Correlation of Cortisol with Non-Invasive Physiological Measures in Response to Exercise

Chad L. Pearson

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Approved by:

A.C. Hackney, Ph.D., D.Sc.

Claudio Battaglini, Ph.D.

J. Troy Blackburn, Ph.D., ATC

ABSTRACT

CHAD L. PEARSON: Correlation of Cortisol with Non-Invasive Physiological Measures in Response to Exercise
(Under the direction of Anthony C. Hackney, Ph.D., D.Sc.)

The present study attempted to find alternative methods to measure training stress when cortisol blood analysis is not available. The relationships during exercise between cortisol and six non-invasive physiological measures (heart rate, lactate, lactate:RPE ratios, oxygen uptake, oxygen pulse, and ratings of perceived exertion) were assessed. Subjects (n = 18) participated in four experimental sessions (40%, 60%, and 80% $VO_{2max,}$ and control). Blood samples were taken pre- and post-exercise along with non-invasive measures being recorded throughout the exercise session. All correlations between cortisol and the non-invasive measures were significant. However, the small variance accounted for between each non-invasive measure and cortisol lead to low predictability. Individually, non-invasive measures are not viable indicators of training stress. However, exploratory step-wise multiple regression analysis revealed that when combined, blood lactate and lactate:RPE ratio are highly predictive of cortisol, suggesting that in combination they may be a plausible alternative to assess training stress.

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CHAPTER I

BASIS FOR STUDY

Introduction

Cortisol is a glucocorticoid that is released from the adrenal cortex in response to emotional or physical stress (e.g., exercise). During prolonged exercise, cortisol has several physiological actions; a key one being to help maintain blood glucose levels. These actions include, promoting the breakdown of tissue amino acids which are then used by the liver in gluconeogenesis; stimulating the breakdown of free fatty acids from adipose tissue; stimulating gluconeogenesis; and blocking entry of glucose uptake into cells (Powers & Howley, 2007). Numerous studies have shown that cortisol responses to exercise are directly related to the intensity of the exercise (Davies & Few, 1973; Few, 1974; Hartley et al., 1972; Hill et al., 1955).

It is generally known that exercise is taxing on the body and as a result, physical stress (and perhaps emotional) levels are increased. Higher stress levels from exercise training may lead to overtraining as many studies have previously reported. For example, Fry et al. (1991) asserted that frequent intensive training without sufficient recovery was the main cause of overtraining. Similarly, Snyder et al. (1995) found that extended periods of intense training without adequate rest-recovery resulted in overtraining. Kuipers and Keizer (1988) also found that excessive bouts of high intensity exercise interjected into training resulted in overtraining.

In addition, overtraining can lead to a host of other health-medical problems for an athlete or active person. Studies have linked overtraining to a multitude of setbacks including immune-suppression and injury. For example, one study found that overtrained compared to non-overtrained athletes possess fewer neutrophils which can lead to more upper respiratory tract infections (URTI) (Pederson et al., 1996). Furthermore, compared to non-overtrained swimmers, overtrained swimmers showed less concentration of immuglobulin A which is used to fight URTIs (MacKinnon & Hooper, 1994). Other studies have also found that overtraining leads to a higher incidence of musculoskeletal injuries (Hess et al., 1989; Renstrom & Johnson, 1985). Therefore, the monitoring of stress levels to effectively prevent problems that stem from overtraining in athletes and active individuals may assist coaches and exercise specialists. Coaches and exercise specialists would be equipped to better monitor exercise training that could lead to overtraining and problems related to overtraining.

The importance of stress monitoring has been highlighted by numerous studies (Foster, 1998; Urhausen et al., 1995; Urhausen & Kindermann, 2002). Furthermore, these early studies have prompted a recent scientific review article that summarized and confirmed that the aversion of overtraining and achievement of optimal training rely greatly on the monitoring of stress levels during and in response to exercise (Kellman, 2010). In efforts to monitor stress, various methods have been devised involving measurement of responses by different systems of the body. The endocrine system is no exception. More specifically, cortisol is considered one of the gold standards concerning hormonal markers for monitoring stress during exercise (Kindermann et al., 1982; Shephard & Sidney, 1975).

Typically, cortisol is monitored via blood sampling (veni-puncture); however, this is an invasive procedure that can create further stress in an individual. Emotional stress caused by anxiety, which needle sticks can introduce, has been shown to increase cortisol levels (Lader, 1983). Consequently, misinterpretations of data are possible if cortisol increases are not due to the exercise protocol but instead are due to a person's emotional stress that is a result of a fear of needles. As a result, obtaining blood samples is not always a practical approach for examining cortisol or monitoring exercise training stress. However, the effectiveness of cortisol as a stress marker should not be disregarded. Instead, it should be considered whether there are other means to monitor exercise training stress that relate to cortisol and may be used as effectively as blood cortisol analysis for the monitoring of exercise stress.

Several non-invasive physiological measures (e.g., heart rate (HR), ratings of perceived exertion (RPE)) exist that can be measured to potentially monitor exercise training stress levels. Given that these measures increase with increasing exercise intensities (Bowen, 1903; Eston & Williams, 1988; Knox, 1940), as does cortisol, one could question whether there is a significant and strong relationship between cortisol responses and these measures. If so, one of these measures or a similar variable could serve as a substitute marker for cortisol when trying to monitor exercise training stress.

Surprisingly, few to no studies have assessed the relationship of cortisol with such non-invasive measures in response to exercise as it might occur in a training session for endurance athletes. Endurance athletes (marathon runners, triathletes, distance swimmers) are of special concern because they are some of the most susceptible to overtraining and the development of the Overtraining Syndrome (Kellman, 2010).

Purpose

The purpose of the study was to determine if select physiological non-invasive measures; HR, RPE, oxygen uptake (VO_{2max}), oxygen pulse ($VO_2 \cdot bpm^{-1}$), blood lactate (La; finger tip sampling) and the La:RPE ratio had relationships with the cortisol response to exercise. If so, the intent was to identify which of these measures had the strongest relationship with cortisol and could then potentially serve as a substitute indicator of exercise training stress.

Research Hypotheses

H1. There will be a significant positive correlation between the post-exercise change in blood cortisol levels and HR.

H2. There will be a significant positive correlation between the post-exercise change in blood cortisol levels and RPE.

H3. There will be a significant positive correlation between the post-exercise change in blood cortisol levels and percentage maximal oxygen uptake (VO_{2max}).

H4. There will be a significant positive correlation between the post-exercise change in blood cortisol levels and oxygen pulse in response to exercise ($VO_2 \cdot bpm^{-1}$).

H5. There will be a significant positive correlation between the post-exercise change in blood cortisol levels and blood lactate (La).

H6. There will be a significant positive correlation between the post-exercise change in blood cortisol levels and the La:RPE ratio.

Definition of Terms

Adrenocorticotropic Hormone (ACTH) – Hormone that is released by the anterior pituitary and causes adrenal cortex to release cortisol (Powers & Howley, 2007).

Corticotropic Releasing Hormone (CRH) – Hormone released by the hypothalamus which stimulates secretion of ACTH by the anterior pituitary (Powers & Howley, 2007).

Cortisol – Glucocorticoid that is secreted by the adrenal cortex during times of physical and psychological stress (Powers & Howley, 2007).

Hypothalamic-Pituitary Adrenal (HPA) axis – System including the hypothalamus, pituitary and adrenal glands that responds to physical and emotional stress. Includes the hormones, corticotrophin-releasing hormone (CRH), adrenocorticotropin hormone (ACTH) and cortisol (Dallman, 1993; Tharp, 1975).

Lactate:RPE ratio – Physiological variable that has been shown to identify overtraining in endurance athletes (Snyder et al., 1993).

Maximal oxygen uptake (VO_{2max}) – Maximum amount of oxygen uptake during strenuous and exhaustive exercise (Powers & Howley, 2007).

Overtraining Syndrome (OTS) – Stress-related disorder that disrupts the physiological functions of body while also impairing physical adaptations to training, and psychological and immunological function (Angeli et al., 2004).

Oxygen Pulse ($VO_2 \cdot bpm^{-1}$) – Physiological variable that measures the amount of oxygen uptake per heartbeat that is reflective of myocardial oxygen uptake (Astrand et al., 2003).

Rating of Perceived Exertion (RPE) – Perceptual effort ratings used to measure physical exertion during exercise (Borg, 1982).

Limitations

1. The findings are only applicable to endurance male athletes, 18-30 years of age with moderate to high levels of fitness that train on a cycle ergometer or bicycle.
2. The relationships between cortisol and non-invasive measures were only assessed in response to the exercise (immediate post-exercise) and not during recovery.
3. It was assumed that subjects answered all questions regarding this study in a truthful fashion.
4. Presents results were only applicable to a single exercise bout.

Delimitations

1. Emotional stress levels will be assessed using a questionnaire before each session.
2. Subject population will consist of male endurance athletes, 18-30 years of age with moderate to high levels of fitness.
3. All exercise sessions will occur at the same time of day to account for the influence of circadian rhythms on cortisol levels.
4. Carbohydrate intake will be monitored through a food diary that will determine if sufficient carbohydrates ($\geq$50% of diet) are consumed by subjects.
5. No strenuous activity within 24 hours of VO_{2max} test.
6. Subjects will report to VO_{2max} test, 4 hours post-prandial.
7. No non-steroidal anti-inflammatory drugs (NSAIDS), alcohol, or caffeine will be consumed $\leq$ 8 hours before VO_{2max} test.

Significance of Study

The present study served as an exploratory study and assessed any relationship between cortisol and the non-invasive physiological measures. Any significant results may warrant further studies. Therefore, this study served as a first step towards future studies that assess the relationship between cortisol and non-invasive physiological measures during an actual endurance exercise training program.

Validity of any of these physiological measures as viable substitutes for blood cortisol measurements would potentially result in a less-invasive and a more practical method of monitoring exercise training stress. This means the use of less invasive techniques will result in less need for blood sampling of subjects or athletes along with less biochemical-based laboratory analysis. Therefore, it may be possible for coaches or trainers to more quickly and easily monitor the level of training stress in their athletes.

If found to be valid, the less invasive physiological measurements could easily be used at practice or competitions. Consequently, training intensities based on stress levels will allow coaches to devise training regimens that allow a more optimal training for their athletes; thus, potentially allowing for better performance and fewer injuries in the athletes who are being monitored.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The review of literature will cover research areas that are important for understanding the present research study. To begin, the pathway of cortisol release through the HPA axis will be summarized. Next, the studies examining the variable release of cortisol will be explored. Furthermore, the background studies investigating the non-invasive measures (e.g., RPE, HR) will be reviewed. Also, the very few studies examining the correlations between cortisol and the non-invasive measures will be discussed. However, it is important to note that few studies have assessed the relationship of cortisol and non-invasive physiological measures. Finally, the conditions of overreaching and overtraining in athletes and active individuals will be discussed.

Pathway of Cortisol Release

The complete pathway of cortisol release is referred to as the hypothalamic-pituitary-adrenal (HPA) axis. The initial stimulus comes from stressful event(s) which may include bone breaks, burns, or exercise (Powers & Howley, 2007). The stressful event signals an increase in cortisol levels (Powers & Howley, 2007).

The pathway of cortisol release begins in the hypothalamus. The hypothalamus secretes cortisol releasing hormone (CRH). The release of CRH then stimulates the release of adrenocorticotrophic hormone (ACTH) via the anterior pituitary gland. The release of

ACTH then produces a signal that results in an increased release of cortisol via the adrenal cortex (Powers & Howley, 2007). A negative feedback loop circulates from the adrenal cortex back to the pituitary gland and hypothalamus. As cortisol levels increase to appropriate levels that are dependent on the initial stimulus, the negative feedback loop is activated and ultimately results in lower levels of cortisol release (Powers & Howley, 2007).

Physiological Purpose of Cortisol Release

As was mentioned above, cortisol release is driven by a variety of factors including bone breaks, burns and exercise (Guyton, 2006). In the case of exercise which lowers blood glucose levels, cortisol release helps maintain blood glucose levels (Brooks et al., 2000). More specifically, cortisol achieves this purpose by a range of methods including: promoting the process of gluconeogenesis via the promotion of tissue protein break-down that results in free amino acids, stimulating the release of free fatty acids from adipose tissue, activating liver enzymes that are used in gluconeogenesis, and forcing the body to rely more on free fatty acids for energy by preventing the entry of glucose into tissues (Powers & Howley, 2007; Tharp, 1975).

Furthermore, after exercise, cortisol release is needed for tissue repair (Powers & Howley, 2007). The release of cortisol for tissue repair is summarized by the general adaptation syndrome (GAS) (Selye, 1976). Cortisol is also thought to help replenish glucose and glycogen replenishment during recovery after exercise (Brooks et al., 2000).

Release Variability of Cortisol

The level of cortisol release is primarily influenced by two factors during exercise; exercise intensity and training status of an individual (Galbo et al., 1977; Kuoppasalmi et al., 1980; Tabata et al., 1990). Other factors that influence cortisol release include circadian rhythms and diet (Thuma et al., 1995). Each factor will now be discussed in more detail.

Concerning exercise intensity, cortisol release has been shown to be intensity-dependent (Davies & Few, 1973). A threshold exists and has to be reached before increased levels of cortisol are evident (Few, 1974; Hill et al., 2008; Luger et al., 1987). More specifically, one study performed by Davies and Few (1973) used exercise work-loads ranging from light to near maximal for one hour and found that the threshold intensity is in the range of sixty percent of a person's maximal oxygen uptake (VO_{2max}). Other studies have supported this assertion (Hill et al., 2008; Luger et al.; 1987, Sutton, 1978; Sutton et al., 1969). On the other hand, other studies did not observe a threshold-intensity or found no increases or non-significant increases in cortisol with increasing exercise intensities (Duclos et al., 1997; Farrell et al., 1983). However, these studies may not have controlled for important factors including circadian rhythm, exercise training background or timing of blood sampling (Hill et al., 2008).

Furthermore, it has been found that exercise intensities below 50% of an individual's VO_{2max} will result in a decrease or no change in cortisol levels (Davies & Few, 1973; Deuster et al., 1989; Hill et al., 2008). Both an increase in the rate of cortisol removal from the blood or a decrease in the rate of cortisol secretion are two possible explanations as to why cortisol levels decrease at lower exercise intensities (Davies & Few, 1973).

A person's training status also influences the levels of cortisol release. At absolute workloads, endurance-trained compared to sedentary individuals display less stimulation of the hypothalamic-pituitary-adrenal axis which results in less release of cortisol (Bloom et al., 1976; Luger et al., 1987; Sutton, 1978). Viru (1992) attributed this observation to an augmentation in cellular resources due to training which largely eliminates a necessity for large quantities of body reserves of fuel substrates. Viru (1992) went on to explain that the homeostasis of the individual is less likely to change and therefore less cortisol will be needed to maintain a balance.

Concerning relative submaximal and maximal workloads, few studies have assessed the differences between trained and untrained subjects. Sub-maximally, higher levels of cortisol were seen in trained versus untrained when compared at the same relative strenuous workload (Bloom et al., 1976; Hartley et al., 1972). Another study found no changes in cortisol levels when comparing relative workloads between trained and untrained subjects (Sutton et al., 1969).

Background on Non-Invasive Physiological Variables

Heart Rate

The utilization of heart rate (HR) to assess physiological function dates back to several centuries (Achten & Jeukendrup, 2003). Since then, HR monitoring has been used in several different applications pertaining to exercise or physical activity. Among these include exercise prescription and detection and prevention of overtraining (Achten & Jeukendrup, 2003; Dressendorfer et al., 1985). Another area that will be discussed and has received considerable attention recently is the concept of heart rate variability. In addition,

limitations are present and should be considered when using heart rate for any of the above applications (Achten & Jeukendrup, 2003; Jeukendrup & Van Diemen, 1998). A detailed look at both the applications and limitations will now be discussed.

Traditionally, an individual's VO_{2max} is used to prescribe exercise intensities along with assessing their level of cardiovascular fitness (Powers & Howley, 2007). However, precise determination of VO_{2max} requires a laboratory setting. Conversely, it has been observed that HR and VO_{2max} demonstrate similar increases during increases in a broad range of sub-maximal exercise intensities (Astrand & Rodahl, 1986; Karvonen & Vuorimaa, 1988). As a result of this observation, a simple method of using heart rate to prescribe exercise intensities has been developed.

Using the heart rate reserve (HRR), or better known as the Karvonen method, a person's sub-maximal exercise intensity can be tracked (Powers & Howley, 2007). The Karvonen method uses an individual's maximal and resting HR to determine their HRR which is then used to determine their target heart rate (THR) range. A good estimation of 60-80% of an individual's VO_{2max} is found in 60-80% of their HRR (Powers & Howley, 2007). However, it should be noted that the use of maximal HR in the Karvonen method can create problems when predicting VO_{2max} values, most notably due to its large standard deviation and therefore is only an estimate (Achten & Jeukendrup, 2003; Davis & Convertino, 1975; Londoree & Moeschberger, 1984).

Additionally, HR has been used to assess levels of lactic acid in the blood which are often used to measure exercise intensity (Halson et al., 2002; Padilla et al., 2001). More specifically, one study suggested a method to track the anaerobic threshold using HR (Conconi et al., 1982). Conconi et al. (1982) provided results on over 200 individuals that

showed a plateau of HR which they claimed represented the point of anaerobic threshold. However, critics claim that the exercise protocol used by Conconi et al. resulted in a natural deflection of HR and therefore was not the lactate threshold point (Jeukendrup et al., 1997). Nonetheless, other studies have shown that HR remains stable throughout an endurance season while the workloads at the anaerobic and ventilatory thresholds increase. Therefore, they contend that one laboratory assessment at the beginning of an athlete's season can be used to assess accurate exercise intensities throughout the entire season (Foster et al., 1999; Lucia et al., 2000).

Heart rate has also been used to diagnose and prevent overtraining (Dressendorfer et al., 1985). Several different studies have used different applications of a subject's HR to assess overtraining status. First, increases in resting HR have been linked to overtraining in athletes (Dressendorfer et al., 1985). Furthermore, decreases in HR during both sub-maximal and maximal exertion have been observed in over-trained subjects (Billat et al., 1999; Costill et al., 1988; Jeukendrup & Van Diemen, 1998). However, it should be noted that sub-maximal HR values during overtraining are controversial as other studies have found no differences between over-trained and normal subjects (Halson et al., 2002; Urhausen et al., 1998).

An area receiving more attention in the recent literature is heart rate variability (HRV). The HRV of a person is defined as the time between beats (Achten & Jeukendrup, 2003). More specifically, HRV is measured by observing the variations in R-R intervals between successive heartbeats (Achten & Jeukendrup, 2003). Application of HRV and overall health and fitness is based on recent studies that show high HRV is linked to high levels of VO_{2max} while low levels are related to increased mortality (Achten & Jeukendrup,

2003; Tsuji et al., 1994). Furthermore, endurance training has been shown to increase HRV by increasing R-R intervals (Melanson et al., 2000). However, more studies are needed before the exact mechanisms of how endurance exercise affects HRV are established (Achten & Jeukendrup, 2003).

Nonetheless, there are limitations to the applications of HR. First, the concept of day-to-day variations should be considered. Studies as early as the 1960s have shown that there are day-to-day variations during maximal exercise and therefore these differences should be taken into consideration when prescribing exercise intensities or when used in other applications. Astrand and Saltin (1961) showed there was approximately a 3 beats per minute difference in maximal heart rate when assessing day-to-day variations.

Furthermore, physiological factors including cardiovascular drift and hydration status should be taken into consideration during any application of HR. More specifically, heat stress which can be caused by high environmental temperatures and prolonged exercise combine to produce a condition known as cardiovascular drift. Cardiovascular drift ultimately leads to a decrease in stroke volume (SV) and consequently an increase in HR (Powers & Howley, 2007). Increases of HR produced by cardiovascular drift should be considered during any prolonged exercise bout in hot climates (Achten & Jeukendrup, 2003). Last, studies that observed dehydration in subjects, have found a decrease in SV which is inherently accompanied by an increase in HR (Saltin, 1964). Therefore, the physiological aspects of cardiovascular drift and hydration status of athletes should be considered in applications of HR.

In summary, exercise prescription and detection/prevention of overtraining are the two most prominent applications of HR. In addition, an area receiving increased attention is

the concept of HRV. It should be noted that studies supporting or refuting the use of HR in these applications are found. Also, limitations exist which are seen in day-to-day HR variations along with the physiological factors of both cardiovascular drift and hydration status.

Lactate

The utilization of blood lactate has many applications in the field of exercise physiology, mainly due to its ease of sampling (Karlsson et al., 1983). Similar to HR, the most prominent of these applications include the areas of exercise prescription, prediction of athletic performance along with overtraining. Thus, the following review on lactate will cover the most relevant research on exercise prescription, performance prediction and overtraining. It will then conclude with possible limitations that lab or field researchers must consider when using lactate values in these areas.

The most prevalent application of lactate analysis in exercise prescription is in the field of endurance exercise. The immense application to endurance exercise is mainly due to the concept of the lactate threshold (LT). The LT has been referred to by many different names including the lactate turning point, aerobic capacity and anaerobic threshold (AT) (Davis et al., 1983; Davies et al., 1970; Wasserman et al., 1973). Regardless of the name, the LT is generally referred to as the point during a graded exercise test or exercise session when the levels of blood lactate increase rapidly and non-linearly (Powers & Howley, 2007). The level of lactate in the body at which this normally occurs is typically around 4 mmol/L (Powers & Howley, 2007). Physiologically, this condition is a result of a greater production of lactate relative to its removal during physical activity (Billat et al., 1996). Ultimately, this physiological phenomenon has led to the possibility of prediction of an endurance athlete's performance along with a valid exercise prescription schedule by their coaches (Billat, 1996).

Research studies have shown the importance of LT in regards to prediction of athletic performance. More specifically, one study found that the LT accurately predicted running performance in both the 10,000 meters and marathon (Allen et al., 1985). Additionally, the use of LT as a performance predictor was validated in the study performed by Hagberg and Coyle (1983). In this study, the performance times of racewalkers were within 0.6% when compared to the speed at which LT was observed (Hagberg & Coyle, 1983).

Furthermore, an athlete's LT can be used to accurately prescribe their individual optimal exercise intensities. Jacobs (1986) argued that endurance athletes who fail to train at their optimal training intensity will not receive the greatest performance benefit possible. A study observing distance runners supported this argument when it was found that those athletes who trained closest to their LT attained a greater improvement in their speed (Sjodin et al., 1982). Additionally, one study found greater improvements in running velocity when exercise prescriptions were based on LT rather than percentage of VO_{2max} (Hollmann et al., 1981).

The application of lactate levels in athletes or active individuals extends to the area of overtraining. Focusing on maximal performance and lactate levels, Urhausen and Kindermann (2002) reported on multiple studies in a variety of sports. In review of these studies, it was found that distance runners, cyclists and swimmers who were being treated for overtraining exhibited lower performances of maximal exertion which were accompanied by lower levels of lactate (Costill et al., 1988; Gabriel et al., 1998; Kindermann, 1986). Further emphasizing the importance of maximal lactate measurements in diagnosis of overtraining, one case study falsely reported a case of endurance improvement due to lower levels of sub-maximal lactate levels. However, upon measuring the maximal lactate level, which was

decreased, it was found that the cyclist was suffering from overtraining (Jeukendrup & Hesselink, 1994).

As was previously mentioned in the last paragraph, an over-trained athlete's decreased sub-maximal lactate levels can mistakenly be interpreted as signs of endurance improvement. Therefore, diagnosis of overtraining can be tricky when considering sub-maximal lactate levels (Hurley et al., 1984; Kindermann, 1986). Consequently, studies have been conducted that utilize additional physiological measures along with lactate in an attempt to provide a better indication of overtraining (Bosquet et al., 2001). However, the validity of these markers has not been determined and thus additional studies are needed in this area to reach a sound conclusion (Bosquet et al., 2001). It should be noted that studies have suggested possible mechanisms to explain these lower levels of lactate as a result of overtraining, including the decreased sensitivity of norepinephrine by muscles that leads to decreased production of lactic acid (Lehmann et al., 1997). However, further studies are needed to clearly link lower sub-maximal lactate levels with overtraining.

Although the application of lactate levels in exercise-based studies is solidified by numerous studies showing its importance, it should be emphasized that limitations do exist. First, lactate is like any other substrate in the blood and its level is dependent on both secretion and removal by the body. Thus, both secretion and removal have to be considered when interpreting lactate levels (Jacobs, 1986). Additionally, environmental factors including both heat and cold have been shown to elevate lactate levels and thus it is important for both researcher and coach to carefully consider these factors (Fink et al., 1975; Jacobs et al., 1985). Furthermore, a diet low in carbohydrates may lead to glycogen stores being depleted which can ultimately lead to decreases in lactate levels (Jacobs, 1981).

Equally important, Jacobs (1986) recommends considering the site of lactate measurements as it can vary at different locations.

In summary, the importance of lactate in the exercise physiology field is seen in exercise prescription and prediction along with the usefulness seen in the detection of overtraining. More specifically, the LT is a good predictor of exercise performance at a variety of running events and has been shown to elicit the most optimal training intensity. Lactate measurements are also useful when there are concerns of overtraining. However, at sub-maximal intensities, it can be tricky to diagnose overtraining as studies have shown that lower levels of lactate are related to both overtraining and improved endurance performance. Last, limitations do exist when using lactate measurements. Both researchers and coaches should consider the secretion and removal of lactate along with any environmental or dietary factors that were discussed above. Sites of lactate measurement should also be considered.

La:RPE Ratio

It should be noted that few studies exist concerning the La:RPE ratio and the usefulness in diagnosis of overtraining. Still, the concept of La:RPE ratios was introduced in the early 1990s in attempts to more easily detect the complicated condition of overtraining. The general concept of La:RPE ratios argues that as an athlete reaches an overtraining state due to too much intensified training without recovery, the levels of lactate will decrease while the RPE levels may increase (Snyder et al., 1993). As a result, the La:RPE ratios will decrease in a state of overtraining. The promise of employing La:RPE ratios in sensing overtraining has been spurred by numerous studies showing its relevance in this area of research.

To begin, the first study conducted by Snyder et al. (1993) showed that both one and two-week periods of intensified training led to a state of overtraining in seven well-trained cyclists. The state of overtraining was shown by the decreases in the La:RPE ratio (Snyder et al., 1993). Furthermore, another study performed by Snyder et al. (1995) confirmed that La:RPE ratios decrease in over-trained athletes when these changes were observed in eight male cyclists. A more recent study that monitored La:RPE ratios in elite middle-distance runners found similar results when these athletes were subjected to periods of intensified training (Garcin et al., 2002). In addition, the value of La:RPE ratios was boosted when the day-to-day variability of the overtraining marker was found to be low during a study testing eight endurance athletes (Duke et al., 2008).

However, critics questioning the validity of La:RPE in detection of overtraining argue that it is difficult to promote overtraining in a laboratory setting and thus impossible to know if decreased La:RPE ratios are truly indicative of overtraining (Foster et al., 1999). The critics conclude that the decreased La:RPE ratios are simply an indication of temporary glycogen depletion (Foster et al., 1999). Another study found that La:RPE ratios showed no significant pattern of decrease in over-trained male triathletes (Coutts et al., 2007). The study concluded that a longer duration of overtraining was needed to determine any usefulness of La:RPE in over-trained endurance athletes (Coutts et al., 2007).

In summary, there are studies showing both validity and invalidity of La:RPE ratios when testing athletes for over-training. Advocates contend that the La:RPE ratio is a legitimate tool that can be used to detect the complex condition of overtraining. However, critics assert that its utilization is unclear and therefore the present studies do not positively

determine a state of overtraining. As a result of these mixed reviews, more studies are needed to assess the worth of La:RPE ratios in the area of overtraining.

O_2 Pulse

Oxygen (O_2) pulse is defined as the amount of O_2 consumed per heartbeat (Astrand, 2003). Studies have shown that O_2 pulse increases with endurance training. The main application of O_2 pulse lies in the area of cardiovascular fitness. Numerous studies have shown its validity in gauging an individual's cardiovascular fitness. As supporting evidence, O_2 pulse has shown promise in estimating stroke volume which is one characteristic that is used as a valid indicator of cardiovascular fitness. In addition, some studies have also shown its usefulness in exercise performance. Studies have shown that O_2 pulse has both an influential role in predicting exercise intensity along with possible worth in determining a subject's anaerobic threshold (AT). A review of the literature backing up these assertions will now be discussed.

To begin, O_2 pulse increases during an endurance training program. Kasch et al. (1973) first observed this improvement in men that participated in an endurance training program. Another study supported these initial findings when rowers were monitored during an endurance training program (Mahler et al., 1985). More recently, these findings have again been backed by a study assessing O_2 pulse values in endurance trained males (Laffite et al., 2003). However, this study concluded that O_2 pulse does not relate to a subject's performance improvements (Laffite et al., 2003).

Additionally, O_2 pulse has been shown to indicate a person's cardiovascular fitness. Wasserman et al. (1999) explain that lower and higher values of O_2 pulse are related to weak and strong cardiovascular systems, respectively. More specifically, lower O_2 pulses are

indications of lower levels of O_2 being delivered to the body while the opposite is true of high O_2 pulses (Wasserman et al., 1999). Another researcher explained that high O_2 pulse values indicate high levels of fitness for elite oarsmen (Hagerman, 1984).

Furthermore, O_2 pulse is used to estimate stroke volume (SV) in subjects. A high SV is important for a high level of cardiovascular fitness (Whipp et al., 1996). Therefore, one study identified the need for estimating SV and found this to be possible using O_2 pulse. Whipp et al., (1996) concluded that O_2 pulse could be used to very accurately predict a measured value of SV. Another study found similar results when it found that SV could be predicted using O_2 pulse during sub-maximal cycle training in both trained and untrained cyclists (Bhambhani et al., 1994). A multitude of other studies have supported these findings (Hossack et al., 1980; Mahler et al., 1985).

Moreover, O_2 pulse values have been shown to be a factor in predicting exercise intensity or determining other performance parameters including AT. However, it should be noted that few studies have been performed in this area of research. Nonetheless, one study measured O_2 pulse in cyclists who exercised 5 hours per day and concluded that O_2 pulse values should be considered when determining a cyclist's exercise intensity (Fellman et al., 2003). Another study showed the importance of O_2 pulse when it concluded that AT could be determined using O_2 pulse (Lehmann & Kolling, 1996). On the other hand, one study suggested that obtaining O_2 pulse values does not provide information on improvements in endurance performance (Laffite et al., 2003). Based on these mixed research findings and lack of studies, more performance studies focusing on O_2 pulse should be conducted to resolve any conflicts between studies.

Similar to other physiological measures, special considerations should be employed when observing O_2 pulse and applying values towards cardiovascular fitness and performance. Both body size measured with body mass index (BMI) and age were found to affect measures in O_2 pulse (Al-Hazzaa, 2001). Furthermore, height and gender were found to influence maximal O_2 pulse in the same study (Jones et al., 1985). More specifically, when looking at gender, the study found that males possess higher maximal O_2 pulses compared to females (Jones et al., 1985).

Overall, studies have shown that O_2 pulse increases with endurance training. Thus, the main application of O_2 pulse lies in the determination of cardiovascular fitness. Focusing on SV and its importance in cardiovascular fitness, O_2 pulse has received attention in its ability to predict SV. Also, studies have shown that O_2 pulse is beneficial in predicting exercise intensities and has been shown to have value in predicting AT. To close, these applications are only helpful if certain factors are considered, including body size, age, height and gender.

Ratings of Perceived Exertion (RPE)

Perceived exertion is defined as the task of sensing bodily feelings during exercise (Noble & Robertson, 1996). The concept of perceived exertion has interested exercise researchers for over half a century as the first studies to measure subjective feelings of perceived exertion were conducted in the early 1960s (Borg, 1962; Eisler, 1962; Hueting, 1965). The interest sparked many studies exploring the concept and in 1982, Gunnar Borg designed a rating scale (6-20 scale) that measured ratings of perceived exertion which is still used today (Borg, 1982). Furthermore, studies indicate that one of the primary applications of RPE ratings is in the area of exercise prescription (Dlin et al., 1984; Mahler et al., 1984).

In this section, the value of RPE in exercise prescription for endurance training events will be discussed. More specifically, the discussion will include not only applications in exercise prescription but also the use of RPE in detection of overtraining. The section will end with limitations in using RPE as a valid exercise prescription tool.

As was mentioned, one of the main utilizations of RPE assessment has been geared towards exercise prescription. The value of RPE in this area of research was first suggested back in the early 1970s (Borg & Linderholm, 1970). Since then, studies have shown that RPE ratings can effectively predict exercise intensities and thus be used for exercise prescription. More specifically, one study validated the use of RPE in predicting correct $\%VO_{2max}$ levels (Dunbar, 1992). The RPE ratings were estimated for both the subject's 50% and 70% VO_{2max} levels. The subjects then reproduced these RPE ratings at their respective 50% and 70% VO_{2max} levels. Thus, the study validated the use of RPE as a helpful tool in predicting exercise intensity (Dunbar, 1992). Another study supported this claim when healthy, fit distance runners showed that RPE ratings could predict exercise prescription with similar accuracy as HR (Eston et al., 1987). Accordingly, RPE is a proven method of identifying correct exercise intensities which can then be used for exercise prescription programs.

In addition, the value of RPE is displayed in the detection of overtraining in endurance athletes. The use of RPE ratings in the detection of overtraining is coupled with lactate levels. Using these measures, a ratio is formed and is known as La:RPE ratios. Decreased levels of these ratios have been shown to indicate overreaching or overtraining (Urhausen & Kindermann, 2002). An initial study found that well-trained cyclists experienced lower levels of maximal workload La:RPE ratios after a two-week phase of

high-intensity interval training. The high-intensity interval ratio levels were lower compared to two weeks of both normal and recovery training (Snyder et al., 1993). A subsequent study found decreased La:RPE ratios in elite middle-distance runners after a phase of high-intensity interval training led to overtraining (Garcin et al., 2002).

Although RPE is proven to be a relatively easy way of determining exercise prescription and diagnosing overtraining, it should be noted that an important limitation arises when applying RPE in these areas. While RPE ratings provide an adequate gauge of measuring exercise intensity that is then useful with exercise prescription, it has also been shown that RPE ratings are only useful at higher exercise intensities (Eston et al., 1987; Smutok et al., 1980). One study observing 10 male runners found that RPE was only reliable if the subject was at least running a pace of 9 km/hr or if the heart rate was above 150 beats per minute (Smutok et al., 1980). Exercise intensity at lower levels resulted in erroneously predicted exercise intensities (Smutok et al., 1980). Another supporting study found similar results when it concluded that RPE ratings of 13 and 17 were much more reliable estimates of exercise intensity versus lower RPE ratings such as 9 (20 point Borg Scale) (Eston et al., 1987).

In conclusion, the use of RPE as a beneficial tool for both exercise prescription and overtraining is supported by numerous studies (Dlin et al., 1984; Mahler et al., 1984). However, it should be noted that some studies have reported validity in using RPE ratings at higher exercise intensities only (Eston et al., 1987; Smutok et al., 1980). Therefore, this should be considered when applying RPE to exercise prescription programs.

VO_2

Oxygen uptake (VO_2) is defined as the amount of O_2 used during physical activity (Brooks et al., 2000). The primary application of VO_2 is focused on prescription of exercise intensity using a person's VO_{2max} as a guideline. By definition, VO_{2max} is the maximum amount of oxygen that an individual's larger muscle groups use during exercise (Brooks et al., 2000). Using VO_{2max}, the $\%VO_{2max}$ levels can be found and used to determine optimal exercise intensities. VO_2 can also be a helpful marker for overtraining in endurance athletes. However, limitations are present which are based on practical utilization of VO_2. Each topic pertaining to VO_2 will be discussed.

To begin, the major value of VO_2 lies in the applicability towards exercise prescription. It has been determined that improvement in VO_{2max} is related to improvement in aerobic capacity or endurance performance (Costill, 1979; Foster, 1983). It has been determined that in order to best improve VO_{2max}, an athlete or active person must train at an optimal intensity of $\%VO_{2max}$ (Powers & Howley, 2007). The studies focusing on improvement in VO_{2max} through training at optimal $\%VO_{2max}$ levels will now be outlined.

It should be noted that each endurance athlete is different and the $\%VO_{2max}$ level most likely to induce the most improvement will therefore also be different (Powers & Howley, 2007). However, generally speaking, an intensity in the range of 80% to 90% of an athlete's VO_{2max} will provide the greatest improvements in VO_{2max} (Powers & Howley, 2007). Additional studies support this claim. Davies and Knibbs (1971) found that healthy male subjects that trained at 80% of their VO_{2max} showed the most improvement in VO_{2max}, whereas subjects that trained at 50% of their VO_{2max} or below did not show improvement. Furthermore, another study found greater improvement in muscle fibers that are critical for

VO_{2max} improvements at an exercise intensity that was between 80% and 90% of a subject's VO_{2max} (Dudley et al., 1982).

In addition, VO_2 levels can be used to help diagnose overtraining in athletes. More specifically, the VO_{2max} is used to help detect signs of overtraining. Maximal O_2 uptake levels declined in trained cyclists after a period of intense training (Jeukendrup et al., 1992). Another study found a 3% decrease in seven of the eight subjects' VO_{2max} levels during intensified training in elite cyclists (Snyder et al., 1995). However, it should be noted that this decrease was not significant. Moreover, one researcher found no differences in VO_{2max} levels during normal or over-trained states (Costill et al., 1988). Consequently, the lack of agreement warrants additional studies to be conducted concerning VO_{2max} levels and overtraining.

Shifting focus to sub-maximal VO_2 levels reveals similar conclusions regarding detection of overtraining. While Costill et al. (1988) found no differences in VO_{2max} levels in an over-trained athlete, sub-maximal VO_2 levels were increased. On the contrary, Jeukendrup et al. (1992) found no significant differences in sub-maximal levels in the over-trained group of male elite cyclists. Analogous to conclusions concerning VO_{2max} levels and overtraining, additional research is needed to draw inferences about overtraining and the effects on sub-maximal VO_2 levels.

The benefits of using VO_2 are apparent when considering exercise prescription and possibly detection of overtraining. However, there are limitations to its utilization in exercise performance based on one critical factor. To gain the most exact VO_{2max} values requires the use of laboratory equipment in a laboratory setting (Powers & Howley, 2007). As a result, athletes that do not have access to these facilities are unable to maximally benefit from

VO_{2max} and VO_2 use as an exercise prescription tool. Thus, VO_2 utilization is not very practical when considering this limitation.

However, indirect methods of VO_{2max} measurement have been developed. One study compared VO_{2max} levels during a 12-minute field performance test and laboratory determined maximal O_2 uptake and found a very close relationship between the two values (Cooper, 1968). Furthermore, a more recent study found that VO_{2max} values obtained by a Cooper Field Test, shuttle run test and sub-maximal cycle test had significant positive correlations with a normal VO_{2max} test performed on a treadmill (Grant et al., 1995). Although these alternative methods reproduce similar VO_{2max} values, it should be noted that these are only estimates.

In summary, VO_2 values are beneficial in providing the optimal exercise prescription for endurance athletes. Also, studies have shown its possible promise in detecting cases of overtraining. Even so, a significant limitation exists in the need to have laboratory equipment to obtain the most accurate VO_{2max} levels that are then used as a guide for prescribing correct exercise intensities. As a result, VO_2 utilization is not very practical. Nonetheless, VO_2 values provide some guidance for athletes and coaches to develop training schedules based on optimal training intensities.

Correlations of Cortisol with Non-Invasive Physiological Measures

Few studies have directly assessed the relationship between cortisol and the selected non-invasive physiological measures. Nonetheless, a discussion will now follow looking at those studies which have taken place. Comparatively, some measures have been observed in

relation to cortisol more than others, having said that some measures may contribute more to this discussion than others.

To begin, the relationship of VO_2 and cortisol is perhaps the most well studied. The first significant study to assess the relationship of VO_2 and cortisol was performed by Davies and Few, (1973). The study showed that cortisol levels begin to rise in subjects at approximately 60% VO_{2max} (Davies & Few, 1973). Further analysis showed that below a subject's 50%VO_{2max}, cortisol levels would decline (Davies &Few, 1973). Inspection of the cortisol concentrations revealed that the increase in levels was due to a rise in secretion and not due to a decrease in the removal of cortisol from the system (Davies & Few, 1973). A recent study found similar findings when it concluded that indeed a threshold-intensity does exist for cortisol secretion and is in the range of 60% VO_{2max} (Hill et al., 2008). The study also found that cortisol levels actually decreased at this 40% VO_{2max} intensity due to a higher rate of removal versus secretion of cortisol (Hill et al, 2008). Therefore, the current literature supports a relationship of increasing cortisol levels and increasing exercise intensity (%VO_{2max}) starting at around 60% VO_{2max}. It should be noted that though these studies support a theory of increase in cortisol with increasing exercise intensity, there were no correlations performed to assess the strength of this relationship.

On the contrary, some studies have not seen a threshold-intensity effect and report no increases in cortisol with increasing exercise intensity. In particular, one study found no significant change in cortisol at 65% or 80% of a person's VO_{2max} (Farrell et al, 1983). A more recent study only found increases in cortisol secretion at 80% VO_{2max} for test subjects (Duclos et al., 1997). Therefore, it can be assumed that no positive correlations were found between %VO_{2max} and cortisol. Differences found between studies may be a result of the

failure to control for the circadian rhythms of cortisol. Thuma et al., (1995) found that cortisol secretion is controlled by an internal circadian rhythm and thus when observing levels these rhythms must be taken into account. A need for further studies is needed to perform actual correlations between cortisol and exercise intensity as well as determine if a threshold-intensity does exist between cortisol and exercise intensity (%VO_{2max}).

In addition, studies have assessed the relationship of cortisol with lactate (La). A relationship between cortisol and La was suggested by Farrell et al., (1983). Farrell et al. (1983) found that La was significantly related to the changes in adrenocorticotropic hormone (ACTH) which controls the release of cortisol from the adrenal cortex. An additional study backed this study when it found positive correlations between lactate and cortisol during incremental exercise testing (Port, 1991). However, a study assessing the direct relationship of lactate on the hypothalamic-pituitary-adrenal (HPA) axis found no direct roles of lactate influencing the release of cortisol (Petrides et al., 1999). Similarly, another study found no relations of lactate to cortisol during high exercise intensities (Kraemer et al., 1989). The mixed reviews concerning the possible influence of lactate over cortisol secretion warrants further studies to fully assess any relationship these two blood markers may possess.

Correlation studies will now be discussed concerning cortisol and ratings of perceived exertion (RPE). To begin, an exploratory study looking at cortisol and its effects on affective measures found a positive correlation between RPE and cortisol (Rudolph & McAuley, 1998). More specifically, RPE scores taken around minute 20 and 30 of a 30 minute exercise bout found positive correlations with 30-minute post-exercise cortisol levels (Rudolph & McAuley, 1998). However, no correlations were performed immediately post-exercise. Another study also found an association of lower RPE values with lower cortisol values

during prolonged running at 70% VO_{2max} (Utter et al., 2004). Though an association was found, no correlations were performed between cortisol and RPE. Utter et al., (2004) proposed that the association of cortisol with RPE may be psychological in origin as cortisol is also secreted in response to emotional stress. The lack of both studies and correlations performed to compare cortisol and RPE values warrants further investigation to directly measure correlations between the two measures.

Few studies have assessed any associations via correlations between cortisol and HR. No studies have assessed the relationship in an exercise setting. Furthermore, of the studies looking at the measures, none have performed correlations. One study found both cortisol and HR increased during 30 minutes of gambling (Meyer et al., 2000). Another study found that the measures increased during a stressful social situation (Rimmele et al., 2007). Although both measures showed significant differences between post-session and pre-session, no correlations were performed between measures (Rimmele et al., 2007). The general exploration of the relationship between the two measures does not extend much beyond the two studies just discussed. Consequently, additional studies are needed and should employ an exercise protocol to apply any findings to the exercise performance and overtraining field.

Shifting focus towards La:RPE ratios and oxygen (O_2) pulse reveals that no studies have assessed the relationship between cortisol and these two measures. The lack of studies between cortisol and La:RPE may be due to the relative "newness" of using La:RPE ratios in exercise performance and overtraining studies. The absence of studies between cortisol and O_2 pulse may be influenced by the fact that researchers may view VO_2 as the better measure to compare with cortisol. Since more information can be obtained using VO_2, O_2 pulse has

not been extensively studied and thus no studies have been performed assessing the relationship between cortisol and O_2 pulse.

Overtraining: Causes, Symptoms and Diagnosis

A general definition of overtraining is a disproportionate amount of endurance training compared with too little recovery time (Lehmann et al., 1993). Overtraining is sub-divided into two separate types (Lehmann et al., 1993). First, short-term overtraining is a result of overreaching or short spans of increased exercise intensity (Halson & Jeukendrup, 2004; Lehmann et al., 1993). Athletes are normally able to recover within a couple of weeks due to short-term overtraining (Lehmann et al., 1993). Long-term overtraining is characterized by longer spans of training schedules that too frequently include high-intensity exercise (Halson & Jeukendrup, 2004; Lehmann et al., 1993). As a result, continued long-term overtraining can lead to the condition known as over-training syndrome (OTS) (Lehmann et al., 1993).

Overtraining by an athlete can lead to many symptoms that can be used to help diagnose overtraining or OTS. However, it should be noted that studies have yet to identify a clear detection tool used for identifying overtraining and OTS and there are requests to improve the quality of future studies (Halson & Jeukendrup, 2004). Nonetheless, symptoms include decreases in mood changes, increased resting heart rate, decreases in lactate production (Halson & Jeukendrup, 2004; Urhausen & Kindermann, 2002). The tools for detecting overtraining have not improved in recent years which have led to several recommendations pertaining to future studies (Urhausen & Kindermann, 2002).

Recommendations include the request for better controlled studies to identify this often times difficult condition to diagnose (Halson & Jeukendrup, 2004).

Summary

To summarize the literature review, cortisol is a hormone that is released in response to stress that includes emotional or physical stress. In response to stress, cortisol is released to assist in maintaining blood glucose in multiple manners including, promoting the breakdown of tissue amino acids that are used for liver gluconeogenesis; helping break down free fatty acids from adipose tissue; stimulating gluconeogenesis; and blocking entry of glucose uptake into cells (Powers & Howley, 2007). Concerning the pathway of cortisol release, the mechanism is explained based on the HPA axis. (Dallman, 1993; Tharp, 1975).

Relative to exercise, the release of cortisol is dependent on the intensity of the exercise. Mixed reviews exist, but the general consensus is that cortisol begins to increase around 50-60%VO_{2max}, but either stays the same or decreases below this threshold (Davies & Few, 1973; Deuster et al., 1989; Hill et al., 2008). Circadian rhythms have also been shown to affect the levels of cortisol (Thuma et al., 1995).

Furthermore, there are non-invasive physiological measures that have been developed or discovered that may help in determining a person's exercise prescription or training status (e.g., overtrained) including RPE; HR; La:RPE ratio; lactate; VO_2 and O_2 pulse. Each of these were discussed along with their main training prescription applications.

Finally, studies have shown that there are correlations between cortisol concentration and the non-invasive variables discussed (Hollander et al., 2003; Port, 1991; Rimmele et al., 2007). However, there are not enough of these studies to accurately and convincingly

determine that a relationship exists between cortisol and the non-invasive measures. Having said this, the risk of overtraining as a result of consistently high stress levels has been identified along with the importance of regulating and monitoring stress (Kellman, 2010; Kuipers & Keizer, 1988). Therefore, more studies, such as the present one, are needed in this area of research.

CHAPTER III

METHODOLOGY

Subjects

Subjects were males and were required to be endurance athletes (distance runners, cyclists, swimmers, etc.) with a moderate to high level of cardiovascular fitness (i.e., involved with aerobic exercise training at least 60 minutes/day for at least 3 times/week). Subjects were recruited from the community of Chapel Hill, North Carolina along with the surrounding areas using a recruitment flyer that explained the details of the study. Subjects were required to complete a written informed consent form before participating in the study. The informed consent form was approved by the institutional review board at the University of North Carolina at Chapel Hill (UNC-CH). In addition, subjects were required to complete a medical history questionnaire and undergo a UNC-CH Department of Exercise & Sport Science physical screening.

Instrumentation

Maximal oxygen uptake (VO_{2max}) and respiratory gases were assessed using a Parvo Medics TrueMax® 2400 Metabolic system (Parvo Medics, Salt Lake City, UT, USA). All exercise sessions were performed using a Lode® electronically braked cycle ergometer (Lode, Groningen, The Netherlands). The HR was recorded utilizing a Polar heart rate monitor (Polar, Lake Success, NY, USA). Height and mass were measured using a stature meter (Perspective Enterprises, Portage, MI, USA) and a Detecto 2381 balance beam scale

(Detecto, Webb City, MO, USA), respectively. Blood lactate analysis was performed using a Vitros DT-60 automated blood analyzer (Johnson and Johnson, New Brunswick, NJ, USA). The RPE was assessed using the Borg 6-20 scale (Borg, 1970). Cortisol analysis was conducted using radioimmunoassay (RIA) procedures (Siemens Healthcare, USA).

Procedures/Protocol

Subjects reported to the UNC-Chapel Hill Applied Exercise Physiology Laboratory for each of the 5 sessions. Prior to the initial visit that included the orientation/VO_{2max} session, subjects were instructed to complete a 3-day food diary log to confirm that they were consuming adequate carbohydrates ($\geq$50% of diet). If it was found that they were not consuming adequate carbohydrates, they were briefed on a diet plan that allowed for sufficient consumption and asked to come back in three days. Diet assessment was properly monitored using the nutrition database on the mypyramid.gov website.

The initial visit consisted of orientation with the equipment along with a VO_{2max} test on the cycle ergometer. Each subject's VO_{2max} was evaluated using an increasing intensity cycling protocol on a Lode electronically braked cycle ergometer. The subject was allowed to adjust the seat height to his comfort for this initial test and future trials. The subject was then allowed to warm-up for 5 minutes. The warm-up also allowed the subject to become acquainted with the motion of the bike. After warm-up, the subject rested on the bike while oxygen uptake was recorded for 4 minutes to confirm that the indirect calorimetry unit was working properly. To begin the VO_{2max} test, the subject began cycling at a power of 50 Watts (W) for 3 minutes. The power was increased by 50 W every 3 minutes until maximal exertion was met. If the subject exceeded 12 minutes, power then increased 25 W every 1

minute, thereafter, until the subject reached maximal exertion. The VO_{2max} test was conducted following a protocol as described by MacDougall, Wenger, and Green (1991). Oxygen uptake and HR were consistently measured throughout the max test. At the end of each test stage, the RPE and HR were assessed. A 5-minute recovery period on the cycle ergometer was allowed after the test. A participant was able to exit from the laboratory after a HR of 100 or lower was obtained. Using VO_{2max} determinants described by Brooks et. al (1999), a test was considered a valid VO_{2max} test if three of the four following criteria were met: plateau of VO_2 with increasing workload, a respiratory exchange ratio (RER) of 1.1 or greater, and an RPE value of 18 or greater, and a age-predicted heart-rate maximum (within 5%). If three of the criteria were not met, the test was considered a VO_{2peak}, however, the subject was still allowed to participate in the study based on these values. To avoid confusion, any references to the maximal performance test will be referred to as VO_{2max} tests and not VO_{2peak}.

Experimental Training Sessions

The experimental training sessions consisted of exercise at 40%, 60%, and 80% of VO_{2max} along with a rest session. The order of sessions was counterbalanced for all subjects in order to prevent an order effect within the data. Training sessions were separated by at least 72 hours. If subjects reported to the laboratory more than +/- 30 minutes of their scheduled exercise session they were asked to reschedule during another appropriate time. To begin each session, subjects filled out REST-Q questionnaires that assessed their stress levels prior to the exercise or rest session. Stress levels that were too high (score $\geq$ 36), resulted in subjects not being allowed to complete the session for that day and reported back

at a future appropriate date. Next, the subject rested for 30 minutes in the supine position. Subjects then warmed up for 5 minutes on the cycle ergometer. Upon warming up and completing 30 minutes of exercise at the selected intensity, subjects again rested in the supine position for 30 minutes before being discharged. Each training session consisted of 30 minutes of exercise at the selected intensity. Respiratory gas measurements were taken at 7-10, 17-20 and 27-30 minutes of each exercise session. The HR and RPE were assessed during every 5th minute of exercise. One session was substituted with rest and served as the control. Figures 1 and 2 illustrate the timelines for the overall study and each experimental session, respectively.

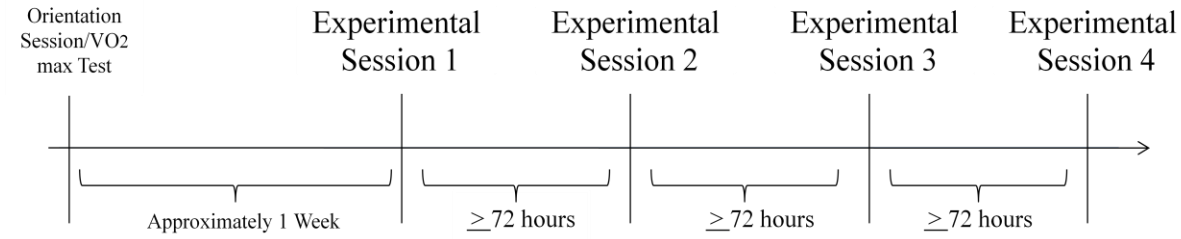

Figure 1. Timeline for overall study protocol.

Blood Collection

Two blood draws for each subject per exercise-rest session was taken using veni-puncture procedures. The first draw was taken after 30 minutes of rest immediately prior to each exercise session. The second draw was taken immediately after 30 minutes of exercise. During the rest session, the same blood sampling procedures were taken. However, 30 minutes of rest replaced the exercise portion of the session. Approximately 3 milliliters (mL) of blood were taken in each draw by a certified phlebotomist.

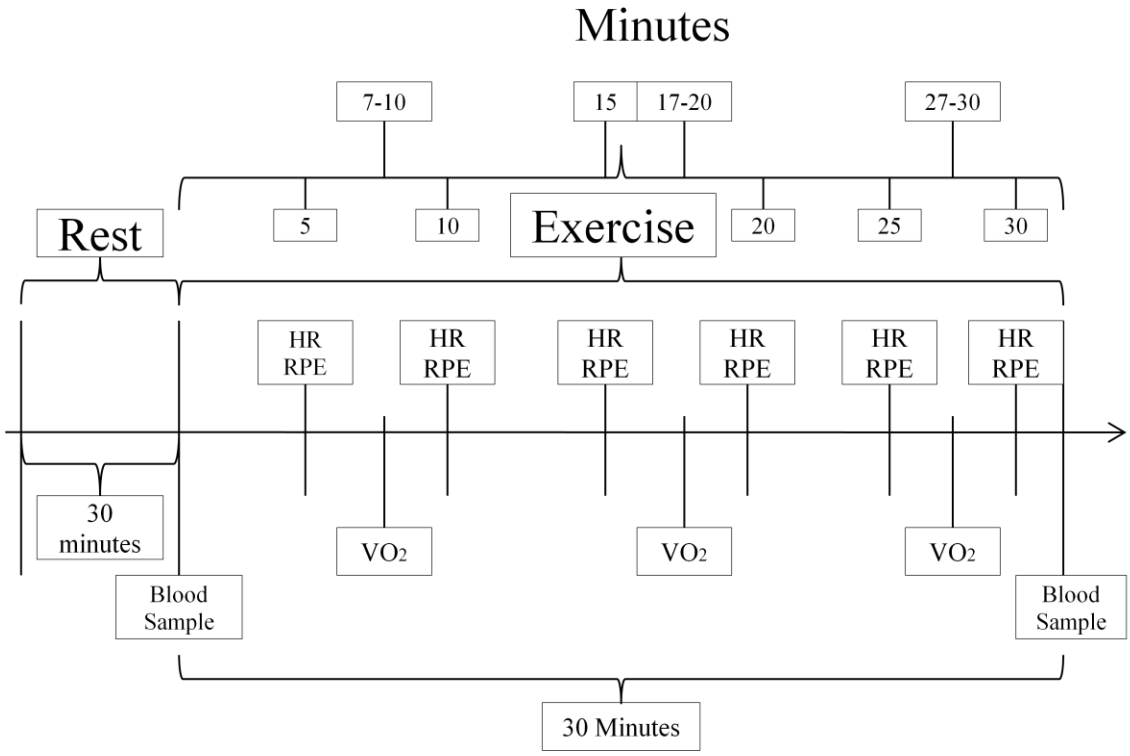

Figure 2. Timeline for each experimental exercise session.

Blood Analysis

Blood cortisol levels were assessed using radioimmunoassay (RIA) technique (Siemens Healthcare, USA). Lactate analysis was conducted using a Vitros DT-60 automated blood analyzer (Johnson and Johnson, New Brunswick, NJ, USA). Details of these procedures appear in the Appendices.

Plasma volume shift measurements were performed to compare any changes from the pre-exercise rest position (supine position) to the post-exercise position on the cycle ergometer. Any changes were assessed using the Dill and Costill (1974) method. For hematocrit measurements, blood was collected in micro-capillary tubes (Fisher Scientific, Waltham, MA, USA) and then centrifuged to isolate the hematocrit. Centrifugation was performed using an Adams MHCT-II centrifuge (Becton, Dickinson and Company, Franklin

Lakes, NJ, USA). Hematocrit measurements were then assessed using a micro-hematocrit measuring tool (International Equipment Company, Needham Heights, MA, USA). Hematocrit was measured in triplicate. Hemoglobin measurements were taken using whole blood and measured with the Stat-Site WT-9" Hemoglobin meter (Stanbio Laboratory, Boerne, TX, USA). Triplicate measurements were also taken for hemoglobin readings.

Statistical Analysis

All statistical tests were conducted using SPSS 18.0 Statistical Analysis Software (IBM, Somers, NY, USA). A 4x2 (trials x sampling time) repeated measures analysis of variance (ANOVA) test determined any significant differences in absolute cortisol levels (post-exercise levels – pre-exercise levels) between each exercise session. Furthermore, for each non-invasive physiological variable, a one-way repeated measures ANOVA was used to determine any significant differences between each exercise intensity. In addition, a one-way repeated measures ANOVA test determined any significant differences between each subject's REST-Q scores between exercise sessions. Any significant ANOVAs were followed by a Tukey post-hoc test to determine the means that were significantly different. The significance level was set at $\alpha \leq 0.05$. Means and standard deviations were determined for height, weight, age, body fat % and VO_{2max}.

Correlations were used to determine any relationships between serum cortisol (Δ cortisol (post-pre)) and each of the non-invasive measures (average response of 30 minutes of exercise) including: HR, RPE, oxygen uptake (VO_2), oxygen pulse ($VO_2 \cdot bpm^{-1}$), blood lactate (La) and La:RPE ratio. More specifically, the relationship was assessed using the relative cortisol changes of each subject ((Δcortisol/resting cortisol levels) x 100% = relative

change). Relationships were assessed using a Pearson Product-Moment correlation coefficient. The correlation analysis assessed the relationship between cortisol levels and non-invasive measure responses across all three exercise intensities (i.e., if n = 20, then "n" for Δ cortisol values and non-invasive variables = 60 due to 3 exercise intensities).

Chapter IV

RESULTS

Subject Characteristics

Eighteen moderate to highly trained endurance male athletes participated in the study. Moderate to highly-trained was defined as participating in endurance-training activities (distance running, swimming, distance cycling, etc.) for at least sixty minutes per day and during at least three days per week during the previous six months. Seventeen of the eighteen subjects completed all aspects of the study with one discontinuing the study after the completion of two sessions. Anthropometric measurements were taken for each subject (n=18) and means for each measurement were as follows: age (years) = 21.4 ± 3.9 years, height (cm) = 177.4 ± 7.3, mass (kg) = 69.8 ± 11.1, body mass index (BMI) (kg/m^2) = 22.1 ± 2.2, body fat (%) = 9.2 ± 2.7. Prior to participating in the study, the subject's carbohydrate intake was monitored and was required to exceed 50% of their daily caloric intake. The mean carbohydrate intake was 55.7% ± 4.6%. All subjects indicated that all pre-session guidelines and restrictions were followed before each study session including the VO_{2max}/orientation session.

VO_{2max} /Orientation Session

The results of the VO_{2max} test are outlined in Table 1. Overall, the results indicate that the subjects possess superior cardiovascular fitness (Wilmore & Costill, 2005).

Measure	Value
Absolute VO_{2max} (L/min)	4.24 ± .83
Relative VO_{2max} (ml/kg/min)	60.88 ± 7.07
Peak Heart Rate (bpm)	192.61 ± 7.11
Peak RPE	18.83 ± 0.71
Peak RER	1.09 ± 0.05
Length of Test (min)	16.11 ± 2.13

Table 1. The VO_{2max} test results are reported as mean values ± standard deviation (SD).

Rest-Q Scores

Rest-Q mean values are found in Table 2. The one-way repeated ANOVA revealed no significant differences in scores between the sessions which suggest that stress levels were successfully low and consistent for each of the sessions.

Measure	Control	40% VO_{2max}	60% VO_{2max}	80% VO_{2max}
Rest-Q Score	8.19 ± 8.96	7.94 ± 6.76	8.62 ± 6.77	7.50 ± 4.59

Table 2. The Rest-Q scores are shown as mean values ± SD.

Plasma Volume Shifts

The shift in plasma volume was assessed from pre-trial to post-trial. The shifts were calculated using both hemoglobin and hematocrit levels taken from the pre-trial and post-trial blood sample. The plasma volume shifts are displayed in Table 3.

Exercise Session	Δ Pre-Trial to Post-Trial (%)
Control	-5.57 ± 5.60
40% VO_{2max}	-10.36 ± 4.06
60% VO_{2max}	-12.01 ± 4.25*
80% VO_{2max}	-12.71 ± 4.76*

Table 3. The plasma volume shifts (%) are reported as mean values ± SD. * denotes significance when compared with control group ($p < 0.05$).

Experimental Sessions

Non-Invasive Physiological Measures

The non-invasive physiological measurements during the exercise experimental sessions are reported in Table 4 (the control session did not have these measures assessed). The three exercise intensities were slightly higher than was originally planned but were still in the range of the proposed intensity increments.

Measure	40% VO_{2max}	60% VO_{2max}	80% VO_{2max}
Heart Rate (bpm)	124.50 ± 12.46*	153.65 ± 12.01**	174.65 ± 12.45
Lactate (La) (mmol/L)	2.58 ± 1.34*	4.53 ± 2.43**	10.24 ± 2.80
La:RPE (%)	55.44 ± 31.63*	72.61 ± 37.34**	127.62 ± 34.92
O_2 Pulse (mL/beat)	14.90 ± 2.74*	17.26 ± 3.06**	19.75 ± 3.71
RPE	9.74 ± 1.93*	12.61 ± 1.87**	16.09 ± 1.10
Relative VO_2 (ml/kg/min)	26.50 ± 3.01*	38.05 ± 4.05**	50.25 ± 4.75
% VO_{2max}	43.81 ± 4.76	62.76 ± 7.51	82.80 ± 5.83
Workload (W)	102.39 ± 20.22	156.71 ± 27.00	195.82 ± 34.40

Table 4. Exercise intensity measurement for overall responses is expressed as mean values ± SD. * denotes significance when compared to the 60% and 80% values ($p < 0.05$). ** denotes significance when compared to the 80% value ($p < 0.05$).

Relationship of Non-Invasive Physiological Measures at Increasing Exercise Intensities

Heart Rate

The mean HR value (bpm) for each exercise session is shown in Table 4. A one-way repeated ANOVA revealed there was a significant difference between each average HR for the three exercise intensities. Post-hoc analysis showed that the 40% mean HR was significantly lower than both HR means at 60% and 80%. In addition, the mean HR at 60% was significantly less than the mean HR at 80%.

Lactate

The mean lactate value (mmol/L) for each exercise session is shown in Table 4. The one-way repeated ANOVA revealed there was a significant difference between mean lactate values between the three exercise intensities. Post-hoc analysis revealed a significant difference shown at the 40% exercise intensity when compared to both the 60% and 80% exercise intensity. Similarly, the 60% exercise intensity displayed a significant difference compared to the 80% exercise intensity.

La:RPE

The mean La:RPE values (%) are shown respectively for each exercise session in Table 4. The one-way repeated ANOVA showed a significant difference of ratio values between the three exercise intensities. Additional investigation using post-hoc methods displayed a significant difference shown between all three exercise intensities. More specifically, the 40% ratio value was significantly less than both the 60% and 80% mean values. Furthermore, the 60% mean ratio value was significantly less than the 80% mean ratio value.

O_2 Pulse

The mean O_2 pulse VO_2 (mL/beat) value for each exercise session is shown in Table 4. The one-way repeated ANOVA displayed a significant difference between mean O_2 pulse values between the three exercise intensities. Post-hoc analysis demonstrated a significant difference in mean values between all three exercise intensities. Thus, the O_2 pulse at 40%

was significantly less than both the 60% and 80% exercise intensity values. Likewise, the 60% O_2 pulse mean value was significantly less than the 80% O_2 pulse mean value.

Rating of Perceived Exertion (RPE)

The mean RPE value for each exercise session is shown in Table 4. A one-way repeated ANOVA showed a significant difference in RPE values between the three exercise intensities. Post-hoc analysis revealed a significant difference in mean values between all three exercise intensities. Therefore, the 40% mean RPE value was significantly less than both the 60% and 80% mean RPE value. Also, the 60% mean RPE value was significantly less than the 80% mean RPE value.

Oxygen Uptake (VO_2)

The relative (ml/kg/min) VO_2 elicited at each exercise session are shown in Table 4. The one-way repeated ANOVA revealed there was a significant difference for the relative VO_2 between the three exercise intensities. Post-hoc analysis showed that the 40% relative VO_2 was significantly less than both the relative 60% and 80%. Furthermore, the relative VO_2 during the 60% exercise session was significantly less than the relative VO_2 at the 80% exercise session.

Cortisol

Absolute Cortisol Changes (Δ)

Absolute (post-pre exercise levels, cortisol Δ (post-pre exercise levels, (µg/dL)) are reported in Table 5. A one way repeated ANOVA showed there were significant differences at both the 60% and 80% VO_{2max} exercise intensities. The greatest margin of absolute increase in cortisol was seen at the 80% VO_{2max} intensity. The control session also displayed

a significant change, specifically a decrease in absolute cortisol levels. The absolute cortisol values were not corrected for plasma volume shifts.

Relative Cortisol Δ

The relative cortisol changes ((absolute cortisol changes/resting cortisol levels)*100, (%)) for the 40%, 60%, and 80% exercise intensities and control session are displayed in Table 5. The one-way repeated ANOVA showed there were significant differences for the relative cortisol Δ between the three exercise intensities and control session. Further analysis using post-hoc methods revealed that the 40% level was significantly less than both the 60% and 80% exercise intensity levels. In addition, the 60% cortisol change was significantly less than the 80% cortisol change. The control session was significantly less than both the 60% and 80% exercise intensity values. The control session was not however, significantly less than the 40% exercise intensity. The cortisol values were not corrected for plasma volume shifts.

Exercise Intensity	**Pre-Exercise** (μg/dL)	**Post-Exercise** (μg/dL)	**Relative Cortisol Δ (%)**	**Absolute Cortisol Δ (μg/dL)**
Control	11.50 ± 4.59	9.55 ± 3.56	-15.63 ± 15.24	-1.95 ± 1.97*
40% VO_{2max}	13.12 ± 6.06	12.32 ± 5.32	-1.47 ± 34.31**	-0.98 ± 4.99
60% VO_{2max}	12.50 ± 4.38	16.50 ± 4.29	42.00 ± 40.86 ***	4.24 ± 3.78*
80% VO_{2max}	11.33 ± 3.92	21.55 ± 6.13	102.45 ± 74.40	10.14 ± 5.76*

Table 5. Absolute and relative cortisol changes at each exercise intensity and control session. Values are expressed in mean values ± SD. * denotes significance when compared to pre-exercise cortisol levels ($p < 0.05$). ** denotes significance with the 60% and 80% exercise intensity relative cortisol values ($p < 0.05$). *** denotes significance with the 80% intensity relative cortisol value ($p < 0.05$).

Correlations of Cortisol and Non-Invasive Physiological Measures

The Pearson Product-Moment correlation coefficient analysis was assessed using the relative Δ cortisol and non-invasive measure response across all three exercise intensities

(i.e., if n = 18, then "n" for Δ cortisol values and non-invasive measure = 54 due to 3 exercise intensities). The results of those analyses are displayed in Table 6. The highest correlation was seen between cortisol and VO_2 (ml/kg/min) while the lowest was found between cortisol and O_2 pulse (mL/beat). All correlations were significant ($p < 0.01$).

Non-Invasive Physiological Measure	**Relative Cortisol Δ**
Heart Rate (bpm)	r = .551*
Lactate (La) (mmol/L)	r = .556*
La:RPE (%)	r = .482*
O_2 Pulse (mL/beat)	r = .382*
RPE	r = .533*
VO_2 (ml/kg/min)	r = .630*

Table 6. Relationship of cortisol and non-invasive physiological measures using Pearson product- moment correlations. The relationship was assessed using combined cortisol and non-invasive physiological measures across all three exercise intensities. * denotes significance between cortisol and non-invasive physiological measures ($p < 0.01$).

Chapter V

DISCUSSION

Introduction

The primary purpose of the study was to determine if the selected non-invasive physiological measures were related to the cortisol response during exercise by endurance athletes. The secondary purpose was to determine which of these measures had the strongest relationship with the cortisol response. Consequently, it was proposed this measure(s) could potentially serve as a substitute indicator of exercise training stress in endurance athletes. It was hypothesized that there would be a significant correlation between cortisol responses and each of the non-invasive physiological measures, in response to the selected exercise intensities (40%, 60%, and 80% VO_{2max}). The selected non-invasive physiological measures studied were: HR, RPE, VO_2, O_2 pulse, La:RPE ratio and blood lactate concentration.

Non-Invasive Physiological Measures

The subjects displayed normal physiological responses to the selected exercise protocols employed in the study. Though each of the actual exercise intensities (%VO_{2max}) were slightly higher than the pre-set intensities, the responses observed were in a normal range. A discussion concerning these normal responses to exercise follows.

To begin, HR showed expected increases as %VO_{2max} was increased (Table 4). It is generally known that increases in exercise intensity leads to both increases in HR and greater oxygen requirements (VO_2) (Astrand et al., 2003). Thus, the present study's HR and

%VO_{2max} relationship in response to increasing exercise power outputs (i.e., workloads) was normal and expected. The RPE response also showed a progressive increase as exercise intensity was increased. The RPE ratings, which measured the subjective effort of subjects, showed a typical greater demand placed on the subjects with increases in exercise intensity. This finding is also generally known and agrees with the research literature (Powers & Howley, 2007).

In addition, blood lactate concentrations also increased with increasing exercise intensity. The observed lactate response is also characteristic of what is expected with increasing exercise intensity (Brooks et al., 1999). The lactate levels at 40% were below the lactate threshold which was the expected outcome. The 60% VO_{2max} concentration response was close to the lactate threshold intensity that is typically reported in the literature (Gollnick et al., 1986). It is well supported that a person's 60% VO_{2max} level is normally indicative of approximately 4 mmol/L blood lactate (Brooks et al., 1999; Gollnick et al., 1986). The present result of 4.53 ± 2.43 mmol/L was thus appropriate for the 60% exercise intensity. The greatly elevated 80% VO_{2max} lactate results were also expected. It is generally reported that an exponential lactate increase is seen after exceeding an intensity of approximately 60% VO_{2max} (Gollnick et al., 1986). This exponential increase in lactate was observed as lactate levels more than doubled from 60% to 80% VO_{2max}.

The increases in lactate also translated to typical increases in the La:RPE ratio which was evident in findings of this study. The exponential increases in lactate help explain the large increase in La:RPE ratios observed between the 40%, 60% and 80% exercise intensities (i.e., as RPE increased, but in a more linear fashion) occurred. The findings reveal that the ratio nearly doubled from 60% to 80% VO_{2max}, compared to only small increases from 40%

to 60% VO_{2max}. These ratio responses were normal and expected and agree with the literature (Snyder et al., 1993). The O_2 pulse measures showed standard responses as well. The O_2 pulse estimates stroke volume along with the arteriovenous oxygen difference responses to exercise (Laukkanen et al., 2006). Stroke volume and arteriovenous oxygen difference determine the myocardial VO_2 uptake responses to exercise; that is the "work of the heart" (Laffite et al., 2003). Therefore, since overall body VO_2 levels (which are highly related to myocardial VO_2) were observed to increase as exercise intensity increased, O_2 pulse should have increased too. Thus, the increased levels of the O_2 pulse as exercise intensity increased were also expected responses.

Cortisol Responses

The cortisol responses (absolute concentration and relative change values) were typical as compared to similar studies that observed cortisol activity during moderate to prolonged exercise activity (Hackney, 2006). As expected, for the 40% VO_{2max} exercise, cortisol decreased post-exercise versus pre-exercise both in relative and absolute terms (see Table 5). Previous studies have shown that 40% VO_{2max} and lower exercise intensities result in decreases in circulating cortisol levels (Davies & Few, 1973; Hill et al., 2008). It is thought this occurs because removal (metabolic clearance) of cortisol from the blood is faster than the cortisol secretion by the adrenal cortex (Powers & Howley, 2007). The cortisol responses at 60% VO_{2max} (both relative and absolute) were as expected and agreed with the literature too (Davies & Few, 1973; Hackney, 2006; Hill et al., 2008). Unlike the adrenal cortex activity seen at the 40% intensity, there is a higher rate of cortisol secretion versus cortisol removal and thus the blood concentrations increased in response to exercise (Powers

& Howley, 2007). The 80% VO_{2max} cortisol response also displayed an increase as was expected and similar to the 60% intensity (increase circulating levels), as there is a much higher rate of adrenal cortisol secretion versus cortisol removal. These findings were expected when considerations are made concerning the physiological function of cortisol during exercise and the overall physical stress of the respective exercise sessions.

The cortisol responses appear unaffected by pre-existing emotional stress or plasma volume fluid shifts. That is, all pre-exercise REST-Q questionnaire scores (stress evaluation questionnaire) were in the acceptable range for all subjects and thus cortisol levels were not unduly elevated by other stressors outside the exercise protocol. Plasma volume shifts were calculated and based upon responses it was concluded that these shifts also did not affect the observed cortisol responses more so in one exercise session than the other.

Endurance Training Applications

Based on the present results, application suggestions can be made pertaining to endurance training programs. As hypothesized, each non-invasive physiological measure displayed significant correlations with cortisol, and overall, most of the relationships exemplified a large degree of magnitude (i.e., effect size). However, it should be noted that due to the lack of previous studies in this area and the exploratory nature of this study, any recommendations should be viewed as tentative and there is a need for additional studies to support or refute the present findings.

To begin, a significant relationship was observed between cortisol and VO_2 during the exercise sessions ($r = 0.630$). Therefore, according to the present results, VO_2 measurement would be the most accurate way to estimate cortisol responses to exercise (and thus the

physical stress of exercise) in a non-invasive fashion. This relationship was expected as it is commonly known that cortisol responses show a strong relationship to increasing work rates that elicit an increasing level of VO_2. Cortisol is released in order to maintain blood glucose levels which tend to decrease due to the greater utilization of glucose as an oxidative energy substrate during exercise (Brooks et al., 2000). However, the prospect of using VO_2 in training programs to easily assess cortisol responses (physical stress) to exercise is minimal due to the impracticality of directly measuring VO_2 in field settings. Direct oxygen uptake (VO_2) measurements require a laboratory setting with sophisticated equipment which likely makes this measurement technique unavailable to most athletes and coaches. On the other hand, it should be noted that previous literature reports a linear relationship exists between VO_2 and HR (Art & Kuipers, 1994). In the present study, a correlation performed between VO_2 and HR was found to be strong ($r = 0.850$). Therefore, this would suggest that HR can be used to estimate VO_2 which could then measure training stress levels. However, the direct correlation between cortisol and HR was $r = 0.551$, and while significant, it was not highly predictive and accounted for only approximately 30% of the variance between the measures. In conclusion, neither assessment of VO_2 (direct or indirect methods [i.e., HR]) provides a valid or practical alternative measure to cortisol when monitoring exercise training stress.

The next closest related measure to cortisol was lactate ($r = 0.556$). The accompanying rise in lactate with cortisol may be explained by a relationship between lactate and the hypothalamic-pituitary-adrenal (HPA) axis. Increases in lactate production have been shown to activate the HPA axis which regulates cortisol secretion (Luger et al., 1987). However, not all studies have reported that lactate directly activates the HPA axis (Petrides et al., 1999). These conflicting findings are somewhat supported by the current findings; that

is, although there was a significant relationship between lactate and cortisol, the level of variance accounted for (~ 31%) was not viewed as extremely large in nature.

Nevertheless, the practicality of blood lactate measurement makes it a viable potential alternative to cortisol, in assessing exercise training stress. Direct measurements of lactate require blood sampling but only a few drops of fingertip blood are needed with hand-held analyzers whereas cortisol analysis requires larger amounts of blood and extensive and demanding laboratory work. The advancement of technology has led to automated lactate analyzers which provide accuracy and short testing delays (only a few seconds) in lactate analysis. Furthermore, it has been shown that a high degree of expertise is not needed when working with these automated lactate analyzers. Karlsson et al. (1983) found there was no difference in lactate values when both an experienced and novice sampler used an automated lactate analyzer. Thus, this may give coaches and athletes a reliable and practical way to approximate cortisol response by using lactate measurements and hence gain valuable insight into an athlete's level of training stress. Although more studies will be needed to fully gauge the lactate and cortisol relationship, the possibility of more easily assessing training stress through lactate concentrations supports the need for those studies.

Heart rate was found to hold only a slightly lower level of a significant relationship to cortisol when compared with previously discussed measures ($r = 0.551$); Tables 4 and 5 show that with exercise, there are both increases in HR and cortisol, respectively. The dual increase in both of these measures may be explained by the relationship seen between catecholamines and cortisol. The catecholamines, epinephrine (E) and norepinephrine (NE) have two roles during exercise. The first is to increase the HR in order to supply the increased need of blood and oxygen to the working muscles but the catecholamines also help

with the maintenance of blood glucose (Brooks et al., 2000). On the other hand, cortisol has been referred to as a permissive hormone which means as a hormone in that it facilitates the actions of other hormones such as E and NE (Powers & Howley, 2007). Cortisol may increase in part to serve in this permissive hormone role for the catecholamines in regulatory efforts to maintain blood glucose levels. This is supported by the fact that the catecholamine and cortisol responses are regulated by a common higher brain center at the hypothalamic level. Thus, the dual increase in HR and cortisol may partly be attributed to the increased levels of catecholamines which influence both increases in HR and blood glucose levels during exercise.

The development of technology for HR monitoring through portable chest monitors has evolved into a common tool used by athletes and coaches to more quickly and accurately assess exercise training intensities (Achten & Jeukendrup, 2003). However, even though HR is practical, it is not very predictive of cortisol and only accounts for ~30% of the variance (as noted earlier). Therefore, individually it does not appear to provide a viable alternative to cortisol when assessing exercise training stress.

As to RPE, there was a weaker correlation between RPE and cortisol, but nonetheless still significant ($r = 0.533$). Tables 4 and 5 show the increases in both RPE and cortisol as exercise intensity increased. Few studies have assessed this relationship, but Rudolph and McAuley (1998) proposed that adrenocortical function does influence RPE responses during exercise. In their study, lower cortisol responses were significantly related to lower levels of RPE responses (Rudolph & McAuley, 1998). A later study found similar results when lower levels of RPE were again related to lower levels of cortisol (Utter et al., 1999). Utter proposed that the connection between cortisol and RPE may be influenced by carbohydrate

availability which affects glucose levels and thus hormonal responses and affective state.

The ease of assessing RPE makes it a desirable and strong candidate to measure in place of cortisol as an indice of exercise training stress. Furthermore, concerning invasiveness, RPE would be the least invasive compared with the other non-invasive physiological measure (e.g., a simple 20 point scale questionnaire is used to assess RPE). Thus, the utilization of RPE would be extremely simple to use by coaches. However, it is critical that athletes honestly assess each measured RPE response to exercise. To keep up with their competition, athletes understand that completing workouts are essential. Problems may arise, when an athlete fears his coach may prematurely end his workout if he shows signs of exercise fatigue and thus this may lead to the athlete not giving accurate RPE ratings (i.e., underrating). As a result, some athletes may report false low RPE values and try to push through workouts which may result in overtraining over time. Still, given the ease of use, RPE measurements could provide a possible simple alternative to blood cortisol sampling for monitoring training stress; however, as with other measures it has low predictive ability.

The O_2 pulse displayed another significant relationship ($r = 0.382$) relative to cortisol. Tables 4 and 5 reveal increases in both O_2 pulse and cortisol, respectively, as exercise intensity increases. It appears no studies have assessed the relationship of O_2 pulse with cortisol in response to exercise. However, an analysis can be made if VO_2 and HR are considered (as O_2 pulse is found by dividing oxygen uptake by the number of heart beats per minute). Observing the present relationship shows that greatest percent increases were seen in VO_2 compared to HR during increasing workloads. Therefore, VO_2 was ultimately

responsible for increases in O_2 pulse. Recalling that VO_2 and cortisol displayed the highest correlation seen during this study helps explain the correlation of O_2 pulse with cortisol.

The ease with which O_2 pulse could be used to assess cortisol has its complications and limitations. Although there was a significant correlation between O_2 pulse and cortisol, there is a difficulty in assessing O_2 pulse. The assessment requires both accurate values of VO_2 and HR and as noted earlier the need for a laboratory to measure VO_2 results in an inconvenience to measure O_2 pulse. Thus, O_2 pulse does not present the most practical method of assessing cortisol response and assessment of training stress.

For the final measure, La:RPE ratio, the correlation with cortisol was $r = 0.482$. The rise in the La:RPE ratio as exercise intensity increased was a result of a greater increase in lactate concentration compared to RPE for each exercise intensity. More specifically, the values at 80% are almost twice those of 60% which is explained by the exponential increases in lactate at the 80% exercise intensity. Therefore, the physiological reasons that explain increases in lactate are applicable to the increases seen in the La:RPE ratio. As was discussed in the lactate section, lactate may influence cortisol levels by affecting the HPA axis (Luger et al., 1987). However, the correlation between cortisol and La:RPE ratios was one of the lowest observed. Reasons for this lower correlation may stem from the fact that cortisol levels decreased at 40% while lactate levels increased thus leading to divergent responses and potentially less of a relationship. Why this did not affect the lactate and cortisol correlation ($r = 0.556$) as much is unclear but it may have to do with the RPE value changes during the exercise effect on the ratio values.

It would seem likely the utilization of La:RPE ratios may prove useful as a substitute indicator of cortisol since it incorporates a physiological and psychological measurement.

More specifically, the ratio includes blood lactate which was the second highest correlation as well a psychological gauge (RPE) of measuring stress which would be beneficial to both coach and athlete. Furthermore, both lactate and RPE are both easily monitored and thus would provide a practical way to assess cortisol levels if the relationship is found to be reliable and valid, which future studies must determine. However, one consideration to keep in mind refers back to the possible inaccurate values of RPE that may be reported by some athletes as noted earlier. Although, it is important to note that in this study the subjects were informed of the critical need to have accurate and unbiased RPE rating to the exercise sessions.

Limitations of Study

The study included potential limitations that could have affected the observed results. First, the study was limited to young male endurance athletes with moderate to high levels of fitness. Thus, it is difficult to generalize the results with other groups of people. Second, prior to each experimental session, it was assumed that the subjects honestly answered all questions regarding stress levels, diet and prior physical activity. It was also assumed that subjects accurately completed the medical history questionnaire. Failure to honestly report pre-exercise behaviors or medical conditions could have affected the study results. An additional factor which may have affected cortisol levels could have stemmed from fear of the veni-puncture procedure. However, this factor was controlled for as much as possible by inserting a catheter prior to each experimental session which reduced the number of needle sticks per session. Furthermore, many of the subjects were experienced with blood sampling and did not find the procedure traumatic. Finally, the present results are only applicable to

single bouts of endurance exercise. Thus, the results are not applicable to overall training periods in endurance athletes.

Summary

To summarize, all the assessed measures had significant correlations with cortisol. As was shown by the Pearson Product Moment correlations, some measures held a stronger relationship with cortisol compared to others. However, none of the measures possessed a strong correlation with cortisol. This is supported by the amount of variance observed between cortisol and each non-invasive measure, as VO_2 which accounted for the most variance was still relatively small (~39%). Therefore, the present results show that though these measures are valid surrogates for exercise intensity, they are not very predictive of cortisol levels and thus may not be very helpful when individually used to assess exercise training stress. However, an exploratory (due to small sample size) step-wise multiple regression analysis was conducted between the non-invasive measures and cortisol and revealed that when blood lactate and La:RPE ratio are combined into a regression model, these measures more strongly relate to and predict cortisol responses to exercise ($R = 0.747$, 55.8% variance, $p \leq 0.001$). Based upon this analysis, and in lieu of the other findings in this study, blood lactate in combination with the La:RPE ratio would appear to serve as the best alternative to cortisol for measuring exercise training stress in endurance athletes to a single exercise session. However, future studies will need to be conducted to substantiate these findings and conclusions.

Chapter VI

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The study observed the relationship between cortisol responses and six non-invasive physiological measures (HR, lactate, lactate:RPE ratios, VO_2, oxygen pulse, and RPE) during exercise. The relationship between cortisol and non-invasive physiological measures were assessed over three experimental exercise sessions (40% VO_{2max}, 60% VO_{2max}, and 80% VO_{2max}). Subjects were male endurance athletes, 18-30 years of age, with moderate to high levels of aerobic fitness.

The Pearson Product Moment correlations revealed significant correlations between cortisol and each of the non-invasive physiological measures. When individually utilized, the non-invasive measures accounted for a very small amount of variance and are therefore not very predictive of cortisol and thus training stress levels. However, the exploratory step-wise multiple regression revealed that combining blood lactate and La:RPE ratio provided a strong predictive level for cortisol and more importantly exercise training stress. Based on this observation and ease of utilization, it is concluded that blood lactate would provide the best alternative to measuring exercise training stress. However, future studies need to confirm these findings and expand upon the research design employed.

Conclusions

H1. *There will be a significant positive correlation between the post-exercise change in blood cortisol levels and HR.* This hypothesis was supported as there

was a significant Pearson Product Moment-correlation coefficient revealed when comparing combined immediate-post exercise cortisol levels and combined HR values of all exercise sessions.

H2. *There will be a significant positive correlation between the post-exercise change in blood cortisol levels and RPE.* This hypothesis was supported as there was a significant Pearson Product Moment-correlation coefficient revealed when comparing combined immediate-post exercise cortisol levels and combined RPE values of all exercise sessions.

H3. *There will be a significant positive correlation between the post-exercise change in blood cortisol levels and the oxygen uptake (VO_2).* This hypothesis was supported as there was a significant Pearson Product Moment-correlation coefficient revealed when comparing combined immediate-post exercise cortisol levels and combined VO_2 values of all exercise sessions.

H4. *There will be significant positive correlations between the post-exercise change in blood cortisol levels and oxygen pulse in response to exercise ($VO_2 \cdot bpm^{-1}$).* This hypothesis was supported as there was a significant Pearson Product Moment-correlation coefficient revealed when comparing combined immediate-post exercise cortisol levels and combined oxygen pulse values of all exercise sessions.

H5. *There will be a significant positive correlation between the post-exercise change in blood cortisol levels and blood lactate (La).* This hypothesis was supported as there was a significant Pearson Product Moment-correlation coefficient revealed when comparing combined immediate-post exercise cortisol levels and combined immediate-post exercise blood lactate values of all exercise sessions.

H6. *There will be a significant positive correlation between the post-exercise change in blood cortisol levels and the La:RPE ratio.* This hypothesis was supported as there was a significant Pearson Product Moment-correlation coefficient revealed when comparing combined immediate-post exercise cortisol levels and combined La:RPE values of all exercise sessions.

Recommendations For Future Studies

Several recommendations for future studies exist. First, future studies should employ a more realistic endurance exercise training protocol that better mimics training cycles of endurance athletes. The present recommendation may provide a more accurate assessment of the relationship between cortisol and the non-invasive measures. Second, the measures should be expanded to better assess the relationship between cortisol and the non-invasive measures. Third, the subject pool should include females along with populations of different fitness and age groups. Fourth, the number of subjects should be increased to help validate the significance of results. Finally, different modes of exercise should be employed. Although, it is thought that these results can be applied to different endurance sports, future studies employing different modes of exercise would help solidify the present findings.

APPENDICES

A. Medical history

B. Physical screening

C. Data collection forms

D. Informed consent form

E. Assay information

APPENDIX A

MEDICAL 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 light-headed 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 ever been treated for or told you might have diabetes? _____ _____
21. Have you ever been treated for or told you might or 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

34. Have you ever been treated for Osgood-Schlatter's disease? _____ _____
35. Have you ever had any injury to your neck involving nerves or vertebrae? _____ _____
36. Have you ever had a shoulder dislocation, separation, or other injury of the shoulder that incapacitated you for a week or longer? _____ _____
37. Have you ever been advised to or have you had surgery to correct a shoulder condition? _____ _____
38. Have you ever experienced any injury to your arms, elbows, or wrists?_____ _____
39. If yes, indicate location and type of injury:_____

_____

40. Do you experience pain in your back? _____ _____
41. Have you ever had an injury to your back? _____ _____

42. If yes, did you seek the advice of a doctor? _____ _____
43. Have you ever been told that you injured the ligaments or cartilage of either knee joint? _____ _____
44. Do you think you have a trick knee? _____ _____
45. Do you have a pin, screw, or plate somewhere in your body as the result of bone or joint surgery that presently limits your physical capacity? _____ _____
46. If yes, indicate where:_____

_____
47. Have you ever had a bone graft or spinal fusion? _____ _____

Activity History

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

Activity	Frequency	Duration	Intensity

54. How would you describe your present state of fitness?
Excellent_____ Good_____ Fair_____ Poor_____
55. Please list the type(s) of work you have been doing for the previous ten years:

Year	Work	Indoor/Outdoor	Location (city/state)

56. Whom shall we notify in case of emergency?
    Name:_____
    Phone: (Home)_____ (Work)_____
    Address:_____
57. Name and address of 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

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 FORMS

Orientation/VO_{2max} Session

Subject Name_____ Subject ID_____

Informed Consent
1. Inform participant of the experimental protocol
2. Make certain that the subject is aware of the possible risks
3. Sign informed consent

Participant Compliance Questions
1. Did subject refrain from strenuous physical activity for 24h prior to $VO_{2\ max}$ testing?
   Yes No
2. Did the subject report to the lab 4 hours post-prandial?
   Yes No
3. Did the subject take NSAIDs, consume alcohol, or caffeine 8 hours prior to testing?
   Yes No

Examinations _____
1. Medical History
2. 12 Lead ECG
3. Physical Examination
4. Blood Pressure_____

Physical Characteristics
1. Age _____ yrs
2. Height _____ cm
3. Mass _____ kg
4. *Percent Body Fat* _____%

   Skinfolds:

   a. Chest (diagonal fold midway between upper armpit & nipple) _____mm
   b. Abdominal (vertical fold; 1 inch to right of navel) _____mm

   c. Thigh (vertical fold midway between kneecap and top of thigh) _____mm

Before VO_{2max} Testing Protocol
1. Set up metabolic system (gas calibration & mouthpiece)
2. Fit electronically-braked cycle ergometer to the participant - record seat position using
   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 light workload
2. 5 minutes of stretching focused on the lower extremities
3. Record resting oxygen consumption to verify values fall within normal range

$VO_{2\ max}$ 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
14. Recovery - reduce resistance and have participant continue pedaling
15. Participant rests (supine) until HR is less than or equal to 100 bpm

Was the total test time equal to or greater than 12 minutes? Test time = _____

Criteria for valid and reliable $VO_{2\ max}$ Test
1. Was there a 150 ml/min or less increase in oxygen consumption in response to an increased workload?
Yes No
2. Did the participant have a maximal RER equal to or greater than 1.1?
RER =_____
3. Did the participant reach age-predicted maximal HR (220-age ± 5%)?
HRmax =_____
4. Did the participant have a RPE equal to or greater than 18?
RPE = _____

Recovery:
1. Reduce resistance and have participant continue pedaling
2. Allow subject to rest (supine) until HR is less than or equal to 100 bpm

Before Subjects Leave Laboratory:
1. Obtain diet 3-day diet record for analysis required before first experimental session
2. Schedule first experimental session

Experimental Exercise Sessions

Subject Name_____ Subject ID_____

Selected Exercise Intensity: _____VO_{2max} Workload: _____W

- Experimental sessions should begin approximately 1 week after the VO_{2max} test session, each experimental session should be separated by $\geq$ 72 hours. Order of sessions will be counterbalanced, experimental sessions should be completed at the same time of day ($\pm$ 30 minutes).

Set-up for experimental sessions

- 1)Turn on (about 30 minutes to warm-up making sure heater is on) and calibrate Parvo system (flowmeter and gas), 2) connect bike to computer, 3) assemble mouthpiece, 4)position RPE board and HR/RPE collection sheet, 5)position fan, 6)locate HR monitor, 7) Adjust seat height on bike to previous selected subject seat height ____cm. 8) Fill ice container for blood samples, 9) Prepare blood collection supplies.

Subject Arrival

- Ask subject the 3 following compliance questions:
  1) Did subject refrain from vigorous activity in the last 24 hours before session? YES NO
  2) Did subject report to the session $\geq$ 4 hours post-prandial? YES NO
  3) Did the subject report to the session $\geq$ 8 hours refraining from alcohol, caffeine, and non-steroidal anti-inflammatory drugs? YES NO
- Administer the REST-Q questionnaire to subject. SCORE_____ (Must be under a score of 36 for the subject to complete session for that day).
- Fit HR monitor to subject. (Do you have a signal?).

Subject Session Protocol

- Supine Rest for 30 minutes.
- 30 minutes of exercise at selected intensity. Intensity _____ VO_{2max} Workload _____W (Provide water for subject if needed).

    - Make sure subject warms up for 5 minutes at 15-20% VO_{2max}, light stretching if necessary.
    - Follow stretching by 3-4 minutes of resting oxygen consumption to make sure values fall within normal range.
- Supine rest for 30 minutes.
- Catheter should be placed during first phase of rest. Blood draws/saliva samples taken immediately after each phase (about 3mL per sample). Use sterile K^2EDTA (purple top) Vacutainer tube: place on ice.
- Make sure subject collects saliva in mouth prior to each sample. (Obtain 0.5-1.0 mL saliva in cup, transfer to polypropylene vial). Use paraffin film if needed.

Exercise Session (HR & RPE Recording)
Intensity: _____VO_{2max} Workload: _____W
0 Minutes HR _____
RPE _____
5 Minutes HR _____
RPE _____
7-10 Minutes (VO_2 Measurement-Remove Mouthpiece After)
10 Minutes HR _____
RPE _____
15 Minutes HR _____
RPE _____
17-20 Minutes (VO_2 Measurement-Remove Mouthpiece After)
20 Minutes HR _____
RPE _____
25 Minutes HR _____
RPE _____
27-30 Minutes (VO_2 Measurement-Remove Mouthpiece After)

30 Minutes HR _____
RPE _____

Post-Session Reminders: 1) Schedule next session, 2) Subject allowed to leave after HR is under 100 bpm.

Experimental Control (Resting) Session

Subject Name_____ Subject ID_____

Control/Resting session will be randomly assigned and may occur at any point in the timeline of the 4 separate experimental sessions. As with the exercise sessions, this session should be spaced 72 hours apart from other sessions and all completed at the same time of day (±30 min) as the other sessions

Administer REST-Q-Sport Questionnaire for Athletes to subject

1. What was the subject's score? _____

The score must be lower than 36 (average of the midpoints of each of the 12 scales; scale ranges from 1 to 6) for the subject to proceed on that day.

Before Starting Control Session
1. Set up blood collection supplies
2. Set up saliva collection supplies

Pre-Rest Procedures
1. The participant will rest in the supine position for 30 minutes
2. While subject is resting, place catheter into arm.
3. After 30 minutes rest, obtain 3-mL of venous blood
4. Place blood into a sterile K^2 EDTA (purple top) Vacutainer® tube; place tube on ice
5. Obtain resting saliva sample (~0.5-1.0 mL); place saliva in polypropylene vial
   If subject has trouble salivating, stimulate through chewing on paraffin film

Resting Protocol
Instead of exercise, subject will rest in supine position for 30 minutes

Immediately Post-Rest
1. Immediately after 30 minute resting/control session, obtain 3-mL of venous blood.
2. Place blood into a sterile K^2 EDTA (purple top) Vacutainer® tube; place tube on ice
3. Obtain saliva sample (~0.5-1.0 mL); place saliva in polypropylene vial
   If subject has trouble salivating, stimulate through chewing on paraffin film
4. Begin supine resting position

Post-Rest Procedures
1. Immediately after 30 minute supine resting session, obtain 3-mL of venous blood.
2. Place blood into a sterile K^2 EDTA (purple top) Vacutainer® tube; place tube on ice
3. Obtain saliva sample (~0.5-1.0 mL); place saliva in polypropylene vial
   If subject has trouble salivating, stimulate through chewing on paraffin film

Before Subjects Leave Laboratory:
1. Schedule next experimental session

APPENDIX D

INFORMED CONSENT

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Participants
Social Behavioral Form

IRB Study #__09-1704_____
Consent Form Version Date: 9/28/20010

Title of Study: The Relationship between Plasma and Salivary Cortisol Levels in Response to Different Exercise Intensities

Principal Investigator: Dr. Anthony C. Hackney
UNC-Chapel Hill Department: Exercise and Sport Science (EXSS)
UNC-Chapel Hill Phone number: 919-962-0334
Email Address: ach@email.unc.edu

Faculty Advisor: Dr. Anthony C. Hackney
Study Contact telephone number: 919-962-0334
Study Contact email: ach@email.unc.edu

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this research study is to learn about the relationship between blood and saliva cortisol levels at rest and in response to different exercise intensities to determine if salivary concentrations can be an accurate method to assess the body's stress response to exercise.

You are being asked to be in the study because you are an endurance-trained male athlete.

Are there any reasons you should not be in this study?

You should not be in this study if you do not exercise regularly; consume a diet chronically low in carbohydrates; have a prior history of hormonal disorders; have a mental illness; or engage in chronic non-steroidal anti-inflammatory (i.e. ibuprofen) drug use.

How many people will take part in this study?

If you decide to be in this study, you will be one of approximately twelve people in this research study.

How long will your part in this study last?

You will be asked to come to the Applied Physiology Laboratory (APL) in Fetzer Gymnasium for approximately 90-120 minutes on 5 separate occasions. These testing sessions will be separated by a minimum of 72 hours. More specific scheduling will be done after you agree to participate in the study, although each session will occur at the same time of day (within 30 minutes). After your final exercise session, your participation in the study will be completed.

What will happen if you take part in the study?

You will be asked to report to the Applied Physiology Laboratory (APL) in Fetzer Gymnasium at the University of North Carolina-Chapel Hill on five separate occasions. You should maintain a consistent food intake throughout the duration of your participation. Also, you are asked to come to the laboratory having not exercised for 24 hours, not eaten for the previous 4 hours, and having consumed no caffeine or alcohol for 8 hours prior to the session. You must wear athletic clothing and shoes, or appropriate cycling gear to each exercise session. A locker room will be provided should you need to change clothes.

First visit to the laboratory:

1. During the first visit, you will learn specific details about the study, read and sign the informed consent form, and have a brief discussion on dietary intake and training history. You will also complete a questionnaire to measure your stress levels. You will then undergo a familiarization process with the bike apparatus, blood and saliva collection procedures, and have your height, and body weight recorded. You will also have your body fat percentage measured using skinfold calipers.
2. Next, your blood pressure will be taken, and then you will have 10 electrodes placed on your chest in order to acquire a 12-lead resting electrocardiogram to determine if cardiovascular problems exist.
3. If the ECG recording is normal, you will be properly fit with a mouthpiece (used to measure your respiratory gases), and a heart rate monitor. A resting oxygen uptake will be assessed to make sure that the values are normal and the metabolic system is functioning properly. If everything is normal, you will be instructed to warm up on the bike for 5 minutes at a very light workload. This light warm-up will be followed by 5 minutes of stretching.
4. After the warm-up, you will perform a bicycling graded exercise test, to determine your maximal aerobic capacity ($VO_{2\ max}$). During the test, your heart rate, respiratory gases, and perceived exertion will be monitored continuously. The incremental exercise test will begin at a workload previously determined by your training history. The workload will

increase at the end of 3 minute intervals (stages) until you feel you can no longer continue.

5. After successful completion of the aerobic capacity test, you will cool down and be allowed to leave the laboratory after your heart rate is less than 100 beats per minute (bpm).
6. Your initial visit to the laboratory will last approximately 90-120 minutes.

Second, Third, Fourth, and Fifth Visits to the Laboratory:

7. On a separate day, at least 72 hours but no more than approximately 7 days, following your previous visit, you will again report to the laboratory for either a resting session (control) or to perform your first 30 minute exercise session at one of the randomly selected intensities. You will report to the laboratory for this test at the same time of day as your first visit (±30 minutes). During this testing session, you will have 3 blood draws performed and 3 saliva samples taken. All of the blood draws will be performed by a certified and experienced professional. The blood and saliva samples will be temporarily kept cool and then stored until the study is complete. After completion of this study, all of the samples will be destroyed.
8. First, you will rest in a supine (lying down, face-up) position (to control for posture) for 30 minutes, after which the pre-exercise saliva and blood samples will be taken. Approximately 1 teaspoon (3cc) of blood and saliva will be collected with each respective sample (3 total samples during each exercise session).
9. After the 30 minute resting period, the first blood and saliva sample will be taken. Next, you will be asked to put on the heart rate monitor around your chest in order to measure your HR throughout the trial. Finally, your resting heart rate and blood pressure will be taken.
10. During each of the separate exercise trials, you will be asked to warm-up for 5 minutes on the bike and stretch. You will then cycle at one of the randomly selected intensities, at approximately either 40%, 60%, or 80% of your maximal aerobic capacity. Your heart rate will be monitored every 5 minutes. You will also have wear the mouthpiece and nose-clip (as with the first session) to monitor your respiratory gases every 10 minutes. You will be able to drink water as needed. The duration of the exercise will be 30 minutes.
11. Immediately after exercise, a second blood and saliva sample will be collected. After the samples are collected, you will be allowed to actively cool down on the bike and then rest.
12. Thirty minutes after the post-exercise blood and saliva samples are taken (Step 9 above), the final blood and saliva samples will be collected, after which you will be allowed to leave the laboratory.
13. During the resting, control session, you will have 3 blood draws performed and 3 saliva samples taken, just as with the exercise sessions. However, instead of the exercise session, you will simply rest. This session serves as a control.
12. Each of these sessions should take approximately 90-120 minutes.
13. This process will be replicated for each of the sessions (40%, 60%, 80% intensity or control resting session), which will be separated by a minimum of 72 hours.

What are the possible benefits from being in this study?

- Research is designed to benefit society by gaining new knowledge.
- You may also expect to benefit by participating in this study by receiving a free physical exam, and resting ECG. You will also get to measure your aerobic capacity (maximal oxygen uptake) that can be used to aid you in any future training. The study will also provide you with your ventilatory threshold, which can also be a tool to be used in training. The serum and saliva cortisol values will also be made available to you after the study.
- This study may help to provide a better understanding of associations between serum and salivary cortisol levels in response to exercise. Both athletes and researchers could benefit from this method's non-invasive, more feasible, and practical alternative to blood sampling if the relationship between these two methodologies are understood and can be properly applied to research design and methodology

What are the possible risks or discomforts involved from being in this study?

- There is a possibility that you will experience some light-headedness, dizziness, or fainting due to maximal and exhaustive exercise. Additionally, there is a rare possibility that you will experience a heart attack or even death. However, due to your physically active status, the risk of catastrophic events is minimized. ECG and blood pressure measurements will be taken before tests to ensure proper heart functioning. The pre-screening physical exam that includes basic measurements of pulmonary, circulatory, and orthopedic function will help detect any abnormalities that may result in termination of your participation in the study. At least two individuals certified in CPR, AED, and first-aid will be present during the entire test, and a proper emergency protocol is established if there is an emergency situation.
- There is risk of bruising or infection with all blood draws. These blood draws will be administered by an experienced professional, certified in phlebotomy. Proper first-aid procedures after the blood draws will also be followed in order to minimize infection and risk of bruising.
- There is a likelihood that you will experience muscle soreness and general fatigue following exercise sessions. This discomfort will be minimized allowing at least 72 hours to pass in between sessions. Also, a proper warm-up and stretch period prior to exercise bouts will help to minimize soreness. No soreness that inhibits daily activities is likely to occur
- There is also a rare possibility that any latent illness may become evident from the exercise or blood work.
- There may be uncommon or previously unknown risks. You should report any problems to the researcher.

How will your privacy be protected?

- Every effort will be made to protect the confidentiality and privacy of the data obtained from you as a subject participating in the study in this study. You will assigned an ID number and all data entered into computer will not be identifiable by your name.
- Only the principal investigator and the faculty advisor will have access to the names and the data of each of the subjects.

- All information will be stored in locked file cabinets and secure password-protected, computer files.
- In the event that the study is published, no association will be made between reported data and your name.

You will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, University of North Carolina at Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. However, by signing this form, you do not give up any of your legal rights.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will not receive anything for taking part in this study.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions, complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research participant?
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

- -

Title of Study: The Relationship Between Plasma and Salivary Cortisol Levels in Response to Different Exercise Intensities

Principal Investigator: Dr. Anthony C. Hackney

Participant's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____ _____
Signature of Research Participant Date

_____
Printed Name of Research Participant

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____
Printed Name of Research Team Member Obtaining Consent

APPENDIX E

ASSAY INFORMATION

Serum 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 125I 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.

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