, at inactive tissues, to keep more in the blood. This all occurs as exercise is being performed, and is the main function of cortisol at that time (Brooks et al., 2005). Gluconeogenesis also takes place during exercise. This glucose being put into the blood is more for maintaining glucose delivery to the brain and central nervous system than the working muscles. Once exercise has ended, cortisol continues to

operate by increasing the breakdown of muscle tissue. This frees amino acids into the blood which can then be used in the liver for gluconeogenesis, or for rebuilding of proteins, and muscle remodeling. The protein breakdown in the muscle is occurring at damaged proteins. It does not destroy strong proteins, only clears away those that are less useful for muscular function, so newer stronger muscle can be built. This function is what supposedly keeps cortisol levels elevated for up to 2 hours into recovery (Viru & Viru, 2004).

Cortisol levels in trained individuals are typically lower than those in their untrained counterparts. Trained individuals will also experience a more attenuated response by cortisol as intensity and duration of exercise increase when compared with an untrained individual. There are an increased number of receptors and a heightened sensitivity for the hormone (Brooks et al., 2005) meaning there is a need for less of the hormone. Furthermore, the exercise itself will be less of a stressor to the body.

B. Free Testosterone

1. Function

Testosterone is a steroid, produced primarily in the Leydig cells of the testes. Small amounts are also secreted by the zona reticularis and zona fascisulata in the adrenal gland. Like cortisol, testosterone is regulated by a negative feedback loop. Stimulation begins in the hypothalamus to the anterior pituitary to the testicles to eventual release in the blood. Testosterone follows a circadian rhythm, and has its highest concentrations in the blood overnight (McArdle, Katch, & Katch, 2001). Once in the blood, testosterone is carried by binding proteins, primarily sex hormone binding globulin (SHBG), any that is not bound to a carrier is qualified as free testosterone. This is the biologically active form that interacts with target tissues (Hackney, 1996).

The functions of testosterone can be qualified as either androgenic or anabolic (Figure 3). Androgenic functions include the development of secondary masculine sex characteristics and reproductive function. Anabolic activity is primarily related to the growth and development of tissue (Tortora & Derrickson, 2006).

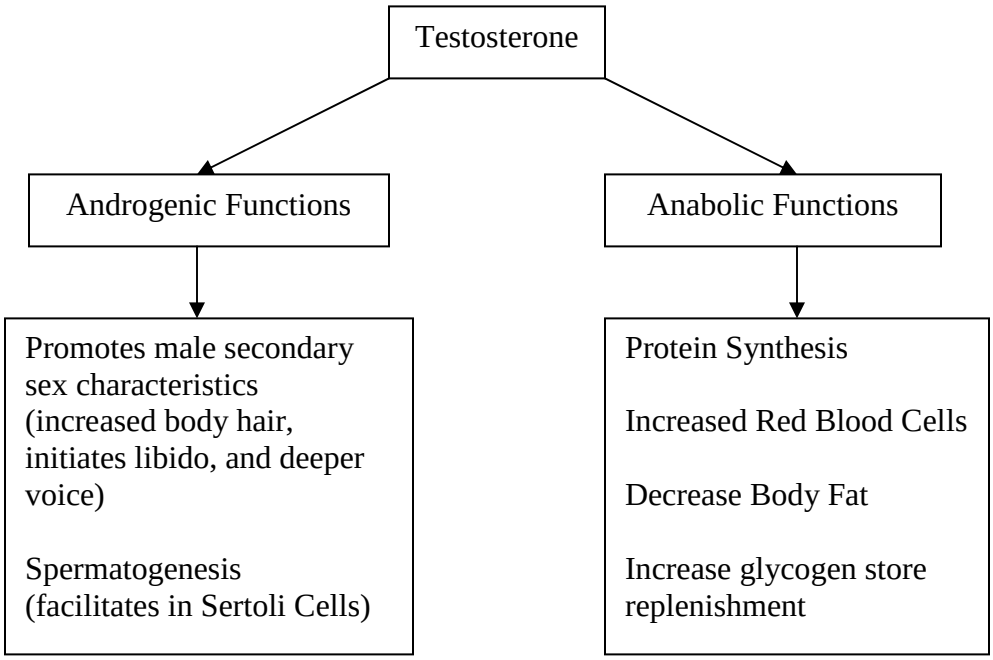

Figure 3. Anabolic and androgenic functions of testosterone (McArdle et al. 2001).

2. Dietary Impact

Anderson et al. (1986), analyzed the effects of two different diets on seven healthy men (age 22-43) with highly controlled isocaloric diets, of high-CHO (70% CHO, 10% protein), and high-protein (35% CHO, 44% protein) both for 10 days. They found that total testosterone concentrations (ng/dl) increased significantly (28%) during the high-CHO diet in comparison to the high-protein diet. Sex hormone binding globulin also increased and was 39% higher, on average, during the high-CHO diet. This later change would impact on free testosterone level (i.e., likely decreases).

Tegelman et al. (1986) had ten women and six men eat a decreased caloric diet for 3 days, then 7 days with only 50g CHO (fasting), followed by 3 days back at their normal diet. Venous blood was drawn immediately before and after the fasting period, as well as one week after fasting. During the fast, they found a significant increase in sex hormone binding globulin (SHBG), which could explain the decrease in the free testosterone percentage found. The total testosterone levels remained unchanged; leading the authors to believe there was a reduction in gonadal production. They were, however, unable to identify a mechanism for the increased levels of SHBG.

3. Exercise Response

The testosterone response to exercise is dependant on intensity and duration of the exercise. During short term maximal exercise, there will be an increase in testosterone, due probably to changes in hemoconcentration, or a decreased metabolic clearance rate (MCR) with increased production. With submaximal exercise at low intensities, there will be a very minor response; however, at workloads $\geq$ 60% of one's maximal oxygen consumption, testosterone will respond more robustly (Brooks et al., 2005). At a moderate intensity lasting 45-90 minutes testosterone levels will progressively increase. When this exercise is continued beyond 90 minutes research has shown no change or very small decreases. Finally, with moderate exercise to exhaustion or for longer than 2 hours, testosterone will initially rise, but decreased circulating levels are found upon completion of the activity (Hackney, 1996). The mechanisms for the various responses with submaximal exercise for extended durations are not clearly understood, it is assumed that no individual factor is responsible. Multiple factors that could be involved include: hemoconcentration, a decrease in production and secretion, which could be due to decreased testicular blood flow, a

decrease in hepatic blood flow which could lead to diminished hepatic clearance. Any of these may contribute to increased testosterone levels. These elevated levels could be reduced by uptake of testosterone by skeletal muscle (Hackney, 1996). Galbo (1983) suggested the decrease in serum testosterone following prolonged exercise could be due to a decreased secretion rate because of a diminished testicular blood flow, elevated body temperatures, or an increased concentration of prolactin in the blood (prolactin suppress gonadal function).

Testosterone is used in the muscle, as it aids in protein synthesis. Its primary anabolic effect is enhancing protein synthesis. Testosterone also may increase the ability of the muscle to refill its glycogen stores after reduction or depletion during exercise. This is done through the increased activity of the muscle glycogen synthetase (Urhausen et al., 1995).

The levels of testosterone are generally found to be lower at a resting state in trained individuals in comparison to untrained individuals. Hackney (1996) found through serial blood sampling that trained subjects had only 40-80% of the levels of their matched untrained counterparts. Also, as one becomes more trained, the response to exercise will decrease (Brooks et al., 2005). The mechanisms responsible for the decrease with training could include alterations in the hormonal production rates, MCRs, and binding protein concentration (Hackney, 1996).

C. Free Testosterone to Cortisol (fTC) Ratio

1. Function/Purpose

In 1986, Adlercreutz et al. executed and analyzed four biochemical (biomarker) tests: plasma testosterone to cortisol ratio, plasma free testosterone to cortisol ratio (fTC ratio), serum sex hormone binding globulin (SHBG) and growth hormone, and saliva testosterone to

cortisol ratio. The protocol consisted of two subject groups, no size given, and 2 weeks of training. One week of normal training, a second week of "very intense" training, no additional details. Upon completion the subjects were categorized into three groups, non-overstrain, overstrain (defined by a decrease in the fTC ratio of >30% or $<0.35x10^{-3}$) and uncertain. The fTC ratio was negative for all of the subjects with no signs of overstrain, positive in all but one classified as overstrain, and all uncertain. All of the other tests provided false positives, especially the saliva testosterone to cortisol ratio. From this, they suggested using plasma fTC ratio as a training biomarker; they also suggested using SHBG for interpretation, because diet may considerably influence values of the ratio alone.

Daly, Seegers, Rubin, Dobridge, and Hackney (2005) researched the possibility of a negative relationship between testosterone and stress hormones. They had 22 trained males perform a max test, return to the laboratory at a later date in a rested and fasted state, rest for 30 minutes in a supine position, placed a catheter in an antecubital vein, and drew a resting blood sample. The subjects then completed a run to volitional fatigue at 100% of their ventilatory threshold (±3%). Another blood sample was taken at volitional fatigue, as well as at 30, 60, and 90 minutes into recovery. Finally, the subjects returned for a resting sample 24 hours after the initial sample. They found that cortisol can lead to a physiological reduction of circulating testosterone. The potential mechanism was considered a direct inhibition of steroidogenesis at the Leydig Cell, and/or a central or peripheral disruption of the hypothalamic-pituitary-gonadal axis.

Brownlee, Moore, and Hackney (2005) followed up Daly's research by looking at the relationship between cortisol and testosterone (total and free) in men at rest and in recovery. They had 45 healthy, active males, 10 hours fasted, give a resting blood sample

(venipuncture technique), then exercise (run, row, or cycle) at 65-75% of VO_{2max} for 60-90 minutes, and another blood sample was taken 1 hour into recovery. After 48-96 hours, the subjects would return to the laboratory and repeat the process. They found a negative relationship between cortisol and total testosterone in recovery, but not at rest, suggesting a cortisol threshold must be met before it can influence testosterone. There was also a positive relationship between cortisol and free testosterone in the recovery samples. The mechanism for this relationship is also unclear. They suggest it could be several factors. It could be the result of increased production from the adrenals. The affinity of binding hormones may also have been reduced, leaving a greater amount unbound, or free. Finally, cortisol and testosterone are structurally similar and can bind to the same carrier proteins. With an elevated level of cortisol, it is possible that the competition for carriers left a greater amount of testosterone in a free state.

Hormones are a valuable tool in assessing how the body is responding to various activities; due to the responsibility they have in mobilizing systems to maintain homeostasis. The hormones of cortisol and testosterone both respond to training, and their individual functions, and relationship to each other have been previously identified. The fTC ratio may be able to inform through analysis how the body is responding to training levels, and possibly identify the beginnings of maladaptations (Viru & Viru, 1999).

2. Implications in Training

Athletes train at high intensities for great volumes in an effort to induce an adaptation. Kuipers et al. (1998), suggested an inverted U-shaped relationship between training volume and performance. A balance is necessary for positive adaptations to occur, and the identification of a hormonal marker indicating when enough recovery has not taken

place would be exceedingly beneficial, since a decrement in performance of only 3% can be the difference between winning and finishing last.

The free testosterone to cortisol (fTC) ratio, in theory, provides a snapshot of the anabolic, or catabolic state of an individual, because of the anabolic properties of testosterone and the catabolic tendencies of cortisol. This ratio has been suggested as an indicator of training adaptation, or when training has exceeded the amount to effectively induce adaptation (Urhausen et al., 1995). His research revealed a multitude of studies that could not agree on a consistent analysis as to the efficacy of the fTC ratio as a useful biomarker for training.

Lehmann's model (Figure 4) represents potential mechanisms for the development of overtraining. Some research has shown that the fTC ratio may be a reliable indicator of an imbalance between training/load and recovery time (Hug, Mullins, Vogt, Ventura, & Hoppeler, 2003; Banfi & Dolci, 2006; Flynn et al., 1994; Lac & Maso, 2004; Vervoorn et al., 1991). The ability to identify a training imbalance with a biomarker like fTC ratio could be beneficial in preventing progression of a catabolic state to a detrimental point, and athletic performance decline.

Specifcally, in 2004, Lac and Maso, analyzed various possible biomarkers for monitoring an individual's training progress. Hormonal levels were considered key measurements, since they are metabolic regulators. One biomarker they looked at closely was the fTC ratio. As noted earlier, cortisol stimulates numerous effects on the body including gluconeogenesis. This can result in a catabolic effect because it is utilizing amino acids for activities other than protein synthesis. Cortisol increases with heavy exercise and

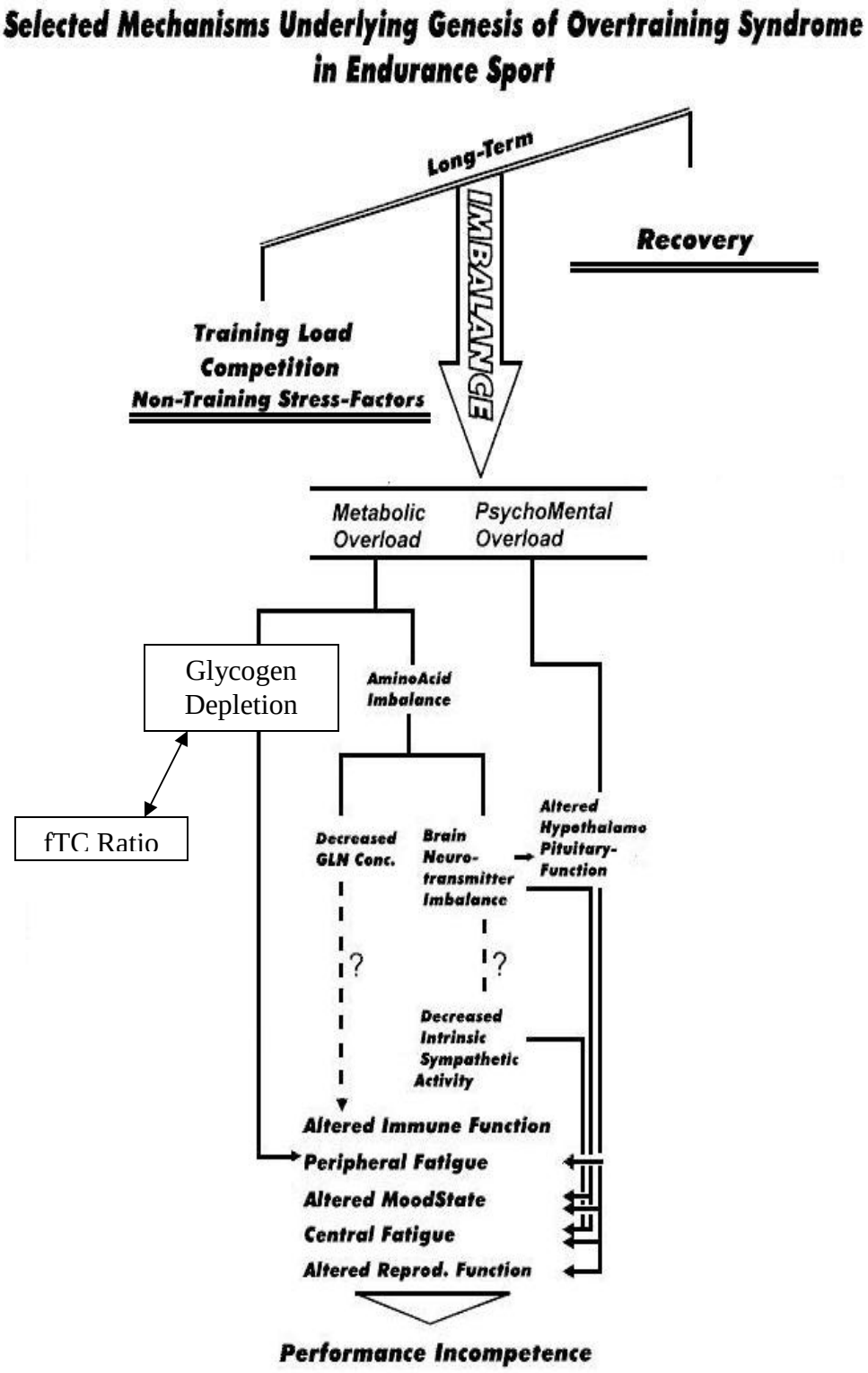

Figure 4. Selected potential mechanisms in the development of the overtraining syndrome in endurance athletes (adapted from Lehmann et al., 1993).

can be chronically elevated with heavy training loads. This can lead to positive adaptations, but eventually the negative impacts can override the positive with excessive protein catabolism. Testosterone levels are also affected with training. Testosterone exerts a positive effect on restocking glycogen stores and on muscle protein synthesis following long exercise. Intense endurance training has been shown to cause a decrease in testosterone (see earlier discussion). Therefore, these researchers suggested monitoring the ratio between free testosterone and cortisol may be beneficial for monitoring a training regimen.

In 1994, Flynn et al., examined the hormonal responses to changes in training loads. They looked at eight cross country runners, and five swimmers throughout a collegiate season. Venous blood samples were taken at four times, at the beginning of the season, the middle, then pre-taper and post-taper, based around the conference championships. The samples were collected after fixed exercise at intensities of 75% and 90% of preseason VO_{2max} for the swimmers, respectively. The training did not alter cortisol, testosterone, or free testosterone significantly from the beginning to the end of the season. There was a significant decrease in testosterone during the increased training segments in swimmers. Even with a significant increase in the training workload, there was no significant alteration in this ratio. The researchers questioned the usefulness of the fTC ratio in its ability to monitor training programs.

III. Exercise

A. Dietary Influence

Costa, Jones, Lamb, Coleman, and Williams (2005), tested the effects of a high-CHO diet (12 g/kg bw/day) compared to a self-selected diet on 32 healthy male triathletes. Each subject was randomly assigned to one of the diet groups and instructed to maintain normal

training throughout the study. In addition, they each ran for 1 hour at 70% of VO_{2max}. The subjects followed this training for 6 days, only coming into the laboratory on days 1, 4, and 6 to provide a saliva sample before and after their 1 hour run. At the end of the study, the high-CHO group had significantly lower cortisol levels, and higher glucose levels than the self-selected group. They concluded that with sufficient consumption of CHO, the body can maintain adequate blood glucose levels and prevent substantially elevated cortisol levels during exercise.

In 1988, Costill et al., examined the effect of increased training volume on glycogen content and how it affected performance with self-selected diets. Subjects consisted of 12 male collegiate swimmers who had completed six months of intense training prior to the study. The training volume was ultimately doubled form normal training (4,266 to 8,970 m) for 10 days at 94% of VO_{2max}, with subjects maintaining diet records on the first and last 2 days of training (days 1, 2, 9, and 10). Muscle biopsies were also taken from the deltoids of each subject to determine glycogen content. Four of the subjects were unable to complete the exercise at the appropriate intensity. These subjects were found to have a lower intake of total calories as well as a decreased CHO component in their diet. They determined that inadequate CHO consumption (5.3 g/kg/day) in the diet can result in decreased glycogen content during intense training, and may ultimately inhibit performance. The other eight swimmers had sufficient calories and CHO (8.2 g/kg/day) and were able to maintain the desired intensity throughout all 10 days.

Galbo et al. (1979) studied the effect of high versus low-CHO diets on hormonal response to prolonged exercise. Seven healthy males ran on a treadmill at 70% of VO_{2max} for 30 minute bouts with 10 minutes of rest between until exhaustion, after 4 days of high

(77% CHO) and low (10.5% CHO) CHO liquid diets. Blood samples were taken at the end of each session, and muscle biopsies of the vastus lateralis were obtained immediately before exercise, and after the first and second run and at exhaustion for each diet. The glycogen content was significantly lower in the low-CHO subjects, and they reached exhaustion 40 minutes sooner than those subjects in the high-CHO group. Cortisol was also significantly higher in the low-CHO group at exhaustion. They concluded that the hormonal response to exercise can be controlled to some degree by the diet preceding exercise. Further, Galbo concluded when insufficient CHO is consumed blood glucose levels will decline more rapidly, leading to a faster response from the stress hormones to restore homeostasis.

At rest, in the post absorptive state, fat oxidation is the primary source of energy for an individual. Even at low intensity exercise fat oxidation supplies the majority of energy; however, around 65% of VO_{2max} the rate of fat oxidation will decline, and carbohydrate oxidation will accelerate. In the later stages of prolonged exercise, glycogen stores will decrease and CHO oxidation may not be able to maintain the rate of energy production necessary. This is why Jeukendrup (2003) examined how a high-CHO diet would affect muscle glycogen content during prolonged, intense exercise. He had 8 well-trained cyclists, follow an 88% CHO diet and cycle for 2 hours per day at 70% VO_{2max} for 7 days. He found that fat oxidation had been reduced by 27%, and the muscle glycogen concentration of the subjects increased to twice the normal resting level (824 mmol/kg of dry mass). This suggested that even with intense exercise, glycogen stores can be maintained with appropriate amounts of CHO in the diet.

In an effort to see CHO consumption affected the occurrence of overtraining in subjects during increased training, Snyder et al. (1995) had eight competitive male cyclists

execute three training periods in an effort to induce overtraining. The training included 7 days of normal training (12.5 hr/wk), 15 days of overtraining (18 hr/wk), and 6 days of recovery training (7.5 hr/wk). Diets were assessed once at the end of both the normal and overtraining periods, where the subjects consumed 64.0 and 67.4% CHO. Also, each subject had been given 160 g of liquid CHO to ingest within 2 hours of exercise. All subjects indicated a state of overtraining had been reached, based on five psycho-physiological criteria used. However, none of the subjects had significantly different levels of muscle glycogen. Snyder concluded that low glycogen levels may be responsible for fatigue, but another mechanism must be responsible for overtraining state (i.e., Overtraining Syndrome).

Tsai et al. (1993) implemented a crossover design to see if short-term changes in diet would affect basal anabolic and catabolic hormone levels. They had six healthy individuals (two women, four men), complete three tests after three dietary conditions. The first was the control diet, what they normally ate (45% CHO), another was a low-CHO diet (15% CHO), and finally high-CHO (55% CHO). After three days of diet intervention, each subject came in and performed a cycle test at 80% VO_{2max} for 20 minutes, or until perceived exhaustion (RPE of 19 or 20). They found no changes in the circulating concentrations of the hormones. There was, however, a performance effect. After the control and low-CHO diets, only four and two subjects, respectively, completed the cycle test. While the diet intervention may not have been enough to affect the hormones, they suggested the decrease in CHO consumption was enough to limit the exercise capacity of the subjects.

B. fTC Ratio Response

1. Short-Term Studies

Filaire et al. (2002) looked at the effect of intensive training on certain factors including cortisol and testosterone, and how long any changes in these factors remained during recovery. They had 12 national level road cyclists participate in a 4 day training camp that consisted of exercise for 4 hours a day. Salivary samples were obtained at 8:00 a.m. each morning of the training camp, and for the three mornings after. There was a significant increase in cortisol during the most intense training day, coupled with a significant decrease in testosterone, and a significant decrease in the fTC ratio. They noted that only peripheral neuro-endocrine markers were affected, and that performance was unchanged, suggesting that it is important with individuals who already have high workloads to monitor hormonal parameters during recovery to monitor training adaptations.

Kokalas, Tsalis, Tsigilis, and Mougios (2004) examined the effect of three different training programs on anabolic and catabolic hormones in rowers. They had six healthy male rowers, participate in a counterbalanced study; in which they perform each of three types of training on successive days. The training included endurance, intervals, and resistance work. Blood samples were taken at 8:00 a.m., 9:00 a.m., and 1:00 p.m. the day before training began to serve as baseline, resting levels. Blood samples were drawn at the same time each day of training, which corresponded with immediately before, after and 4 hours after exercise training. Cortisol and testosterone were only significantly altered during the endurance training; both increased, and testosterone had returned to below resting levels within 4 hours, while cortisol had not. They suggested monitoring hormones as a means of tracking training to confirm the desired adaptations are occurring with endurance training.

Vaananen, Vasankari, Mantysaari, and Vihko (2004) chose to examine the effect of consecutive days of cross-country skiing on hormonal responses within nine healthy males. Each subject skiied 50 km on consecutive days beginning at 11:00 a.m. Antecubital vein blood samples were obtained prior to the exercise (9:45-10:30 a.m.), after exercise (1:30-3:00 p.m.) and 2 weeks after day 2 (2:00 p.m.). Testosterone and cortisol both showed significant changes, with testosterone decreasing by 20% after day 2, and cortisol increasing 2.2 and 2.6 fold, day 1 and 2, respectively. Both hormones had returned to resting levels 2 weeks later. The researchers concluded that with consecutive days of intense exercise, an individual should be aware of the potential for hormonal overreaching.

2. Long-Term Studies

Soccer is not an endurance sport, but Banfi and Dolci (2006) used it to track the training of 32 professional soccer players over two seasons. They collected blood samples at four times during the first season, and 5 times in the second. The samples were collected 1) at the start of training, following 2 months of no competition, 2) at the start of initial competitions, 3) at the start of the second competitive portion of the season, and 4) at the end of the second competition portion. The additional measure ((5) the second season) included the start of the training the next season. No significant changes in the fTC ratio were found, however, there were trends in the data with the ratio decreasing during the competitive portions. The researchers concluded that the fTC ratio is a viable marker, and can be applied in soccer even though it has a generally lower risk of overtraining than endurance sports. The ratio was still thought to be helpful in identifying an imbalance between training and recovery.

Hug et al. (2003) tested 11 endurance-trained cyclists at the end of their competitive season in a 6 week program to evaluate the role of hormones in training and overtraining. These subjects, in addition to 80% of their normal training habits, three times a week, cycled for 30 minutes under normoxic (560 m elevation) or simulated hypoxic (3200 m elevation) conditions. Blood samples were taken from an antecubital vein before the training period and at the end of each training week before breakfast. No significant changes were found in cortisol, testosterone, or the fTC ratio. Nonetheless, the authors still suggested, the ratio may be beneficial in monitoring the balance between anabolic and catabolic activity during training.

In 2006, Purge, Jurimae, & Jurimae, tested 11 elite male rowers seven times over a 24 week preparatory period prior to competition. A blood sample was obtained through an antecubital vein at the beginning of the preparatory period, and every 4 weeks during the 24-week period. Weekly training consisted of 90% prolonged low-intensity exercise (swim, bike, run, row) with some strength at about 50% of 1-RM doing 50-100 repetitions. The other 10% was resistance training at approximately 75% of 1-RM of 8-12 repetitions for four sets. Both basal cortisol and testosterone were significantly related to the mean training volume, and testosterone was significantly higher during weeks 4, 8, and 20. This suggested a high level of anabolism was occurring, with elevated levels of cortisol implying that sufficient proteolysis was providing the amino acids necessary for muscle growth. The researchers also concluded that cortisol and testosterone were more sensitive to changes in training volume than growth hormone, making them the more specific hormone to monitor for training adaptations.

Vervoorn et al. (1991) used six national level rowers to observe the fTC ratio over a span of 9 months (December to August). Blood samples were collected from an antecubital vein between 8:00 and 10:00 a.m. at rest every 5 weeks. In addition to those samples, 2 weeks of training camp exposure resulted in three more samples every 4 days. There were no significant changes in the fTC ratio, nor was there even a trend toward a change. There was, however, a great deal of variation they attributed to seasonal hormonal changes. The researchers suggested that at a decrease greater than 30% the fTC is not an indication of overtraining, but an identification that there is an imbalance between training loads and recovery time. Such an imbalance can lead to an increased risk of overtraining according to Lehmann et al. (1993).

IV. Summary

It has been established that cortisol and testosterone are both affected by exercise, exercise training, and diet. Cortisol has catabolic characteristics that if left unbalanced could be detrimental to performance. Testosterone promotes anabolic properties that are critical to the stability of the muscular system as well as other tissues. Any imbalance between these two hormones could be a sign of negative progress, or potential danger with a training program relative to the balance between training load and recovery time.

The fTC ratio appears to be a legitimate measure of that balance. However, because both hormones are affected by diet, that component must be considered when utilizing this ratio. The dietary studies discussed above suggest that the components of one's diet affects glycogen availability, alters stress hormones, and ultimately could decrease the ability to maintain performance.

The exercise studies examined here-in, showed elevated cortisol and decreased testosterone levels with high intensity training. The majority of these studies were long-term with no dietary component manipulation. Even the few short-term studies identified showed similar trends. However, the limited number of short-term training studies are not conclusive suggesting further work is warranted. Identifying a tool that can reveal an imbalance in training and recovery would be very beneficial, but that tool can only be properly analyzed if the changes are actually due to the training volume, and not an unbalanced diet. Evidence shows that research needs to be done to determine if the CHO consumption in one's diet is affecting the ratio, or if it is indeed a response to the exercise alone.

CHAPTER III

METHODOLOGY

Methods

The subjects in this study visited the laboratory on five separate occasions. On the initial or orientation visit, the subjects signed the informed consent, underwent a medical and physical exam to confirm they were able participate, and executed a peak oxygen uptake (VO_{2peak}) test. The next three visits were the actual testing sessions where they performed 60 minutes of cycling at approximately 75% of their VO_{2peak}. Blood samples (3 ml) were taken immediately before and after each training session. On the final visit, no exercise took place. The subject returned 24 hours after the completion of the previous day's training and a resting blood sample was taken. All blood samples were analyzed for free testosterone and cortisol. Those measures were used to determine the fTC ratio.

Subjects

The subjects consisted of 20 males between the ages of 18 and 45. Each was moderately to highly trained in endurance sports. Each potential subject was informed about the possible risks and experimental protocol. An informed consent for each individual was signed before participation began. To be included in this study, the individuals had to train at least 5 days per week for a minimum of 60 minutes. They were also involved in competitive endurance sporting events. History of an infection in the six week period prior to beginning the study, or a history of current or chronic medical condition or musculoskeletal injury

would have prevented inclusion in this study. The use of any medication, including non-steroidal anti-inflammatory drugs (NSAIDs) would also have resulted in exclusion from the study.

Physical restrictions during the study were as follows: 1) refrain from strenuous activity 24 hours before the VO_{2peak} test, 2) minimal physical activity (defined as a low intensity or easy workout) two days before the training sessions began, 3) no activity the day before training sessions, and 4) during the training sessions subjects were to refrain from any additional activity outside of the study. The subjects also agreed to refrain from eating, smoking, and drinking alcohol and/or caffeine in the eight hours before the training sessions. The subjects followed a randomly assigned diet of low CHO or control CHO. The control CHO group consumed a carbohydrate supplement, Polycose®, on the day before the first training session and each subsequent day of training. The low CHO group was given instruction regarding maintenance of approximately 40% CHO consumption. They were given three 237 ml bottles of Boost High Protein® to drink each day of the low-CHO diet. The control-CHO group data were archived from collection in a previous research study (Duke, 2008). Both groups maintained daily records of all foods consumed each day of the study from the day prior to the first training session through the final visit to the laboratory. Compliance was checked by monitoring resting RER and blood glucose levels.

Instrumentation

The subject's characteristics were measured using a portable stadiometer (Perspectives Enterprises, Portage, MI) for height and a mechanical scale (Detecto, Webb City, MO) for body mass. Body fat was measured by one technician through select skinfolds with Lange skinfold calipers (Beta Technology, Inc., Santa Cruz, CA). The respiratory gases

obtained during the VO_{2peak} were measured with the Parvo Medics TrueMax 2400 Metabolic System (Parvo Medics, Salt Lake City, UT). The incremental test to determine VO_{2peak} was performed on a Lode electronically braked cycle ergometer (Lode, Groningen, The Netherlands). Heart rate and ratings of perceived exertion were determined using a Polar telemetry system (Polar Electro, Inc., Lake Success, NY) and Borg's original 6-20 rate of perceived exercise scale (RPE). Nutritional analysis was determined using Nutritionist Pro (Version 3.2.0, Stafford, TX) software.

Hematocrit and hemoglobin were both measured from whole blood. The Adams MHCT II microhematocrit centrifuge (Becton Dickinson, Franklin Lakes, NJ) was used to separate cells from plasma, and an International Microcapillary Reader (International Equipment Company, Needham Heights, MA) was used to determine the hematocrit measures. A STATSite® M^{Hgb} Hemoglobin Meter (Stanbio Laboratory, Boerne, TX) was used to determine hemoglobin. The plasma was used in radioimmunoassay hormonal analysis once all subjects completed the protocol to determine free testosterone and cortisol levels. Once the whole blood parameters were obtained, the whole blood was placed in an IEC Centra-8R refrigerated centrifuge (International Equipment Company, Needham Heights, MA). The centrifuge was spun at 3,000 rpm to separate the plasma. The plasma was then transferred to micro-storage tubes where it was stored in a sub-zero freezer at -78 degrees Celsius until the experimental protocol was completed.

Protocol

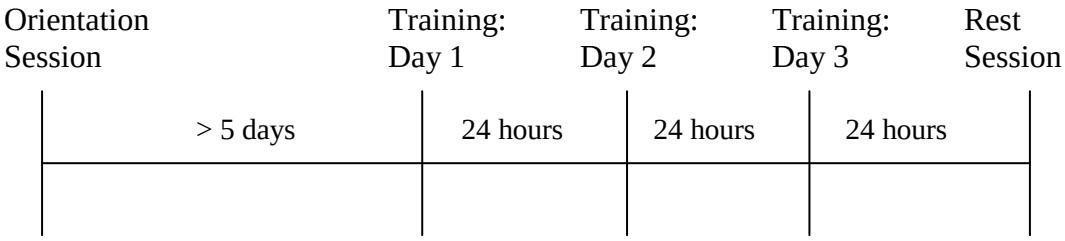

Figure 5. Overview of experimental training protocol (adapted from Behr, 2007).

Orientation Session. The initial session took place at the Applied Physiology Laboratory where the subject arrived a minimum of two hours post-prandial. The subject was informed about all potential risks involved with the study and the protocol was thoroughly explained. Once all questions were answered, the subject signed the informed consent and underwent a medical and physical screening to confirm the subject was healthy and could participate. The medical screening included a 12-lead electrocardiogram to identify cardiac abnormalities. After clearance, the subject's height, body mass, age, and body fat percent, were determined.

The subject was then prepared for the peak oxygen uptake (VO_{2peak}) test. The test was an incremental cycle ergometer test to volitional fatigue. The cycle was set for the comfort of the subject at both the seat and handlebars, and the marks were recorded for later use. The subject then had a ten minute warm-up. The first 5 minutes consisted of cycling at a workload of 50 watts (W), and the second 5 minutes were for the subject to stretch with a focus on his lower body. Following the warm-up, the subject was seated on the cycle and oxygen uptake (VO_2) was measured for four minutes to ensure the subject had followed the dietary guidelines. To confirm the subject had complied, RER could not be greater than 0.83 at rest.

Once the resting VO_2 measure had been completed the actual protocol of the test began. The incremental stages were 3 minutes in duration for the first four stages. The subject began pedaling at 50W and that workload increased by another 50W with each additional stage. After the fourth stage, incremental increases were decreased to 25W and the duration became 1 minute per stage. This continued until the subject reached volitional fatigue, and could no longer continue (MacDougall, Wenger, & Green, 1991). Oxygen uptake was monitored the entire time, while HR and RPE were collected at the end of each stage. The subject chose an active or passive recovery, but was not permitted to leave the laboratory until his HR had returned below 100 beats per minute (bpm). Determination of whether the test was a true peak was based on several criteria. The test was considered legitimate if the subject's respiratory exchange ratio (RER) was greater than or equal to 1.1, HR was within 5% of age-predicted maximal heart rate, and RPE was 18 or higher (MacDougall et al., 1991). If a subject was unable to complete a true peak test, they were re-tested a week later. This session was used in determining the workload for the subject during the training sessions.

Before the subject left, he was randomly assigned to either the control or low CHO group. He then received instruction dependent on grouping. The control CHO group participants received a canister of Polycose® glucose polymer (Ross Laboratory, Columbus, OH). They were instructed to consume 25% of the canister on the day before their first training session as well as 25% each day of training. They returned the empty canister at the final session. The low CHO group participants were given direction on how to limit their carbohydrate consumption to 40% of their caloric intake. They were also given Boost High Protein® as a supplement, and consumed three 237 ml bottles beginning the day before the

training sessions and each day of training. Both groups were instructed to maintain a daily record of all foods and beverages consumed from the day before their first training session through their final training session, and brought the log to the final session (Simonsen et al., 2000). During this period, the subjects were contacted and reminded of the importance of keeping accurate and complete logs.

Training Sessions. The training sessions consisted of three consecutive visits to the Applied Physiology Laboratory where the subject cycled for 60 minutes at 75% of his VO_{2peak}. A linear regression equation was obtained by plotting the oxygen consumption versus each corresponding workload from the VO_{2peak} test. This equation was used to calculate the workload for these training sessions (MacDougall et al., 1991).

The subject returned to the laboratory a minimum of 5 days and a maximum of 4 weeks, after the orientation session, after at least an 8 hour overnight fast. The training sessions were made at the same time, between 6 a.m. and 10 a.m. for each session. Physical activity practices were to be altered according to the following guidelines: two days before training session I, an easy day of training was to be completed, and the day before the training session was a day of rest, no training. Each subject also maintained a food record for everything consumed on the day before and each day of the training sessions.

Once the subject arrived at the laboratory, he laid in a supine position for 10 minutes after which a 3 mL blood sample was drawn using the standard venipuncture technique. The blood went directly from the syringe into a sterile K^2EDTA Vaccutainer® tube, and was put on ice. The subject then began a 10 minute warm-up on the cycle ergometer. The first 5 minutes consisted of the subject pedaling at a workload of 50W. The second 5 minutes were reserved for the subject to stretch with a focus on the lower body. Following the warm-up,

the subject sat on the cycle ergometer, while VO_2 was measured for 4 minutes. The subject then cycled for 60 minutes at a workload eliciting approximately 75% of his VO_{2peak}. The intensity and duration of the training sessions were designed in an effort to represent a training session executed by an endurance athlete.

During the training sessions the subject was encouraged to drink water ad libitum. Heart rate and RPE were monitored continuously and recorded every 10 minutes, starting from minute 0. Oxygen uptake was also monitored periodically. It was recorded for 4 minutes at the end of every 15 minute interval (i.e. minutes 16-20, 36-40, 56-60).

Once the 60 minutes had been completed the subject remained seated on the cycle ergometer where another 3 mL blood sample was drawn following the same procedures as the resting draw. The subject selected a passive or active recovery and was not permitted to leave until his HR had returned below 100 bpm.

Rest Session. The subject's final visit to the Applied Physiology Laboratory occurred 24 hours after the completion of the third training session. After arriving at the lab, the subject laid in a supine position for 10 minutes. The final 3 mL resting blood draw was collected with the standard venipuncture technique, transferred directly from the syringe to a sterile K^2EDTA Vaccutainer® tube and put on ice. The subject returned the food record that had been maintained during the study, and the empty Polycose® canister, if in the control group.

Blood Procedures

Hematocrit. Resting and post-training hematocrit were determined in triplicate from whole blood samples. The samples from the whole blood were used in 75mm microhematocrit capillary tubes (Fisher Scientific International, Inc., Albany, NY), sealed

with Critoseal (Krakeler Scientific, Inc., Albany, NY) and were spun for 3 minutes in a microhematocrit centrifuge to separate the plasma from the separated particles. The tube was then placed on an International Microcapillary Reader to determine hematocrit levels.

Hemoglobin. Resting and post-training hemoglobin were determined in duplicate from whole blood samples using a STATSite® M^{Hgb} hemoglobin meter. Twenty microliters (µL) of whole blood was placed into meter, which is a reflectance photometer. It uses the azide-methemoglobin chemical reaction for color development on the test area and the wavelength of the illuminating light to determine the reading. This meter uses a Light Emitting Diode (LED) as its light source. All calculations are performed automatically as the STATSite® M^{Hgb} hemoglobin meter is a microprocessor-controlled device.

Plasma Volume Shift. It is expected that exercise resulted in a plasma volume shift. Dill and Costill's (1974) equation to determine the change in plasma volume with hematocrit and hemoglobin was used. Hormonal concentrations were examined to the degree of fluid shift effects seen within the plasma volume.

Hormonal Analysis. The plasma samples were analyzed with hormone specific radioimmunoassay procedures for both free testosterone and cortisol (Siemens Medical Diagnostics, Los Angeles, CA). The sensitivity for cortisol and free testosterone assays were 0.2 µg/dL and 0.15 pg/mL, respectively. The coefficient of variation was calculated for each and is reported in the results.

Data Analysis

All analysis were conducted with a statistical software package (SPSS version 15.0, Chicago, IL) and presented in mean ± standard deviation (SD). A 2x4 mixed model analysis of variance (ANOVA) was done to determine the effect of the training protocol on the fTC

ratio at rest. Between groups ANOVA was executed in analyzing the dietary protocols, and their effectiveness. Additional ANOVAs were executed to determine the differences between groups within VO_2, HR, RPE, and VO_{2peak} test results, with differing levels for these repeated measures variables. Any significant findings were investigated with Tukey post hoc tests to determine where the significance existed. The significance level was set at $\alpha \leq 0.05$.

CHAPTER IV

RESULTS

Subject Characteristics

Table 1 displays the physical characteristics for both groups, values are reported as mean ± standard deviation (SD). No significant differences were detected between the groups.

	Age (yr)	Height (cm)	Mass (kg)	Body Fat (%)	N
Control-CHO	27.08±5.84	179.43±6.79	75.00±7.29	12.07±3.33	12
Low-CHO	24.00±3.38	179.98±5.62	74.73±7.90	9.83±3.21	8

Table 1. Physical characteristics (mean±SD) of control-CHO and low-CHO groups.

Peak Test Data

There were no significant differences found between the two groups in peak oxygen consumption (VO_{2peak}), absolute or relative, maximal respiratory exchange ratio (RER), heart rate (HR), rating of perceived exertion (RPE), test duration, or maximal Watts output. The complete data set for the peak testing for both groups is found in Table 2.

	Control-CHO	Low-CHO
VO_{2peak} (L/min)	4.52±0.76	4.58±0.41
VO_{2peak} (ml/kg/min)	60.10±6.56	61.94±8.57
Max RER	1.19 ± 0.07	1.16 ± 0.06
Max HR (bpm)	188.41±8.84	191.00±8.92
Max RPE	19.29±0.72	19.25±0.70
Test Duration (Minutes)	18.91±2.50	17.68±1.62
Max Workload (Watts)	350.00±47.67	315.62±37.64

Table 2. VO_{2peak} test data (mean ± SD) for the control-CHO and low-CHO groups.

Dietary Analysis

The analysis of the diets indicated there was a significant difference ($p \leq 0.0001$) in the percentage of carbohydrate consumption between the groups. The control and low-CHO groups consumed 58.45 ± 4.91 and 31.92 ± 2.46 percent of their daily dietary intake in CHO, respectively. The low-CHO group consumed a lesser amount of CHO than was prescribed, providing a greater effect. The total mean calories (kcal) per day consumed during the study (4 days) by the control group was 3226.67 ± 389.78, and in the low-CHO group was 2804.12 ± 604.91. There was no significant difference found in the caloric intake between the groups.

Training Analysis

Oxygen Consumption (VO_2)

Training Session 1: The initial training session elicited a slightly greater VO_2 response from the control-CHO group at the 20, 40, and 60 minute points. However, these were not significantly greater than those of the low-CHO group. The control-CHO group's mean VO_2 decreased as the training session progressed. Conversely, the low-CHO group experienced an increase in the level of oxygen consumption from the beginning to the end of

the session; however, neither of these changes were significant. The control-CHO group worked at 73.44% of their VO_{2peak}, with the low-CHO group at 69.79% VO_{2peak}, these intensities were not significantly different. Table 3 identifies all VO_2 measures as mean ± SD for all training session values at the 20, 40, and 60 minute time point.

	Training 1		Training 2		Training 3	
Minute	Control-CHO	Low-CHO	Control-CHO	Low-CHO	Control-CHO	Low-CHO
20	3.36±0.59	3.19±0.61	3.36±0.55	3.19±0.50	3.28±0.56	3.13±0.56
40	3.32±0.51	3.16±0.46	3.36±0.46	3.15±0.32	3.28±0.51	3.16±0.36
60	3.28±0.46	3.24±0.41	3.28±0.48	3.39±0.36	3.23±0.50	3.26±0.32

Table 3. Mean (± SD) VO_2 data for subjects at 20 minute intervals during 3 training sessions.

Training Session 2: Training session 2 followed the same pattern as session 1, with no significant differences in VO_2 measures. The control-CHO group showed consecutive decreasing measures of VO_2, while the low-CHO group had a 40 minute measure lower than the 20, but at 60 minutes being greater than both previous measures (Table 3). The control-CHO and low-CHO groups worked at 73.74% and 70.81% of their VO_{2peak} values, respectively. These differences were not significantly different.

Training Session 3: The overall trend of the first two sessions continued through training session 3 within and between the groups. The control-CHO group had a decrease in VO_2 with each measurement time, and the low-CHO group showed increased measures with each time point (Table 2). The groups also trained at similar intensities, with the control-CHO group at 72.17%, and the low-CHO group at 69.50% of their VO_{2peak} values. There were no significant differences between the two groups in any of these measures.

Heart Rate (HR)

Training Session 1: Figure 6 illustrates the changes in the heart rates of each group during the session. All HR responses were substantially elevated by the exercise, but there were no significant differences in HR between the two groups during this session.

Training Session 2: Training session 2 resulted in higher heart rates for the low-CHO group in comparison to the control-CHO group, at each time point, however the differences were not significant. The control-CHO group experienced increasing HR throughout the exercise, but maintained an overall lower rate than the low-CHO group.

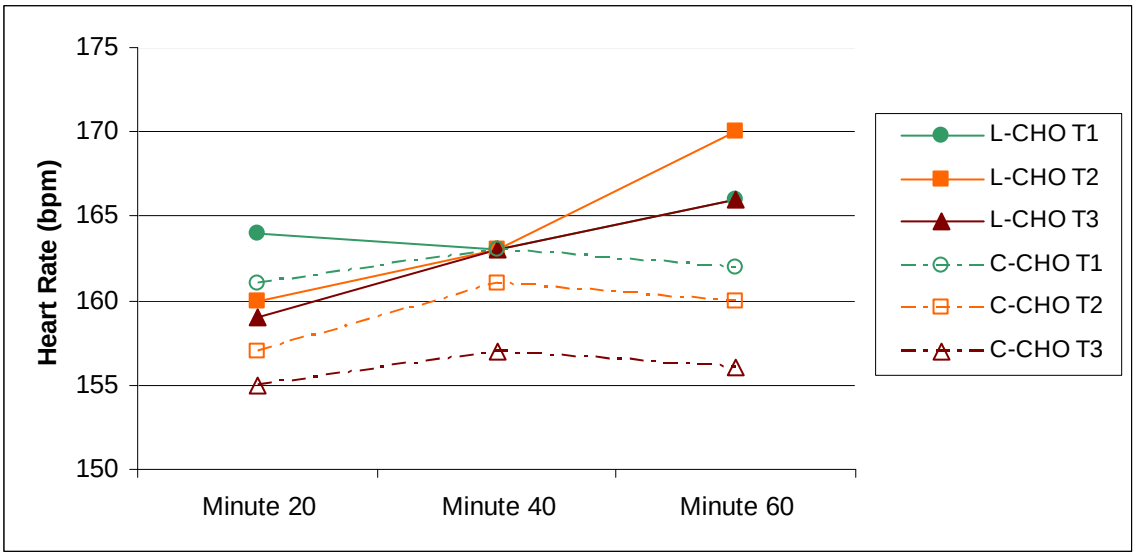

Figure 6. Mean HR values at 20, 40, 60 minutes of Training Sessions 1-3 for both groups.

Training Session 3: The same trend as in training sessions 1 and 2 was found in training session 3. The control-CHO group had lower HR (Figure 1) than the low-CHO group at each time point. These differences were not statistically significant, however, at the 60 minute point, this trend approached significance between the groups (p=0.099).

Rate of Perceived Exertion (RPE)

Training Session 1: Table 4 displays the differences in RPE responses between the training sessions and groups. Both groups had substantial elevations in RPE in response to

the exercise, and displayed gradual increases over time. The low-CHO group response, at 60 minutes, was significantly greater than the control-CHO group response ($p=0.036$). No other significant between group differences were found.

Training Session 2: There were no significant differences between the RPE levels of the groups during training session 2. The low-CHO group, however, tended to have higher measures at each time point, than the control group similar to session 1.

Training Session 3: The final training session elicited the same general responses as the previous sessions. The low-CHO group had slightly higher RPE values than the control-CHO group. There only significant difference between the groups was at the 60 minute response ($p= 0.010$).

	Training 1		Training 2		Training 3	
Minute	Control-CHO	Low-CHO	Control-CHO	Low-CHO	Control-CHO	Low-CHO
20	14.4±1.4	14.7±1.1	14.6±1.4	15.3±1.3	14.4±1.6	15.0±0.9
40	16.1±1.8	16.6±1.5	15.7±1.4	15.8±0.9	15.0±1.5	16.3±1.3
60	16.0±1.7*	17.6±1.1*	16.0±1.4	17.0±0.5	15.0±1.7*	17.0±1.1*

Table 4. RPE measures for control-CHO and low-CHO groups at 20, 40, 60 minutes for training sessions 1-3. * significant difference within respective groups ($p < 0.05$).

Hormonal Analysis

Cortisol

Training Session 1: During the initial training session, the cortisol (μg/dL) levels rose significantly in response to exercise in both of the groups ($p \leq 0.001$). However, there was no significant difference between the groups in the magnitude of the responses. All data, in mean ± SD, can be found in Table 5.

Training Session 2: A similar exercise induced effect was observed in session 2. Likewise, training session 2 also had no significant differences between the group responses.

Training Session 3: A significant difference (p=0.030) between the two groups was found at the resting level (Pre 3) in this session, with low-CHO having higher cortisol levels than the control-CHO group. The low-CHO group also had a significantly higher (p=0.037) levels than at training session 1 Pre measurements (i.e., Pre 1 vs. Pre 3). Again both groups increased slightly during the exercise session, however, this time the response was primarily seen in the control-CHO group.

Rest: The Rest session cortisol level was significantly greater in the low-CHO group than the control-CHO group. Also, the Rest in the low-CHO group was significantly greater (p=0.035) than the resting value in session 1 (Pre 1) for that group. Finally, the control-CHO group displayed no significant change in the Pre 1 to 3 versus the Rest cortisol values.

It should be noted that the coefficient variation for between and within assay replicates for the radioimmunoassay analysis was less than 10%. This supports the validity of these hormonal findings.

		Control-CHO	Low-CHO
Training 1	Pre	19.69 ± 6.11	24.07 ± 9.13
	Post	27.64 ± 5.79	29.16 ± 9.84
Training 2	Pre	19.59 ± 4.00	24.43 ± 7.09
	Post	25.51 ± 7.66	30.22 ± 7.05
Training 3	Pre	19.26 ± 5.09	27.62 ± 7.42*
	Post	22.47 ± 8.25	27.89 ± 6.75
Rest	Rest	19.40 ± 4.40	27.64 ± 7.91*

Table 5. . Mean (± SD) cortisol (µg/dL) data for each training session. * significant difference from Pre 1 ($p < 0.05$).

Free Testosterone

Training Session1: The complete testosterone data for the training sessions can be found in Table 6. There were no significant differences between the groups for testosterone during training session 1. Both the control and low-CHO groups had increases in response to exercise during the session, but these changes were non-significant.

Training Session 2: The responses to exercise in this session were similar to session 1. Also as in session 1, there were no significant differences between the groups during this training session.

Training Session 3: The resting (Pre 3) testosterone levels for training session 3 were lower for both the control-CHO (-5.66 pg/ml) and low-CHO (-2.75 pg/ml) groups from those observed in training session 1 Pre levels ($p > 0.05$). Slight increases were experienced for both groups during the training session due to the exercise. There were no significant differences, however, between the groups in the magnitude of the responses.

Rest Session: There were no significant differences between the two groups at the final resting measurement. The control-CHO experienced a final measure less than their own training session 1 Pre-level (-3.58 pg/ml), while the low-CHO group was substantially lower than their session 1 Pre measure (-7.93 pg/ml) (Table 6). These reductions in the hormonal levels did not reach statistical significance.

It should be noted that the coefficient variation for between and within assay replicates for the radioimmunoassay analysis was less than 10%. This supports the validity of these hormonal findings.

		Control-CHO	Low-CHO
Training 1	Pre	24.66 ± 15.20	21.96 ± 5.40
	Post	32.71 ± 10.49	25.11 ± 8.86
Training 2	Pre	22.75 ± 10.00	20.33 ± 3.83
	Post	34.40 ± 13.70	23.23 ± 9.26
Training 3	Pre	19.00 ± 8.52	19.21 ± 5.62
	Post	29.65 ± 11.30	22.08 ± 9.11
Rest	Rest	21.08 ± 7.86	14.03 ± 4.85

Table 6. Free testosterone (pg/ml) data for each training session.

Free Testosterone to Cortisol Ratio (fTC ratio)

Training Session 1: During training session 1, both the control-CHO group and the low-CHO group showed a significant decrease in the fTC ratio ($p=0.020$) following the exercise. There were, however, no significant between groups differences in the response. The complete data is presented as mean ± SD in Table 7.

Training Session 2: The control-CHO group experienced a small increase in the ratio (0.27 nm/L), while the low-CHO group experienced a minor decrease (0.10 nm/L) following the exercise in this session. These changes in response to the exercise were not, however, significant. Also, there were no significant differences between the groups.

Training Session 3: Increases in the ratio were experienced in both groups during the final training session. These increases were not significant and did not differ between the groups. However, the resting (Pre 3) levels for both groups was significantly lower ($p=.014$) from their same respective measure at training session 1 (Pre 1).

Rest Session: At the final experimental session, the fTC ratio of the control-CHO group rose slightly from the previous days resting (Pre 3) value, while the low-CHO group experienced a slight decrease, these changes were not significant.

When examining all the resting measurements alone (Pre 1-3, Rest), a significant main effect for time was observed ($p=0.008$) with the values decreasing from Pre 1 to Rest. However, close examination of the individual group responses revealed that in the low-CHO group the resting measures substantially decreased over time (Pre 1 versus Rest), while those of the control-CHO group only decreased slightly.

		Control-CHO	Low-CHO
Training 1	Pre	1.90 ± 1.98	1.53 ± 1.30
	Post	1.54 ± 0.58	1.27 ± 0.89
Training 2	Pre	1.54 ± 0.93	1.21 ± 0.67
	Post	1.81 ± 0.84	1.11 ± 0.78
Training 3	Pre	1.44 ± 1.20	1.04 ± 0.72
	Post	1.79 ± 0.69	1.16 ± 0.80
Rest	Rest	1.49 ± 0.91	0.82 ± 0.71

Table 7. Mean (± SD) free Testosterone to Cortisol ratio data for each session.

Due to the variance and differences observed between the groups at the training session 1 Pre measure and the tendency noted above for the low-CHO to display substantial reductions over time, an analysis of covariance (ANCOVA) was also conducted. The training session 1 Pre resting values of the respective groups were used in the ANCOVA as covariates. Figure 7 illustrates the results of the analysis. This alternative analysis revealed a significant interaction effect for the resting ratio changes between the groups ($p=0.022$). The low-CHO group experienced a decrease at every measurement, and the final Rest measure was significantly lower than their own training session Pre 1 value ($p<0.0001$). The change over time for the control-CHO was not significant. Furthermore, at the final measure comparison (Rest) the low-CHO values were significantly less than that of the control-CHO groups ($p=0.021$).

The effect size of the fTC ratio was calculated with the Cohen's d statistic for the significant difference in the low-CHO group ($d = 0.678$) from Pre 1 to Rest, and between the groups at Rest ($d = 0.821$). Any d value for effect size greater than 0.600 is considered a large effect size, confirming the significant differences found in the fTC ratio (Cohen, 1988).

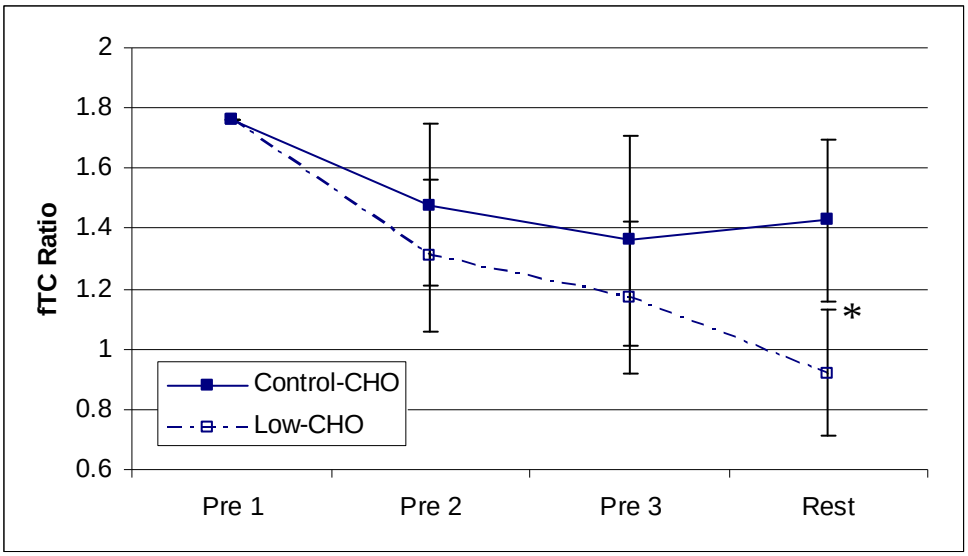

Figure 7. Plot of ANCOVA results for Pre measures of fTC ratio (mean ± SE) of both groups. * significant difference from Pre 1 within low-CHO group and between both groups ($p < 0.05$).

CHAPTER V

DISCUSSION

The purpose of this study was to examine the effect that CHO consumption has on the free testosterone to cortisol (fTC) ratio during an intense microcycle of exercise training. The research question being investigated was: Does the quantity of CHO consumed affect the amount of decrease in the fTC ratio response to such intensive training? The posed hypothesis was that the low-CHO diet would produce a greater reduction in the fTC ratio than a control-CHO diet.

This chapter will be organized into four sections. I will begin by discussing the treatments applied. Secondly, I will review the effect of the treatments on the resting fTC ratio. Followed by an explanation of why I believe these responses were seen, and finally, what kind of practical application the information from this study provides to the scientific community as well as individuals involved in endurance training activities.

Section I: Treatments

The dietary CHO content of the two groups were significantly different from one another, which was the dietary treatment desired. The low-CHO group consumed a diet significantly lower (31.92%) in carbohydrates than the control-CHO (58.45%) group. There was no significant difference in the total number of daily calories consumed between the groups, only the composition of the calories.

The low-CHO group consumed 23% less CHO during the study period, than in their normal diets which was significantly different. The total daily caloric consumption, however, was not significantly different within the subjects in each group for the before study and study periods. Therefore the differences between groups were of a significantly different dietary composition only and strongly indicating that the dietary treatment was effectively achieved.

The other treatment effect being pursued in this study was an intense microcycle of exercise training. The groups both worked near 70% of their peak oxygen consumption, specifically, the control-CHO and low-CHO groups were at 73.1% and 70.0% of VO_{2peak} over the three training sessions, respectively. The individual intensities selected for each subject was based upon their ventilatory threshold from the VO_{2peak} test results, which is why there was a slight variance between the groups in intensity. Both groups demonstrated high RPE values suggesting these intensities were highly demanding workloads. Their HR also demonstrated that both groups were working at strenuous levels; specific data is available in Figure 6 in Chapter 4. The measures every 20 minutes of each of these variables indicate that the subjects were performing a high intensity exercise bout, but at relative steady state responses (see Table 3, 4, Figure 6). There were no significant differences between the groups during the training sessions, except for RPE values at the 60 minute mark on sessions 1 and 3, when the control-CHO group was lower, suggesting perhaps those subjects were becoming more accustomed to the exercise. Overall it appears both groups worked at similar levels for each of their individual days of training, and experienced nearly the same level of physiological responses. It is interesting to note that following the completion of the study,

many of the subjects commented that the training intensity was very demanding and not something they would do on multiple days of their own personal training regimes.

Section II: Resting Free Testosterone to Cortisol (fTC) Ratio Response

The resting fTC ratio significantly decreased from the training session 1 Pre to the Rest session within the low-CHO group. There was no significant difference in the control-CHO group ratio throughout the training. This suggests that a 3-day microcycle of high intensity exercise can induce a decrease in this ratio if sufficient levels of CHO (~30% daily intake) consumption in the diet are not maintained. The control-CHO group demonstrated that by maintaining adequate levels of CHO (~60%) in their diet that even with difficult training on consecutive days, the fTC ratio can be maintained within a normal range. Lehmann et al. (1993) suggest maintaining the ratio is indicative of a healthy balance between training load and recovery which is critical to optimal training and adaptation in athletes. The reduced ratio response of the low-CHO group matches trends found by other researchers investigating this ratio in response to exercise training (Filaire et al., 2002; Vaananen et al., 2004; Banfi & Dolci, 2006; Costa et al., 2005). These studies include both short- and long-term training studies. However, these studies did not control for diet to the same extent as in the current study. The similarity of ratio response between the current and other studies provides confidence that these present responses were not random chance, but were a result of the experimental treatments.

Section III: Why the Decrease in the fTC Ratio at Rest

It has been suggested that in men, changes in the fTC ratio are driven by the action of free testosterone, and in women, cortisol is the more dominant hormone (Urhausen et al., 1995). This present study appears to follow suit as most of the change was observed in free

testosterone. Specifically, in the low-CHO group, both hormones changed from training session Pre 1 measure to the Rest measure 3 days later, but cortisol increased (14.8%) while free testosterone decreased (36.1%).

Why was resting cortisol elevated over the course of the study? Upon review of Figure 8, it appears the increases in cortisol in response to exercise during the first two training sessions were so great there was not enough time to recover between training sessions and the resting levels (Pre 3, Rest) were not able to return to a normal range.

Metabolically, the low-CHO diet in combination with the exercise would have prevented glycogen stores from being completely resynthesized between each training session. This has been shown in the classic work by Costill in the 1970s 1970s (reviewed in, Brooks et al., 2005). This potentially drove a faster and more robust response from the fuel mobilizing hormones, including cortisol (Galbo et al., 1983). Experimentally Galbo et al. (1979) found that with several days of a low-CHO diet, during exercise, the resulting compromised glycemia levels induced a much more rapid and robust response from cortisol than with a high-CHO diet. This cortisol effect is known to persist for some time into the recovery from exercise as much as 24 hours (Hackney, 2006).

It is important to know that cortisol is also known to have a suppressive effect on the testosterone steroidogenesis process. Therefore, with perpetually elevated levels of cortisol in response to exercise, and in the recovery from exercise, there would be a potential for inhibition on testosterone production (Cumming, Quigley, & Yen, 1983; Brownlee et al., 2005).

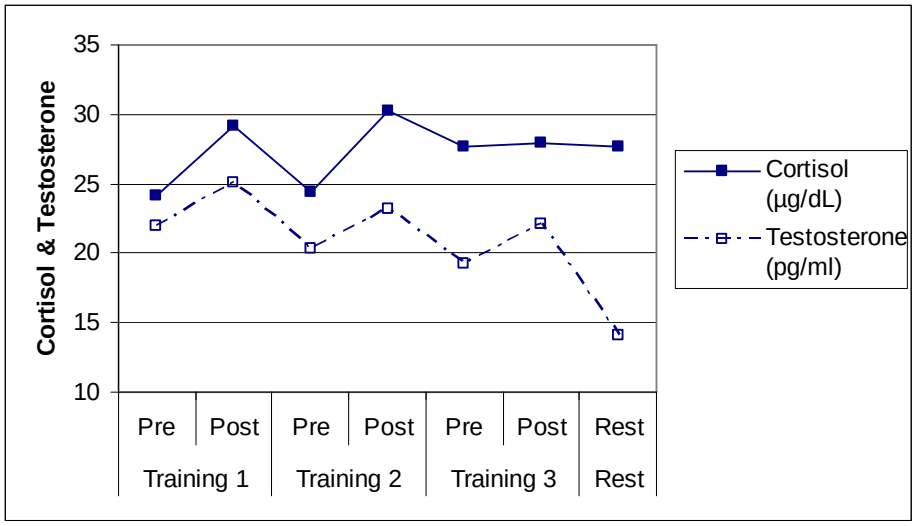

Figure 8. Mean cortisol and free testosterone response during the training sessions and rest in the low-CHO group.

In Figure 8 you see, the exercise during the training sessions caused testosterone to increase slightly relative to the resting (Pre) levels before each exercise session in the low-CHO group. These effects became mitigated over time and by the final training session; the exercise induced elevation barely brought the hormone levels back to the original resting value (Pre 1). This reduction in free testosterone was influential in the ratio being significantly suppressed. Cortisol, as a previously stated, inhibits testosterone production, and with the increase witnessed in that hormone (Figure 8), it was likely responsible, in part, for the depressed levels of testosterone in the low-CHO group. This is supported by the fact that the change in cortisol levels during the study (Pre 1 vs. Rest) and the reduction observed in the fTC ratio (from Pre 1 to Rest) show a highly negative correlation ($r = -0.763$, $p=0.0277$), even though there are only 8 subjects in the low-CHO group (i.e., ↑ cortisol →↓ fTC ratio).

Additionally, factors beside cortisol may have influenced the testosterone levels too. With the low-CHO diet, increased levels in the sex hormone binding globulins may have occurred (Anderson et al., 1987) reducing the bioavailability of free testosterone, since more

of it may have been in the bound form. With less of the hormone in the blood helping with protein synthesis and resynthesizing glycogen stores (Hackney, 1996), when exercise began, the stress response would potentially have been accelerated instead of attenuated, producing even more cortisol (Hackney, 2006). Thus, perpetuating the cycle of suppressed testosterone with elevated cortisol, and adding to a growing disparity with each training session.

Testosterone may also have been decreased as a result of a reduction in the pituitary gonadotropes (LH, FSH) as a result of the training sessions, which stimulate testosterone production. These hormones were not measured in this study. In men, longer training cycles are typically needed to cause reductions in these hormones, which has been established in previous published research (Hackney, 1996). Finally, testosterone may also have been suppressed by prolactin, another stress related hormone. In men, prolactin is known, (when at hyperprolactinemic levels) to effect of the gonadotropes at the testis, inhibiting testosterone production. These hyperprolactemic men have been shown to display significantly suppressed testosterone levels (Hackney, Dobridge, & Wilson, 2000). In an ancillary project in this current study, prolactin levels were examined in some of the subjects (data not reported here). While the levels of this hormone did increase, they were not at levels indicative of a hyperprolactinemic state. Therefore, prolactin may have been responsible for some of the decrease in testosterone levels, but was likely not a key factor in the overall suppression.

Section IV: Practical Application

This study has shown that endurance athletes need to be aware of the effect one's diet can have on their ability to maintain normal endocrine status during high level of training. With the high volume of training required to be competitive, it is imperative that glycogen

stores be replenished between each training bout. With more sufficient dietary CHO (~60%) intake, the subjects illustrated more of an adaptation hormonally rather than an imbalance, as has been seen in the literature previously (Jeukendrup, 2003). The current findings suggest when appropriately fueled, the body is capable of performing high intensity exercise for at least several consecutive days (i.e., three) without a negative hormonal response.

The fTC ratio has also been suggested as a biomarker for overreaching and overtraining in endurance activities. Adlercreutz et al. (1986) suggested that a decrease in the ratio greater than 30% indicates a state of overreaching-overtraining. In this study, with the low-CHO diet there was a decrease of 43% in the ratio, while the control-CHO group only experienced a 3% decrease, suggesting the low-CHO subjects may have been experiencing at least overreaching. Lehmann et al., suggest this represents an imbalance between training load and recovery. Such an imbalance places the athlete at great risk for developing the Overtraining Syndrome and ending their competitive season (Lehmann et al., 1993). It is not possible from the current data to determine if the low-CHO subjects were truly overreaching or overtraining as this diagnosis requires more medical evaluation than this study intended. But, if the fTC ratio is a valid biomarker for such training stress indications, then the current data suggest as little as 3 days of hard training without adequate carbohydrate can be potentially detrimental-risky to an athlete. The current findings strongly support that it is critical therefore, to control for diet if this ratio is going to be utilized for diagnostic or informative measures of overreaching or training balance status, otherwise the results could be misleading.

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