Influence of Exercise Training on the Free Testosterone to Cortisol Ratio

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ABSTRACT

Joseph W. Duke, Jr.: The Influence of Exercise Training on the Free Testosterone to Cortisol Ratio.
(Under the direction of Anthony C. Hackney, Ph.D)

The purposes of this investigation were to determine if the free testosterone to cortisol ratio (fTC) is a reliable marker that can be used to monitor an athlete's training and possibly diagnose overtraining and/or the Overtraining Syndrome. The investigator also wanted to see if a 3-day intense micro-cycle of training causes an athlete to become overtrained. Twelve highly trained male endurance athletes cycled for 60 minutes at 75% of their VO_{2peak} for 3 consecutive days. Blood samples were taken immediately pre and post-exercise and were analyzed for free testosterone and cortisol from which the fTC ratio was calculated. Results of this investigation suggest that the fTC ratio is a reliable marker as it did not significantly change during the training. Similarly, the results also suggest that 3 days of intense training may not be enough to cause an athlete to become overtrained.

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CHAPTER I

BASIS FOR STUDY

Introduction

Athletes must train extremely hard in order to reach their competitive potential. Each athlete must constantly push their body further and further in an attempt to get the most out of themselves. However, there is a very fine line between training hard and training too much. In order to provide a stimulus for an adaptation one must challenge the homeostatic balance within the athlete (i.e., overreaching). However, if the athlete pushes him or herself too hard and does not allow adequate regeneration time they can become overtrained. If adequate rest is still not allowed the athlete can enter something referred to as the Overtraining Syndrome (OTS), which is chronic overtraining. The Overtraining Syndrome can last for months and is not easily reversed (3, 6).

Overtraining and OTS are common fears of athletes and coaches alike. This fear is most likely due to its prevalence in sport and because the central symptom of overtraining is decreased performance (1). Overtraining and OTS may present in a variety of symptoms (1, 3). Fry et al. (3) has organized them into four categories: 1) alterations in physiological function and decreases in performance, 2) psychological symptoms, 3) immunological dysfunction, and 4) biochemical alterations. Overtraining can occur in all sports; however it is thought to occur most frequently in endurance sports because of the high volume and intensity of the training necessary to succeed (2). Studies

have shown that at any given time about 7-20% of elite athletes may show symptoms of overtraining (2) and it is likely that all athletes will enter a state of overtraining or OTS at some point in their career (1, 3). Unfortunately, despite its prevalence in sport, there lacks a determinative diagnostic tool (1).

There in lies a conundrum; how hard can an athlete push him or herself without developing OTS? Where is this "fine line" and how does an athlete or coach know when they are at it? For a number of years, blood hormone concentrations have been proposed as having diagnostic value in quantifying training intensity (1, 4). Specifically, Adlercreutz et al. (5) suggest the free testosterone to cortisol ratio (fTC) as a possible indicator of the anabolic-catabolic balance in the athlete. If an athlete has an upset in the anabolic-catabolic balance at the skeletal muscle (i.e., excessive catabolism) they may be unable to adapt to the training and their performance may become compromised (1, 5). If there is a decrease of 30% or more in the fTC ratio then the athlete may be overtrained (3, 5).

Free Testosterone to Cortisol Ratio

The free testosterone to cortisol ratio (fTC) is the ratio of blood levels of unbound or free testosterone and cortisol in their molar concentrations. It is usually expressed as a value times 10^{-3} (i.e. 0.35 x 10^{-3}) in order to obtain numbers easier to use. Adlercreutz et al. (5) suggests that a decrease of 30% or more in the ratio, as well as a value of 0.35 x 10^{-3} or less, signifies overtraining and an increased catabolic state. Jurimae et al. (11) also suggests a 30% or more decrease in fTC may suggest an inappropriate level of anabolic to catabolic hormonal balance.

Most of the research conducted monitoring of the fTC ratio has looked at its fluctuations during a competitive season or an intense period of training. In one particular study the fTC ratio was monitored during 7 weeks of a competitive rowing season (8). The 7-week period consisted of regattas, intense training camps, and regeneration (i.e., rest) periods. The fTC ratio showed a decrease during the regattas and intense training camps, whereas during regenerative training periods, the fTC ratio began to increase and return towards normal levels. Similarly, Vervoorn et al. (9) monitored the fTC ratio during a rowing season. The investigators also found the fTC ratio to decrease in response to intense training and regattas and increasing towards normal levels during period of rest and regeneration training (9).

An increased state of catabolism has also been demonstrated following a single bout of intense exercise. Specifically, the fTC ratio was monitored after a prolonged sculling session (10). Subjects in this study rowed at roughly 75% of the anaerobic threshold for approximately 2 hours. Blood samples were taken pre exercise, immediately post exercise, 30 minutes, 60 minutes, and 120 minutes post exercise. There was a decrease in the fTC ratio immediately post exercise, followed by a further decrease at 30 minutes post exercise. At 60 minutes, there was a progressive return to normal values by 120 minutes post exercise. This study shows that an acute bout of intense exercise has an acute affect on the fTC ratio in athletes. Duke et al. (11) found similar results in a case study performed on a highly trained long-distance runner. Following 82.2 minutes of running at approximately 70% of his VO_{2max}, the fTC ratio decreased immediately post exercise. Approximately three hours after exercising, the fTC ratio began to increase towards normal levels.

Studies have shown that acute and prolonged exercise has an affect on the fTC ratio. There have been a number of studies conducted in which the fTC ratio was monitored during a season or intense period of training. These studies were conducted over a long period of time ranging from seven weeks to nine months. Also, some studies have monitored the effect of an acute bout of exercise on the fTC ratio. However, few if any studies have monitored the fTC ratio for a period of time between these two extremes. Specifically, the effect of a short-term, intensive micro-cycle of training on the fTC ratio has not been studied.

Purpose

The purpose of this study was to determine the effects of a short-term intensive period (micro-cycle) of training on the fTC ratio. In order to ascertain this effect, subjects underwent three days of controlled, intensive exercise sessions with the fTC ratio assessed at 24 hour intervals after each session. The study was designed to attempt to answer the question - Does the fTC ratio return to "normal" levels 24-hours after each exercise session, or will it remain suppressed in relation to pre-study levels?

Research Hypotheses

1. The resting fTC ratio levels at 24-hr following each exercise session will be significantly suppressed from resting levels obtained prior to the study period.

Definition of Terms

1. Overreaching – A short-term increase in training intensity or volume. This is done in an attempt to provide a stimulus for adaptation. This can result in overtraining and/or OTS (3).
2. Overtraining – A short term condition that is the result of an imbalance in training and recovery. The main symptom of this is a decrease in performance (6).
3. Overtraining Syndrome (OTS) – Chronic overtraining resulting in a prolonged period of fatigue and decreased performance. Unlike overreaching and overtraining, OTS cannot be easily reversed (3, 6).
4. Cortisol – A glucocorticoid hormone secreted from the zona fasciculata of the adrenal cortex. Cortisol release is stimulated by adrenocorticotropin hormone (ACTH) from the anterior pituitary (7).
5. Testosterone – An androgenic and anabolic hormone secreted mostly from the Leydig cells of the testis and in small amounts from the adrenal cortex. Testosterone release is stimulated by luteinizing hormone (LH) from the anterior pituitary (7).
6. Free Testosterone to Cortisol Ratio (fTC) – The ratio of the unbound or free portion of the hormone testosterone to cortisol.

Assumptions

1. Subjects refrained from any additional exercise in addition to what is being prescribed to them.

2. Subjects consumed an adequate amount of carbohydrates throughout the exercise trials.
3. Subjects consumed the proper amount of polycose glucose polymer.

Delimitations

1. Male subjects between 18 and 45 years of age were recruited from the University of North Carolina at Chapel Hill, and surrounding areas.
2. Subjects were healthy, moderate to highly trained endurance athletes.
3. Subjects reported to exercise after an overnight fast of at least 8 hours and refrain from any exercise in addition to what was performed during each exercise trial.

Limitations

1. Results can only be generalized to healthy, moderately to highly endurance trained adult males between 18 and 45 years of age.
2. Although subjects will be asked to maintain a high carbohydrate diet, consume a polycose glucose polymer solution, and refrain from exercise in addition to what they are completing during exercise trials, there could be an occasional violation of these guidelines that may be undetectable.

Significance of Study

At present there is no reliable biological marker of exercise training intensity (1). As a result of this, coaches and athletes constantly use trial and error to determine if a training regime is effective. Using this method, athletes run the risk of becoming

overtrained or even developing the OTS. If there were a reliable biological marker to monitor during training, the risk of becoming overtrained could be severely reduced.

Some studies have suggested that the fTC ratio could be a useful marker of training intensity and of the anabolic and catabolic balance within the body (1, 5). A number of studies have monitored the fTC ratio throughout a season or intense period of training (8), however limited studies have been done on the effect of repeated intense training during back-to-back days (i.e., training micro-cycle). Certainly few studies if any have monitored the fTC ratio on a daily basis over a short period of time during intense training. Therefore, this study was designed to examine and monitor the effect of three days of intense exercise training on the fTC ratio. This study will contribute to the knowledge of the fTC ratio and help to elucidate its potential use as a biological marker of training intensity.

CHAPTER II

REVIEW OF LITERATURE

Introduction

This review of literature covered research over a range of topics. First, basic endocrinology was discussed, focusing on the primary role, function, purpose, and types of hormones. Following this brief overview, the two hormones cortisol and testosterone were examined. This portion of the review was separated into research that deals with the response of each hormone to an acute bout of exercise and those studies that investigate the response to chronic exercise. Next, the concepts of overtraining and OTS were explained in detail. Following the discussion on overtraining and OTS, research investigating the fTC ratio was reviewed. Lastly, this chapter closes with a brief summary of what has been discussed.

Hormones

Hormones are chemical messengers that regulate physiological and metabolic functions, as well as, maintain homeostasis and adapt to stress (13, 14). Hormones can be made up of a variety of different substances including: proteins, amino acids, cholesterol, steroids, and glycoproteins (7). The process in which a hormone brings about a specific physiological response can be very complicated and a detailed discussion is beyond the scope of this review. However, it is important to understand some of the

basics. Hormones bind to specific receptor molecules and then initiate a cascade of events that results in a specific response (7). Some hormones also travel in the blood bound to proteins, which affects the bioavailability of the hormone since only the portion that is unbound or free is biologically active (7).

Hormones can be grossly categorized as catabolic and anabolic (5, 7, 14) based upon their primary physiological actions. The catabolic hormones include cortisol, epinephrine, norepinephrine, and glucagon (13). The anabolic hormones include testosterone, insulin, and growth hormone (13). However, this investigation will be measuring cortisol and testosterone as a catabolic and anabolic index, respectively. Therefore the next sections of this review will focus on these two hormones.

Cortisol

Function. Cortisol release is stimulated by a wide array of stimuli and possesses a large range of functions (7, 12, 14, 17). Being a "stress" hormone, cortisol increases as a response to any and all stresses. These include affect, exercise, temperature, negative energy balance, and altitude (14). The functions of cortisol include: stimulation of gluconeogenesis by the liver, stimulation of the glucose-alanine cycle, decreased glucose use by cells, protein breakdown, increased free amino acid pool, stimulation of erythropoiesis, and anti-inflammatory effects (4, 12, 14).

During exercise cortisol can respond to hypoglycemia (14). In an attempt to correct this, cortisol increases blood glucose levels indirectly by increasing lipoylsis and directly stimulating gluconeogenesis at the liver (14). Cortisol also inhibits the uptake of glucose by skeletal muscle (4) and has an inhibitory affect on protein synthesis during exercise (12), which may carry late into recovery. Although decreased protein synthesis

may seem less than ideal, the protein breakdown stimulated by cortisol after exercise is vital to adaptation (12). Cortisol breaks down physiologically exhausted elements of protein structures, which allows for new, stronger proteins to be built (13).

Acute Exercise. A large volume of literature has been completed in regards to the response of cortisol to exercise. Davies and Few (15) investigated the cortisol response of ten subjects to a light load (<50% VO_{2max}) and a heavy load (60-90% VO_{2max}). Nine of the ten subjects were measured at both workloads. The subjects exercised for a one-hour period and had multiple blood samples taken (three samples during the 45-60 minutes pre-exercise, at ten minute intervals during exercise, and at minutes 2, 10, 30 and 60 of recovery). Results suggested that the increase in cortisol was connected with the intensity of exercise performed. The investigators stated that a critical threshold of ~60% of VO_{2max} must be met for cortisol to increase.

Kindermann et al. (16) measured cortisol following 50 minutes of aerobic exercise at the anaerobic threshold (i.e., 4 mmol/L of lactate) and anaerobic exercise (156% of maximal capacity) to exhaustion. Cortisol increased 35% after the anaerobic exercise, with 12% of that increase occurring during the recovery period. There was a 54% increase observed after the aerobic exercise. The authors concluded that the increase in cortisol is affected by intense prolonged exercise. In conjunction with an intensity threshold, the duration of exercise may determine the final concentration of cortisol following exercise (i.e., cortisol continues into recovery after short-duration, high intensity exercise similar to what was done in the above study).

Cortisol also increases as the duration of exercise increases. Inder et al. (17) examined the effect of prolonged exercise on cortisol, adrenocorticotropin hormone

(ACTH) and corticotrophin releasing hormone (CRH) (17). Six highly trained male triathletes performed 60 minutes of cycling at 70% of their VO_{2max}, and then the workload was increased by 25W every two minutes until volitional fatigue was reached. Blood samples were taken at time points –30, 0, 30, 60, 75, and 90. The results showed that prolonged exercise caused an increase in cortisol, as well as, ACTH and CRH. The results also showed that cortisol continued to increase into recovery.

Numerous textbooks and review articles have reported and summarized the above findings. For example, McMurray and Hackney (14) stated that a critical intensity of 50-60% of VO_{2max} must be reached for cortisol to be increased. These authors also stated that the final cortisol levels attained during exercise are dependent on the total duration of the exercise bout. Therefore, short-duration, high intensity exercise may provoke a cortisol response that is not seen until some time into recovery. Galbo (18) states that cortisol increases as the intensity and/or duration is increased and that the increased levels may continue well into recovery. Viru and Viru (4, 12) agree with these conclusions about cortisol secretion during and after exercise.

Chronic Exercise. Studies investigating the cortisol response to chronic exercise training suggest that basal levels do not change after training (14). However, there appears to be a change in the cortisol response to an acute bout of exercise following chronic training (4, 14, 18). When comparing an athlete at an absolute workload or intensity before and after training, the cortisol response will be decreased (4). This is due to an increase in the intensity threshold (4, 18). In other words, the intensity is less stressful on the body after training. However, during exercise at maximum and/or above maximal levels after training results in a more pronounced cortisol response when

compared to pre-training concentrations (4, 18). It is thought that there is some adrenal gland hypertrophy with training, thereby increasing its capacity to secrete cortisol (4, 18). Similarly, there may be an increase in target tissue sensitivity and receptor number (14, 18).

Testosterone

Function. Testosterone is a steroid hormone synthesized from cholesterol and is controlled by a negative feedback loop via the hypothalamic-pituitary-testicular axis (19). Like other steroid hormones, a fraction of the circulating testosterone is bound to a carrier protein called sex hormone binding globulin (SHBG) (14). The part that is not bound to this protein is referred to as free or unbound and is the biologically active portion (14). Approximately 3-4% of the total testosterone is in the unbound or free form (14). Therefore, total testosterone concentration can remain unchanged, but if SHBG increases or decreases then the biologically active portion can be affected. Testosterone has many physiological roles within the body, all of which can be placed into two categories: androgenic and anabolic (19).

The androgenic effects of testosterone are beyond the scope of this review and study, but it is useful to understand their importance. For example, testosterone has an effect on the reproductive organs, the promotion of secondary sex characteristics, and masculinizing the brain and promotion of aggressiveness (20). Testosterone's effect on the reproductive organs in males includes the formation of reproductive ducts, external genitalia, and the growth and maturation of internal and external genitalia (20). Testosterone is also required for normal spermatogenesis in males (19, 20). The

development of secondary sex characteristics like deep voice, chest and pubic hair are also a result of the increase in testosterone during adolescence (20).

The principle anabolic functions of testosterone include: growth of long bones during puberty and protein synthesis (19). It is a potent anabolic hormone and by activating specific genes on the target tissue (i.e., skeletal muscle) it enhances transcription of mRNA molecules, which results in increased protein synthesis (19, 20). However, the specific type of protein synthesized is dependent upon the target tissue. At the skeletal muscle testosterone promotes the formation of the contractile proteins actin and myosin (21).

Acute Exercise. Wilkerson et al. (22) investigated the testosterone response of five male subjects during submaximal exercise and following maximal exercise. The investigators had each subject exercise for 20 minutes at a range of intensities (i.e. 30, 45, 60, 75, and 90% of VO_{2maz}) and then had them perform a maximal test after one minute of rest. Blood samples occurred at rest and during minutes 9, 14, and 19 minutes of exercise and then at minute 4 of recovery. The results show that testosterone increases after exercise, but indicate that the increase in testosterone concentration during and following all submaximal bouts and maximal tests could be attributed to changes in plasma volume (i.e., hemoconcentration). The investigators suggested that short duration submaximal exercise may not cause an increase testosterone concentration.

Sutton et al. (23) measured testosterone levels in 14 male rowers and 11 swimmers (7 male, 4 female) following 60 minutes of maximal exercise. The exercise for the rowers consisted of running and calisthenics, while the swimmers swam and did an all-out 800-meter run at the end. For all subjects, the 60 minutes of maximal exercise

resulted in a significant increase in testosterone levels after the exercise. This investigation also measured four untrained subjects before and after 20 minutes of submaximal cycle ergometry. These subjects also had an increase in testosterone. The peak testosterone values were reached at 20 minutes and returned to normal after 60 minutes of recovery. However, this investigation did not correct for changes in plasma volume after exercise and into recovery.

Galbo et al. (24) measured testosterone during graded and prolonged exercise in 8 healthy male students. The subjects ran repeated bouts of 20 minutes at approximately 76% of VO_{2max}. Testosterone significantly increased after 40 minutes of total work, but began to decline at 60 minutes and continued to decline well into recovery. These results suggest that testosterone may decrease when exercise lasts 60 minutes or more. The investigators suggest that this may be due to a decrease in testicular blood flow during exercise similar to a reduction in hepatic blood flow.

Chronic Exercise. Hackney et al. (25) investigated ten male subjects (5 highly trained endurance athletes and 5 untrained) under resting conditions. The results of this investigation showed that highly trained male endurance athletes have a significantly lower basal level of total testosterone, as well as, free testosterone levels that trended toward being significantly lower.

Similarly, Hackney et al. (26) measured the basal total and free testosterone levels in 53 endurance-trained males and 35 age-matched sedentary males. The hormonal evaluations were determined after a 24-hour control period. The results of the study showed that the endurance trained men had a significantly lower basal total and free testosterone level than the sedentary men. However, the testosterone levels in the

endurance-trained men were within the normal clinical range, but at the low end of this range. The authors concluded that the decreased basal testosterone levels were a result of the chronic endurance training and an alteration in the hypothalamic-pituitary-testicular axis function. It is presently unclear whether the decreased levels are beneficial or may have a negative affect on the anabolic processes in men.

After training, there appears to be an attenuation of the testosterone response to submaximal exercise (14). However, the testosterone response to incremental exercise to maximal appears to be more pronounced, although starting from a lower resting level after training (4).

Overtraining and the Overtraining Syndrome

Reviewing the literature in the area of overtraining and the Overtraining Syndrome (OTS) is very challenging. Although there is a large body of literature on the topic, original studies are few and far between. Those studies that are original in nature are usually done after the fact and after overtraining occurred. This is due to the ethical issues that deal with intentionally causing a subject to become overtrained. Therefore, most of the research discussed in this section is from review articles that have discussed the topic from a theoretical point of view.

Basics of Exercise Training. The purpose of exercise training is to make the body more efficient and able to handle the stress that exercise places upon it. In order for this to be accomplished athletes must undergo a large volume of intense training to stimulate the plethora of adaptations that occur as a result of training. However, this is not done

without some risk. Exercise training and recovery must be balanced to maximize the benefit of the training program and reduce the risk of overtraining.

In order to stimulate the adaptation processes to occur one must upset the homeostatic balance within the body (3). This can be achieved by increasing training volume, intensity or some combination of the two and is often referred to as the overload principle (3). After the body is overloaded there is immediate fatigue and a decrease in performance. The body will then enter what is referred to as the resistance stage (27). During this phase the body attempts to handle and adapt to the stressor. The adaptation phase occurs next and it is during this phase that the body attempts to reestablish homeostasis (27). This process is referred to as the general adaptation syndrome (27). The one caveat of this response is that if the stress (exercise) introduced is too stressful the body will not adapt and the individual may become overtrained.

In order to maximize the potential benefits and reduce the risks of overtraining coaches sometimes use periodization (28). Periodization is a structuring of a training program into long, medium, and short cycles of training (e.g., macro-cycle, meso-cycle, micro-cycle) in order to help to organize the training plan (28).

For athletes the annual training program is often separated into three macro-cycles (preparatory, competitive, and transition) and then further subdivided into meso-cycles and finally micro-cycles (28). A common recommendation is that there are four micro-cycles (ordinary, development, shock, and rehabilitation) in each meso-cycle and that the athlete needs to be completely recovered from the meso-cycle before moving onto the next (28). It appears coaches and athletes often neglect this recommendation and move forward before recovery is achieved and as mentioned earlier, inadequate rest or

regeneration can result in the athlete becoming overtrained (3, 6). When an athlete becomes overtrained they become unable to adapt to the exercise. Prolonged or chronic overtraining can result in a condition called the Overtraining Syndrome (OTS). The Overtraining Syndrome has a myriad of symptoms and can last for months at a time (3).

Basics of Overtraining. Overtraining is a complicated topic that has been studied for many years. When overtraining was first being studied it was separated into two categories based on the symptoms being displayed by athletes: sympathetic and parasympathetic overtraining (3). Sympathetic overtraining was characterized by an increase in resting heart rate, decrease in body mass, disturbed sleep, and a decrease in appetite, whereas the symptoms of parasympathetic overtraining were a little more subtle and included such things as progressive anemia and digestive disturbances (3). Parasympathetic overtraining was also thought to be associated with neuroendocrine system exhaustion (3).

Although the symptoms of parasympathetic overtraining seem less severe, it is thought that it reflects a more advanced state of overtraining and sympathetic overtraining is thought to reflect a prolonged stress response that precedes exhaustion (3). Research has suggested that endurance athletes are more likely to have the parasympathetic form, while speed and power athletes display more symptoms of the sympathetic form (3). Unfortunately, as more research has been done in this area it appears that overtraining is a far more complicated subject (3). Athletes were showing symptoms from both forms of overtraining, which made it hard to classify them into one of the two categories. There was also a lack of evidence supporting the fact that the symptoms of the two categories were separate (3).

Currently the terminology of parasympathetic and sympathetic overtraining is being used much less in the literature and overreaching, overtraining, and OTS are becoming more widely used (3). Contemporary thought is that the symptoms lie on a continuum. The first set of symptoms are a result of a homeostatic imbalance from day-to-day training. With continued hard training more complex symptoms can develop and chronic conditions can eventually occur (3).

One of the consistent consequences of overtraining is hypothalamic-pituitary axis dysfunction (3). This axis regulates most of the major endocrine glands and can affect the body in a wide array of ways. Although it is not an exhaustive description, Figure 1 displays some physiological functions controlled by the hypothalamic-pituitary-adrenal axis (HPA axis) and the hypothalamic-pituitary-testicular axis (HPT axis) that can be affected when the axis is not functioning properly, as can happen as a result of overtraining.

Symptoms. Overtraining and the OTS can present in a plethora of different symptoms. As was mentioned in the previous chapter, the major symptoms can be classified into four categories (3). Although it is by no means an all-inclusive list, Table 1 below displays the four categories and the symptoms that occur within each.

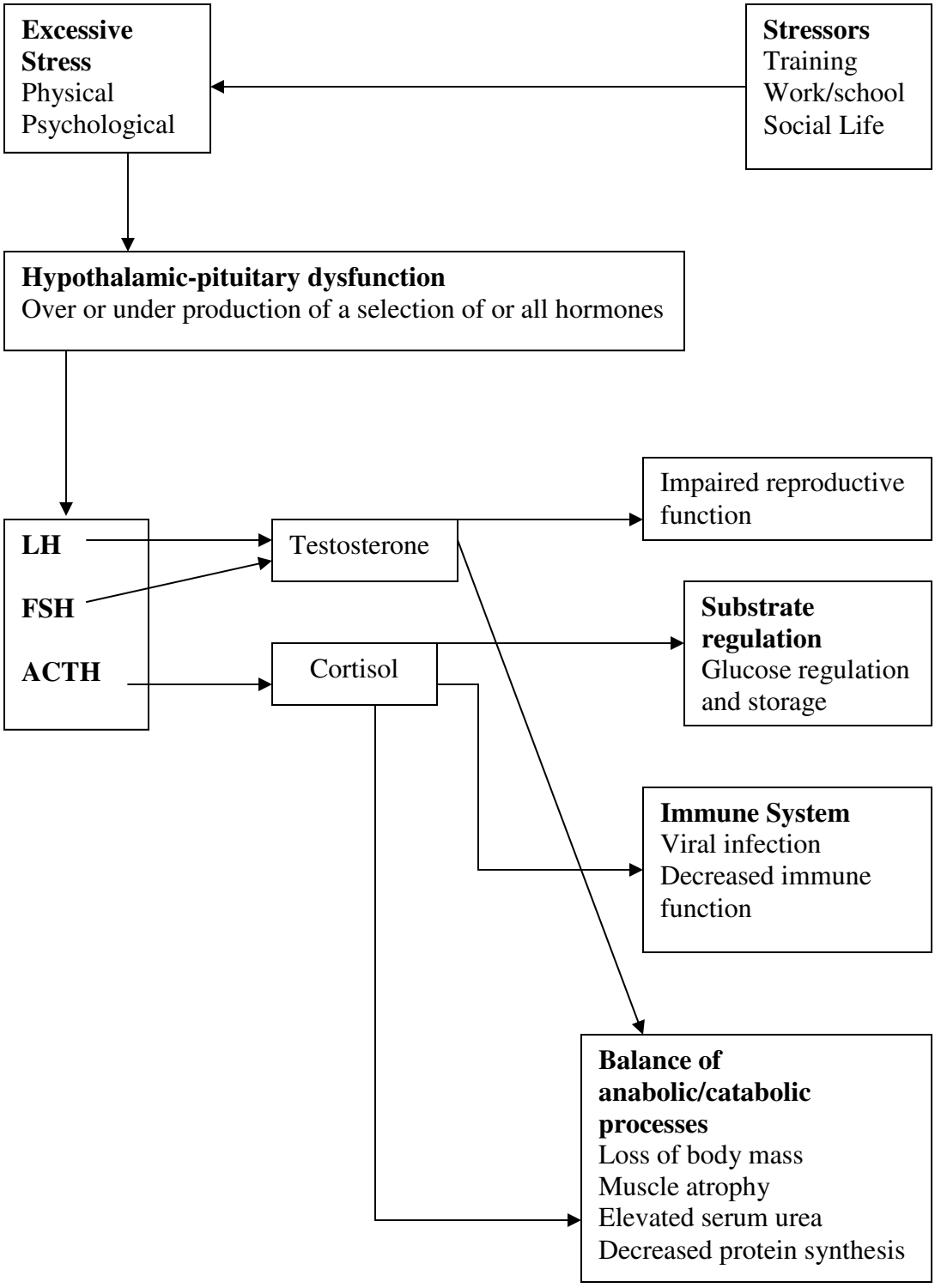


Figure 1. Hypothalamic-pituitary axis function following chronic exposure to stress can result in dysfunction and cause an upset in regulation of other glands and the physiological processes they regulate (adapted from Fry et al. [3]).

Table 1. The major symptoms of overtraining separated into the four major categories (adapted from Fry et al. [3] and Angeli et al. [29]).

Physiological/performance	
Decreased performance	Decreased body fat
Decreased muscular strength	Increased VO_2 at submaximal loads
Muscle soreness	Changes in heart rate
Prolonged recovery periods	Loss of appetite
Chronic fatigue	
Psychological	
Feelings of depression	General apathy
Difficulty concentrating	Emotional instability
Fear of competition	Excitation
Restlessness	
Immunological dysfunction	
Increased susceptibility to infection	Increased severity of minor infections
Decreased functional activity of neutrophils	Decreased total lymphocyte counts
Reduced response to mitogens	Decreased production of immunoglobulins
Biochemical alterations	
Decreased hemoglobin	Negative nitrogen balance
Increased urea levels	Decreased free testosterone levels
Decreased ratio of free testosterone to cortisol ratio of more than 30%	Elevated cortisol levels

Free Testosterone to Cortisol Ratio

Development of the Ratio. As discussed above, training that is too strenuous can lead to overtraining and/or OTS. Likewise it was discussed above that overtraining and OTS is thought to be at least partly a result of an upset in the anabolic and catabolic balance at the skeletal muscle (1, 2, 3, 5). Adlercreutz et al. (5) suggest that there are several physiological tests that can diagnose overtraining, but they all detect overtraining

once it is already present. Therefore, the investigators set out to develop a test that could aid in the monitoring of training, as well as, diagnose overtraining.

To do so two groups of long distance runners were studied; one group was training normally and the other was training intensely for one week. Following the training, the athletes were split into three categories (i.e., non-overstrain, overstrain, and uncertain) based on the results of various physiological tests. The investigators then performed the following hormonal tests: plasma testosterone to cortisol and free testosterone to cortisol ratio, serum SHBG and growth hormone, and saliva testosterone to cortisol ratio. It was found that the free testosterone to cortisol ratio was the best test. The criteria set by the investigators to determine if an athlete was overtrained was a decrease of 30% or more in the ratio or a value of 0.35×10^{-3}. The results of the physiological tests showed that the free testosterone to cortisol ratio was negative (i.e., less than a 30% decrease or a value greater than 0.35×10^{-3}) for all those athletes that showed no other symptoms of overtraining (5).

Based on the results of the study discussed above, other researchers have used the criteria set by Adlercreutz et al. (5). These studies will be discussed in more detail in the sections that follow.

Long-term Studies. The fTC ratio has been used to monitor athletes in training during an athletic season or competition period. Urhausen et al. (8) monitored testosterone, cortisol, and urea in nine elite rowers (6 male and 3 female) during a seven-week period of intense training and competition. From the testosterone and cortisol data, the total testosterone to cortisol ratio was calculated. During the seven weeks, the rowers underwent various training camps, regattas, and regeneration periods and blood samples

were taken on a weekly basis. The results of this study suggest that during a period of intense training there is an increased catabolic state in the athletes. This was evidenced by a decrease in the total testosterone to cortisol ratio, as well as, an increase in urea. It was also demonstrated that the regeneration periods appeared to increase the total testosterone to cortisol ratio thereby reducing the anabolic-catabolic imbalance in the athletes.

Vervoorn et al. (9) conducted a similar study involving rowers, however they monitored the fTC ratio throughout an entire rowing season (i.e., 9 months) leading up to an Olympic Games. Blood samples were collected under resting conditions after a test was completed on a rowing ergometer. This test occurred approximately every five weeks and consisted of a 3-minute standard warm up followed by a 1-minute rest period, 5 minutes at a load corresponding to the athletes' anaerobic threshold (i.e. 4 mmol lactate) followed by a 2-minute rest, and then a 2-minute all-out bout where the athlete attempts to record as many revolutions as possible. The results of this study were very similar to that of Urhausen et al. (8). During intense training camps there was a decrease in the fTC ratio, usually due to a decrease in free testosterone. Following the training camps was a regeneration period, which typically resulted in the fTC ratio increasing towards normal values.

Tyndall et al. (30) measured cortisol, testosterone, and insulin in elite swimmers. The investigators took blood samples from the 19 subjects (9 males, 10 females) during the off-season, after 9 weeks of training 5500 m/day, and then after 9 weeks of training 8300 m/day. The most important result of this study was that testosterone levels decreased during intense training in the male athletes. Compared to initial values the

estimated total testosterone to cortisol ratio changed less than 1% after 9 weeks of swimming 5500 m/day, however it changed approximately 20% following 9 weeks of swimming 8300 m/day.

Although all three studies discussed above found a decrease in the fTC or total testosterone to cortisol ratio following intense training only two had a decrease of 30% or more in the fTC ratio or the total testosterone to cortisol ratio (8, 9). Urhausen et al. (8) found a decrease of approximately 31% in the total testosterone to cortisol ratio, but had athletes that were still able to put forth high-level performances. Similarly, Vervoorn et al. (9) found a 30% decrease in the fTC ratio, but found no other symptoms of overtraining present. The investigators suggest that a consistently lowered fTC ratio (i.e., for a number of weeks) value may be needed before the athlete becomes overtrained (9).

Short-term Studies. The fTC has been shown to respond after a single exercise bout. Jurimae et al. (10) studied 12 national caliber male rowers after an intense bout of skulling. The subjects rowed for approximately 2 hours at approximately 75% of their anaerobic threshold, which is approximately 50% of VO_{2max}. The investigators took blood samples immediately pre-exercise, post-exercise, 30 minutes, 60 minutes, and 90 minutes post-exercise. The fTC ratio decreased from pre to post-exercise and decreased further after 30 minutes of recovery before returning to approximately normal values. However, these changes were not statistically significant. The investigators proposed that an intensity greater than 75% of the anaerobic threshold may be necessary to elicit a significant change in the fTC ratio. Although no statistical significance was observed this study demonstrates that the fTC ratio does respond to a single bout of prolonged exercise.

Urhausen and Kindermann (31) measured cortisol, testosterone (T), sex hormone binding globulin (SHBG), and then approximated the free portion of testosterone (based upon T/SHBG values) and the total testosterone to cortisol ratio in nine male athletes before and after a triathlon competition. Blood samples were taken one day before, immediately after the competition, one, two, three, and four days after the competition. Cortisol significantly increased from one day before to immediately after the triathlon. This increase in cortisol caused the total testosterone to cortisol ratio to decrease at approximately the same magnitude. Interestingly, T/SHBG was relatively unchanged immediately post-competition, but began to decrease one day after and remained decreased for the remaining days of the investigation. This change in the estimated free portion of testosterone (T/SHBG) was due to an increase in SHBG and not an increase in testosterone, which remained relatively unchanged throughout the investigation. Although cortisol and the total testosterone to cortisol ratio returned to normal values by one day after the competition, the decrease in T/SHBG illustrates a possibly decreased anabolic ability following prolonged intense exercise.

Duke et al. (11) completed a case study investigating the 24-hour response of the fTC ratio to an acute bout of intensive running. The athlete ran on a treadmill at approximately 70% of his VO_{2max} for 82.2 minutes (i.e., until exhaustion). During exercise, blood samples were taken every 15 minutes and then at 0.5, 1, 1.5, and 2 hours after exercise was ceased. After two hours of recovery, blood samples were taken every hour until the 24-hour period was completed. There was a substantial decrease in the fTC ratio immediately post-exercise that continued for approximately three hours, after which the fTC ratio began to increase towards normal values.

Jurimae et al. (10) found approximately a 20% decrease in the fTC ratio immediately following two hours of submaximal skulling. Similarly, Urhausen and Kindermann (31) found approximately an 82% decrease in the total testosterone to cortisol ratio following a triathlon competition. Duke et al. (11) found the fTC ratio to decrease by approximately 69% following submaximal running to exhaustion. Although, the later two studies found a decrease in the fTC or total testosterone to cortisol ratio that was greater than 30% the investigators did not report the subjects as being overtrained. In all three investigations discussed above the ratio returned towards normal values within 24 hours of cessation of the exercise. Based on these results it would appear that a single bout of intense exercise might not be enough to cause an athlete to become overtrained and that many intense sessions are needed.

Criticisms of the Ratio. Banfi and Dolci (32) suggest that using absolute values of the fTC ratio (i.e., 0.35×10^{-3} and a decrease of 30% or more) is not useful. The authors analyzed samples collected in professional male soccer players over the course of two seasons. Values obtained throughout the season were compared to pre-season values. In this study the authors found no significant differences between season values and pre-season values. The authors concluded that a classification method using the values of the fTC ratio may be more advantageous in comparison with symptoms and risk levels.

Viru and Viru (4) are critical of the reliability of the fTC ratio. The co-authors suggest that pronounced increases in training volume and intensity may induce variable changes in cortisol and testosterone. The authors also suggest that diverse changes may exist within a group of athletes performing a similar training regime, as these hormonal

changes are merely a response to the stimulus placed on the individual and each athlete may respond different. Viru and Viru (4) also state that no strict empirical evidence exists that suggests a decreased fTC ratio is associated with a decrease in performance.

Summary

Hormones are chemical messengers that regulate many physiological systems within the body (13, 14). Exercise and exercise training causes a change in most of the hormones within the body (14). Two such hormones are cortisol and testosterone.

Cortisol increases with intensity and duration (4, 12, 13, 14, 15, 16) however there exists a critical intensity below which an increase will not be seen (15). The testosterone response to exercise appears to be unclear, as some studies have shown it to increase (4, 14, 22, 23) while others have suggested it may decrease with prolonged exercise (14, 24). Both hormones appear to respond to exercise training (4, 14). The cortisol response to the same absolute workload is less after training; however, the response to maximal exercise is greater following exercise training allowing the athlete to exercise at a higher workload (4, 18). The most pronounced changes in testosterone concentration after training is the decreased basal levels (4, 19). The testosterone response to submaximal exercise is attenuated (14), however is more pronounced after maximal exercise following exercise training (4).

Overtraining and the OTS appear to be prominent in sport (1) and can negatively affect a wide variety of physiological systems and areas of athletic performance (3, 6). Unfortunately there lacks a reliable biological marker of overtraining and OTS, however the fTC ratio has shown some promise as such a marker (1). The fTC ratio has been shown to decrease following intense exercise training (8, 9, 10, 11, 22). The fTC ratio

has also been shown to “rebound” towards normal levels when a regeneration (i.e., rest) period is given to the intensely training athlete (8, 9). However, many of these studies were done during an athletic season with relatively infrequent blood sampling. Most studies were obtaining blood samples anywhere from once a week to five times a week. There are, however, few or no studies that investigate the response of the fTC ratio to an intense micro-cycle of exercise training.

CHAPTER III

METHODOLOGY

The experimental procedures of this study required the subjects to visit the laboratory five times (see Figure 2 below). The first visit was an "orientation" session that consisted of the subject signing an informed consent form, collecting subject characteristics, a physical examination, and a peak oxygen uptake (VO_{2peak}) were obtained. The following three visits were submaximal cycle exercise sessions. These cycle exercise sessions were 60 minutes in duration and at a workload calculated to elicit approximately 75% of VO_{2peak}. Blood samples (3 mL) were taken prior to and immediately following each of the cycle exercise sessions. These blood samples were analyzed for cortisol and free testosterone, from which the fTC ratio was calculated. During the fifth and final visit to the laboratory only a resting blood sample was obtained. There was no exercise done during this last visit to the laboratory.

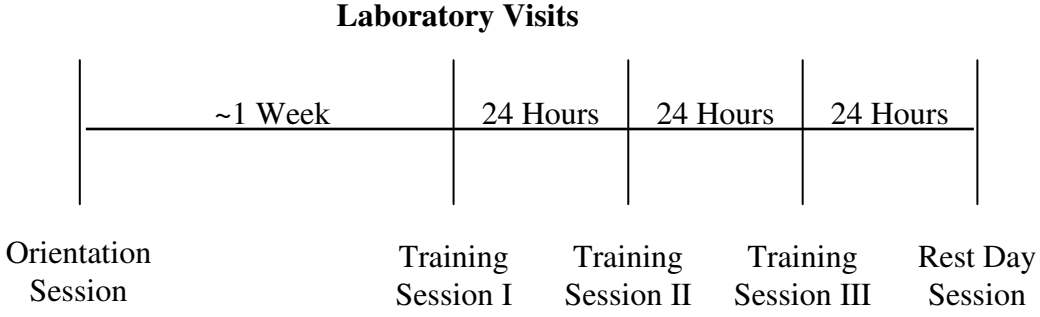


Figure 2. Above is an overview of the experimental protocol (adapted from Behr [33]).

Subjects

Twelve highly trained male endurance athletes between ages 18 and 45 years, were postulated as the necessary sample size for this study. In order to participate, subjects must have been training a minimum of five days per week for 60 minutes or more. Potential subjects must also have been involved in competitive endurance sporting events. The experimental protocol and possible risks were thoroughly explained to the subjects and an informed consent was signed prior to participation in the study. Exclusion criteria included: history of any current or chronic medical condition or musculoskeletal injury, history of an infection in the six week period prior to the commencement of the study, the use of any medication, including non-steroidal anti-inflammatory drugs (NSAIDs).

Subjects refrained from strenuous activity 24 hours prior to the VO_{2peak} assessment. Additionally, subjects performed only minimal physical activity two days before beginning the training sessions and refrained from any activity the day before the training sessions. During training sessions the subjects performed no other physical activity than what is prescribed in the study. Finally, subjects also refrained from eating, smoking, and consuming alcohol and/or caffeine in the eight hours prior to the training sessions. Subjects also consumed a carbohydrate supplement, Polycose®, on the day proceeding the first training session and on each of three training days.

Instrumentation

Subject height was determined using a portable standiometer (Perspectives Enterprises, Portage, MI) and body mass using a mechanical scale (Detecto, Webb City,

MO). Peak oxygen uptake was obtained by using an incremental, continuous cycling test on a Lode electronically braked cycle (Lode, Groningen, The Netherlands). Respiratory gases obtained during the VO_{2peak} test, as well as, during the submaximal training sessions were measured via a Parvo Medics TrueMax 2400 Metabolic System (Parvo Medics, Salt Lake City, UT). Heart rate was monitored using a Polar telemetry system (Polar Electro Inc., Lake Success, NY). Ratings of perceived exertion was determined by using Borg's original 6-20 rate of perceived exercise scale (RPE) (34). Select skin folds (i.e., abdomen, midaxillary, chest, and suprailliac) was measured using Lange skinfold calipers (Beta Technology, Inc., Santa Cruz, CA).

Hematocrit was measured from whole blood using an Adams MHCT II microhematocrit centrifuge (Becton Dickinson, Franklin Lakes, NJ) and an International Microcapillary Reader (International Equipment Company, Needham Heights, MA). Hemoglobin was also measured from whole blood using a Milton Roy Spectronic 1201 flow through cell spectrophotometer (Milton Roy, Ivyland, PA). Following the measurement of these blood parameters, whole blood was placed into an IEC Centra-8R refrigerated centrifuge (International Equipment Company, Needham Heights, MA) and then spun at 3,000 *g* to remove the plasma. The plasma was then aliquoted into micro-storage tubes and placed into a sub zero freezer for storage. The plasma was then used in radioimmunoassay hormonal analysis, which took place after all subjects had completed the full experimental protocol.

Protocol

Orientation Session. Each participant arrived at the Applied Physiology Laboratory two or more hours post-prandial. They were informed of the possible risks of participation in the study and the experimental protocol was thoroughly explained to them. The participant then signed a written informed consent statement and underwent a medical screening. This screening included a 12-lead electrocardiogram and a physical screening (i.e., blood pressure, follow-up questions regarding family and personal illness history, previous orthopedic injuries, etc.) to determine if the subject was fit to participate. Following the medical screening, subject characteristics were obtained. These characteristics included height, body mass, and age. Percent body fat was estimated using a sum of skin folds table (35).

After subject characteristics had been collected, the VO_{2peak} was determined using an incremental, continuous cycling protocol on an electronically braked cycle ergometer. The seat height and handle bar angle were adjusted to the comfort of each subject and was noted and recorded. Adjustment of the cycle was followed by a light 10-minute warm-up. The first 5-minutes of the warm-up consisted of cycling at a workload of 50 watts (W). The remaining 5-minutes consisted of stretches selected by the participant and focusing on the lower body. The subject then returned to a seated position on the cycle ergometer. Resting oxygen consumption (VO_2) was recorded for four minutes in order to ensure that the subject had followed the dietary guidelines.

The VO_{2peak} exercise protocol began with the subject cycling at a resistance of 50W for 3-minutes. Resistance was increased by 50W every three minutes for the next nine minutes (i.e. four stages of 3-minutes, increasing by 50W each). After the fourth 3-

minute stage, resistance was increased by 25W and stage length decreased to 1-minute. These stages continued until volitional fatigue occurred (36). Oxygen consumption and heart rate (HR) were monitored continuously throughout the exercise. Heart rate was recorded at the end of each stage. Rate of perceived exertion (RPE) was also recorded at the end of each stage. After the conclusion of the exercise, subjects were allowed to recover actively or passively. However, subjects were not allowed to leave the laboratory until their HR was below 100 beats per minute (bpm). The VO_{2peak} was considered valid if three of the following criteria were met: the participant achieved a respiratory exchange ratio (RER) of 1.1 or higher, a HR greater than or equal to the age predicted maximum HR for the subject (220 – age ± 5%), and must report an RPE of 18 or greater (36).

At the end of the orientation session, the subject were given a canister of Polycose® glucose polymer (Ross Laboratory, Columbus, OH) and given instructions for its consumption. The day before beginning the training sessions, each subject consumed 25% of the canister (approximately 87 grams). Subjects then consumed an additional 25% of the canister on each of the training session days and then returned the empty canister at the final visit to the laboratory to ensure compliance.

Sessions I-III: Training Sessions. For each of the training sessions the subject cycled for 60 minutes at a workload that will elicit 75% of VO_{2peak}. This workload was calculated using a linear regression equation obtained from plotting the oxygen consumption against each corresponding workload from the orientation session VO_{2peak} test (36).

No less than five days after completing the VO_{2peak} test and orientation session, the participant returned to the Applied Physiology Laboratory in the morning between

6:00 a.m. and 10:00 a.m. to begin the three consecutive training sessions. Every participant came to the laboratory at the same time for each of the three training sessions and blood samples were taken at the same time each day. Participants arrived after an overnight fast of at least 8 hours. Two days prior to beginning the training sessions, the subjects were instructed to complete an easy day of training. The day before beginning the trials the subjects were instructed to take a rest day of no training. Subjects also compiled a food diary during the day before beginning the training sessions and on each of the training session days.

After arriving to the laboratory, the subjects assumed a supine position and rested for ten minutes. After resting, a 3 mL blood sample was obtained using a standard venipuncture technique. The blood sample were then transferred from the syringe into a sterile K^2 EDTA (purple top) Vaccutainer® tube and put on ice. The subjects then completed a ten-minute warm-up consisting of five minutes of cycling at 50W and five minutes of stretching focused on the lower body. After completing the warm-up the subjects returned to the cycle and resting VO_2 was collected for four minutes. Subjects then cycled for 60 minutes at the previously determined workload to elicit 75% of VO_{2peak}. This exercise intensity and duration was chosen to mimic an intense training session that would be performed by a highly trained endurance athlete.

Heart rate and RPE were monitored throughout the training sessions and recorded every ten minutes beginning at 0 minutes. Oxygen consumption was monitored throughout the training sessions and recorded for four minutes every twenty minutes (i.e. minutes 16-20, 36-40, and 56-60)(see Figure 3). The subject was allowed to ingest water *ad libitum* throughout all training sessions.

Immediately after the cessation of exercise, the participants returned to the supine position and a 3 mL blood sample was obtained using the same procedures used prior to commencing exercise. The subjects were permitted to recover either actively or passively and were allowed to leave the laboratory after their HR was less than 100 bpm.

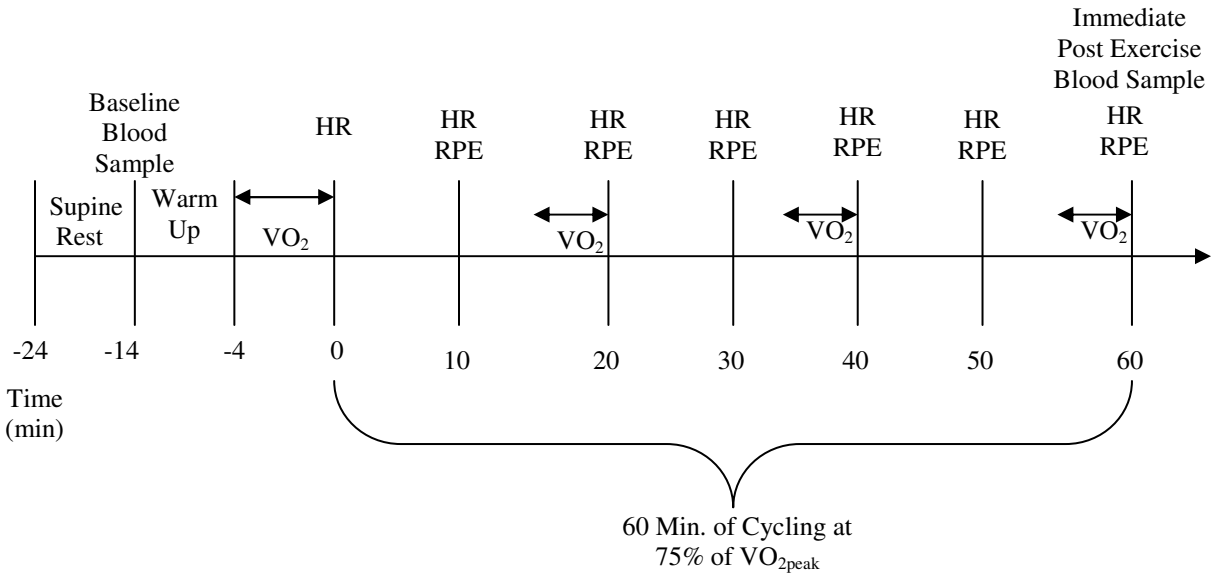


Figure 3. Overview of the protocol for the training sessions (Sessions I-III) is presented above (adapted from Behr [33]).

Session: Rest Day. Approximately 24 hours after completing Session III the subjects returned to the Applied Physiology Laboratory for a final resting blood sample. Subjects came into the laboratory at the same time as they had for the previous three sessions in order to obtain the resting blood sample at approximately the same time of day. The subjects assumed a supine position and rested for ten minutes. After the rest period, a 3 mL blood sample was obtained using a standard venipuncture technique. The

blood was transferred into a sterile K^2 EDTA (purple top) Vaccutainer® tube and immediately put on ice.

Blood Procedures

Hematocrit. Resting and post-exercise hematocrit (Hct) values were obtained in triplicate from the whole blood samples. Blood was drawn up into 75 mm microhematocrit capillary tubes (Fisher Scientific International Inc., Hampton, NH) and sealed using Critoseal (Krakeler Scientific, Inc., Albany, NY). The capillary tubes were then be placed into a microhematocrit centrifuge and spun for three minutes. After being spun, the tubes were removed from the centrifuge and placed on a hematocrit reader to determine the percentage of formed elements in each sample. A mean value was computed from the triplicate samples and used in data calculations.

Hemoglobin. Resting and post-exercise hemoglobin (Hb) values were obtained in triplicate from whole blood samples using a cyanmethemoglobin technique. Five mL of cyanmethemoglobin was dispensed into each test tube. Twenty microliters (μL) of whole blood was pipetted into the test tubes. Each test tube was then covered using parafilm and allowed to rest for three minutes.

After three minutes, the samples were individually read on a spectrophotometer at a wavelength of 540 nM. Hemoglobin concentration was calculated using the absorbance versus concentration relationship (Beer's Law) devised for standard hemoglobin concentration. A mean value was computed for each triplicate hemoglobin sample (resting and post-exercise).

Plasma Volume Shift. Changes in plasma volume due to exercise were calculated using Hct and Hb and the equation of Dill and Costill (37). Plasma volume changes were reported so as to indicate the effect of exercise induced fluid shifts on hormonal concentration.

Hormonal Analysis. Stored plasma samples were analyzed for both cortisol and free testosterone using a radioimmunoassay (RIA) protocol specific to each hormone. The details of these assays are reported in Appendix E. The sensitivity for the cortisol and free testosterone assays were 0.2 μg/dL and 0.15 pg/mL respectively. The coefficient of variation was calculated for each assay and reported in the results section of this study.

Data Analysis. Data analysis was performed using a computer based statistical software program (SPSS version 14.0, Chicago, IL). All data results are reported as mean ± standard deviation (SD).

To determine the effect of the training sessions on the resting value of the fTC ratio (hypothesis 1) a one-way (4 levels) within ANOVA was used. If a significant F-ratio was observed then a Tukey post hoc test was computed to determine which means were different from one another.

The cardiorespiratory responses to the training sessions (i.e., VO_2, HR, and RPE) were compared using a totally within 3X2 analysis of variance (ANOVA). If a significant F-ratio was observed then a Tukey post hoc test was computed to determine which means were different from one another. All ANOVA analysis and post hoc testing have a statistical significance set at $\alpha = 0.05$.

CHAPTER IV

RESULTS

Subject Characteristics

Twenty-seven highly trained male endurance athletes were recruited for participation in this study. From these 27 individuals 12 completed the experimental protocol. Table 2 displays the physical characteristics of these subjects. The other 15 recruited athletes were unable to complete the protocol due to scheduling, time commitments, or did not meet the inclusion criteria.

Table 2. Physical characteristics of the subjects (n = 12). Values are mean ± standard deviation (SD).

Age (yr)	**Height** (cm)	**Mass** (kg)	**Body Fat** (%)
27.1 ± 5.8	179.4 ± 6.8	75.0 ± 7.3	12.1 ± 3.3

All subjects were exercising for a minimum of five days per week for 60 minutes or more. Subjects were also participating in or training for competitive endurance sporting events. The subjects were active in a variety of endurance sporting events including running, cycling, or triathlons. Additionally, all subjects complied with the experimental guidelines during the three training sessions (Sessions I-IV). See the Methods chapter for more details about these guidelines.

VO_{2peak} Testing

Subjects completed the VO_{2peak} testing approximately one to four weeks prior to the training sessions. In order for a VO_{2peak} test to be considered valid a number of criteria needed to be met (36). Of the 12 subjects, ten of them met all of the criteria for a valid test. Of the two who did not meet the criteria, one subject did not reach the RER requirement (1.08 versus 1.10) and the other did not reach the HR criteria (171 bpm versus age predicted max of 197 bpm). The results of the VO_{2peak} tests are displayed below in Table 3.

Table 3. Results of the VO_{2peak} test for all subjects (n = 12). Values are mean ± SD. Abbreviations are defined in the text.

Measure	Value
VO_{2peak} (L/min)	4.52 ± 0.76
VO_{2peak} (mL/kg/min)	60.1 ± 6.6
Max RER	1.20 ± 0.07
Max HR (bpm)	192.1 ± 6.5
Max RPE	19.3 ± 0.7
Test Duration (min)	18.9 ± 2.5
Max Workload (W)	350.0 ± 47.7

Training Sessions

All 12 subjects completed three training sessions separated by 24 hours (I-III). Each subject also participated in a resting session (Rest Day Session - IV) that took place 24 hours following the third training session. Similarly, all subjects complied with pre-training session guidelines established by the investigator (see Methodology chapter).

Cardiovascular-Respiratory Responses. Each training session consisted of 60 minutes of cycling at a workload prescribed to elicit 75% of VO_{2peak}. The actual mean session workload was 217.1 ± 34.1 W, which elicited a mean VO_2 of 3.33 ± 0.51 L/min,

3.34 ± 0.49 L/min, and 3.27 ± 0.52 L/min for sessions I, II and III respectively. This corresponded to 73.8 ± 6.6%, 74.2 ± 7.7%, and 72.7 ± 7.6% of VO_{2peak} for sessions I, II, and III respectively (See Table 4).

The VO_2 data shows that the subjects were cycling at a physiological steady state, as there was not a significant main effect for time (i.e., VO_2 at 20 minutes was not different than VO_2 at 40 or 60 minutes). Similarly, there was not a significant main effect for session nor was there a significant interaction effect (See Table 4).

The results of the HR data show that there was a significant main effect for session, and for time; however there was not a significant interaction effect. Tukey HSD post hoc analysis for the main effect of session showed that the HR response to session I was greater than that of session III ($p = 0.002$). Post hoc analysis for the main effect of time showed that HR at minute 10 was significantly lower than the HR at minutes 30, 40, 50, and 60 ($p = 0.011, 0.006, 0.011, 0.041$, respectively).

There was a significant main effect for session and for time and a significant interaction effect for rating of perceived exertion (RPE). Tukey HSD post hoc analysis showed that the RPE values for all time points in sessions I and II were not statistically different. However, RPE values at minutes 40, 50, and 60 in session I were significantly greater than the RPE score at the same time points in session III ($p = 0.004, 0.0001, 0.008$). Similarly, the RPE values at minutes 50 and 60 for session II were significantly greater than those at the same time points in session III ($p = 0.0008, 0.008$). However, the largest mean difference was approximately 1.5 units on the original 6-20 point Borg scale. This corresponds with a difference in perceived exertion of "somewhat hard" and "hard."

Table 4. VO_2, HR, and RPE responses for sessions I, II, and III are displayed as mean ± SD. Dashed line (---) indicates that the parameter was not recorded at this time point. Post hoc analysis on the main effect for time for HR showed the HR at minutes 20, 30, 40, 50, and 60 were significantly higher than the HR at minute 10. † Indicates a significantly different RPE value compared to the same time points in session I and ‡ indicates a significantly different RPE value compared to the same time points in session II.

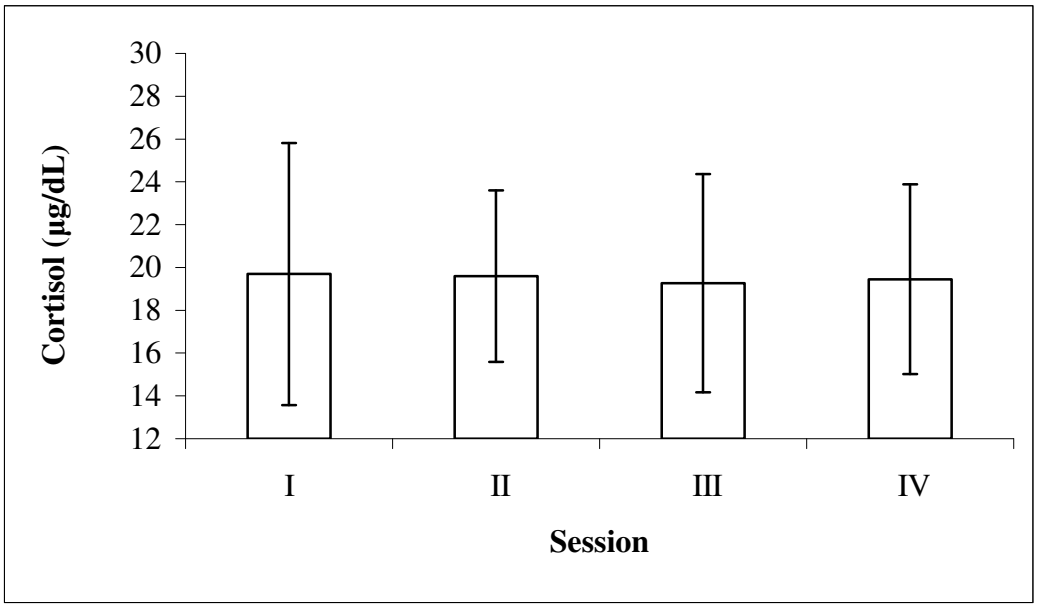
		Time (minutes)						
	Measure	**Rest**	**10**	**20**	**30**	**40**	**50**	**60**
Session I	**VO_2** (L/min)	0.35 ± 0.05	---	3.37 ± 0.59	---	3.32 ± 0.51	---	3.29 ± 0.47
	HR (bpm)	61.4 ± 9.4	157.8 ± 18.5	161.9 ± 17.4	163.8 ± 15.0	163.7 ±13.4	163.8 ± 14.4	162.3 ± 13.9
	RPE	---	13.8 ± 1.2	14.5 ± 1.4	15.3 ± 1.5	16.1 ± 1.8	16.3 ± 1.7	16.0 ± 1.7
Session II	**VO_2** (L/min)	0.35 ± 0.06	---	3.37 ± 0.55	---	3.36 ± 0.47	---	3.29 ± 0.49
	HR (bpm)	61.9 ± 10.0	152.8 ± 16.6	157.9 ± 15.1	161.5 ± 14.3	161.2 ± 12.2	161.1 ± 13.3	160.8 ± 13.6
	RPE	---	13.8 ± 1.3	14.7 ± 1.4	15.3 ± 1.4	15.8 ± 1.5	16.0 ± 1.3	16.0 ± 1.5
Session III	**VO_2** (L/min)	0.36 ± 0.05	---	3.29 ± 0.57	---	3.28 ± 0.52	---	3.23 ± 0.5
	HR (bpm)	61.3 ± 9.4	151.8 ± 16.5	155.3 ± 15.0	156.3 ± 13.3	157.8 ± 13.5	156.8 ± 15.5	156.0 ±13.7
	RPE	---	13.7 ± 1.9	14.4 ± 1.7	14.7 ± 1.4	† 15.0 ± 1.6	† ‡ 14.8 ± 1.4	† ‡ 15.0 ± 1.7

During the training sessions the subjects lost a substantial amount of plasma volume. Mean plasma loss was -17.5 ± 6.6%, -17.0 ± 4.6%, and -16.8 ± 6.4% for training sessions I, II, and III respectively ($p = 0.931$). Statistical analysis showed that plasma volume loss did not significantly differ between training sessions. Similarly, there was not a significant change in pre-exercise (resting) plasma volume between trials ($p = 0.627$). The mean pre-exercise plasma volume changes were -3.0 ± 6.4%, -3.6 ± 5.1%, and -4.1 ± 6.9% for sessions I to II, I to III, and I to IV respectively.

Hormonal Response

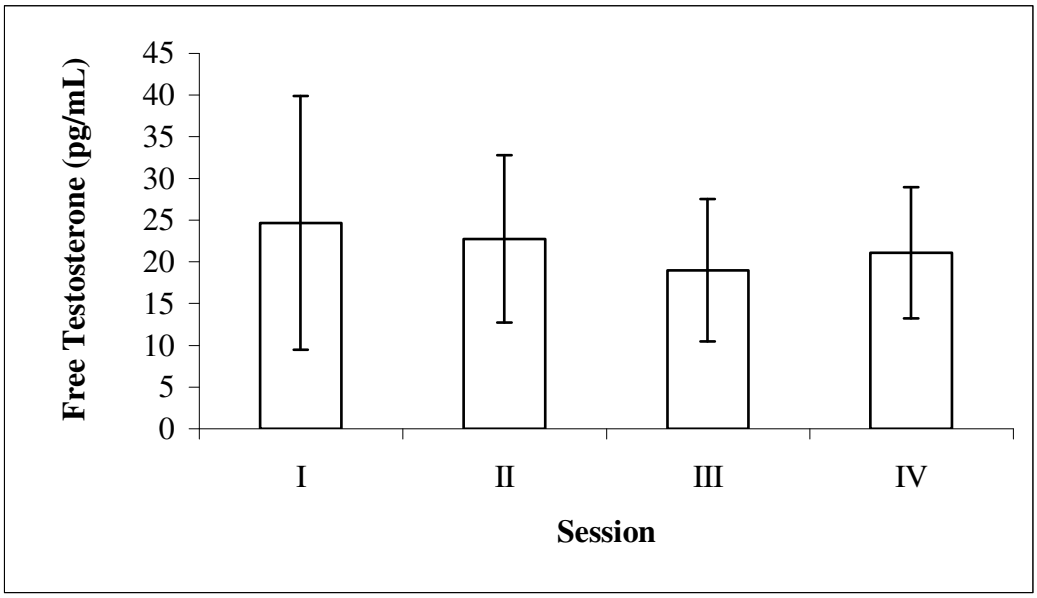
Cortisol. The resting concentrations of cortisol (i.e., pre-exercise) during sessions I, II, III, and IV did not show a significant difference ($p = 0.947$, see Figure 4). However, the cortisol concentration pre- and post-exercise (3X2 ANOVA) showed there was a significant main effect for session and for time ($p = 0.008$ and $p = 0.018$, respectively), as well as, a significant interaction effect ($p = 0.037$) (See Table 5). In sessions I and II there was a significant increase in cortisol concentration pre- to post-exercise. However, the post-exercise cortisol concentration in session III was significantly lower than that of session I.

Figure 4. Mean ± SD of the pre-exercise cortisol concentration in each training session (n = 12). A repeated measures ANOVA did not show a significant difference in resting concentrations between sessions (p = 0.947).



Free Testosterone. There was not a significant difference in the resting concentration (i.e., pre-exercise) of free testosterone during sessions I, II, III, and IV (p = 0.129) (See Figure 5). However, the free testosterone concentration pre and post-exercise (3X2 ANOVA) showed that there was a significant main effect for session and for time (p < 0.0005 and p = 0.022, respectively), but no interaction effect (p = 0.516) (See Table 5). The Tukey HSD post hoc analysis on the main effect for session showed the free testosterone concentration in sessions I and II were significantly greater than that of session III (p = 0.037, 0.042). The Tukey HSD post hoc analysis on the main effect for time showed that the post-exercise blood sample was significantly greater than that of the pre-exercise (p < 0.0005) (See Table 5).

Figure 5. Mean ± SD of the resting free testosterone concentration prior to each training session (n = 12). A repeated measures ANOVA did not show a significant difference in concentrations between sessions (p = 0.129).



Free Testosterone to Cortisol Ratio. There was no significant difference in resting concentration (i.e., pre-exercise) between sessions I-IV (p = 0.254) (see Figure 6). Similarly, the 3X2 ANOVA on the pre- to post-exercise response to the fTC ratio showed there to be no significant main effect for session or for time (p = 0.797 and p = 0.750, respectively), but an interaction effect that was approaching significance did occur (p = 0.051) (See Table 5). This shows that the greatest difference in pre- to post-exercise comparisons (session I) was almost significantly different.

Table 6 shows the fTC ratio values after they have been corrected for changes in plasma volume. The plasma volume corrections were applied in an attempt to discern by what means the fTC ratio was being affected (i.e., production-clearance vs. hemoconcentration). The 3X2 ANOVA on the pre- to post-exercise response showed that there to be no significant main effect for session or for time (p = 0.739 and p = 0.582,

respectively), but there was a significant interaction ($p = 0.044$). The Tukey HSD post hoc analysis showed there was a significant decrease in the fTC ratio pre- to post-exercise in session I.

Figure 6. Mean ± SD of the resting free testosterone to cortisol ratio value prior to each training session (i.e., pre-exercise) ($n = 12$). A repeated measures ANOVA did not show a significant difference in concentrations between sessions ($p = 0.254$).

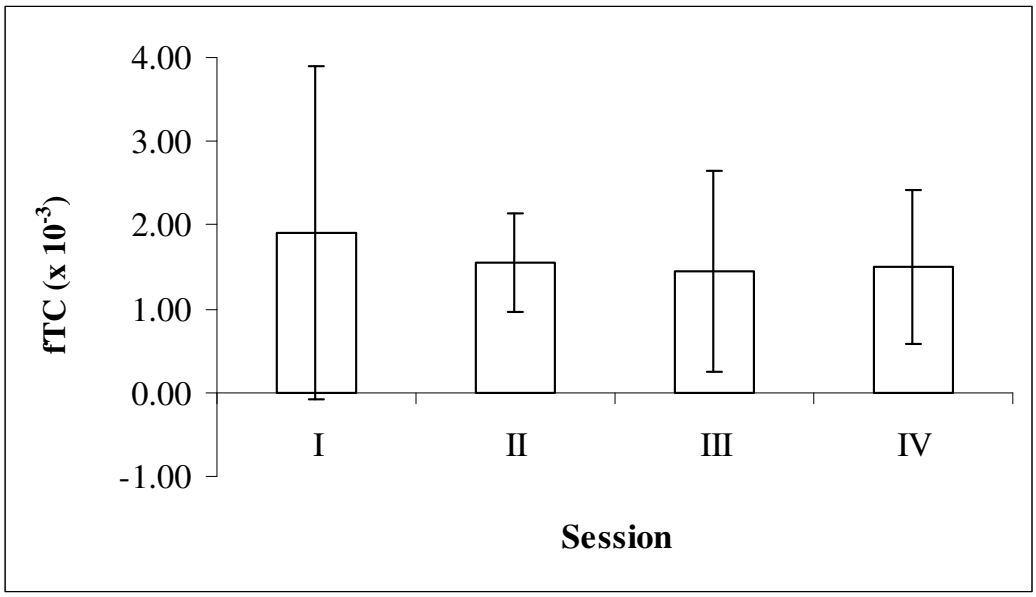


Table 5. Mean ± SD pre-exercise and post-exercise values of cortisol, free testosterone, and the fTC ratio. * Indicates a significantly higher post-exercise value ($p < 0.005$) and † indicates a significantly lower post-exercise value compared to session I for cortisol. ‡ Indicates a significantly higher post-exercise value ($p < 0.0005$). Post hoc analysis on the main effect for session for free testosterone showed that there was a significantly higher concentration in sessions I and II when compared to session III for free testosterone.

Session	**I**		**II**		**III**		**IV**
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**
Cortisol (μg/dL)	19.7 ± 6.1	* 27.6 ± 5.8	19.6 ± 4.0	* 25.5 ± 7.7	19.3 ± 5.1	† 22.5 ± 8.3	19.4 ± 4.4
Free Testosterone (pg/mL)	24.7 ± 15.2	‡ 32.7 ± 10.5	22.8 ± 10.0	‡ 34.4 ± 13.7	19.0 ± 8.5	‡ 29.7 ± 11.3	21.1 ± 7.9
fTC (x 10^{-3})	1.91 ± 1.99	1.55 ± 0.58	1.55 ± 0.94	1.82 ± 0.84	1.45 ± 1.21	1.79 ± 0.69	1.49 ± 0.91

Table 6. Mean ± SD pre-exercise and post-exercise values for the fTC ratio after a correction for the changes in plasma volume that occurred during each session. * Indicates a significantly lower post-exercise value ($p = 0.044$).

Session	**I**		**II**		**III**		**IV**
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**
fTC (x 10^{-3})	1.91 ± 1.99	* 1.27 ± 0.47	1.55 ± 0.94	1.57 ± 0.74	1.45 ± 1.21	1.51 ± 0.62	1.49 ± 0.91

CHAPTER V

DISCUSSION

Introduction

The purpose of this study was to determine the effect of three days of intense exercise training (i.e., a training micro-cycle) on the fTC ratio. The study was designed to help determine the acute effect of exercise on the fTC ratio and also whether the ratio would return to "normal" levels by 24-hour after each exercise session. Similarly, the investigator wanted to determine if three days of intensive exercise would be stressful enough to cause the athletes to enter an excessive catabolic hormonal (i.e., maladaptive) state. It was hypothesized that the fTC ratio would significantly decrease following each day of exercise. Figure 7 displays in a generalized fashion the projected fTC ratio results hypothesized.

This chapter begins with a discussion about the training session responses. In this first section the VO_2, HR, and RPE responses to the exercise sessions are discussed. Next the hormones cortisol and free testosterone are considered. This section includes discussion on the resting values and exercise responses of each hormone. Finally, the analysis focuses on the results and implications of the fTC ratio. The chapter concludes with a brief summary of the findings and overall implications of this study.

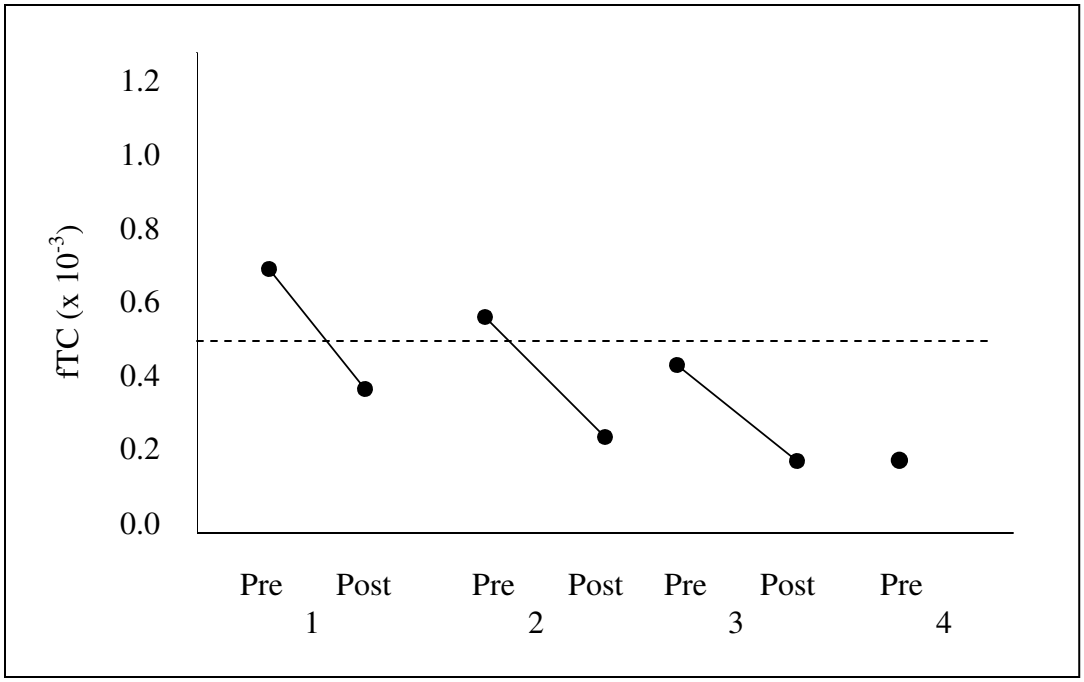


Figure 7. The projected results of the fTC ratio. The dashed line signifies a decrease of 30% from the initial pre-exercise value (i.e., before commencement of the sessions).

Training Sessions

As was reported in the previous chapter, the workload (held constant at each session) that the subjects cycled at elicited 73.8 ± 6.6%, 74.2 ± 7.7%, and 72.7 ± 7.6% of VO_{2peak} for sessions I, II, and III respectively, which is slightly lower than the goal intensity of 75% of VO_{2peak}. A regression equation was used to predict the workload that would elicit 75% of VO_{2peak}, however the ventilatory threshold was taken into consideration before the subject began their training sessions and workload prescriptions adjusted slightly. This adjustment was to ensure that the subject could cycle for the prescribed 60 minutes and repeat the exercise three consecutive days. Specifically, if the workload predicted by the regression equation was above the ventilatory threshold then the subject might not have been able to reach a steady state. If this had occurred, it is likely that the subject could not complete the

prescribed exercise sessions. Similarly, the investigator adjusted the workload during the first training session in order to keep the VO_2 as close to the prescribed level as possible. Any adjustments made to the workload during training session I were repeated in sessions II and III to ensure the workloads were identical for all three sessions.

As can be seen from the findings in the Results chapter (Table 4), VO_2 did not significantly differ within or between the sessions. Thus, while the intensity was slightly below the desired (~2.3 to 0.8%), the level of intensity was constant across the three exercise sessions. However, RPE and HR did vary within and between sessions. Heart rate was significantly lower at 10 minutes into exercise than at minutes 30, 40, 50, and 60 of exercise (i.e., consistent finding across all three sessions). This most likely occurred due to a series of events termed "cardiac drift." During prolonged exercise blood is shunted away from the core to the periphery in order to dissipate heat. This causes plasma volume to decrease and venous return to decrease, thereby decreasing stroke volume. In order to maintain cardiac output, HR must increase (38). Furthermore, HR responses in session I were significantly greater than that of session III. This may be attributable to the slightly greater loss in plasma volume during session I versus session III ($-17.5 \pm 6.6\%$ and $-16.8 \pm 6.4\%$ respectively). Interestingly enough, the subjects reported that session III was the easiest of the three (see below).

Ratings of perceived exertion were higher during the second half of each of the three training session. This indicates that the subjects perceived the exercise to be more stressful as time proceeded. By the end of exercise in each session, RPE had reached a rating of approximately 15-16. This level of rating is indicative that the subjects are performing demanding exercise. In a somewhat similar fashion to the HR findings, results show that RPE

was lower during session III compared to session I and II, which is consistent with the subjects reporting session III being the easiest (subjectively determined by asking subject upon completion of exercise).

It is important to recognize that the exercise workload requirement of the subjects was held exactly constant at each of the three sessions. The finding of slightly lower HR and RPE responses in session III suggests the subjects were accommodating and adapting to the demands of the training. In other words, it appears they were not being "too stressed" by the training and compromised in some way.

While there were some statistically significant differences between select variables in each of the training session, essentially the desired "treatment effect" of the experiment design was achieved. That is, the subjects completed three consecutive days of intensive training at approximately 75% of VO_{2peak} in a controlled and exactly replicated manner.

Cortisol

The mean pre-exercise cortisol concentrations were consistent with expected values for normal adult males in the morning according to a standard clinical reference (39). Similarly, studies conducted on athletes of comparable training level and ages have also found resting cortisol values that are in agreement with those seen in the present study (17, 40, 41).

Cortisol levels increased immediately post-exercise in sessions I and II, but not in session III. For an increase in cortisol to be seen an intensity threshold of approximately 60% of VO_{2max} must be met (15). As previously discussed, the mean intensity that subjects in the present investigation exercised at was 73.6 ± 7.1% of VO_{2peak} (mean of all three

sessions), which exceeds the intensity needed. The post-exercise cortisol concentrations (22-27 μg/dL) were comparable to those found in other research studies following similar bouts of exercise. Inder et al. (17) had 6 highly trained male triathletes cycle for 60 minutes at approximately 70% VO_{2max} and had a post-exercise cortisol concentration of approximately 28 μg/dL. Daly et al. (41) had 22 highly trained male endurance athletes run to volitional fatigue at approximately 100% of their ventilatory threshold. The average time to fatigue was 84.8 minutes. Immediately post-exercise these subjects had cortisol concentrations of approximately 24 μg/dL. For Daly et al.(41), the exercise mode was different and duration was longer than in the present investigation, but the exercise intensities were similar between the two studies.

Although the workloads in the present study were identical for all three training sessions, the cortisol response to session III differed from the previous two sessions. This is consistent with subjects reporting session III to be the easiest (see comments above). Some researchers have suggested that multiple consecutive days of intense exercise can have a rapid effect on the hypothalamic-pituitary-adrenal axis and thereby affecting cortisol release (42, 43). This is illustrated by Fellmann et al. (42) who monitored the cortisol concentration in 11 males during a 6-day Nordic ski race. The researchers found pre- and post-competition cortisol concentrations to be significantly lower during the second half of the race (i.e., days 3-6) despite the intensity and duration of competition being similar. This significantly lower cortisol concentration was present despite there being no difference in circulating ACTH concentration between the days. The researchers suggest that a decrease in adrenal sensitivity to ACTH may be the gland's way of adapting to repetitive or prolonged stress.

Free Testosterone

The mean pre-exercise concentrations of free testosterone fell outside what clinical reference textbooks site as the average concentration of free testosterone in adult males (50-210 pg/mL) (39). However, endurance trained male athletes have been shown to have a lower concentration of free testosterone than untrained adult males of the same age (19, 25, 26). For example, Hackney et al. (25) reported endurance-trained athletes with mean resting free testosterone concentrations of approximately 17.3 pg/mL (or less than 50% of typically clinical values), which is similar to the present findings.

Exercise caused the concentration of free testosterone to significantly increase in each of the training sessions. This result has been demonstrated in the literature by several investigators. Wilkerson et al. (22) found total testosterone to increase following 20 minutes of submaximal treadmill running of varying intensities. The investigators suggested that all increases in testosterone could be attributed to hemoconcentration of the blood. Similarly, Vogel et al. (44) found free testosterone to increase from 10.3 ng/dL to 12.9 ng/dL following 45 minutes of submaximal cycling. The investigators speculate that the increase in free testosterone was due to an increase in catecholamine stimulus to the testis, hemoconcentration, and/or a decrease in metabolic clearance rate due to decreased hepatic blood flow. However, the investigators stated that the exact mechanism for the observed increase could not be determined from the data obtained in the study.

Although an increase in testosterone following exercise has been demonstrated, other aspects of the literature are equivocal on this point. That is, some studies show no change in testosterone concentration following prolonged exercise, while others show a decrease in concentration. Jurimae et al. (10) had elite rowers skull for 2 hours at 75% of their anaerobic

threshold (~50-55% of VO_{2max}) and found no change in free testosterone concentration immediately post-exercise. Galbo et al. (24) found testosterone to decrease following 60 accumulated minutes of running at approximately 76% of VO_{2max}.

There was a significant main effect for session for free testosterone. Post hoc analysis showed that free testosterone concentration in session III was significantly lower than sessions I and II. Although there was not a significant difference in pre-exercise free testosterone concentrations between sessions, there is a trend towards a decrease (i.e., session I = 24.7 ± 15.2, session II = 22.8 ± 10.0, session III = 19.0 ± 8.5 pg/mL; $p = 0.129$). Free and/or total testosterone has also been shown to decrease during times of intense training. Urhausen et al. (8) monitored the resting concentration of total testosterone and cortisol during 7 weeks of intense row training. These investigators found that total testosterone decreased during times of intense training or competition. They found total testosterone to decrease from 21.1 ± 1.5 nmol/L to 18.6 ± 2.1 nmol/L, which is a similar magnitude as the present investigation. Urhausen and Kindermann (31) found that total testosterone decrease one day after a triathlon competition (~ 177 minutes in duration). The free testosterone hormonal portion was not measured, but was calculated using total testosterone and sex hormone binding globulin (SHBG) values. The calculated free testosterone (index) was found to significantly decrease following the triathlon competition.

These investigators interpreted these changes in testosterone as being the consequence of the exercise stress (8, 31). Physiologically the body reduces testosterone production and secretion due to inhibitory actions at the central and peripheral component of the hypothalamic-pituitary-gonadal regulatory axis (8, 31). Some investigators see such actions as a necessary energy conservation measure and recovery-regeneration step in

athletes following periods of intensive training or demanding competition (45). While there was a trend towards lowered resting testosterone levels in the present findings, the changes were not significant and suggest the training did not reach the level necessary to provoke this stress response.

Free Testosterone to Cortisol Ratio

As previously discussed, the resting cortisol and free testosterone concentrations were within the expected range for athletic males, thereby making the ratio values within the normal expected range of values. Furthermore, the ratio values found are in general agreement with those reported in the literature (10, 30).

There were slight, but not significant changes pre- to post-exercise in the fTC ratio in each of the training sessions. Following session I the fTC ratio decreased from 1.91 ± 1.99 to 1.55 ± 0.59. However, following sessions II and III the fTC ratio increased from 1.55 ± 0.94 to 1.82 ± 0.84 and 1.45 ± 1.21 to 1.79 ± 0.69 respectively. Previous literature has shown the fTC ratio to respond to an acute single bout of exercise typically resulting in reduced values. Specifically, Jurimae et al. (10) showed the fTC ratio to decrease from approximately 1.25 to 1.1 following skulling at 75% of the subjects' anaerobic threshold. Although the intensity in the latter study was considerably lower than that of the present investigation this ratio change is of similar magnitude as seen in session I. Daly et al. (41) found approximately a 55% reduction in the fTC ratio (calculated from mean data) following running to volitional fatigue at a similar intensity to the current study. Similarly, Duke et al. (11) found approximately a 75% reduction in the fTC ratio following running to volitional fatigue at approximately 70% of VO_{2max}. Reductions in the fTC ratio are thought to reflect a shift towards a more catabolic hormonal status (5, 21; see later discussion).

To the investigator's knowledge there has been no literature showing an increase in the fTC ratio following an acute bout of exercise. However, such a finding was seen in response to exercise in sessions II and III of the present study. Thus, this may be new and novel research findings. Converse to the point made above, an increase in the fTC ratio may reflect a shift towards a more anabolic hormonal status (5, 21). This would suggest the subjects where in a more constructive-adaptive state in the immediate period following the exercise sessions. This interpretation is one postulated by Viru and Viru (12). However, this is speculative since no direct outcome measures of protein turnover were measured in the present study. It is important to note, that in the current study the fTC ratio values were initially not corrected for changes in plasma volume shifts. When this correction was done (see Table 6 in Results) the direction of change following exercise in sessions II and III was reversed (i.e., no change rather than increase) and would agree with changes reported in the literature. Regrettably, it is unclear in some of the studies in the literature reporting reduced fTC ratios after exercise whether plasma volume shift corrections were performed by the investigators.

Despite the fact that there were small changes in the fTC ratio that occurred in response to exercise, the ratio returned to pre-session levels 24-hours following each training session. Although the exercise was intense enough to elicit an acute change in the fTC ratio the physiological stress placed on the subjects was not great enough to cause the ratio to remain disturbed to the 24-hour point. Similarly, the fTC ratio recovery pattern (i.e., returning to pre-exercise levels) followed the same manner in response to each of the three training sessions. This suggests the fTC ratio is stable and undisturbed when being examined across a short-term micro-cycle of training. In other words, the fTC ratio appears to be a

reliable measure. This result may give strength to other studies that have found a significant change in the fTC ratio during intense training and competitions (i.e., if it does display a change 24-hours post exercise then there has been a substantial alteration in the hormonal status of an individual). Thus, demonstrating stability and reliability in this marker may show that when a significant change is seen (in other future studies), it is reflecting a meaningful physiological event. Some researchers have been critical of the fTC ratio and have suggested that intense exercise training may not bring about a substantial and consistent change in the fTC ratio (4). That is, the ratio may not respond similarly to the same bout of exercise and/or may respond differently in individuals undergoing the same exercise training. The current findings would not seem to support this assertion.

Similar to the present findings, other researchers have found the fTC ratio (or total testosterone to cortisol ratio) to change and then return to normal values following intense bouts of exercise. Urhausen and Kindermann (31) measured the total testosterone to cortisol ratio one day before and immediately after a triathlon competition. They found the ratio to significantly decrease (i.e., approximately 82%) immediately post-competition, however the ratio returned to pre-competition values one day into recovery. Likewise, Duke et al. (11) found the fTC ratio to change to an acute bout of running and then return to normal values after 24 hours of recovery. Both of these studies placed a large amount of physiological stress on the subjects causing the fTC ratio to dramatically change (reduce), but in each case the ratio returned to normal 24 hours following the exercise. These study results, along with the current findings, suggest that a more prolonged period of intense exercise, or more than three days of intensive training may be needed to elicit a long term, pronounced change in the resting fTC ratio value. However, conversely Daly and associates (41) found the fTC

ratio to remain slightly decreased (approximately 8% less) 24 hours following running to volitional fatigue when compared to pre-exercise values.

Summary and Significance

It was first proposed in the 1980's that the fTC ratio might be a meaningful reflection of the anabolic and catabolic hormonal status (balance) in skeletal muscle. Furthermore it was proposed that this ratio may change in response to exercise and exercise training (5, 8, 18). In theory it is hypothesized that an excessive increase in muscle catabolic activity creates an imbalance in protein breakdown versus synthesis (21), which may not allow optimal exercise training adaptations to occur at the cellular level (1, 5). Thus, if this type of hormonal environment (i.e., increased catabolism and decreased anabolism) is prolonged it may lead to a decrease in athletic performance and result in the athlete perhaps becoming overtrained (1, 3). To this date, there are still many researchers who are advocates of using the fTC or total testosterone to cortisol ratio as a viable biological marker of exercise training. Many of these researchers feel that the ratio is indicative of whether exercise training is being efficacious or not (see review article [14]). There are, however, some who suggest that the measure lacks validity or that some researchers have perhaps "over" interpreted its importance (12). However, the intent of the present study was not to examine whether the ratio truly reflects accurately the internal milieu of cellular events. The validity of this measurement for representing cellular status must be addressed by others and was beyond the scope of this study.

The current findings are important for a number of reasons and provide meaningful contributions to the research literature. The training load was not intense enough to cause a

change in the resting fTC ratio value. The training utilized, however, was very representative of the training loads typically employed by persons involved with athletic training. The finding of no changes in the fTC ratio demonstrate that the ratio is stable and reliable in response to such training loads. As discussed above, other studies also have found the fTC ratio to return to normal resting values following acute intense exercise (11, 31). Collectively, this may suggest that more prolonged, intense training may be needed to cause a true upset in the anabolic-catabolic balance at the skeletal muscle (as assessed by the fTC ratio). Three days of intensive training does not seem to be sufficient to induce such changes. One point important to emphasize, is the fact that the subjects in this study maintained a well-balanced diet and consumed high amounts of carbohydrate. This is critical because low carbohydrate consumption exacerbates the cortisol response to exercise (14) and thus could provoke more dramatic and persistent changes in the fTC ratio (18).

To conclude, if the ratio is a valid biological marker, then the current data suggest that athletes and coaches can utilize such three day micro-cycles within their training regimes without excessive concern that this could be too demanding and counter productive. It would appear that findings of changes during intense periods of training during an athletic season may be more meaningful now that the stability and reliability of the fTC ratio has been demonstrated. One criticism of the fTC ratio has had to do with the lack of stability within the measurement (4). That is, some have suggested that the response of the fTC ratio are unique to the individual and two athletes may not have the same responses of the ratio to an identical training load. However, the current results would discount that concern and imply that the fTC ratio is stable and reliable measure.

CHAPTER VI

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

The purposes of this investigation were to determine if the free testosterone to cortisol ratio (fTC) is a reliable marker that can be used to monitor an athlete's training and possibly diagnose overtraining and/or the Overtraining Syndrome. The investigator also wanted to see if a three-day intense micro-cycle of training causes an athlete to become overtrained. Twelve highly trained male endurance athletes cycled for 60 minutes at 75% of their VO_{2peak} for three consecutive days. Blood samples were taken immediately pre- and post-exercise and were analyzed for free testosterone and cortisol from which the fTC ratio was calculated.

The intensity that the subjects cycled at was 73.6 ± 7.1% of VO_{2peak} (mean of all three sessions) and based on the results the subjects were exercising intensely and the desired treatment effect was obtained. VO_2 did not differ between or within the three sessions. However, HR and RPE varied within and between the sessions. HR and RPE increased as the time progressed in each session. Similarly, session III appeared to be the least stressful session when compared to sessions I and II. Cortisol significantly increased pre- to post-exercise in sessions I and II, but not session III. Similarly, free testosterone increased significant pre- to post-exercise in each session. However, the resting concentrations of these hormones did not significantly differ between sessions I, II, III, and IV. The fTC ratio also did not significantly change pre- to post-exercise, but some slight changes did occur.

However, when slight changes occurred the fTC ratio returned to "normal" levels as the resting concentration of the fTC ratio did not significantly differ between the sessions (I vs. II-IV).

Results of this investigation suggest that the fTC ratio is a reliable measurement as it did not significantly change during the training. This may indicate that the fTC ratio may be useful in the monitoring of training, as well as, the diagnoses of overtraining. Results also suggest that a three-day intense micro-cycle of normal exercise training was not enough stress to cause the subjects to become overtrained. Therefore, coaches may be able to prescribe this type of micro-cycle to their athletes without fear of causing them to become excessively stressed.

Conclusions

The investigator hypothesized that the resting fTC ratio levels at 24-hr following each exercise session will be significantly suppressed from resting levels obtained prior to the study period. Based on the results of the present study the investigator rejects this hypothesis.

Recommendations

The following recommendations are made relative to future research work studying the fTC ratio and exercise;

- Have subjects perform more than three days of intensive training to determine if 4, 5, 6 …etc. days causes persistent ratio disturbances

- Follow the subjects with serial blood sampling through a 24 hours post-training period to determine exactly how long it would take the fTC ratio to return to baseline after exercise
- Add additional hormonal measurements that reflect anabolic-catabolic status of the body, such as growth hormone, dehydroepiandrosterone (DHEA), and catecholamines

APPENDICES

A. Medical and Training History

B. Physical Screening

C. Data Collection Sheets

D. Assay Information

APPENDIX A

Medical and Training History

Department of Exercise and Sport Science
Medical History

Subject ID: _____ Telephone: _____

Address: _____

Occupation: _____ Age: _____

YES NO

Patient History

1. How would you describe your general health at present?
   Excellent _____ Good _____ Fair _____ Poor _____
2. Do you have any health problems at the present time? _____ _____
3. If yes, please describe: _____
_____
4. Have you ever been told you have heart trouble? _____ _____
5. If yes, please describe: _____
_____
6. Do you ever get pain in your chest? _____ _____
7. Do you ever feel lightheaded or have you ever fainted? _____ _____
8. If yes, please describe: _____
_____
9. Have you ever been told that your blood pressure has been elevated? _____ _____
10. If yes, please describe: _____
_____
11. Have you ever had difficulty breathing either at rest or with exertion? _____ _____
12. If yes, please describe: _____
_____
13. Are you now, or have you been in the past 5 years, under a doctor's care for any reason? _____ _____
14. If yes, for what reason? _____
_____
15. Have you been in the hospital in the past 5 years? _____ _____
16. If yes, for what reason? _____
_____
17. Have you ever experienced an epileptic seizure or been informed that you have epilepsy? _____ _____
18. Have you ever been treated for infectious mononucleosis, hepatitis, pneumonia, or another infectious disease during the past year? _____ _____
19. If yes, name the disease. _____
20. Have you been treated for or told you might have diabetes? _____ _____
21. Have you been treated for or told you might have low blood sugar? _____ _____
22. Do you have any known allergies to drugs? _____ _____

23. If so, what? _____
_____
24. Have you ever been "knocked out" or experienced a concussion? _____ _____
25. If yes, have you been "knocked out" more than once? _____ _____
26. Have you ever experienced heat stroke or heat exhaustion? _____ _____
27. If yes, when? _____
_____
28. Have you ever had any additional illnesses or operations (other than childhood diseases)? _____ _____
29. If yes, please indicate specific illness or operations: _____
_____
30. Are you now taking any pills or medications? _____ _____
31. If yes, please list: _____
_____
32. Have you had any recent (within 1 year) difficulties with your:
    a. Feet _____ _____
    b. Legs _____ _____
    c. Back _____ _____

Family History

33. Has anyone in your family (grandparent, father, mother, and/or sibling) experienced any of the following?
    a. Sudden death _____ _____
    b. Cardiac disease _____ _____
    c. Marfan's syndrome _____ _____

Mental History

34. Have you ever experienced depression? _____ _____
35. If yes, did you seek the advice of a doctor? _____ _____
36. Have you ever been told you have or has a doctor diagnosed you with panic disorder, obsessive-compulsive disorder, clinical depression, bipolar disorder, or any other psychological disease? _____ _____
37. If yes, please list condition and if you are currently taking any medication.

Condition	Medication

Bone and Joint History

38. Have you ever been treaed for Osgood-Schlatter's disease? _____ _____
39. Have you ever had any injury to your neck involving nerves or vertebrae? _____ _____
40. Have you ever had a shoulder dislocation, separation, or other injury of the shoulder that incapacitated you for a week or longer? _____ _____
41. Have you ever been advised to or have you had surgery to correct a shoulder condition? _____ _____

42. Have you ever experienced any injury to your arms, elbows, or wrists? _____ _____
43. If yes, indicate the location and type of injury: _____
44. Do you experience pain in your back? _____ _____
45. Have you ever had an injury to your back? _____ _____
46. If yes, did you seek the advice of a doctor? _____ _____
47. Have you ever been told that you injured the ligaments or cartilage of either knee joint? _____ _____
48. Do you have a trick knee? _____ _____
49. Do you have a pin, screw, or plate somewhere in your body as a result of bone or joint surgery that presently limits your physical capacity? _____ _____
50. If yes, indicate where: _____
_____
51. Have you ever had a bone graft or spinal fusion? _____ _____

Activity History

52. During your early childhood (to age 12) would you say you were:
Very active_____ Quite active_____ Moderately active _____ Seldom active____
53. During your adolescent years (age 13-18) would you say you were:
Very active_____ Quite active_____ Moderately active _____Seldom active ____
54. Did you participate in:
a. Intramural school sports? _____ _____
b. Community sponsored sports? _____ _____
c. Varsity school sports? _____ _____
d. Active family recreation? _____ _____
55. Since leaving high school, how active have you been?
Very active ____ Quite active _____ Moderately active ____ Seldom active_____
56. Do you participate in any vigorous activity at present? _____ _____
57. If yes, please list:

Activity	Frequency	Duration	Intensity

58. How would you describe your present state of fitness?
Excellent _____ Good _____ Fair _____ Poor _____
59. Please list the type(s) of work you have been doing for the previous ten years:

Year	Work	Indoor/Outdoor	Location (city/state)

60. Whom shall we notify in case of emergency?
    Name: _____
    Phone (Home): _____ (Work) _____
    Address: _____
61. Name and address or personal physician: _____
_____
_____

All of the above questions have been answered completely and truthfully to the best of my knowledge.

Signature: _____ Date: _____

APPENDIX B

Physical Screening

Examination status: Approved Disapproved

Department of Exercise and Sport Science
Physical Examination Screening

Name: _____ Age: _____ Gender: _____

Please respond to each of the following in writing.

Pulse rate and regularity: _____ ECG Interpretation: _____

Blood Pressure:
Supine: _____ Sitting: _____ Standing (Left side): _____

Squat: _____ Standing (Right side): _____

Marfan Syndrome evaluation: (Δ BP, Physical Char.) _____

Palpation of Pulses: Carotid: _____ Radial: _____ Pedal: _____

Auscultation of the Lungs:
Back: Lower: _____ Middle: _____ Upper: _____

Front: Middle: _____ Upper: _____

Auscultation of Heart Sounds (Supine, Standing, Squatting)
Non-Specific HS: _____/_____/_____

Murmur: ____ Gallop: ____ Click: ____ Rub: ____ Click w/ Murmur: ____

Bruits: Carotid: _____ Abdominal: _____

Edema: Abdominal: _____ Calf: _____ Pedal: _____

Tenderness: Abdominal: _____ Other: _____

Xanthoma or xanthelasm: _____

Medical/Family History:
High Blood Pressure: _____ Diabetes: _____ CHD/CAD: _____

Last examination w/ physician: _____
Medications (prescription/ counter): _____

Examiner: _____ Date: _____

APPENDIX C

Data Collection Sheets

Orientation Session

Informed Consent

1. Inform participant of the experimental protocol
2. Make participant aware of the possible risks
3. Sign informed consent

Participant Compliance Questions

1. Did the participant perform strenuous physical activity for 24 hours prior to VO_{2peak} testing:
   Y N
2. Did the participant eat, smoke or consume alcohol or caffeine 2 hours prior to testing:
   Y N

Examinations _____

1. Medical Examination
2. 12 Lead EKG
3. Physical Examination

Physical Characteristics

1. Sex _____
2. Age _____ yrs
3. Height _____ cm
4. Weight _____ kg
5. Percent Body Fat _____%; Skinfolds:
   a. Chest _____mm
   b. Ilium _____mm
   c. Abdomen _____mm
   d. Axilla _____mm
   e. Sum of Skinfolds _____mm

Before VO_{2peak} Protocol

1. Set up metabolic system (calibrate, mouthpiece, etc)
2. Fit cycle ergometer to the participant - record seat position using flexible tape (line tape up from black cross bar on back of seat to the clear piece of tape on the cycle)
   Seat height: _____ cm
3. Fit polar heart rate (HR) monitor to participant
4. Make sure polar heart rate monitor picks up signal
5. Place RPE scale near cycle ergometer/explain RPE to participant

Warm Up

1. 5 minutes of cycling at 50W
2. 5 minutes of stretching focused on the lower body
3. Record resting oxygen consumption for 4 minutes (have participant sit on bike) – headpiece (NOT mouthpiece) is mandatory for all MAX tests

VO_{2peak} Protocol

1. Stage 1: 50W for 3 minutes →HR _____; RPE _____
2. Stage 2: 100W for 3 minutes →HR _____; RPE _____
3. Stage 3: 150W for 3 minutes →HR _____; RPE _____
4. Stage 4: 200W for 3 minutes →HR _____; RPE _____
5. Stage 5: 225W for 1 minute →HR _____; RPE _____
6. Stage 6: 250W for 1 minute →HR _____; RPE _____
7. Stage 7: 275W for 1 minute →HR _____; RPE _____
8. Stage 8: 300W for 1 minute →HR _____; RPE _____
9. Stage 9: 325W for 1 minute →HR _____; RPE _____
10. Stage 10: 350W for 1 minute →HR _____; RPE _____
11. Stage 11: 375W for 1 minute →HR _____; RPE _____
12. Stage 12: 400W for 1 minute →HR _____; RPE _____
13. Increase workload until volitional fatigue → add more stages if necessary (record to right)
14. Recovery - reduce resistance and have participant continue pedaling
15. Participant rests (supine) until HR is less than or equal to 100 bpm

Prior to Participant Exiting – Give canister of polycose supplement. Instruct the participant as follows:

1. Consume 25% of the canister throughout the day before Session II
2. Consume 25% of the canister throughout the day of Session II
3. Consume 25% of the canister throughout the day of Session III
4. Consume 25% of the canister throughout the day of Session IV
5. Instruct the participant to bring the empty canister with them for Session V
6. Instruct the participant to write down everything they eat and drink on the day preceding Session II, as well as on the days of Session II, III and IV

Criteria for valid VO_{2peak} Test

1. Did the participant have a maximal RER equal to or greater than 1.1? RER = _____
2. Did the participant reach age predicted maximal HR (220-age ± 5%)? HR_{max} = _____
3. Did the participant have a RPE equal to or greater than 18? RPE = _____
4. Was the total test time equal to or greater than 12 minutes? Test time = _____

Power Output Estimation for Intensive Training Trials

1. VO_{2peak} = _____
2. Peak Workload = _____
3. Workload that corresponds to 75% VO_{2peak} = _____

Training Sessions I-III

Special Notes

1. Session II must start at least one week after determination of VO_{2peak}
2. Sessions II-IV must be spaced 24 hours apart, at or about the same time each day

Participant Compliance Questions (some only apply to Session II)

3. Did the participant perform an easy day of training 2 days prior to the start of session I? Y N N/A
4. Did the participant perform activity the day prior to Session I? Y N N/A
5. Did the participant consume 25% of the polycose supplement the day prior to the Session? Y N
6. Has the participant been sick in the 6 week period prior to testing? Y N N/A
7. Did the participant eat, smoke or consume alcohol or caffeine 8 hours prior to testing? Y N
8. Has the participant taken any NSAIDS in the past 24 hours? Y N

Before Starting Exercise Protocols for Sessions II-IV

6. Collect the Food Diary from the participant
7. Set up metabolic system (calibrate, mouthpiece, etc) NOTE: use only the mouthpiece for the submaximal exercise sessions.
8. Set up blood supplies
9. Set up cycle ergometer to previously recorded seat height: _____ cm
10. Fit polar heart rate (HR) monitor to participant
11. Make sure polar heart rate monitor picks up signal
12. Place RPE scale near cycle ergometer

Exercise Protocol for Sessions II-IV

1. The participant will rest in the supine position for 10 minutes
2. Obtain 3-mL of venous blood using the standard Venipuncture technique
3. Placed blood into a sterile K_2 - EDTA (purple top) Vacutainer® tube
4. Place tube on ice immediately
5. 10 minute warm up
    a. 5 minutes of cycling at 50W
    b. 5 minutes of stretching focused on the lower body
6. Record resting oxygen consumption for 4 minutes with participant seated on bike (remove mouthpiece after sampling)
7. Cycle for 60 minutes at the previously determined workload that elicits 75% of VO_{2peak}
    a. Min 0 → HR _____; RPE _____
    b. Minute 10 → HR _____; RPE _____
    c. Minutes 16-20 → VO_2 measurement (remove mouthpiece after sampling)
    d. Minute 20 → HR _____; RPE _____
    e. Minute 30 → HR _____; RPE _____
    f. Minutes 36-40 → VO_2 measurement (remove mouthpiece after sampling)
    g. Minute 40 → HR _____; RPE _____
    h. Minute 50 → HR _____; RPE _____

i. Minutes 56-60 → VO_2 measurement (remove mouthpiece after sampling)
j. Minute 60 → HR _____; RPE _____

8. Recovery - assist participant off bike, and have them rest (supine)
9. Obtain 3-mL of venous blood using the standard Venipuncture technique
10. Placed blood into a sterile K_2 - EDTA (purple top) Vacutainer® tube
11. Place tube on ice immediately
12. Participant rests (supine) until HR is less than or equal to 100 bpm

Rest Day

Special Notes
1. Sessions IV must occur ~24 hours after session IV

Participant Compliance Questions
1. Did the participant eat, smoke or consume alcohol or caffeine 8 hours prior to testing? Y N
2. Has the participant taken any NSAIDS in the past 24 hours? Y N
3. Did the participant consume 25% of the polycose supplement the day prior to the Session? Y N

Before Starting Protocols for Sessions V
1. Collect the Food Diary from the participant
2. Set up blood supplies

Session V Protocol
1. No exercise will be performed on this day
2. The participant will rest in the supine position for 10 minutes
3. Obtain 3-mL of venous blood using the standard Venipuncture technique
4. Placed blood into a sterile K_2 - EDTA (purple top) Vacutainer® tube
5. Place tube on ice immediately

APPENDIX D

Assay Information

Cortisol Assay Procedures

All components must be at room temperature (15-28º C) before use.

1. **Plain Tubes:** Label four plain (uncoated) 12 x 75 mm polypropylene tubes T (total counts) and NSB (non-specific binding) in duplicate. Because non-specific binding in the Coat-A-Count procedure is low, the NSB tubes can be omitted without compromising accuracy or quality control.
   Coated Tubes: Label twelve Cortisol Ab-Coated Tubes A (maximum binding) and B through F in duplicate. Label additional Cortisol Ab-Coated Tubes, in duplicate, for controls and patient samples.

Calibrators	µg/dL	nmol/L
A (MB)	0	0
B	1	27.6
C	5	138
D	10	276
E	20	552
F	50	1380

2. Pipet 25 µL of the zero calibrator A into the NSB and A tubes. Pipet 25 µL of each remaining calibrator, control, and patient samples into the tubes prepared. Pipet directly to the bottom. It is good practice to use a disposable-tip micropipette, changing the tip between samples, in order to avoid carryover contamination.

3. Add 1.0 mL of ^{125}I Cortisol into every tube. Vortex. Laboratories equipped with a reliable pipettor-diluter may handle steps 2 and 3 simultaneously. No more than 10 minutes should elapse during the dispensing of the tracer. Set the T tubes aside for counting at step 6; they require no further processing.

4. Incubate for 45 minutes at 37º C. Use a water bath; neither an oven nor a heat block is suitable. Longer incubation periods will not significantly affect the assay.

5. Decant thoroughly. Removing all visible moisture will greatly enhance precision. Decant the contents of all tubes (except the T tubes) using a foam decanting rack, and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbent paper to shake off all residual droplets.

6. Count for 1 minute in a gamma counter.

Free Testosterone Assay Procedures

All components must be at room temperature (15-28º C) before use.

1. **Plain Tubes:** Label four plain (uncoated) 12 x 75 mm polypropylene tubes T (total counts) and NSB (non-specific binding) in duplicate. Because non-specific binding in the Coat-A-Count procedure is low, the NSB tubes can be omitted without compromising accuracy or quality control.
   Coated Tubes: Label twelve Free Testosterone Ab-Coated Tubes A (maximum binding) and B through F in duplicate. Label additional Cortisol Ab-Coated Tubes, in duplicate, for controls and patient samples.

Calibrators	pg/dL	pmol/L
A (MB)	0	0
B	0.55	1.9
C	2.5	8.7
D	9	31
E	25	87
F	50	173

2. Pipet 50 µL of the zero calibrator A into the NSB and A tubes. Pipet 25 µL of each remaining calibrator, control, and patient samples into the tubes prepared. Pipet directly to the bottom. Do no attempt to dilute patient samples expected to contain high concentrations in the zero calibrator (since dilution shifts the equilibrium between free and bound testosterone, the assay system cannot be expected to maintain linearity under dilution. It is good practice to use a disposable-tip micropipette, changing the tip between samples, in order to avoid carryover contamination.

3. Add 1.0 mL of ^{125}I Free Testosterone into every tube. Vortex. Laboratories equipped with a reliable pipettor-diluter may handle steps 2 and 3 simultaneously. No more than 10 minutes should elapse during the dispensing of the tracer. Set the T tubes aside for counting at step 6; they require no further processing.

4. Incubate for 4 hours at 37º C. The rack should be covered in parafilm to prevent evaporation. Use a water bath; neither an oven nor a heat block is suitable. Longer incubation periods will not significantly affect the assay.

5. Decant thoroughly. Removing all visible moisture will greatly enhance precision. Decant the contents of all tubes (except the T tubes) using a foam decanting rack, and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbent paper to shake off all residual droplets.

6. Count for 1 minute in a gamma counter.

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