THE RELATIONSHIP BETWEEN TRAINING LOAD, CORTISOL AWAKENING RESPONSE AND ORTHOSTATIC HEART RATE

Travis Anderson

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Masters of Arts in the Department of Exercise and Sport Science (Exercise Physiology).

Chapel Hill
2016

Approved by:

Anthony C. Hackney

Claudio L. Battaglini

Edgar W. Shields

Amy R. Lane

ABSTRACT

TRAVIS ANDERSON: The Relationship Between Training Load, Cortisol Awakening Response and Orthostatic Heart Rate
(Under the direction of Anthony C. Hackney)

The cortisol awakening response (CAR) is used as a marker of stress, however it is unknown whether CAR will vary in response to acute training load. The orthostatic heart rate test (OHR) is commonly used by athletes, but the evidence for its use is not well established. Therefore, the purpose of this study is to investigate the relationship of CAR and OHR with acute training loads (TRIMP). TRIMP, CAR and OHR data were collected in endurance athletes ($n = 15$) during two weeks of training. No significant relationships were found between any variables and TRIMP (all: $p > 0.05$). The lack of relationships between CAR and OHR suggest these biomarkers are not useful for tracking TRIMP in endurance athletes. However, when accounting for distance, significant relationships were found between CAR and OHR with TRIMP. CAR and OHR can be used to track training load in endurance athletes, as long as both internal and external loads are accounted for.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the extraordinary support of many people. Dr. Hackney, from the genesis of this project you have guided me with a steady hand and I am so appreciative of the time and knowledge you have invested in me. To my committee members, Dr. Battaglini, Dr. Shields and Ms. Lane, your valuable insight and suggestions have improved this study immensely. I must also acknowledge my classmates and now good friends for always being there to talk to and laugh with as we traveled on this journey together. Natalie: thank you for keeping me grounded, smiling and moving forward, and being so understanding through the sometimes-awkward sample collections. Lastly, I cannot thank my family enough for always being there to support my life goals, even from the other side of the world.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic Hormone
ANS	Autonomic Nervous System
AUC_g	Area Under the Curve relative to the ground
AUC_i	Area Under the Curve relative to the increase
CAR	Cortisol Awakening Response
CRF	Corticotropin-Releasing Factor
ELISA	Enzyme Linked Immunosorbent Assay
FOR	Functional Overreaching
HFP	High-frequency Power
HPA-axis	Hypothalamic-Pituitary-Adrenal axis
HR	Heart Rate
HR_{max}	Maximal Heart Rate
LFP	Low-frequency Power
NFO	Non-functional Overreaching
OHR	Orthostatic Heart Rate
OR	Overreaching
OT	Overtraining
OTS	Overtraining Syndrome
PNS	Parasympathetic Nervous System
RPE	Rate of Perceived Exertion
SNS	Sympathetic Nervous System
VO_2max	Maximal Volume of Oxygen Consumption

CHAPTER ONE

Introduction

Endurance running is one of the oldest and most enjoyed sports worldwide. Within the United States of America, the number of participants completing endurance running events has increased 300% since 1990 (*2015 State of the Sport - U.S. Race Trends*, 2015). In 2013, a peak participation rate in endurance running events ranging from 5 km to the marathon was observed, with over 19 million finishers, followed by a growth in participation rates in 2014 in both the half and full marathon distances (*2015 State of the Sport - U.S. Race Trends*, 2015). The demographics competing in these are of course separated from the elite, world-class runners, who have been consistently breaking endurance running records, and separate again from the ultra-endurance athletes competing in events such as the Leadville Trail 100 mile event.

In order to improve and compete in these endurance-running events, whether as an amateur or professional, some degree of physical training is required. Although not all participants will be engaged in serious training programs, it is well known that in order to improve physiological systems, training is essential. This training must address within it what is termed the "Overload Principle" (Huston, Puffer, & Rodney, 1985). This is necessary to ensure a sufficient stress is placed on one's physiology and promote, what Hans Selye in his classic General Adaptation Syndrome model refers to as, resistance (Selye, 1956). Within exercise physiology, the compensations to these training overloads are biological adaptations and are specific to the stress placed on the body (Fry, Steinacker, & Meeusen, 2005). The applied overload will result in fatigue and decreased performances from a single training session,

training day, or short training block (Meeusen et al., 2013). Exhaustion and fatigue will result in cellular adaptations (or mal-adaptations), but with adequate rest can result in adaptations that result in improved performances (Fry, 2005).

If the degree of overload is too great and insufficient time is granted to the recovery (adaptation) process, an athlete may experience the overtraining syndrome (OTS) (Urhausen & Kindermann, 2002). Colloquially, the term overtraining is often used to denote any period with an accumulation of training volume that results in reduced performances (Kreher & Schwartz, 2012). However, the terminology used by Kreider, Fry, and O'Toole (1998) have been widely adopted for the discussion of the overtrained state developing and consists of distinctions between functional (FOR) and non-functional overreaching (NFO), overtraining (OT) and OTS. Importantly, overtraining (OT) and overreaching are terms that refer to processes, whereas OTS is the resultant condition (Fry et al., 2005) and has been estimated to occur in up to 60% of elite endurance runners throughout their careers (Morgan, O'Connor, Sparling, & Pate, 1987).

There is an inherent definitional limitation in determining whether an athlete is in the FOR or NFO or OT stage, due to the need to measure the duration of rest required to achieve previous performance levels. This is clearly a less than ideal situation, as withdrawing an athlete from training and competition to assess their recovery results in an extensive and cumbersome testing procedure and loss of training time. Therefore, a biomarker that is only detectable once an athlete is overtrained serves no practical purpose for the prevention of OTS; rather it only serves as a measure of confirmation that an individual is overtrained. As such, there is a need for a test that can identify increased risk of OTS as a function of training load to serve as an early detection and warning system (Meeusen et al., 2013). Since athletes and coaches often attempt to employ the largest training loads possible in an effort to achieve the greatest degree of

adaptations, biomarkers must be sensitive enough to detect small day-to-day training load variations and provide information regarding the physiological state of the athletes. A useful biomarker for the assessment of overtraining must therefore be specific, sensitive, accurate, reliable and predictive. Whilst having a single all encompassing test to diagnose OTS would be ideal, and many researchers continue this quest for the 'holy grail' of biomarkers, the variable nature of OTS lends itself towards multiple tests (Meeusen et al., 2013) and a potential OTS risk factor composite score being necessary.

The Hypothalamic Hypothesis is perhaps one of the most studied hypotheses of overtraining (Fry et al., 2005) and contends that OT results in a dysregulation of the hypothalamic-pituitary-adrenal axis (HPA-axis). This is evidenced by a multitude of studies that demonstrated changes in adrenocorticotropic hormone, cortisol and testosterone in OTS. Unfortunately, multiple studies show contradictory responses in these variables, both in direction and magnitude of response, which is likely due to the lack of consistency in defining the state of overreaching (FOR/NFO) or OT observed (Meeusen et al., 2013). One method of assessing the HPA-axis is known as the cortisol awakening response (CAR) and is a marker of the change in cortisol concentrations from the time of awakening to 30-45 minutes post-awakening (Hucklebridge, Mellins, Evans, & Clow, 2002). Although this marker has been often used in HPA-axis activity in psychological research (Clow et al., 2010), there are limited reports of its use in measuring the physical stress associated with physical training.

A second overtraining hypothesis is known as the Autonomic Nervous System hypothesis, and contends that OT results in an imbalance in the ANS, specifically between the sympathetic and parasympathetic divisions (Lehmann, Foster, Dickhuth, & Gastmann, 1998). Although these imbalances are often evidenced by the use of heart rate variability measurements,

heart rate measurements prior to and following an orthostatic stress provides a simple and easily accessible method for the assessment of ANS activity. Currently employed by many athletes and coaches, the use of orthostatic heart rate measurements (OHR) in athletes are less well documented in the scientific literature.

Statement of Purpose

With the above points in mind, the purpose of this study was to assess whether training loads observed during regularly scheduled training periods in endurance athletes can be assessed via the cortisol awakening response or orthostatic heart rate. Furthermore, this study will provide insight into the relationship between the cortisol awakening response and autonomic nervous system activity as assessed via the orthostatic heart rate test in response to variable training loads.

Research Questions

RQ1. Does the cortisol awakening response correlate with acute training loads, during a regular two week training period, as measured and expressed as:

   a. Area under the curve; or

   b. Magnitude of change?

RQ2. Does the orthostatic heart rate test correlate with training load changes, during a regular two-week training period?

RQ3. Does the orthostatic heart rate test correlate with cortisol awakening responses, expressed as:

   a. Area under the curve; or

   b. Magnitude of change?

Research Hypotheses

H1. The cortisol awakening response will be positively correlated with training loads, measured as both area under the curve and magnitude of change.

H2. The orthostatic heart rate test will be positively correlated with training load, measured as area under the curve and magnitude of change.

H3. The cortisol awakening response and orthostatic heart rate test will be positively correlated for all variables.

Assumptions

Since this study employed no intervention or manipulation of training loads, many assumptions were made. Firstly, it was assumed that participants were accurately and honestly recording their training session information, including their rate of perceived exertion. It is also assumed that participants were in compliance and strictly adhering to saliva collection and heart rate assessment procedures and specimen storage protocols.

Definition Of Terms

Cortisol – The major glucocorticoid hormone in humans, released from the adrenal cortex.

Cortisol Awakening Response – The increase in cortisol concentration that accompanies the awakening process.

Heart Rate Variability – The variation in beat-to-beat heart rate measurements, often used as a measure of cardiac autonomic regulation.

Heart Rate Reserve – The difference between maximal and resting heart rate values.

Orthostatic Heart Rate – The increase in heart rate that accompanies movement from a supine to standing position, often recorded as both the peak and average responses.

Delimitations

Participants were delimited to endurance trained runners, with a competitive running history of >1 year who were currently training for and competing in endurance running events >5 km. Participants were delimited to runners who train regularly (≥5 days per week) and consistently alternate training loads. An attempt was made to recruit only athletes who are willing to have several training sessions in the two-week period of sessions considered to be 'hard' (>15) based on the 6-20 Borg Rate of Perceived Exertion.

Limitations

Due to the observational nature of the study design, there were clear, inherent limitations. Firstly, since training volume was not manipulated, variation existed, both in the timing, duration and intensity of the training sessions that were considered to be 'hard' by participants. Secondly, due to individual variation in competition schedules, stage of training or periodization model, participants were in varying phases of fitness development and have varying degrees of training history, both acutely and chronically. Although participants were asked to maintain their regular consistent diet throughout the course of the study, the manipulation of dietary factors may have affected the cortisol awakening responses.

Significance

It is known that CAR is a strong potential indicator of the development of burnout, overtraining and overreaching. However, what is currently unknown is whether CAR is a sensitive enough marker to respond to daily training loads in trained endurance athletes, or whether any variations in CAR agree with daily variations in ANS activity, measured via the orthostatic heart rate test. Therefore, it is necessary to investigate the day-to-day variations in

CAR and the relationship with training load, measured by both objective and subjective measures.

If overreaching and overtraining are to be avoided, along with the accompanying physiological and psychological complications, a comprehensive approach to biomarkers and subjectivity must be applied. As discussed earlier, the current method of assessing training loads and subsequent risk of developing overtraining symptoms is rather axiomatic: if one trains until one develops symptoms of overtraining; then the training load was too great. What is needed is a biomarker that can not only provide physiological evidence that one is overtrained, but a number of physiological measurements that are collectively robust yet sensitive enough to monitor training loads and prevent the development of overtraining syndrome (Urhausen & Kindermann, 2002).

CHAPTER TWO

Review of Literature

The following review of literature will begin by addressing the physiological systems of particular interest to this research project; namely, the hypothalamic-pituitary-adrenal axis of the endocrine system and the autonomous nervous system. The review will then discuss the current state of overtraining research, followed by a more detailed analysis of the Hypothalamic Hypothesis and ANS Hypothesis. The review will then conclude with an assessment of the current state of the proposed monitoring procedures: the cortisol awakening response and orthostatic heart rate test.

Hypothalamic- Pituitary-Adrenal Axis

Metabolic functions within the body are controlled by multiple interacting biological systems. One such system is the neuroendocrine system, which is responsible for regulating the production and secretion of hormones from endocrine glands. These hormones act throughout the body to perform specific functions at multiple sites and are often grouped by their structure and/or function. One group of hormones are known as the glucocorticoids, named after their effect on blood glucose, release by the adrenal cortex and their steroidal carbon structure (Brooks, Fahey, & White, 1996). The predominate glucocorticoid hormone in humans is cortisol and its production and secretion is stimulated by a cascade of events that occur in response to both psychological and physiological stress (Hackney, 2006). When the hypothalamus is stimulated to secrete corticotropin releasing factor (CRF), the anterior pituitary gland is subsequently stimulated to secrete adrenocorticotropin hormone (ACTH). ACTH then circulates

and stimulates the adrenal cortex to secrete cortisol; this interaction is known as the hypothalamus-pituitary-adrenal (HPA) axis. Cortisol secretion follows a diurnal pattern, often related with mealtime increases, with the greatest resting concentrations being present early in the morning at approximately 0800 (Hellman et. al 1970). One mechanism for activating the cascade for cortisol release is lowered blood glucose levels. During exercise, as glucose is progressively removed from the blood for subsequent energy liberation, blood glucose levels begin to decline. Proceeding exercise, cortisol has been shown to remain elevated for almost two hours into recovery (Duclos, Corcuff, Rashedi, Fougere, & Manier, 1997), but the time of this post-exercise elevation is highly dependent on the duration (Viru, Karelson, & Smirnova, 1992) and intensity of the exercise (Davies & Few, 1973; Hackney, 2006; Hill et al., 2008). Moreover, the age and fitness capacity of the individual (Traustadottir, Bosch, & Matt, 2005) and the presence of a competitive environment (Obminski et al., 2002; Viru et al., 2007) will also impact the level of cortisol release. Of course, due to the naturally occurring cyclical changes in resting cortisol levels, the duration of cortisol elevation recovery is also related to the time of day the exercise occurs (Brandenberger & Follenius, 1975) as well as potential seasonal influences that lead to variations in cortisol concentrations (Gouarné, Groussard, Gratas-Delamarche, Delamarche, & Duclos, 2005). During the recovery process, the continued elevation in cortisol concentration is thought to be a dominant contributor to restore blood glucose (Brooks et al., 1996).

Autonomic Nervous System

The Autonomic Nervous System (ANS) transmits signals from the central nervous system (CNS) to the periphery and as the name implies, is subconsciously controlled. The ANS controls many physiological systems throughout the body, including heart contractility force and

rate, vasodilation and vasoconstriction, smooth muscle contractility, lung airway dilation, endocrine release (e.g. pancreas and adrenal medulla control) and hepatic gluconeogenesis/glycogenolysis (Freeman, Dewey, Hadley, Myers, & Froelicher, 2006). The ANS can be divided into two separate branches, distinct from each other both anatomically and physiologically (Freeman et al., 2006). The parasympathetic nervous system (PNS) branch is mostly implicated in the digestion and recovery processes and is primarily facilitated by the tenth cranial nerve, known as the Vagus nerve (Brooks et al., 1996). All preganglionic axons in the ANS utilize the neurotransmitter acetylcholine (ACh) in signal transmission at neural synapses. The postganglionic nerve fibers within the PNS also utilize ACh as a neurotransmitter to affect the target tissue. In contrast, the sympathetic nervous system (SNS) is involved in the classic "flight or fight" response (Curtis & O'Keefe, 2002) and primarily utilizes the neurotransmitter norepinephrine (NE) in the postganglionic fibers to affect changes at the target. Most ANS controlled organs are under control of both the parasympathetic and sympathetic branches and the action at the particular site is a result of both predominance of one branch and relative proportion of receptors types. The balance between PNS and SNS activity is termed vagal tone and it has been proposed that exercise induced bradycardia in athletes is partially due to an increased Vagal tone to the sinus node (Achten & Jeukendrup, 2003). During exercise, sympathetic action will predominate and at the cessation of exercise, sympathetic activation will decrease over time (inversely proportional to fitness level), in favor of greater parasympathetic activity (Freeman et al., 2006).

Overtraining

The first phase of overtraining syndrome (OTS) development is a phase known as functional overreaching (FOR). FOR is characterized by a decrease in performance and affective

state, but these decrements can be mediated and reversed with sufficient rest within a two-week period (Meeusen et al., 2013). If the athlete is unable to regain their pre-overreached performance within the two weeks of rest, they are then deemed to be in the non-functional overreaching (NFO) phase (Meeusen et al., 2013). The continuation of training beyond the NFO phase will continue to see performances decrease and more severe decrements in psychological afferent state, immune function and other manifestations. Although difficult to categorize, and no current definition contains strict parameters for declaring an athlete has entered this stage, athletes with the aforementioned condition are deemed to be in the overtrained state (OT). The process of OT will result in the condition known as OTS. This condition can often require weeks or months of rest and recovery prior to the athlete being able to return to training. As Meeusen et al. (2013) recognizes, the use of the term 'syndrome' indicates that this condition is a result of multiple factors and not solely based on exercise (i.e. psychology, capacity to resist fatigue etc. will contribute to the condition). OTS has previously been labeled as the 'unexplained underperformance syndrome' due to the inability to identify a plausible cause; this implies that other factors must be ruled out that may explain the underperformance of persons, including disease states (Meeusen et al., 2013). Although not an exhaustive list, practitioners must be aware of asthma, thyroid disease, adrenal disease, diabetes or infections which all may explain underperformance (Kreher & Schwartz, 2012), prior to considering OTS.

Coaches will often utilize periods of overreaching to induce large compensations (Huston et al., 1985), referred to as super-compensations, and improved performances. For example, a study by Le Meur et al. (2013) induced a period of overreaching in twenty-three trained male triathletes and compared their performances after a tapering period to athletes that underwent a regular training cycle. After the two-week taper, the overreached athletes showed performance

improvements of 7.9 ± 2.4% over pre-training levels, compared to only 3.9 ± 4.6% in the control group (Le Meur et al., 2013). However, some researchers advise against this practice due to the inherent unpredictability of developing overtraining symptoms (Brittenham, Cioroslan, & Davis, 1998 via Urhausen & Kindermann, 2002; Urhausen & Kindermann, 2002). Therefore, training blocks aimed at overreaching an athlete must be closely monitored to ensure the athlete does not reach the non-functional overreaching phase.

Clearly there are enormous difficulties in utilizing these definitions, since there seems to be no specific breakpoint between the conditions, and therefore suggests an oversimplification (Meeusen et al., 2013). It may be best to view these conditions on a continuum, with each definition placed at varied points, based on individual physiology and psychology (Fry, Morton, & Keast, 1991).

A further difficulty in defining these terms is the inconsistency in the literature, with some studies reporting changes in certain variables, whilst other studies reveal contradictory findings. This seems to be related to the individual nature of overtraining and the lack of a gold standard measure for diagnosing an athlete with OTS (Meeusen et al., 2013). Since the differences between overtraining and FOR or NFO are based on the time required to return to pre-diagnosis performance, specific diagnoses are only possible in a retrospective manner. That is, waiting to assess the length of time for the athlete to reach full recovery and retroactively diagnosing the athlete (Kreher & Schwartz, 2012). If the time to recovery takes less than 14-21 days, the athlete would be diagnosed with NFO, compared to being diagnosed with OTS if the time to recovery is longer than 21 days (Meeusen et al., 2013). However, some researchers also emphasize the need to report the severity of symptoms in diagnosing OTS in future research (Pyne & Martin, 2011). No matter, in very few other debilitating conditions is a diagnosis

withheld until the athlete is no longer incapacitated. For an elite athlete whose life, livelihood and often identity is tied to athletic structure, this seems to present an incredible additional burden and is often rejected by athletes and coaches due to the possibility of detraining (Le Meur et al., 2013). However, there are some potential warning signs associated with the development of overtraining that one may use to identify an athlete at high risk of overtraining. These include but are not limited to: an increased training load without adequate recovery, sleep disturbances, altitude exposure, heat injury episode, a severe 'bonk' during training or competition or a high degree of training monotony (Kreher & Schwartz, 2012).

Although the development of our current understanding of overtraining is relatively recent, the concept of excess work leading to reductions in performance or productivity is well known, and has previously been referred to as "burnout, staleness, failure adaptation, under recovery, training stress syndrome and chronic fatigue" (Kreher & Schwartz, 2012). In fact, the modern understanding of burnout shares many similarities with OTS (Selänne, Ryba, & Leppäluoto, 2013). For example, both burnout and OTS show decreased performance at work/athletic performances, increases in tiredness and irritability, experience sleep disturbances, increased risk of infection and sickness and cardiovascular changes (Selänne et al., 2013).

Overtraining is not limited to the athletic field, but is also just as applicable to other physically demanding occupations such as the military. In 2011, Tanskanen and associates investigated the effects of an 8-week basic training course for new recruits in Finland. Basic training within the military traditionally incorporates both a high physical load and a change in environment and routine, possibly adding additional stressors to the soldiers. Tanskanen et. al found that following this eight-week period, 33% of the conscripts were identified as being overreached. This was indicated by their fulfillment of three of five criteria: decreased physical

performance, increased perceived exertion, unable to participate in physical testing due to illness, increased emotional or somatic symptoms, or a high incidence of absence due to illness (Tanskanen et al., 2011).

The specific rates of overtraining are difficult to quantify, due to many of the limitations in study design and definitions. However, it has been proposed that >60% of elite male and female runners may experience overtraining throughout their careers (Morgan, O'Connor, Ellickson, & Bradley, 1988; Morgan, O'Connor, Sparling, & Pate, 1987). Malone (2014) showed that a 6-week intensive basketball training schedule resulted in 50% of the athletes developing symptoms of overtraining, while a study of elite soccer players in Belgium showed 60% of athletes overreaching (Naessens, Chandler, Kibler, & Driessens, 2000). Within this group, 20% of the athletes were diagnosed as being overtrained over the course of the season (Naessens et al., 2000). In a study by Kentta, Hassmen, and Raglin (2001) an overall incidence of staleness in 37% of Swedish youth athletes was recorded, but the authors noted that the rate was varied by sport type. Individual sports showed a higher incidence of overtraining, at 48%, compared to 30% of athletes in team sports (Kentta et al., 2001). The greatest incidence was surprisingly found in badminton players (94%), suggested to be related to the frequency of competition (Kentta et al., 2001).

Many studies have attempted to discover a marker or system of markers that can adequately diagnose the OTS. However, although many biological markers exist, particularly hematological markers, such as creatine kinase (Halson, 2014) and liver enzymes such as AST, ALP, and ALT (Wallace, Slattery, & Coutts, 2014), none have been found to be able to detect overtraining or overreaching consistently (Meeusen et al., 2013). However, these biomarkers

may prove useful in the exclusion of disease states; a necessary part of OTS diagnosis (Meeusen et al., 2013).

To assess whether an athlete has incurred a performance decrement, oftentimes, physical assessments are proposed. This is logical, since the presence of performance decrements are a requirement of the OTS diagnosis. One such test requires an athlete to complete "two incremental, maximal exercise tests separated by 4 hours" (Meeusen et al., 2004). When tested by Meeusen et. al in 2004, the researchers showed that the second bout of exercise resulted in a decrease of 3% between bouts prior to the start of a physically intense training camp. This was compared to after the camp, where a 6% decrease was found in NFO athletes and an 11% decrease in the OT athletes. Moreover, the test was able to reveal hormonal differences between NFO and OT athletes that would not have been elucidated in a single exercise bout. In 2010, Schmikli et al. attempted to validate field test use for diagnosing overtraining. The researchers used a multi-stage shuttle test and a Zoladz test on soccer and middle-distance athletes respectively, and found the athletes with performance decrements (i.e. overreached athletes) also displayed negative mood states and decreased cortisol levels at rest.

There are several hypotheses put forward in an attempt to explain the etiology of OTS. For a more extensive review of each hypothesis, Kreher's 2012 review article is recommended. Suffice to say however that each hypothesis has associated strengths and weaknesses. Of particular interest to this review and the proposed research study are the biomarkers associated with the Hypothalamic Hypothesis and the Autonomic Nervous System Hypothesis.

Hypothalamic Hypothesis

The formulation of the hypothalamic hypothesis was likely fueled by an equine study which showed that increased levels of intensive training reduced the adrenal cortex reaction to an

ACTH stress test (Persson, Larsson, & Lindholm, 1980). Since this early investigation, salivary cortisol levels have been extensively studied in overreaching and overtraining athletes. Soon after this hypothalamic disruption was identified in equine subjects, Barron, Noakes, Levy, Smith, and Millar (1985) investigated the phenomena in overtrained athletes. The researchers studied six marathon competitors, training for a 92 km race. The athletes all followed individual training programs, running between 110-190 km per week. The athletes were tested multiple times over a four-month period, including prior to a standard 42 km race. The researchers found that the four athletes presenting symptoms of OT had significantly lower ACTH, growth hormone and cortisol levels compared to their non-OT peers following administration of intravenous insulin. However, these values returned to regular levels within four week of rest and became indistinguishable from the non-OT values (Barron et al., 1985).

As described earlier, NFO and OTS can be differentiated by a two-session exercise testing protocol (Meeusen et al., 2010). The most sensitive measures that were able to differentiate the two conditions were the ACTH and PRL responses in the second exercise bout, with NFO responses being greater than the blunted responses seen in the OTS athletes (Meeusen et al., 2010). In agreement with this blunted response, a previous investigation demonstrated that exhaustive exercise resulted in a blunted β-endorphin pituitary response to exogenous human CRH in well-trained athletes (Keizer, Platen, & Koppeschaar, 1991).

The HPA-axis dysregulation can be viewed on a continuum with non-distinct phases of "disturbance, adaptation, and maladaptation" (Meeusen et al., 2013). As such, evidence exists for a biphasic response of the HPA-axis to overreaching, and overtraining (Fry et al., 2005). During regular training (overload), both cortisol and ACTH will increase with training and is a regular stress response (Hackney, 2006). However, during overreaching, a desensitization of the adrenals

will occur, producing a lower cortisol response to increased levels of ACTH and CRH (Roberts, Wessely, Chalder, Papadopoulos, & Cleare, 2004). The development of OT state has been characterized as a continued decline in cortisol levels, followed by a significant decrease in central endocrine factors (i.e. ACTH) (Steinacker, Lormes, Reissnecker, & Liu, 2004). This is in fact similar to what previous studies have suggested occurs within the autonomic nervous system.

Researchers have contended that there is a distinct difference between what has been termed the sympathetic (Basedow) and parasympathetic (Addison) overtraining syndrome (Lehmann et al., 1998). Interestingly, sympathetic overtraining syndrome (a predominance of sympathetic nervous system activity), appears to occur more often in anaerobic type sports, although the late Dr. Manfred Lehmann infers this is a more rare occurrence (Lehmann, Foster, & Keul, 1993). This increase in sympathetic activity will increase catecholamine production and secretion, resulting in a down-receptor phenomena decrease in β2 receptor expression and subsequently reduced catecholamine sensitivity (Fry, 2005). Parasympathetic OTS however is characterized as a reduction in sympathetic activation (Lehmann et al., 1998) and has been suggested to occur in endurance athletes more so than strength athletes (Lehmann et al., 1993). This will result in decreased HPA drive, decreased catecholamine secretion and decreased β2-receptor expression – all resulting in decreased performances (Fry, 2005). This is agreeable with the ACTH and cortisol response variations between OR and OT discussed above and may serve to explain the variations seen in ANS studies.

Nonetheless, the feasibility of using peripheral hormonal data is questionable, since other biomarkers (interleukin-6, insulin-like growth factor 1, leptin and insulin) will affect the HPA-axis and therefore interrupt the metabolic role of the HPA-axis (Steinacker et al., 2004).

Meeusen et al. lists several issues with our current method of assessing OTS through hormonal data and among other requirements, suggests that food intake, diurnal responses, menstrual cycle and repeatability need to be addressed.

A study of salivary cortisol has shown elevated levels in collegiate swimmers who experienced overtraining during a regular competitive season (O'Connor, Morgan, Raglin, Barksdale, & Kalin, 1989). However, this investigation occurred at a time prior to a more definitive use of overtraining terminology, and so the criterion for diagnosing OT in this investigation is questionable. Lehmann et al. found small decreases in cortisol and aldosterone levels at rest and following maximal exertion after increases in training volume over the course of 3 weeks (Lehmann et al., 1992). The highly trained athletes did not however present any cortisol changes in response to the change in intensity of training (Lehmann et al., 1992). Interestingly, only the increase training volume resulted in decreased performances and increases in feelings of exhaustion and fatigue (i.e. overtraining symptoms) whereas the increases in intensity of training did not (Lehmann et al., 1992). This was confirmed in another study by the same research group, when they found greater indices of overtraining with increases in training volume when compared to increases in intensity (Lehmann, Wieland, & Gastmann, 1997).

Some researchers have suggested that cortisol measurements alone are not sufficient for addressing training status. Therefore, the ratio of testosterone to cortisol (T:C) was used as an indicator of the catabolic and anabolic processes within the body (Adlercreutz et al., 1986). In the investigation of military conscripts mentioned above, researchers found biomarker changes, including increased basal cortisol, greater basal levels of serum sex-hormone binding globulin, and a decreased T:C in overtrained participants compared to their non-overtrained peers (Tanskanen et al., 2011). The mode of exercise must be considered, since weightlifting for

extended periods has not resulted in decreased T:C (Scanlan, Wen, Tucker, & Dalbo, 2014), although in a recent study of American football players undergoing an intensive weightlifting program that resulted in performance improvements, this ratio did decrease (Wallace, Slattery, & Coutts, 2009). Although some endocrinologists viewed the T:C ratio as an over simplistic application, this variable does consistently alter as a result of overtraining (Fry et al., 2005) although some have argued that the T:C ratio is only an indicator of physiological strain, rather than a method for determining OTS (Meeusen et al., 2013). Again, differences in testing procedures and sensitivity of the instruments, coupled with definitional differences, have potentially led to inconsistent findings.

Autonomic Nervous System Hypothesis

The ANS Hypothesis states that OTS is a result of an imbalance in the autonomic nervous system (Lehmann et al., 1998). Specifically, an imbalance occurs between the sympathetic and parasympathetic branches of the ANS. One method of assessing autonomic function is to assess the variations in beat-to-beat measurements, termed heart rate variability (HRV). It has been shown in both clinical and experimental settings that parasympathetic activity is a major contributor to the high-frequency power (HFP) component of the HRV power spectrum, whereas the low power spectrum (LFP) is more controversial, possibly representing sympathetic tone or a combination of parasympathetic and sympathetic influences (Achten & Jeukendrup, 2003).

In support of the ANS Hypothesis, previous investigations have observed ANS responses to non-physical stressors. For example, the impact of a mild real life stressor was assessed by Lucini, Norbiato, Clerici, and Pagani (2002) and showed that in response to an upcoming university examination, individuals showed parasympathetic withdrawal and increased

sympathetic activity at rest. Likewise, Dishman et al. (2000) demonstrated that individuals with an increased perception of recent emotional stress presented increased vagal modulation. Other psychological research has shown that PTSD patients have an altered autonomic cardiac response, with higher resting heart rate and lower HRV (Cohen et al., 2000). A specific analysis of the HRV power spectrum revealed decreased parasympathetic activity and increased sympathetic activation, which is consistent with other psychological stressors (Cohen et al., 2000).

Investigations of ANS changes to exercise stress have shown similar results. In a study of cyclists undergoing a 7-day intensive training period, an increase in HRV was observed (Halson, 2003). This particular disruption of the ANS implied parasympathetic dominance over sympathetic input. Uusitalo, Uusitalo, and Rusko (2000) investigated cardiovascular changes in female athletes as a result of resistance training. Using HRV measurements, the researchers found increases in the low-power spectrum HRV, suggesting increases in sympathetic activity. Moreover, the HRV in the standing position was shown to decrease after the overtraining period and indicated, “pronounced vagal withdrawal” (Uusitalo et al., 2000). However, this finding was not consistent in the five overtrained athletes from the study, as one athlete showed increases in very-low power spectrum analysis with decreases in the other spectrums.

Changes in these parameters are not universally observed. For example, after a 6-day intensive training camp for elite canoers, which resulted in decreased performances (i.e. indicative of overreaching), no changes were observed in HRV, including measurements taken during an orthostatic tilt test (Hedelin, Kentta, Wiklund, Bjerle, & Henriksson-Larsen, 2000). Yet, the same author, in a case study of an elite junior cross-country skier, showed an increase in HFP after a period of decreased performances suggesting parasympathetic modulation. This

change in HRV was reversed in this individual, only after a two-month recovery period (Hedelin, Wiklund, Bjerle, & Henriksson-Larsen, 2000). Furthermore, in a study of twelve severely overtrained male and female athletes, Hynynen and colleagues showed that overtraining led to autonomic disturbances during awakening, but not during sleep (Hynynen, Uusitalo, Konttinen, & Rusko, 2006). Specifically, researchers found a decreased LFP in the overtrained athletes when awakening compared to the control group (Hynynen et al., 2006). The authors recognized that previous investigations have shown hyperactivity of the HPA-axis in burnout patients as a response to awakening (Greaves‐Lord et al., 2007); these results suggest then, that the awakening response could be a key period for the monitoring of ANS activity.

Biomarkers

Cortisol Awakening Response

As discussed earlier, cortisol follows a diurnal pattern, with the greatest levels often observed in the morning upon waking (Hellman et al., 1970). As such, the rate of this morning increase can be an important indicator of HPA-axis activity (Schulz, Kirschbaum, Prüßner, & Hellhammer, 1998). The cortisol awakening response (CAR) is a measure of cortisol changes upon awakening, and represents a period of increasing cortisol concentrations, peaking at approximately 30-45 minutes after waking (Hucklebridge et al., 2002). CAR is not directly related to cortisol secretion throughout the remainder of the day, thereby leading researchers to suggest CAR to be initiated by the waking process through activation of the hypothalamic suprachiasmic nucleus and fine-tuned by the sympathetic nervous system (Clow et al., 2010). Interestingly, in a study using pharmacological interventions, Schmidt-Reinwald et al. (1999) showed varied responses of CAR based on suppression of specific points in the HPA-axis. These

results demonstrated that CAR was perhaps more related to adrenal function than hypothalamic or pituitary responses.

One of the first investigations of CAR was from Prussner's research team in 1995, who observed that levels of free cortisol doubled within minutes of waking up (Prussner, Kirschbaum, & Hellhammer, 1995). Then, in 1997, the same research group observed the stability of CAR across several groups (elderly, adult and children) and concluded that CAR is a stable (intra-individual) measure of HPA-activity (Pruessner et al., 1997). Since these early investigations, CAR has often been used in psychobiological research as a measure of HPA-axis activity and status (Clow et al., 2010).

An investigation by Wust et. al (2000) confirmed what was alluded to in previous investigations in regards to the robustness of the CAR. The researchers found that age, the use of oral contraceptives or an alarm clock did not significantly impact CAR. They did find that smoking and sleep duration did have a significant impact, although accounted for ~1% of the variance. There were significant gender interactions, but again, this was a small effect accounting for only 3% of the variance. The authors concluded that none of the aforementioned variables would "have a considerable impact on free levels (of cortisol) after awakening" (Wust, Wolf, et al., 2000). Confirming these results, Kudielka and Kirschbaum (2003) found no effect of menstrual cycle, or habitual smoking or between men and women. In this investigation, health status, age and awakening time, did however effect the response (Kudielka & Kirschbaum, 2003). Likewise, Bouma, Riese, Ormel, Verhulst, and Oldehinkel (2009) found no effect of gender or menstrual cycle on CAR in adolescent aged participants. However, oral contraceptives did appear to show a slightly blunted effect in women. Hucklebridge et al. (2002) found no effect of postural differences in cortisol awakening response, providing evidence that it is not the act of

standing after waking that affects cortisol, but rather a physiological response to internal stimuli. A recent study by Smyth, Thorn, Hucklebridge, Evans, and Clow (2015) confirmed what was previously termed the latency period in the CAR. This period is characterized by a non-linear rise over approximately the first 10-minute period after waking, prior to a linear increase towards the peak value at approximately 30 minutes after waking (Smyth et al., 2015). Poor adherence to saliva collection practices therefore can influence the results, and provide overestimations of the CAR (Smyth et al., 2015). Given these parameters, it is reasonable to state that CAR, measured with strict reference to the time of awakening, may provide a cost effective, robust, consistent (Wust, Wolf, et al., 2000) and ecologically valid (Smyth et al., 2015) method of assessing HPA-activity.

With the aforementioned commonalities between burnout and overtraining, an analysis of the relationship between CAR and burnout is warranted. Pruessner, Hellhammer, and Kirschbaum (1999) investigated this relationship when they assessed the CAR of teachers who were determined to be in either low or high burnout categories according to survey measures. Teachers categorized in the high burnout group showed decreased CAR compared to their non-burnout peers (Pruessner et al., 1999). In fact, this effect was shown to be independent of perceived stress (Pruessner et al., 1999), opening the possibility that CAR could discriminate actual and perceived stress levels of individuals. A dexamethasone test was also included in this investigation and although the administration of dexamethasone suppressed CAR in both groups, it did not suppress cortisol to the same degree in high stress teachers compared to low stress counterparts (Pruessner et al., 1999). Grossi et al. (2005) also demonstrated a significant relationship between burnout and CAR. However, in their investigation, high burnout participants showed greater initial level of cortisol, leading to a greater area under the curve

(AUC; Grossi et al., 2005). The differences in the methodology for determining burnout and differences in occupation could be at least partially responsible for the reversal of the CAR responses between these two studies, although this may also be reflected in the adrenal disturbance, adaption, maladaptation progression model that was previously elucidated (Meeusen et al., 2013).

In a critical study by Schlotz and colleagues, a significant difference between CAR on workdays and weekend days was observed (Schlotz, Hellhammer, Schulz, & Stone, 2004). Even when controlling for the time of awakening (i.e. time of collection) weekend days were shown to produce a lower CAR. Moreover, and in contrast to the study by Pruessner et al. (1999), the perceived workloads of participants was correlated with the degree of weekday CAR increases. This suggests that CAR is at least partially representative of both the neuroendocrine stress system's adaptation to chronic demands, and anticipatory responses to the upcoming demands of the day. In fact, CAR has been described as a "boosting" mechanism, to prepare an individual for the upcoming demands, whilst still reflecting experiences from the prior day (Adam, Hawkley, Kudielka, & Cacioppo, 2006a).

Employing CAR as a marker of physical stress, Minetto et al. (2008) studied soccer players during an intensive training camp. Fifteen elite players completed all testing battery protocols before and after the seven-day intensive training program, which included a counter-movement jump and a 20 m multi-stage shuttle test (beep test). The athletes were also required to produce salvia samples immediately upon waking, and again 15 minutes and 30 minutes after waking, for two days, both before and after the seven days of training. The results clearly demonstrated that in response to the one week of intense training, CAR, as measured by the AUC increased (511.9 $\pm$ 92.1 nmol/l pre vs. 612.3 $\pm$ 119.8 nmol/l post, $p = 0.034$). The absolute

increase in CAR was also significantly increased (12.4 $\pm$ 2.4 nmol/l pre vs. 16.4 $\pm$ 3.3 nmol/l post, p=0.004); however, the authors noted that only some participants were classified as CAR responders. The researchers therefore concluded that CAR is a sensitive marker for training induced stress.

Prior to these findings, a study of triathletes conducted by Gouarné et al. (2005) showed a significant effect of training on CAR. However, the authors noted that since the triathletes in their investigation showed a similar pattern of CAR to the OTS participants, it could be concluded that CAR is not an appropriate measure for the determination of OTS. Although this is plausible, their investigation included only two OTS participants. Moreover, the evaluation of these athletes as having OTS is questionable and does not meet the criteria (time to recovery) as discussed earlier. Strahler, Ehrlenspiel, Heene, and Brand (2010) offered the suggestion of a potential habituation effect, as the martial arts athletes in their study did not show a CAR response commensurate with increased trait anxiety leading up to a major competition (Strahler et al., 2010). This was somewhat confirmed in a recent study of professional swimmers preparing for a career affecting competition, when they showed no differences between CAR responses on competition and control days, even though the AUC for total cortisol concentrations in the swimmers were found to be greater on competition days (Díaz et al., 2013). It is possible that the swimming event that occurred later in the day was too far removed from the CAR (0700 h vs. 1400 h), thus leading to non-significant results. Although not statistically significant, the authors did find a negative relationship between mood states and CAR, measured as the area under the curve, relative to the initial value (AUC_i;r = -0.55, p = 0.07) and as measured as the area under the curve relative to the ground (AUC_g; r = -0.59, p =0.55) (Díaz et al., 2013).

Orthostatic Heart Rate Test

Orthostatic tolerance is a measure of the degree of orthostatic stress required to induce arterial hypotension (Hainsworth, 2000). An orthostatic test therefore increases gravitational forces and reduces venous return, thereby reducing stroke volume. Since blood pressure (BP) is proportional to the product of cardiac output and total peripheral resistance (TPR), in order to maintain BP, an increase in either heart rate (HR) or TPR is required.

It is well known that increases in HR can be due to either vagal withdrawal or increased sympathetic activity (Allen & Crowell, 1989). In a fascinating study using pharmacological interventions to blunt sympathetic and parasympathetic responses, researchers confirmed that heart rate is a significant contributor to the maintenance of blood pressure during orthostatic tests (Convertino & Sather, 2000). Moreover, the researchers suggest that sympathetic activation, rather than parasympathetic withdrawal, is a predominate contributor to the maintenance of blood pressure during an orthostatic stress test (Convertino & Sather, 2000). The monitoring of HR changes during orthostatic tests can therefore provide insight into ANS activity.

The vasovagal response (i.e. the dilation of arterials and slowing of heart rate) has been observed to occur in response to severe emotional stress (van Lieshout, Wieling, Karemaker, & Eckberg, 1991). This is often clinically observed through an orthostatic test, sometimes resulting in syncope, which some researchers have suggested may be an important test for affects of patients who have previously been abused (Rice & Records, 2006). In fact, physically abused pregnant women, a state with obvious emotional stress, showed an altered vagal response to an orthostatic challenge test (Rice & Records, 2006).

Orthostatic tolerance has been shown to be significantly lower in adolescents diagnosed with chronic fatigue syndrome (Stewart et al., 1999). Similarly, Wyller et al. (2014) found

greater orthostatic intolerance in those patients with chronic fatigue syndrome when compared to healthy controls. The authors note that this was likely due to either sympathetic predominance, withdrawal of parasympathetic control, or both (Wyller et al., 2014). These confirm the results from previous investigations of adolescents with chronic fatigue syndrome (Wyller, Barbieri, Thaulow, & Saul, 2008; Wyller, Saul, Amlie, & Thaulow, 2007). Lucini et al. (2002), in the aforementioned study of students preparing for an exam, found that in response to standing, students showed lower sympathetic cardiac control, possibly as a result of increased cortisol levels leading to a permissive action in the periphery (Lucini et al., 2002).

The Olympic Cross Country Skiing Sports Medicine book by Stalder et al. (2016) discussed the orthostatic heart rate test as a "(simple), cardiac autonomic function test that can be used in the field." The protocol required athletes to lie supine for 5-10 minutes wearing a digital HR monitor, prior to recording resting HR (HR_{supine}). The participants would then stand for 3 minutes and record the peak HR (HR_{peak}, occurring within the first 30 seconds) and average HR of the final 2 minutes of standing (HR_{stand}) (Stalder et al., 2016).

Rusko provides interpretations on the data from the OHR protocol, which is very similar to the protocol employed in the current study. An increased sympathetic drive will present increases in HR_{supine} and HR_{stand}, whereas parasympathetic dominance will show decreases in these values (Stalder et al., 2016). HR_{peak} (the HR obtained within 15 seconds of standing) is primarily parasympathetically controlled (i.e. vagal withdrawal) (Stalder et al., 2016). In view of these interpretations, practitioners have a convenient tool available for ANS monitoring and potential early warning system for OR and OT.

The earliest published use of the orthostatic heart rate assessment associated with over training was a study conducted by Rusko, Härkönen, and Pakarinen (1994). Cross-country skiers

conducted an orthostatic heart rate test four times over a thirteen-week period, whilst training volume was consistently increased, resulting in decreased aerobic capacity, measured by maximal volume of oxygen uptake. The researchers found that both 15 s and 120 s HR increased from week 4-9, but 120 s HR decreased thereafter. Other overtraining research protocols that utilized an orthostatic test, noted that overtrained athletes had a greater resting HR, and an attenuated HR response to the tilt test (Uusitalo et al., 2000). However, as this response was also seen in the non-overtrained athletes, the authors concluded that it was likely due to plasma volume increases with training (Uusitalo et al., 2000).

CHAPTER THREE

Methodology

Participants

Prior to this study being conducted, full Institutional Review Board approval was obtained. Competitive endurance trained participants (aged 18-35) were recruited from running teams and clubs from Central North Carolina and surrounding areas. Participants were required to have had at least one year of competitive endurance running experience, and were required to be currently training ≥5 days per week for endurance running events >5 km in distance. Participants were also required to complete all of their training with a digital, chest strap heart rate monitor. At the first meeting, participants were informed of the benefits and risks of the study, and signed a Written Informed Consent Form (Appendix 1) after having it verbally reviewed with them to ensure adequate understanding of the study requirements. All participants then completed a full Medical History Questionnaire (Appendix 2). Exclusion criteria included participants who are currently or may begin smoking, a history of endocrine disorders (e.g., Addison's Disease, Cushing's Syndrome etc.), currently taking or undergoing hormone therapy or using anti-inflammatory medication during the course of the investigation. Attempts were made to recruit subjects who were performing a wide variety of training loads to allow for a heterogeneous data sample.

Power Analysis

The sample size proposed is based on a previous investigation examining the cortisol awakening response in trained athletes. Using the results (means ± SD: pre-training 12.4±2.4

nmol/l, 16.4±3.3 nmol/l) published in a study by Minetto et. al (2008), effect size of 0.5, a power of 0.80 and alpha level of 0.05, a sample size of 27 was calculated. The addition of thirteen additional participants allows for a 32.5% subject mortality and non-compliance (Wahbeh, Kishiyama, Zajdel, & Oken, 2008).

Instrumentation

The height (cm) and weight (kg) of each of the participants was determined using a stadiometer (Perspective Enterprises, Portage, MI) and digital scale (Health-o-meter, McCook, IL). Psychological stress was measured by the 52 question Recovery-Stress Questionnaire for Athletes (REST-Q, Appendix 3) (Kellmann, 2001). Rate of perceived exertion was measured during the training period using the Borg RPE 6-20 Scale (Borg, 1970). Heart rate (HR) was constantly monitored throughout the individual training sessions using an individually owned and commercially available heart rate monitor. Saliva samples were collected via the passive drool technique, using sterile polypropylene scintillation vials. Biochemical analysis were conducted using Enzyme Linked Immunosorbent Assays (ELISA) (American Laboratory Products Company, Salem, NH, USA). Data was recorded and organized using Microsoft Excel 2011 (Microsoft Corporation, Seattle, WA, USA). All statistical procedures were performed with SPSS Statistics (version 21.0; IBM, Armonk, New York, USA).

Procedures

Subjects were asked to report to the Applied Physiology Laboratory (APL) for the initial visit, where participants had the opportunity to read and hear the Informed Consent form (Appendix 1) read aloud. Participants were then required to confirm their understanding verbally and sign a written Informed Consent form.

Each participant was then given time to complete a full Medical History Questionnaire. Subjects had their height and weight recorded, prior to starting the instructional process, which included a comprehensive review of the data collection forms that were provided to each subject (Appendix 4). Subjects were instructed on proper guidelines for saliva collection and conducting the OHR test (Appendix 5 and 6 respectively). The subjects were then required to demonstrate proficiency of these techniques, by actively demonstrating a simulated waking process and data recording. In addition, subjects were asked to complete a food frequency questionnaire, which asked questions relative to the types and frequency of foods eaten over the previous month.

Exercise Training

Throughout the duration of the study, participants were asked to maintain their current, regular diet. No intervention into their current training regime was employed, however subjects were asked to limit monotony in their training. Subjects completed three, baseline days, in which they were asked to collect all data but refrain from exercising. From thereon, subjects underwent their regularly scheduled training and competitions, completing each with a commercially available digital heart rate monitor (chest strap). Subjects were asked to record their training and competition data, by recording the time of exercise, the duration of exercise, distance covered, average HR over the session, and session rate of perceived exertion (RPE) based on the Borg 6-20 scale (Appendix 7) on the training record form provided (Appendix 4). Subjects were also asked to record any illness or unusual dietary changes throughout the two-week period, as well as any non-running related exercise.

Cortisol Awakening Response

Throughout this regular training period, participants were asked to produce saliva samples (Appendix 5) for the analysis of the cortisol awakening response (CAR). Subjects were

asked to, immediately upon waking and prior to standing (C_0), produce a 1 mL saliva sample via the passive drool technique. Participants, during the instructional period within the initial visit, were verbally told to, and visually shown a 1 mL sample size, prior to being asked to produce a 1 mL sample.

Participants were then asked to complete their regular morning routine, until they produce a second, 1 mL sample 30 minutes after the first sample (C_{30}). No eating, drinking or brushing of the teeth was permitted during this 30 minute time period. Participants were asked to record the time of awakening and time of saliva collection on the provided data collection sheets. The saliva samples were stored in a commercial freezer at approximately -4 °C. Subjects returned the samples to the Applied Physiology Laboratory at UNC Chapel Hill, no more than 48 hours after collection. Therefore, subjects returned samples every two days, for the course of the two-week period.

Orthostatic Heart Rate

The assessment of orthostatic heart rate was based on previous research by Stalder et al. (2016), and incorporated recommendations for orthostatic measurements by Lance et al. (2000), Irvin and White (2004) and O'Donnell, Badrick, Kumari, and Steptoe (2008). Immediately following the production of the initial saliva sample, subjects were asked to follow the OHR procedures (Appendix 6) first lying supine for 5 minutes. Following this supine rest, subjects measured their 15-second HR via the radial palpation technique (OHR_{supine}). Subjects recorded this value, prior to moving to the standing position. Fifteen seconds after standing, a second 15-second HR was taken (OHR_{peak}) and recorded, and a third, 15-second HR was measured after 2 minutes of standing (OHR_{stand}). The difference in measurements between OHR_{supine} and OHR_{peak} will be called $Supine_{\Delta}$, and the difference between OHR_{peak} and OHR_{stand} will be called $Stand_{\Delta}$.

The area under the curve will be determined geometrically relative to the ground (i.e. relative to a 0 value; AUC_g) and relative to OHR_{supine} (i.e. increase measure; AUC_i).

Recovery-Stress Questionnaire

Subjects were asked to complete a Recovery-Stress Questionnaire for Sport (REST-Q Sport) at the conclusion of the baseline period, and twice each training week i.e. day 3, 6, 10, 13 and 17. Subjects were asked to answer questions related to: general stress, emotional stress, fatigue, and physical recovery, amongst others, in relation to the previous three days. Decreases in affective state scores, as given by the REST-Q, may reveal OR or OTS and therefore require further scrutiny for that particular individual (Appendix 8).

Biochemical Analysis

Once received in the APL at UNC, saliva samples were processed and stored at -80 °C until analysis. Saliva samples were centrifuged at 23 °C at 3000 rpm for 1 minute. Samples were analyzed in duplicate for cortisol concentration via Enzyme Linked Immunosorbent Assay (ELISA) (American Laboratory Products Company, Salem, NH). Standard data reductions were employed that satisfy manufacture standards.

CAR was calculated using both magnitude of change (CAR_Δ) and AUC. CAR_Δ was calculated as:

$$CAR_\Delta = [C_{30}] - [C_0]$$

Area under the curve was calculated geometrically as both AUC_g and AUC_i.

Research Design and Statistical Analysis

Statistical analysis was performed by computer-based statistical software (SPSS; version 21). Means and standard deviations for age, height, weight, years of running experience and total training distance were calculated. This study is a single group, quasi-observational design. The significance level for all statistical procedures was set *a-priori* at $\alpha < 0.05$. Training loads were assessed via training impulse based on heart rate reserve (Equation 1).

Equation 1. Calculation of Training Load

$$\text{Training Load} = \text{Exercise Duration} \times [(HR_{ave} - HR_{rest})/(HR_{max} - HR_{rest})]$$

RQ1. Does the cortisol awakening response correlate with training load changes, during a regular two week training period, as measured by:

a. Area under the curve; or
b. Magnitude of change?

For each subject, the greatest and lowest training loads (excluding rest days) each week were selected for analysis. The high and low training loads were required to be separated by >5 RPE points to be considered valid for analysis. The difference scores and ratios between these high and low training loads were calculated for each week and then averaged between the two weeks of training. For each day selected, the saliva samples for the following day were analyzed. Again, a difference score and ratio between the associated high and low loads CAR responses (CAR_{Δ} , AUC_g, AUC_i) were averaged across the two weeks. The training load and CAR difference scores and ratios were analyzed via Pearson-Product moment correlation.

RQ2. Does the orthostatic heart rate test correlate with training load changes, during a regular two-week training period?

Training loads were selected as in RQ1. For each day selected, the OHR ($Supine_{\Delta}$, $Stand_{\Delta}$, AUC_g and AUC_i) for the following day were analyzed. A difference score and ratio between each response was averaged across the two weeks. The training load and OHR ratios were analyzed via Pearson-Product moment correlation.

RQ3. Does the orthostatic heart rate test correlate with cortisol awakening responses, measured as:

a. Area under the curve; or
b. Magnitude of change?

A Pearson-Product moment correlation was computed for each CAR and OHR variable pairing.

CHAPTER FOUR

Results

Subject Characteristics

In the recruitment for this study, 14 running clubs and groups from central North Carolina were contacted, and disseminated information about the study, as well as several exercise science classes at UNC; with the addition of study advertisements, which resulted in approximately 4500 endurance running athletes being exposed to the information on the study. In addition, approximately 150 endurance athletes were recruited directly and in-person, occasionally multiple times. From these recruitment avenues, 59 athletes who met the inclusion/exclusion criteria expressed direct interest and were contacted to set up initial sessions. Due to the relatively high subject burden, study length (17 days), and specific exclusion criteria, 20 subjects were enrolled in the study, with 15 subjects completing the study requirements. One subject dropped out due to sustaining an injury prior to the start of the study protocol, 2 subjects dropped out due to logistical constraints, and 2 subjects were excluded due to excessive participation in non-running endurance exercise (swimming and resistance training). Subject demographics and training history are displayed in Table 1.

Table 1. Subjects characteristics and training history (means±SD)

	Males (n = 9)	Females (n = 6)	Total (n = 15)
Age (yrs)	23.7±4.9	23.2±3.7	23.5±4.4
Height (cm)	173.6±6.4	169.5±3.5	172.0±5.7
Mass (kg)	69.1±8.7	56.0±7.3	63.9±10.4
Training Frequency (#/wk)	5.8±1.3	5.3±0.8	5.6±1.1
Training Distance (km/wk)	74.2±18.8	42.6±12.0	61.6±22.6
Competition Frequency (#/yr)	5.2±1.2	3.0±1.3	4.3±1.6
Competition Distance (km/competition)	26.1±17.1	20.1±2	23.7±13.3

Training Loads

Subjects showed great inter-variability between training sessions, in both duration and distance (range: 17-340.2 mins; 3.2 – 50.1 km). Means (±SD) for training duration, distance, RPE and average HR (HR_{ave}) between high and low training days are displayed in Table 2. Calculated TRIMP scores on high and low training days were significantly different from each other ($p < 0.001$). Although one athlete competed during the study period (5 km event), that day was not selected for analysis as either a high or low training load day.

Table 2. Training characteristics of high and low training days (mean±SD)

	Duration (mins)	Distance (km)	RPE (6-20)	HRave (bpm)	TRIMP
High	83.7±74.2*	14.4±9.6*	14.3±1.8*	153.7±13.9*	175046.9±99759.4*
Low	35.2±13.6	6.7±3.1	11.7±1.9	145.6±19.6	56969±21412.7

*Indicates significant difference between high and low training days ($p<0.01$).

Biomarker Analysis

Cortisol Awakening Response Analysis

All biochemical assays were completed with an analysis of intra-assay coefficient of variations of < 2.4% and all control samples within manufacturers acceptable ranges. The inter-assay coefficient was calculated at 6.7%. Salivary samples were all collected at the proposed time intervals (C_0: immediately after waking; C_{30}: thirty minutes after initial sample; ±1 min). CAR responses across the study were typical and expected; salivary cortisol was significantly elevated at C_{30} relative to C_0 (38.2±18.4 ng/mL vs. 52.4 ±20.4 ng/mL; $p < 0.001$).

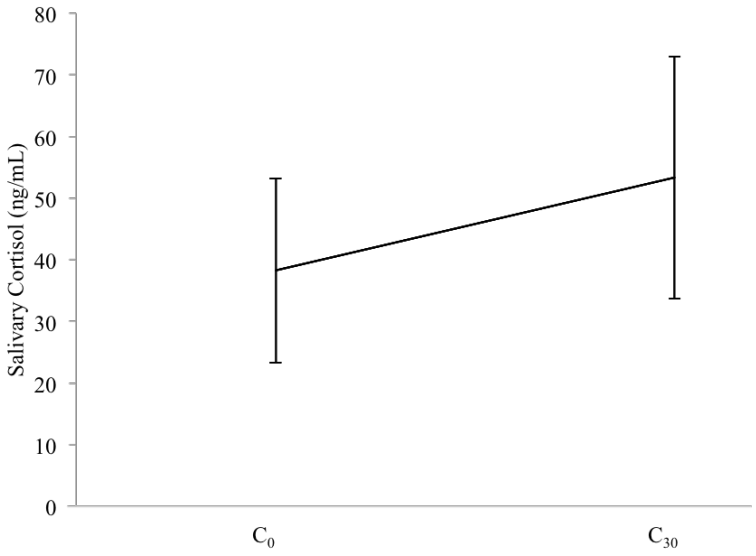

Figure 1. Main effect of cortisol responses across the study immediately after waking (C_0) and after 30 minutes (C_{30}) (means±SD)

No significant differences were observed between high and low training load days for either C_0 (39.3±19.3 ng/mL vs. 37.2±17.5 ng/mL; p=0.541) or C_{30} (51.5±20.8 ng/mL vs. 53.3±19.9 ng/mL; p=0.620; Figure 2).

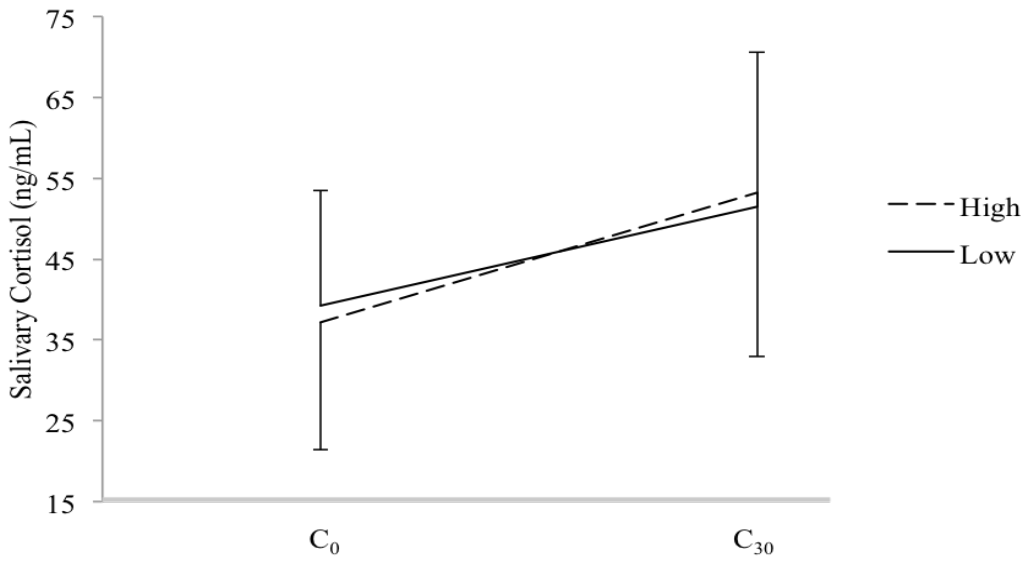

Figure 2. The cortisol awakening responses following high and low training load days (means±SD)

No significant differences were observed between CAR AUC_g or AUC_i (see Methods chapter, page 33; 1356.9±494.0 vs. 1360.7±528.5 ng/mL p=0.965; and 241.1±265.3 vs. 182.8±289.2 ng/mL p=0.355, respectively), or between CAR_Δ (16.1±17.7 ng/mL vs. 12.2±19.3 ng/mL; p=0.355) scores between high and low training days.

Orthostatic Heart Rate Analysis

As displayed in Figure 3, both high and low training days showed a significant increase at OHR_{peak} compared to OHR_{supine} (high: 54.4±7.8 bpm vs. 68.7±10.9 bpm; low: 54.4±7.6 vs. 66.7±11.1 bpm; p<0.001), with no difference between OHR_{peak} and OHR_{stand} following high (66.5±10.5 bpm; p = 0.237) or low (66.8±10.9 bpm; p = 0.914) training days.

No significant differences were observed between high and low training days for OHR_{supine} (p=1.0), OHR_{peak} (p=0.166), or OHR_{stand} (p=0.801).

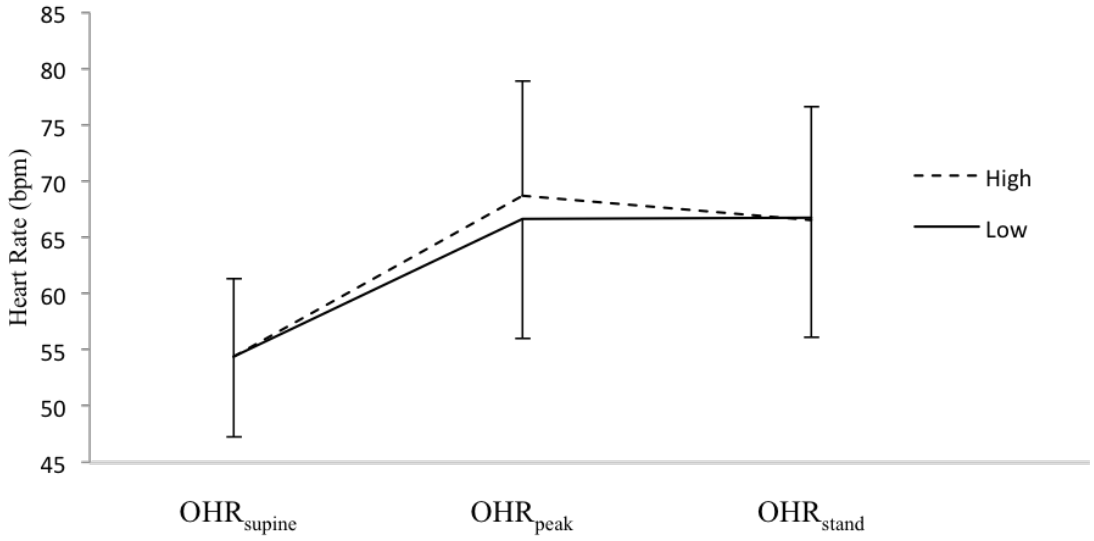

Figure 3. Orthostatic heart rate following high and low training days (means±SD)

No significant differences were observed for OHR AUC_g (p=0.710) or AUC_i (p=0.778). Neither $Supine_\Delta$ or $Stand_\Delta$ were significantly different between high and low training load days (p=0.277 and p=0.098 respectively).

Correlational Analysis

CAR responses to high and low training load days, as measured by AUC_g, did not show a significant relationship with TRIMP, either as a ratio (p = 0.228) or difference score (p = 0.579). When measured as AUC_i, CAR was again not significantly related to TRIMP as a ratio (p = 0.303) or difference score (p = 0.201). No relationship was observed between CAR_Δ and TRIMP as a ratio (p = 0.303) or difference score (p = 0.201) (see Table 3).

Table 3. Relationships between CAR and TRIMP ratio and difference scores

	Ratio		Difference	
	r	p	r	p
AUC_g	-0.227	0.288	-0.105	0.579
AUC_i	-0.194	0.303	-0.240	0.201
CAR_Δ	-0.194	0.303	-0.240	0.201

No relationships were observed between OHR AUC_g or AUC_i and TRIMP when analyzed as ratios ($p = 0.430$, $p = 0.511$) or difference scores ($p = 0.602$, $p = 0.344$). Neither $Supine_\Delta$ or $Stand_\Delta$ were significantly related to TRIMP as ratios ($p = 0.905$, $p = 0.872$) or difference scores ($p = 0.387$, $p = 0.463$, see Table 4).

Table 4. Relationships between OHR and TRIMP ratio and difference scores

	Ratio		Difference	
	r	p	r	p
AUC_g	-0.150	0.430	-0.099	0.602
AUC_i	-0.125	0.511	0.179	0.344
$Supine_\Delta$	-0.023	0.905	0.164	0.387
$Stand_\Delta$	-0.035	0.872	-0.139	0.463

CAR and OHR were also not significantly related for any variables (Table 5).

Table 5. Relationships between the CAR and OHR ratio and difference scores

			Cortisol Awakening Response					
			AUC_g		AUC_i		CAR_Δ	
			Ratio	Diff	Ratio	Diff	Ratio	Diff
Orthostatic Heart Rate	AUC_g	Ratio	0.014		-0.029		-0.029	
		Diff		0.073		-0.098		-0.098
	AUC_i	Ratio	-0.248		-0.100		-0.100	
		Diff		-0.172		-0.027		-0.027
	$Supine_\Delta$	Ratio	-0.289		-0.035		-0.035	
		Diff		-0.140		-0.011		-0.011
	$Stand_\Delta$	Ratio	0.140		-0.243		-0.243	
		Diff		-0.179		-0.036		-0.036

Recovery Stress Questionnaire for Sport

Repeated measures analysis of variance revealed no significant difference across the study for REST-Q scores, compared to baseline scores (all $p > 0.141$), or between each time point ($p = 0.401$).

Exploratory Analysis

Due to the novelty of this research, additional exploratory analysis was completed. Independent t-tests revealed significant differences between males and females for C_{30} following low training days ($p = 0.017$), and C_0 following high training days ($p = 0.033$), although all cortisol values were trending towards significance ($p < 0.109$). From this analysis, differences between males and females were also assessed for training load variables, and it was discovered that males ran greater distances, both on high (17.5±10.6 vs. 9.1±4.0 km; $p = 0.024$) and low training load days (8.1±2.8 vs. 4.5±1.7 km; $p < 0.001$). Therefore, a partial correlation, controlling for distance was used to assess the relationships between ratios and differences scores of CAR and OHR variables and training load. The partial correlation revealed a significant relationship between CAR AUC_g and TRIMP ratios ($r = 0.753$, $p = 0.012$) and a significant negative relationship between OHR $Stand_{\Delta}$ and TRIMP ($r = -0.675$, $p = 0.032$).

CHAPTER FIVE

Discussion

Introduction

The purpose of this study was to assess the relationships between the cortisol awakening response (CAR) and training loads during a regular two-week training period in endurance runners. The relationship between a practical orthostatic heart rate test and training load and CAR were also assessed. These variables were assessed in an attempt to identify a potential biomarker for assessing physiological readiness in endurance athletes, allowing coaches and athletes to modify training loads to avoid the deleterious effects of the overtraining syndrome. It was hypothesized that the ratio and difference scores of high to low training loads (TRIMP) would be positively related to CAR and OHR, for both the area under the curve relative to the ground (AUC_g) and increase (AUC_i), as well as the magnitude of change scores for all variables (CAR_Δ, $Supine_\Delta$, $Stand_\Delta$). However, no significant relationships were found for any variables with TRIMP, or between CAR and OHR.

Athlete Characteristics

Since previous research has demonstrated little difference in CAR between males and females (see: Chapter II), the present study was not delimited based on sex. As such, 9 males and 6 females participated in the present study and showed significant differences between TRIMP scores on the high training load days ($p = 0.048$) and trending significance on the low training load days ($p = 0.062$). This may have been a result of the longer distances run by males on both

the high (17.5±10.6 km vs. 9.1±4.0 km; $p = 0.024$) and low (8.1±2.8 km vs. 4.5±1.7 km; $p < 0.001$) training load days. This variation in distance most likely resulted from the heterogenous sample, with competition distances ranging from 5 km to ultra-marathons, with two male subjects regularly competing in 50 km events, while most female subjects competed in half-marathon (23km) or less. These competitive focus differences would dramatically influence the training distances employed by the athletes.

Cortisol Awakening Response

The cortisol awakening responses observed in the present study were lower to those observed in previous investigations. In 2008, Minetto et. al reported pre-training CAR_{Δ} values of 34.2±6.6 prior to a fatiguing week of training and 45.2±9.1 ng/mL post training, compared to CAR_{Δ} in this study of 12.1±1.5 ng/mL for the low training days and 16±2.4 ng/mL following high training load days. Likewise, AUC values in the present study were lower than Minetto et al. (2008) who measured 1412.3±254 ng/mL*30 pre-training, and 1689.3±330.5 ng/mL*30 post-training, compared to the present study: 1356.9±494.9 ng/mL*30 for low training load days and 1360.7 ±528.5 ng/mL*30 for high training load days. However, Wust, Wolf, et al. (2000) examined CAR in 509 adults on two consecutive days, in an attempt to calculate normal values and found concentrations at C_0 of 41.7±17.2 ng/mL and C_{30} of 63.3±25.2 ng/mL, similar to the cortisol concentrations following the baseline days in the present study (C_0: 38.6±16.8 ng/mL; C_{30}: 55.4±19.9 ng/mL).

The reduced magnitude of change from C_0 to C_{30} relative to other investigations is possibly a result of delimiting sampling to only two time points (i.e., 0 and 30 minutes after waking). Some studies have shown that cortisol will not peak until 45 minutes after waking (Evans, Hucklebridge, Loveday, & Clow, 2012; Wolfram, Bellingrath, & Kudielka, 2011),

although there is significant agreement that the peak cortisol response tends to occur at approximately the 30 minute point (Pruessner et al., 1997; Schlotz et al., 2004; Wust, Federenko, Hellhammer, & Kirschbaum, 2000; Wust, Wolf, et al., 2000) with many studies choosing to measure only C_0 and C_{30} (Adam, Hawkley, Kudielka, & Cacioppo, 2006b; Alderling, Theorell, Torre, & Lundberg, 2006; Eller, Netterstrøm, & Hansen, 2006; Gonzalez, Jenkins, Steiner, & Fleming, 2009; Greaves‐Lord et al., 2007; Kallen et al., 2008; O'Donnell et al., 2008; Therrien et al., 2008; Wahbeh et al., 2008; Wichers et al., 2007). Even so, recent recommendations are to sample every 15 minutes for the first hour waking in order to capture the peak response (Stalder et al., 2016). There is clearly a cost-benefit analysis to more frequent sampling, both financially and in terms of dramatically disrupting the normal morning routines of athletes. However, the lack of a relationship, particular between CAR_Δ and other variables, could also be a result of the peak cortisol response not being captured by the present sampling methodology.

The lack of a significant relationship between high and low training load day TRIMP scores and CAR was however unexpected. Therefore, it may be appropriate to speculate that CAR is an extremely robust measure of HPA-activity that remains uninfluenced by acute exercise bouts. However, this finding seems to be contradictory to previous investigations that have found variability in CAR in response to several types of global stresses such as chronic fatigue (Roberts et al., 2004), work-related stress (Schulz et al., 1998), burnout (Sonnenschein et al., 2007), and periods of intense exercise (Minetto et al., 2008). Therefore, it is reasonable to suspect a mediating or moderating factor that was previously unaccounted for was resulting in the lack of a significant relationship.

Since there was such a wide range of training distances, on both high and low training load days, it was proposed that the actual distance run during the training session, independent

from the physiological load as calculated by TRIMP, was a factor that may have influenced the relationship. External load, defined as "the work completed by an athlete measured independently of his or her internal characteristics" (Wallace et al., 2009) is often seen as the 'dose' and the subsequent internal load ('response') acts as the stimulus for training adaptation (Viru & Viru, 2000). In this dose-response relationship, it is often assumed that the internal load of the athlete, as measured by session RPE (sRPE), HR or a number of TRIMP models, is reflective of the external load placed on the athlete. Whilst a different exercise modality than the present study, Scanlan et al. (2014) found that while the internal and external training loads were significantly correlated in a group of basketball athletes, due to low shared variability (r^2 = 0.14-0.38), they were measures of "separate constructs" that provide unique feedback to coaches (Scanlan et al., 2014). Wallace et al. (2014) also recently investigated the relationship between measures of internal and external training load quantification and actual 1500m performance in trained endurance runners. Although the authors did find a relationship between performance and Bannister's TRIMP model, a stronger relationship was observed with a measure of external training load, known as a running training stress score (rTSS). This measure of external load can be considered a derivative of a training impulse score (TRIMP), but uses external (e.g. running velocity), as opposed to internal (HR) variables to calculate load (Skiba, 2006). By assessing the distance completed during the training sessions, considering the duration was already accounted for in TRIMP, the velocity of the athletes (as in rTSS) could be included in the analysis. External training loads such as training stress scores (TSS) are commonly used in cycling, where the power output of athletes is used to quantify TSS, although its use in long distance running is less well established. Even so, it has been suggested that both the internal and external training loads are necessary to establish an accurate representation of total training stress (Halson, 2014), and

perhaps the uncoupling of the internal and external components could be an indicator of fatigue in athletes (Pyne & Martin, 2011 via Halson, 2014).

To test the hypothesis that external loads were contributing to the responses, a partial correlation between TRIMP ratios and CAR and OHR responses the following day, while controlling for distance, was employed. Consequently, significant moderate to strong relationships were observed between CAR AUC_g ratios and $Stand_\Delta$ difference scores and TRIMP (see: Chapter IV). It appears therefore, that CAR is not only sensitive to the physiological, internal load of the exercise session as calculated by TRIMP (i.e. duration and heart rate during the exercise), but also the external load of the exercise (i.e. distance). In other words, a high TRIMP that was produced during only a short running distance, or a low TRIMP produced over a greater distance, alters the total load on the athlete and correspondingly affects the CAR response. This is a reasonable suggestion, since acute cortisol responses to exercise are mediated by both the intensity and duration of the training session (McMurray & Hackney, 2000). Although TRIMP scores accounted for the average HR (intensity) and the duration of the session, it is likely that the actual intensity, or total training stress, was not captured by these two variables and the training distance also needs to be considered when determining the intensity of the session, even in steady state endurance exercise.

It was originally hypothesized that because endurance runners typically complete long duration steady state exercise that only vary in intensity between individual training sessions, the heart rate response to the session would capture the training load. However, it may be possible that it is not only the duration of the session that is important to consider, but also acceleration changes over the course of the training session that are not captured by the average HR; thereby influencing the distance run, and affecting the total training load. It may also be possible that low

internal load, longer distance training sessions result in a greater variability in terrain, influencing the neuromechanical demands on the athlete and increasing the total load, which is not subsequently captured by just duration and average HR. Controlling for speed during the training sessions would therefore be an important factor if using CAR to track acute responses to exercise.

Orthostatic Heart Rate

Resting heart rates (e.g., supine) were bradycardic, as expected of endurance athletes whom likely have physiological adaptations consistent with the athletic heart syndrome (Huston et al., 1985). As the protocol for measuring orthostatic heart rate in this investigation is novel, it is difficult to compare the results to previous investigations. However, in a study by (Uusitalo et al., 2000), female endurance athletes resting heart rates were tracked over the course of an intensive training period and showed no difference from baseline measures. This is consistent with the present findings, with athletes supine HR not being significantly different between high and low training days (54.4±7.8 bpm vs. 54.4±7.6; $p = 0.932$). As expected, HR increased at OHR_{peak} in response to the increased orthostatic stress, but no differences were observed between high and low training days. It was expected that HR would decrease at OHR_{stand}, as the initial sympathetic response induced by the increased gravitational load was adjusted. However, this did not occur and only a small, insignificant decrease in HR was observed amongst subjects following low training load days, with no difference on high training load days. In a study of cross-country skiers, Rusko et. al used digital monitors to track both HR and HRV across a training period, and found the peak HR after standing was far greater, even at baseline, than observed in this investigation (>90 bpm vs. 68.7 bpm). In fact, the peak heart rate responses in the present study were similar to those observed by Rusko at OHR_{stand}. It is possible that the 15

seconds of palpation employed in this study to measure heart rate was; a) not a sensitive enough measure to detect decreases in heart rate following 2 minutes of standing, and/or b) occurred at a time that did not capture the actual peak increases and decreases that were occurring amongst subjects.

Interestingly, as with CAR responses to training load, OHR seems to also be mediated by the distance of the training session. When controlling for distance, the change in HR from OHR_{peak} to OHR_{stand} ($Stand_{\Delta}$) is negatively correlated with training load from the previous day (see: Chapter IV). Therefore, as training load increases, the ability of HR to decrease following an initial increase from standing is also diminished. This suggests that following high training loads, athletes present a lower vagal tone and increased sympathetic activity, and that change is detectable by a simple palpated HR test. Again, if OHR is to be utilized as a method of tracking training load, the neuromechanical load (or speed, in endurance runners), is necessary to consider. There was also a significant difference found between males and females for $Stand_{\Delta}$ following both high ($p = 0.004$) and low ($p = 0.024$) training load days, but this difference is likely an artifact of the varying distances run by males and females on high and low training days. The lack of a relationship between CAR and OHR suggests that these two phenomena are independent of each other, and although both may be responsive to training load, the measurements cannot be directly substituted for each other.

Limitations

There were significant limitations encountered throughout this study. Firstly, it must be noted that training loads were calculated as:

$$\text{Training Load} = \text{Duration} \times HR_{ave} \times \text{RPE}$$

This is in comparison to the training load equation presented in Chapter III. The choice to include RPE in the training load equation as opposed to RPE as a method of differentiating high to low training loads was due to; a) a lack of training loads that were separated by >5 RPE points, and b) high/low RPE scores resulting in training load ratios that were inverted. Consequently, two ratios and difference scores were calculated for each subject (Week 1 and Week 2). An initial investigator error in the calculations of training load resulted in the above training load equation being used as opposed to the training load equation based on a percentage of heart rate reserve, as initially proposed. As a consequence, three days selected for the CAR analysis should be considered as the second highest training load from that week, as opposed to the highest, with the average variation between the proposed and actual training load being 0.65%. Even so, clear separation between high and low training loads were achieved. Additionally, TRIMP scores calculated as initially proposed would result in significant multicollinearity, since OHR_{supine} would be used to calculate heart rate reserve values. The lack of variability in OHR_{supine} throughout the study would result in the same variables being incorporated into both TRIMP and OHR AUC_g, AUC_i, and $Supine_{\Delta}$, and would result in interpretation of OHR data difficult compared to the actual TRIMP scores utilized. Even so, when statistically analyzing the results using the initially proposed TRIMP model, no significant differences were observed.

A second limitation is related to the logistics of the saliva sample collections. Several occasions, both accidental (e.g., forgetting samples) and planned with the subject, resulted in some saliva sample processing being delayed for up to 48 h beyond the proposed 48-72 h window. Since all samples were within physiological ranges, and both the C_0 and C_{30} samples for each subject on each day were processed at the same time, it is unclear how much of an

impact this had on biochemical analysis. Additionally, a recent review of the literature has suggested that two-sample CAR protocols (C_0 and C_{30}) may lead to "erroneous conclusions" since peak cortisol responses may occur as late as 45 minutes after waking (Stalder et al., 2016). The authors suggest multiple sampling across the waking period in order to appropriately capture the peak response. Unfortunately this recommendation also contributes to increased subject burden and reduces the practicality of such a measure in elite athletes.

A significant limitation of the study was the reliance on subjects to strictly follow saliva and heart rate collection procedures. Subjects were reminded of guidelines at sample collections and given guidance based on individual feedback, but due to the unsupervised nature of this data collection, it is possible that subjects consumed food or drink, or did not adhere to the timeline of saliva collection. Additionally, the food frequency questionnaire data was not analyzed due to lack of Institutional Review Board approval, so it is possible that dietary components influenced the CAR responses.

Since only 15 subjects participated in the present study, and *a priori* power analysis calculated 27 subjects were required to meet statistical power, it is possible that the lack of significant relationships, as initially propose, were due to being statistically underpowered. However, due to the variation in TRIMP calculation, the data was organized in a manner that allowed each subjects' data from week 1 and week 2 to be included in analysis, resulting in n = 30 for all correlational analysis, potentially minimizing the effect of a small sample size. Moreover, the lack of significant findings, both between high and low days for biomarkers and correlational analysis, may have been due to the relatively low difference between high and low training loads performed by the athletes in the free-living environment. Although significantly different from each other, the RPE scores for high and low training load days suggest that the

high load days were perhaps not of a great enough intensity to invoke a large, detectable physiological change,.

Although REST-Q data was collected and analyzed, and no significant changes were observed across the study, it must be noted that each subject was on their own training schedule, making the analysis of the lack of change in REST-Q averages difficult to interpret. Even so, when qualitatively assessing each participants REST-Q scores, no large changes appeared to occur throughout the study, suggesting all athletes were neither over-reaching nor over-training.

Strengths

Even considering the aforementioned limitations, the present study gives valuable insight in the use of both CAR and OHR in monitoring acute training load in athletes. Furthermore, the relationship between CAR and OHR with TRIMP, accounting for external training loads suggests that the incorporation of both of these techniques may be a valuable method of measuring athletes' responses to specific training loads.

The primary strength of utilizing CAR, is that it presents an objective physiological maker that reflects HPA-axis status. Moreover, since monitoring cortisol is easily performed through saliva samples, there is a relatively low subject burden, as opposed to resting blood draws, or hormonal analysis following exhaustive exercise tests. However the 30-minute window between saliva samples requires athletes to refrain from consuming food and drink, reflecting a potentially large disruption in an athlete's morning routine, and reflecting in a post-completion survey which showed athletes were unlikely to want to continue to collect saliva in this fashion to track training load. However, it must be noted that the current sample of athletes were amateur competitors, with limited vested interest in race performances compared to Olympic-level or professional athletes. If future studies are able to further demonstrate the use of CAR in athlete

monitoring, the 30-minute window of inconvenience each morning may reflect a small burden relative to the loss of livelihood, which may occur due to the development of OTS.

In contrast to CAR, OHR is a very simple test, with little subject burden, reflected by several athletes expressing they were likely or very likely to continue to collect HR data each morning to monitor training load. Again, future studies will need to confirm the relationship observed in the exploratory analysis conducted, but OHR may reflect a cost-effective, low-burden test, which may be able to inform athletes and coaching staff of the athlete's response to the previous days training and provide a foundation for exercise prescription for the upcoming day.

The methodology employed in the present study allows for a 'real-world' analysis of these biomarkers and their relation to training load. The training loads observed were all sessions that would have occurred regardless of the study, with athletes already following a personal periodized training program, likely designed to avoid the development of OTS. This is in comparison to an interventionist approach to the problem, which may induce training loads the athlete is drastically unaccustomed to. Although this laboratory-based study is necessary, the current approach to the research question is far more reflective of the manner in which OTS develops, with only small overloads accumulating with an increase in training frequency. Therefore, if OTS is to be avoided, small variations in training load need to be detectable by whichever biomarker is proposed. This study showed that CAR and OHR may be sensitive enough to detect changes in both the internal and external training load, as typically induced by athletes during regular training periods.

The present study also demonstrated the practicality of saliva and HR data collection over the course of a 17-day period. Previous investigations have collected saliva for only select days

throughout the study, which Stalder et al. (2016) have proposed as a potential limitation in monitoring CAR. The non-discriminatory manner in which saliva was collected demonstrates the feasibility of tracking CAR throughout extensive periods for future research, or during periods of intensive training where coaches and athletes may be more interested in monitoring athlete responses to training. The only limitation to monitoring CAR each day is related to the time and financial expense of doing so. However, due to the relatively low time investment and inexpensive salivary cortisol assay kits, CAR is a far more practical biomarker to monitor compared to blood serum or plasma hormones.

Future Research

Based on the results of the present study, there is sufficient basis for the continued investigation of CAR and OHR as potential biomarkers for the monitoring of acute training load and subsequent use in preventing OTS from developing. As such, future research should use laboratory controlled training loads to assess acute responses to variations in training loads and confirm the findings from the present study. This would also ensure standardization of high and low loads applied to athletes. Additionally, due to the sex-related differences, a controlled training load should be used to assess actual differences in CAR and OHR responses, between males and females. Future research should also directly assess whether internal and external training loads contribute to acute CAR and OHR responses, by controlling distances between high and low internal training loads (i.e. manipulating gradation during the exercise protocol). Additionally, a real-world approach to the study should also be employed using multiple training load stress scores that measure both internal and external training loads. Lastly, studies should assess whether the present relationships exist within other endurance sports such as cycling, swimming and triathlon, or within team based sports.

Conclusion

The results of the recent study reject all proposed research hypotheses. There was no relationship, positive or negative, between CAR and OHR and TRIMP, suggesting that neither CAR nor OHR is a valid means for assessing acute responses to training load. Therefore, at this time, the use of either CAR or OHR as measured by radial palpation cannot be recommended to be used by athletes or coaches to monitor training load. However, since exploratory analysis showed strong relationships between CAR and OHR with TRIMP when accounting external load (i.e. distance), it is highly recommended that CAR and OHR continue to be investigated, with a modification in the methodology for quantifying training load. If, as the present results suggest, CAR and OHR are responsive to both internal and external training loads, they may be valuable biomarkers to monitor the total load of an athlete, which would prove valuable in avoiding the development of OTS.

Appendix 1. Written Informed Consent Form

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Participants

Consent Form Version Date: 7/3/2015
IRB Study # 15-1616
Title of Study: The Relationship Between Training Load, Cortisol Awakening Response and Orthostatic Heart Rate
Principal Investigator: Travis Anderson
Principal Investigator Department: Exercise and Sport Science
Principal Investigator Phone number: 5417608547
Principal Investigator Email Address: tanders2@live.unc.edu
Faculty Advisor: Anthony C. Hackney, Ph.D., D.Sc.
Faculty Advisor Contact Information: (919) 962-0334

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. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

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 investigate the relationship between hormonal and nervous system measures and training loads of endurance athletes. This study is designed to assess the feasibility of data collection, in the hopes that these measures may be valuable in preventing the Overtraining Syndrome. For this study, you will not be required to change your regular training schedule at all, as we are interested in the changes in the cortisol awakening response and orthostatic heart rate and how they can be applied in a real world setting. You are being asked to be in the study because you are an endurance trained athlete training for and competing in competitions of 5 km distance or greater for more than 1 year, and you use a digital heart rate monitor to train.

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

You should not be in this study if you are currently or may begin smoking, have a history of endocrinological disorders, currently undergoing hormone therapy and/or using, or plan to use anti-inflammatory medication during the course of the investigation.

How many people will take part in this study?

There will be approximately 30 people in this research study.

How long will your part in this study last?

You will be required to take part in a 72-hour period of data collection, in which we may establish a baseline measure, followed by two weeks of regular training and data collection each morning. Each data collection session will take approximately thirty minutes total, with opportunity to partake in limited regular morning activities during this time. You will be required to return saliva samples to the laboratory every 24-48 hours. Specimens will be stored for no longer than six months prior to being analyzed and discarded. Total time commitment over the seventeen-day period will be no greater than ten hours.

What will happen if you take part in the study?

If you agree to take part in this study, you will be instructed on proper procedures for saliva collection, orthostatic heart rate testing and completion of the recovery-stress questionnaire. You will also be introduced to the training log, in which you will record all of your training related information. Following this initial meeting, you will be required to refrain from participating in strenuous physical activity for 72 hours. During this period, you will be required to perform the orthostatic heart rate test, consisting of recording your heart rate immediately upon waking, fifteen seconds after standing, and again after two minutes of standing. You will complete the saliva collection procedures, collecting at least 1 mL of saliva immediately, and again, in a separate container, after thirty minutes. During this thirty-minute period, you are asked to also complete the recovery stress questionnaire, consisting of 53 questions related to your affective state, on a frequency scale of 0-6. You will be able to choose to not answer any question for any reason, at any time.

The following two weeks will see you resume your regular training schedule, again, collecting saliva, conducting the orthostatic heart rate test, completing the recovery-stress questionnaire each morning upon waking, as explained above. During this two-week period, you will also be required to record your training in the supplied training log, providing information on the time of training, duration of session, distance (if available), perceived exertion, and average heart rate.

You will be asked to store the saliva samples in a refrigerator, and return them to the laboratory no later than 48 hours after their initial collection. At the completion of the two weeks you will then return the final samples, training log and questionnaires. Once the biochemical and statistical analysis is complete, you will be contacted and offered the opportunity to review your personal results, as well as the sample results from the entire study.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. The benefits to you from being in this study may include a greater understanding of your personal physiological responses to varying training loads, allowing you to better periodize your training schedule. You may also learn new viable monitoring techniques that you can continue to use beyond this research study.

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

The risks to you are limited, since you are asked to undergo your regular training cycle for which you are already participating in. Therefore, the greatest risks to you are those small, previously assumed risks associated with physical training, including muscle damage and musculoskeletal injury. If potential for overtraining is suspected, researchers will inform you in order to assist in mediating overtraining symptoms. There may also be uncommon or previously unknown risks. You should report any problems to the researcher. Pregnancy tests will be done on all females who might be able to get pregnant at the start of the study. These pregnancy tests will be paid for by the Applied Physiology Laboratory at UNC.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will information about you be protected?

Records will be secured by storing them in locked filing cabinets. All electronic files will be password protected. Only the primary investigator, faculty advisor and research collaborators will have access to data collection forms and any identifying information. ID codes will be used to identify data collections sheets, with no personal information being stored with data records.

Participants 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, UNC-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 example, the FDA) 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. You do not give up any of your legal rights by signing this form.

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 response, 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 be compensated for being in this study.

Will it cost you anything to be in this study?

It will not cost you anything to be 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 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 about the study, 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.

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 2. Medical History Questionnaire

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? _____ _____
If yes, please describe:

3. Have you ever been told you have heart trouble? _____ _____
If yes, please describe:

4. Do you ever get pain in your chest? _____ _____
5. Do you ever feel light-headed or have you ever fainted? _____ _____
If yes, please describe:

6. Have you ever been told that your blood pressure has been elevated? _____ _____
If yes, please describe:

7. Have you ever had difficulty breathing either at rest or with exertion? _____ _____
If yes, please describe:

8. Are you now, or have you been in the past 5 years, under a doctor's care for any reason? _____ _____
If yes for what reason?

9. Have you been in the hospital in the past 5 years? _____ _____
If yes, for what reason?

10. Have you ever experienced an epileptic seizure or been informed that you have epilepsy? _____ _____
11. Have you ever been treated for infectious mononucleosis, hepatitis, pneumonia, or another infectious disease during the past year? _____ _____
If yes, name the disease:

12. Have you ever been treated for or told you might have diabetes? _____ _____
13. Have you ever been treated for or told you might or low blood sugar? _____ _____
14. Do you have any known allergies to drugs? _____ _____
If so, what?

15. Have you ever been “knocked-out” or experienced a concussion? _____ _____
If yes, have you been “knocked-out” more than once? _____ _____
16. Have you ever experienced heat stroke or heat exhaustion? _____ _____
If yes, when?

17. Have you ever had any additional illnesses or operations? (Other than childhood diseases) _____ _____
If yes, please indicate specific illness or operations:

18. Are you now taking any pills or medications? _____ _____
If yes, please list:

19. Have you had any recent (within 1 year) difficulties with your:
a. Feet _____ _____
b. Legs _____ _____
c. Back _____ _____

Family History
20. 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
21. Have you ever experienced depression? _____ _____
If yes, did you seek the advice of a doctor? _____ _____
22. 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? _____ _____
23. If yes, please list condition and if you are currently taking any medication.
Condition:
Medication:

Bone and Joint History
24. Have you ever been treated for Osgood-Schlatter’s disease? _____ _____
25. Have you ever had any injury to your neck involving nerves or vertebrae? _____ _____
26. Have you ever had a shoulder dislocation, separation, or other injury of the shoulder that incapacitated you for a week or longer? _____ _____
27. Have you ever been advised to or have you had surgery to correct a shoulder condition? _____ _____
28. Have you ever experienced any injury to your arms, elbows, or wrists?_____ _____
If yes, indicate location and type of injury:
29. Do you experience pain in your back? _____ _____
30. Have you ever had an injury to your back? _____ _____

If yes, did you seek the advice of a doctor? _____ _____
31. Have you ever been told that you injured the ligaments or cartilage of either knee joint? _____ _____
32. Do you think you have a trick knee? _____ _____
33. 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? _____ ____
If yes, indicate where:
34. Have you ever had a bone graft or spinal fusion? _____ _____

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

Activity Frequency Duration Intensity
40. How would you describe your present state of fitness?
Excellent_____ Good_____ Fair_____ Poor_____
41. Please list the type(s) of work you have been doing for the previous ten years:

Year Work Indoor/Outdoor Location (city/state)
42. Whom shall we notify in case of emergency?
Name:
Phone: (Home) (Work)
Address:
43. 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 3. Recovery-Stress Questionnaire

Recovery-Stress Questionnaire

Subject ID:_____

Date:_____

Please complete this questionnaire each morning, preferably between producing saliva samples. Answer each question as intuitively as possible, in reference to the ***previous 3 days***.

1) … I watched TV

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

2) … I laughed

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

3) … I was in a bad mood

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

4) … I felt physically relaxed

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

5) … I was in good spirits

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

6) … I had difficulties in concentrating

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

7) … I worried about unresolved problems

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

8) … I had a good time with my friends

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

9) … I had a headache

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

10) … I was dead tired after work

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

11) … I was successful in what I did

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

12) … I felt uncomfortable

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

13) … I was annoyed by others

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

14) … I felt down

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

15) … I had a satisfying sleep

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

16) … I was fed up with everything

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

17) … I was in a good mood

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

18) … I was overtired

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

19) … I slept restlessly

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

20) … I was annoyed

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

21) … I felt as if I could get everything done

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

22) … I was upset

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

23) … I put off making decisions

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

24) … I made important decisions

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

25) … I felt under pressure

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

26) … parts of my body were aching

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

27) … I could not get rest during the breaks

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

28) … I was convinced I could achieve my set goals during performance

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

29) … I recovered well physically

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

30) … I felt burned out by my sport

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

31) … I accomplished many worthwhile things in my sport

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

32) … I prepared myself mentally for performance

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

33) … my muscles felt stiff or tense during performance

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

34) … I had the impression there were too few breaks

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

35) … I was convinced that I could achieve my performance at any time

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

36) … I dealt very effectively with my teammates' problems

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

37) … I was in a good condition physically

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

38) … I pushed myself during performance

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

39) … I felt emotionally drained from performance

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

40) … I had muscle pain after performance

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

41) … I was convinced that I performed well

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

42) … too much was demanded of me during the breaks

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

43) … I psyched myself up before performance

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

44) … I felt that I wanted to quit my sport

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

45) … I felt very energetic

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

46) … I easily understood how my teammates felt about things

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

47) … I was convinced that I had trained well

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

48) … the breaks were not at the right times

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

49) … I felt vulnerable to injuries

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

50) … I set definite goals for myself during performance

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

51) … my body felt strong

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

52) … I felt frustrated by my sport

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

53) … I dealt with emotional problems in my sport very calmly

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

Appendix 4. Data Collection Forms

Training Log

Please record details from each training session, even if you complete multiple training sessions per day.
Please include include information that is as specific as possible. Also include any notes from the training session, including but not limited to: type of training (intervals, hills etc.), whether you felt any pain (i.e. injury) during participation, or whether it was a group of solo training session.

Date	Start Time	Duration	RPE	HR average	Distance	Notes

Orthostatic Heart Rate and Cortisol Awakening Response

Please record your HR upon first waking, while lying supine in bed. Then stand for 15s take your HR again, and again after 2 mins of standing
Following this, please produce a cortisol sample (record the time) and produce a 2nd sample 30 minutes later (record the time).

Date (mm/dd/yy)	Time of Awakening (hh:mm)	Supine HR (bpm)	Standing HR 15s (bpm)	Standing HR 2 mins (bpm)	Corstiol 1 (hh:mm)	Cortisol 2 (hh:mm)

Appendix 5. Saliva Collection Procedures

1. Immediately upon waking, and without standing, open the correctly pre-labeled saliva collection container.
2. Produce a saliva sample by allowing saliva to pool in the mouth, prior to drooling the saliva into the collection container. Produce a 1 mL sample.
    a. If necessary, a chewable saliva stimulating aid can be used.
    b. Do not drink water prior to, or during sample collection.
3. Record the time of saliva collection on the supplied form, with time of awakening.
4. Place sample in commercially available freezer.
5. Wait 30 minutes, during which time you can not:
    a. Brush your teeth;
    b. Eat; or
6. Drink; (water is permitted immediately after waking, but not within 10 minutes of second collection).
7. After 30 minutes, open the correctly pre-labeled saliva collection container.
8. Produce a second saliva sample by allowing saliva to pool in the mouth, prior to drooling the saliva into the collection container. Produce a 1 mL sample.
    a. If necessary, a chewable saliva stimulating aid can be used.
    b. Do not drink water during sample collection.
9. Place sample in commercially available freezer.
10. Return samples to the Applied Physiology Laboratory, or by appointment with the research team, within 48 hours of collection.
11. Repeat for three baseline days, and two weeks (14 days) of regular training.

Appendix 6. OHR Procedures

1. Upon completion of the initial saliva collection and recording, lay down in a supine position for 5 minutes.
2. Count pulse via radial palpation technique for 15 seconds. Record value on supplied form.
3. Stand upright, wait 15 seconds, and count pulse via radial palpation technique for 15 seconds. Record value on supplied form.
4. Stand quietly for another 90 seconds.
5. Count pulse via radial palpation technique for 15 seconds. Record value on supplied form.
6. Follow saliva collection procedures.

Appendix 7. Borg Rating Of Perceived Exertion

Borg Rating of Perceived Exertion

6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

Appendix 8. REST-Q Rationale

Subjective Questionnaires

There is a general agreement that OTS is expressed as a decrease in performance, as well as decreases in affective states (Morgan, Brown, Raglin, O'Connor, & Ellickson, 1987). It has been proposed that changes in psychology and affective states may be a "useful indicator" of OR when combined with biological measurements (Halson & Jeukendrup, 2004). Although the use of biomarkers has previously shown inconsistent results, there appears to be a consensus that the use of psychological measures are valuable in appropriately diagnosing OTS (Kreher & Schwartz, 2012). In fact, in a recent systematic review by Viru and Viru (2000), the authors found that subjective measures showed a greater correlation with athlete well being than objective measures. For example, in 1995 Hooper and colleagues assessed staleness in 17 swimmers over a six-month period, and found that the subjective questionnaire categories of sleep, fatigue, stress and muscle soreness accounted for 49%, 78%, 76% of the variance, depending on the stage of training the athletes were in (Hooper, Mackinnon, Howard, Gordon, & Bachmann, 1995). One of the earliest and most widely used subjective questionnaires is the Profile of Mood States (McNair, Lorr, & Droppleman, 1981). In the aforementioned investigation by Diaz and colleagues, the Profile of Mood States was found to be significantly correlated with the average cortisol concentrations across two days of competition in swimmers (Díaz et al., 2013).

In the aforementioned study of overtrained collegiate swimmers, HPA-activity was increased in these athletes, which significantly correlated with negative affective states (O'Connor et al., 1989). Conversely however, this decline in affective state was not present in a more recent study of professional soccer players, even though an intense increase in physical

load resulted in decreased testosterone to cortisol ratio by more than 30%, implying an overtrained state (Filaire, Bernain, Sagnol, & Lac, 2001). The authors noted that a possible explanation for no change in mood state being observed, was the ~72% winning percentage of the team at the time. This result indicates that external factors to physiological load (e.g. psychology and environmental influences) must also be considered when assessing the overtrained state of the athletes.

REST-Q

Developed by Kellmann and Kallus, the Recovery Stress Questionnaire (REST-Q) was developed as a sport specific questionnaire for systematically assessing the recovery and stress states of athletes (Kellmann & Kallus, 2001). Since OTS is developed due to an imbalance of stress and recovery, this makes the REST-Q theoretically ideal for assessing athletes. It has been noted by Gonzalez et al. (2009) that the REST-Q is among a limited number of questionnaires that "attempt to address the full complexities of stress and recovery."

The REST-Q-Sport began as an 86-item questionnaire, building upon the REST-Q with an additional 38 items that were deemed to be sport specific. The Maslach Burnout Inventory (Kallen et al., 2008) served as a model for including selected questions in an effort to assess burnout (OTS) in athletes (Kellmann & Kallus, 2001). Based on reliability data, the questionnaire was then shortened to 76 questions, with 80-question version available for specific research needs. Further development lead to the REST-Q52 Sport widely used today. Based OR and OT being a result of an imbalance and training load, and the inclusion of the recovery component within the REST-Q, it has been declared a more appropriate psychometric for the assessment of the OTS (Kellmann, 2010).

Specifically utilizing the REST-Q Sport, Brink, Visscher, Coutts, and Lemmink (2012) studied elite youth players (mean age of 17 years), over the course of a competitive season. The authors were able to distinguish those athletes who were in the OR stage from healthy peers, as they showed decreased recovery scores two months prior to their diagnosis (Brink et al., 2012). Likewise, Coutts, Wallace, and Slattery (2007) followed sixteen triathletes during their base training period and monitored a variety of physiological, psychological and biochemical measures. The authors found that only the REST-Q scores showed significant differences between the intensified and normal training groups (Coutts et al., 2007).

Kellmann and Gunther (2000) also utilized the REST-Q Sport during an analysis of 11 Olympic level rowers, during their preparation for competition. The results showed that "alteration of extensive endurance training was well reflected in psychological measures" (Kellmann & Gunther, 2000). Moreover, the researchers showed that with increased duration, increased levels of psychological stress and lowered recovery scores were present. The authors also showed two contrasting case studies within their results, where REST-Q scores from 9 days prior to competition were representative of results in the competition (i.e. lower stress and greater recovery scores prior to competition was followed by a higher placing in the competition) (Kellmann & Gunther, 2000).

The REST-Q Sport questionnaire was employed by Meister et. al when a group of professional soccer players were tracked over a 3-week intensive training period. In contrast to the aforementioned studies however, the researchers were not able to find any significant differences between high and low soccer exposure periods (Meister, Faude, Ammann, Schnittker, & Meyer, 2013). The authors therefore concluded that this three-week period did not result in significant physiological stress or lack of recovery (Meister et al., 2013). However it must be noted that the

criteria for determining LE and HE in this study was a defined cut-off point of minutes played per week. Although the HE category was found to be 2-3 times greater than LE (Meister et al., 2013), this may not be a significantly high (or low) load for these athletes.

REFERENCES

2015 State of the Sport - U.S. Race Trends. (2015). Retrieved from: http://www.runningusa.org/statistics

Achten, J., & Jeukendrup, A. E. (2003). Heart rate monitoring: applications and limitations. *Sports Medicine, 33*(7), 517-538.

Adam, E. K., Hawkley, L. C., Kudielka, B. M., & Cacioppo, J. T. (2006). Day-to-day dynamics of experience--cortisol associations in a population-based sample of older adults. *Proceedings of the National Academy of Sciences U S A, 103*(45), 17058-17063.

Adlercreutz, H., Harkonen, M., Kuoppasalmi, K., Naveri, H., Huhtaniemi, I., Tikkanen, H., Remes, K., Dessypris, A., Karvonen, J. (1986). Effect of training on plasma anabolic and catabolic steroid hormones and their response during physical exercise. *International Journal of Sports Medicine, 7 Suppl 1*, 27-28.

Alderling, M., Theorell, T., Torre, B., & Lundberg, I. (2006). The demand control model and circadian saliva cortisol variations in a Swedish population based sample (The PART study). *BMC Public Health, 6*(1), 1.

Allen, M. T., & Crowell, M. D. (1989). Patterns of autonomic response during laboratory stressors. *Psychophysiology, 26*(5), 603-614.

Barron, J. L., Noakes, T. D., Levy, W., Smith, C., & Millar, R. P. (1985). Hypothalamic dysfunction in overtrained athletes. *Journal of Clinical Endocrinolgy and Metabolism, 60*(4), 803-806.

Borg, G. (1970). Perceived exertion as an indicator of somatic stress. *Scandinavian Journal Rehabilitation Medicine, 2*(2), 92-98.

Bouma, E. M., Riese, H., Ormel, J., Verhulst, F. C., & Oldehinkel, A. J. (2009). Adolescents' cortisol responses to awakening and social stress; effects of gender, menstrual phase and oral contraceptives. The TRAILS study. *Psychoneuroendocrinology, 34*(6), 884-893.

Brandenberger, G., & Follenius, M. (1975). Influence of timing and intensity of musclar exercise on temporal patterns of plasma cortisol levels. *Journal of Clinical Endocrinology and Metabolism, 40*(5), 845-849.

Brink, M. S., Visscher, C., Coutts, A. J., & Lemmink, K. A. (2012). Changes in perceived stress and recovery in overreached young elite soccer players. *Scandinavian Journal of Medicine and Science in Sports, 22*(2), 285-292.

Brittenham, G., Cioroslan, D., & Davis, J. (1998). Overtraining: the challenge of prevention. A consensus statement. *Second annual USOC/ACSM human performance summit*.

Brooks, G. A., Fahey, T. D., & White, T. P. (1996). *Exercise Hhysiology: Human Bioenergetics and its Applications*: Mayfield publishing company.

Clow, A., Hucklebridge, F., & Thorn, L. (2010). The cortisol awakening response in context. *International Review of Neurobiology, 93*, 153-175.

Cohen, H., Benjamin, J., Geva, A. B., Matar, M. A., Kaplan, Z., & Kotler, M. (2000). Autonomic dysregulation in panic disorder and in post-traumatic stress disorder: application of power spectrum analysis of heart rate variability at rest and in response to recollection of trauma or panic attacks. *Psychiatry Research, 96*(1), 1-13.

Convertino, V. A., & Sather, T. M. (2000). Effects of cholinergic and beta-adrenergic blockade on orthostatic tolerance in healthy subjects. *Clinical Autonomic Research, 10*(6), 327-336.

Coutts, A. J., Wallace, L. K., & Slattery, K. M. (2007). Monitoring changes in performance, physiology, biochemistry, and psychology during overreaching and recovery in triathletes. *International Journal of Sports Medicine, 28*(2), 125-134.

Curtis, B. M., & O'Keefe, J. H., Jr. (2002). Autonomic tone as a cardiovascular risk factor: the dangers of chronic fight or flight. *Mayo Clinic Proceedings, 77*(1), 45-54.

Davies, C. T., & Few, J. D. (1973). Effects of exercise on adrenocortical function. *Journal of Applied Physiology, 35*(6), 887-891.

Díaz, M. M., Bocanegra, O. L., Teixeira, R. R., Tavares, M., Soares, S. S., & Espindola, F. S. (2013). The Relationship Between the Cortisol Awakening Response, Mood States, and Performance. *The Journal of Strength & Conditioning Research, 27*(5), 1340-1348.

Dishman, R. K., Nakamura, Y., Garcia, M. E., Thompson, R. W., Dunn, A. L., & Blair, S. N. (2000). Heart rate variability, trait anxiety, and perceived stress among physically fit men and women. *International Journal of Psychophysiology, 37*(2), 121-133.

Duclos, M., Corcuff, J. B., Rashedi, M., Fougere, V., & Manier, G. (1997). Trained versus untrained men: different immediate post-exercise responses of pituitary adrenal axis. A preliminary study. *European Journal of Applied Physiology and Occupational Physiology, 75*(4), 343-350.

Eller, N. H., Netterstrøm, B., & Hansen, Å. M. (2006). Psychosocial factors at home and at work and levels of salivary cortisol. *Biological Psychology, 73*(3), 280-287.

Evans, P., Hucklebridge, F., Loveday, C., & Clow, A. (2012). The cortisol awakening response is related to executive function in older age. *International Journal of Psychophysiology, 84*(2), 201-204.

Filaire, E., Bernain, X., Sagnol, M., & Lac, G. (2001). Preliminary results on mood state, salivary testosterone:cortisol ratio and team performance in a professional soccer team. *European Journal of Applied Physiology, 86*(2), 179-184.

Freeman, J. V., Dewey, F. E., Hadley, D. M., Myers, J., & Froelicher, V. F. (2006). Autonomic nervous system interaction with the cardiovascular system during exercise. *Progress in Cardiovascular Diseases, 48*(5), 342-362.

Fry, A. C., Steinacker, J. M., & Meeusen, R. (2005). Endocrinology of overtraining. *The Endocrine System in Sports and Exercise*, 578-599.

Fry, R. W., Morton, A. R., & Keast, D. (1991). Overtraining in athletes. *Sports Medicine, 12*(1), 32-65.

Gonzalez, A., Jenkins, J. M., Steiner, M., & Fleming, A. S. (2009). The relation between early life adversity, cortisol awakening response and diurnal salivary cortisol levels in postpartum women. *Psychoneuroendocrinology, 34*(1), 76-86.

Gouarné, C., Groussard, C., Gratas-Delamarche, A., Delamarche, P., & Duclos, M. (2005). Overnight urinary cortisol and cortisone add new insights into adaptation to training. *Medicine and Science in Sports and Exercise, 37*(7), 1157-1167.

Greaves−Lord, K., Ferdinand, R. F., Oldehinkel, A. J., Sondeijker, F. E., Ormel, J., & Verhulst, F. C. (2007). Higher cortisol awakening response in young adolescents with persistent anxiety problems. *Acta Psychiatrica Scandinavica, 116*(2), 137-144.

Grossi, G., Perski, A., Ekstedt, M., Johansson, T., Lindström, M., & Holm, K. (2005). The morning salivary cortisol response in burnout. *Journal of Psychosomatic Research, 59*(2), 103-111.

Hackney, A. C. (2006). Stress and the neuroendocrine system: the role of exercise as a stressor and modifier of stress. *Expert Review of Endocrinology and Metabolism, 1*(6), 783-792.

Hainsworth, R. (2000). Heart rate and orthostatic stress. *Clinical Autonomic Research, 10*(6), 323-325.

Halson, S. L. (2014). Monitoring training load to understand fatigue in athletes. *Sports Medicine, 44*(2), 139-147.

Halson, S. L., & Jeukendrup, A. E. (2004). Does overtraining exist? An analysis of overreaching and overtraining research. *Sports Medicine, 34*(14), 967-981.

Hedelin, R., Kentta, G., Wiklund, U., Bjerle, P., & Henriksson-Larsen, K. (2000). Short-term overtraining: effects on performance, circulatory responses, and heart rate variability. *Medicine and Science in Sports and Exercise, 32*(8), 1480-1484.

Hedelin, R., Wiklund, U., Bjerle, P., & Henriksson-Larsen, K. (2000). Cardiac autonomic imbalance in an overtrained athlete. *Medicine and Science in Sports and Exercise, 32*(9), 1531-1533.

Hellman, L., Nakada, F., Curti, J., Weitzman, E. D., Kream, J., Roffwarg, H., Ellman, S., Fukushima, D.K., Gallagher, T. F. (1970). Cortisol is secreted episodically by normal man. *Journal of Clinical Endocrinology and Metabolism, 30*(4), 411-422.

Hill, E. E., Zack, E., Battaglini, C., Viru, M., Viru, A., & Hackney, A. C. (2008). Exercise and circulating cortisol levels: the intensity threshold effect. *Journal of Endocrinological Investigations, 31*(7), 587-591.

Hooper, S. L., Mackinnon, L. T., Howard, A., Gordon, R. D., & Bachmann, A. W. (1995). Markers for monitoring overtraining and recovery. *Medicine and Science in Sports and Exercise, 27*(1), 106-112.

Hucklebridge, F., Mellins, J., Evans, P., & Clow, A. (2002). The awakening cortisol response: no evidence for an influence of body posture. *Life Sciences, 71*(6), 639-646.

Huston, T. P., Puffer, J. C., & Rodney, W. M. (1985). The athletic heart syndrome. *New England Journal of Medicine, 313*(1), 24-32.

Hynynen, E., Uusitalo, A., Konttinen, N., & Rusko, H. (2006). Heart rate variability during night sleep and after awakening in overtrained athletes. *Medicine and Science in Sports and Exercice, 38*(2), 313-317.

Irvin, D. J., & White, M. (2004). The importance of accurately assessing orthostatic hypotension. *Geriatric Nursing, 25*(2), 99-101.

Kallen, V., Tulen, J., Utens, E., Treffers, P. D., De Jong, F., & Ferdinand, R. (2008). Associations between HPA axis functioning and level of anxiety in children and adolescents with an anxiety disorder. *Depression and Anxiety, 25*(2), 131-141.

Keizer, H., Platen, P., & Koppeschaar, H. (1991). Blunted β-endorphin responses to corticotropin releasing hormone and exercise after exhaustive training. *International Journal of Sports Medicine, 12*, 97.

Kellmann, M. (2010). Preventing overtraining in athletes in high-intensity sports and stress/recovery monitoring. *Scandinavian Journal Medicine and Science in Sports, 20 Suppl 2*, 95-102.

Kellmann, M., & Gunther, K. D. (2000). Changes in stress and recovery in elite rowers during preparation for the Olympic Games. *Medicine and Science in Sports and Exercise, 32*(3), 676-683.

Kellmann, M., & Kallus, K. W. (2001). *Recovery-Stress Questionnaire for Athletes: User Manual* (Vol. 1): Human Kinetics.

Kentta, G., Hassmen, P., & Raglin, J. S. (2001). Training practices and overtraining syndrome in Swedish age-group athletes. *International Journal of Sports Medicine, 22*(6), 460-465.

Kreher, J. B., & Schwartz, J. B. (2012). Overtraining Syndrome A Practical Guide. *Sports Health: a Multidisciplinary Approach, 4*(2), 128-138.

Kreider, R., Fry, A., & O'Toole, M. (1998). Overtraining in sport: terms, definitions, and prevalence. *Overtraining in Sport*, 309-331.

Kudielka, B., & Kirschbaum, C. (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology, 28*(1), 35-47.

Lance, R., Link, M. E., Padua, M., Clavell, L. E., Johnson, G., & Knebel, A. (2000). Comparison of different methods of obtaining orthostatic vital signs. *Clinical Nursing Research, 9*(4), 479-491.

Le Meur, Y., Pichon, A., Schaal, K., Schmitt, L., Louis, J., Gueneron, J., Vidal, P.P., Hausswirth, C. (2013). Evidence of parasympathetic hyperactivity in functionally overreached athletes. *Medicine and Science in Sports and Exercise, 45*(11), 2061-2071.

Lehmann, M., Foster, C., Dickhuth, H. H., & Gastmann, U. (1998). Autonomic imbalance hypothesis and overtraining syndrome. *Medicine and Science in Sports and Exercise, 30*(7), 1140-1145.

Lehmann, M., Foster, C., & Keul, J. (1993). Overtraining in endurance athletes: a brief review. *Medicine and Science in Sports and Exercise, 25*(7), 854-862.

Lehmann, M., Gastmann, U., Petersen, K. G., Bachl, N., Seidel, A., Khalaf, A. N., Fischer, S., Keul, J. (1992). Training-overtraining: performance, and hormone levels, after a defined increase in training volume versus intensity in experienced middle- and long-distance runners. *Br J Sports Med, 26*(4), 233-242.

Lehmann, M., Wieland, H., & Gastmann, U. (1997). Influence of an unaccustomed increase in training volume vs intensity on performance, hematological and blood-chemical parameters in distance runners. *Journal of Sports Medicine and Physical Fitness, 37*(2), 110-116.

Lucini, D., Norbiato, G., Clerici, M., & Pagani, M. (2002). Hemodynamic and autonomic adjustments to real life stress conditions in humans. *Hypertension, 39*(1), 184-188.

Malone, J. J. (2014). *An Examination of the Training Loads Within Elite Professional Football. (*Doctoral thesis*)*. Liverpool John Moores University, Liverpool, England.

McMurray, R. G., & Hackney, A. C. (2000). Endocrine responses to exercise and training. *Exercise and Sport Science*, 135-161.

McNair, D. M., Lorr, M., & Droppleman, L. F. (1981). *Profile of Mood States* (pp. 1-29). San Diego, CA: Educational and Industrial Testing Service.

Meeusen, R., Duclos, M., Foster, C., Fry, A., Gleeson, M., Nieman, D., Raglin, J., Rietjens, G., Steinacker, J., Urhausen, A. (2013). Prevention, diagnosis, and treatment of the overtraining syndrome: joint consensus statement of the European College of Sport Science and the American College of Sports Medicine. *Medicine and Science in Sports and Exercise, 45*(1), 186-205.

Meeusen, R., Nederhof, E., Buyse, L., Roelands, B., de Schutter, G., & Piacentini, M. F. (2010). Diagnosing overtraining in athletes using the two-bout exercise protocol. *British Journal of Sports Medicine, 44*(9), 642-648.

Meeusen, R., Piacentini, M. F., Busschaert, B., Buyse, L., De Schutter, G., & Stray-Gundersen, J. (2004). Hormonal responses in athletes: the use of a two bout exercise protocol to detect subtle differences in (over)training status. *European Journal of Applied Physiology, 91*(2-3), 140-146.

Meister, S., Faude, O., Ammann, T., Schnittker, R., & Meyer, T. (2013). Indicators for high physical strain and overload in elite football players. *Scandinavian Journal of Medicine and Science in Sports, 23*(2), 156-163.

Minetto, M. A., Lanfranco, F., Tibaudi, A., Baldi, M., Termine, A., & Ghigo, E. (2008). Changes in awakening cortisol response and midnight salivary cortisol are sensitive markers of strenuous training-induced fatigue. *Journal of Endocrinological Investigation, 31*(1), 16-24.

Morgan, W. P., Brown, D. R., Raglin, J. S., O'Connor, P. J., & Ellickson, K. A. (1987). Psychological monitoring of overtraining and staleness. *British Journal of Sports Medicine, 21*(3), 107-114.

Morgan, W. P., O'Connor, P. J., Ellickson, K. A., & Bradley, P. W. (1988). Personality structure, mood states, and performance in elite male distance runners. *International Journal of Sport Psychology*.

Morgan, W. P., O'Connor, P. J., Sparling, P. B., & Pate, R. R. (1987). Psychological characterization of the elite female distance runner. *International Journal of Sports Medicine, 8 Suppl 2*, 124-131.

Naessens, G., Chandler, T. J., Kibler, W. B., & Driessens, M. (2000). Clinical Usefulness of Nocturnal Urinary Noradrenaline Excretion Patterns in the Follow-Up of Training Processes in High-Level Soccer Players. *The Journal of Strength and Conditioning Research, 14*(2), 125-131.

O'Connor, P. J., Morgan, W. P., Raglin, J. S., Barksdale, C. M., & Kalin, N. H. (1989). Mood state and salivary cortisol levels following overtraining in female swimmers. *Psychoneuroendocrinology, 14*(4), 303-310.

O'Donnell, K., Badrick, E., Kumari, M., & Steptoe, A. (2008). Psychological coping styles and cortisol over the day in healthy older adults. *Psychoneuroendocrinology, 33*(5), 601-611.

Obminski, Z., Stupnicki, R., Lerczak, K., Starczewska-Czapowska, J., Olszewska-Letonkiewicz, M., & Hackney, A. C. (2002). Cortisol and testosterone responses to training and competition stress in ice-dance skaters - a case study. *The South African Journal of Sports Medicine, 9*(1), 23-25.

Persson, S., Larsson, M., & Lindholm, A. (1980). Effects of training on adreno‐cortical function and red‐cell volume in trotters. *Zentralblatt für Veterinärmedizin Reihe A, 27*(4), 261-268.

Pruessner, J., Wolf, O., Hellhammer, D., Buske-Kirschbaum, A., Von Auer, K., Jobst, S., . . . Kirschbaum, C. (1997). Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sciences, 61*(26), 2539-2549.

Pruessner, J. C., Hellhammer, D. H., & Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosomatic Medicine, 61*(2), 197-204.

Prussner, J., Kirschbaum, C., & Hellhammer, D. (1995). *Waking up-the first stressor of the day? Free cortisol levels double within minutes after awakening.* Paper presented at the Journal of Psychophysiology meeting.

Pyne DB, Martin DT. Fatigue-insights from individual and team sports. In: Marino FE, editor. Regulation of fatigue in exercise. New York: Nova Science; 2011. p. 177–85.

Rice, M. J., & Records, K. (2006). Cardiac response rate variability in physically abused women of childbearing age. *Biological Research for Nursing, 7*(3), 204-213.

Roberts, A. D., Wessely, S., Chalder, T., Papadopoulos, A., & Cleare, A. J. (2004). Salivary cortisol response to awakening in chronic fatigue syndrome. *The British Journal of Psychiatry, 184*(2), 136-141.

Rusko, H., Härkönen, M., & Pakarinen, A. (1994). 356 Overtraining effects on hormonal and autonomic regulation in young cross-country skiers. *Medicine amd Science in Sports and Exercise, 26*(5), S64.

Scanlan, A. T., Wen, N., Tucker, P. S., & Dalbo, V. J. (2014). The relationships between internal and external training load models during basketball training. *The Journal of Strength and Conditioning Research, 28*(9), 2397-2405.

Schlotz, W., Hellhammer, J., Schulz, P., & Stone, A. A. (2004). Perceived work overload and chronic worrying predict weekend–weekday differences in the cortisol awakening response. *Psychosomatic Medicine, 66*(2), 207-214.

Schmidt-Reinwald, A., Pruessner, J., Hellhammer, D., Federenko, I., Rohleder, N., Schürmeyer, T., & Kirschbaum, C. (1999). The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sciences, 64*(18), 1653-1660.

Schulz, P., Kirschbaum, C., Prüßner, J., & Hellhammer, D. (1998). Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload. *Stress and Health, 14*(2), 91-97.

Selänne, H., Ryba, T., & Leppäluoto, J. (2013). Common features in overtrained athletes and individuals with professional burnout: implications for sports medical practice. *Athletic Insight: The Online Journal of Sport Psychology, 15*(3).

Selye, H. (1956). The Stress of Life. New York: McGraw-Hill.

Skiba, P. F. (2006). Calculation of power output and quantification of training stress in distance runners: the development of the gOVSS algorithm.

Smyth, N., Thorn, L., Hucklebridge, F., Evans, P., & Clow, A. (2015). Detailed time course of the cortisol awakening response in healthy articipants. *Psychoneuroendocrinology*.

Sonnenschein, M., Mommersteeg, P. M., Houtveen, J. H., Sorbi, M. J., Schaufeli, W. B., & van Doornen, L. J. (2007). Exhaustion and endocrine functioning in clinical burnout: an in-depth study using the experience sampling method. *Biological Psychology, 75*(2), 176-184.

Stalder, T., Kirschbaum, C., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., . . . Hellhammer, D. H. (2016). Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrinology, 63*, 414-432.

Steinacker, J. M., Lormes, W., Reissnecker, S., & Liu, Y. (2004). New aspects of the hormone and cytokine response to training. *European Journal of Applied Physiology, 91*(4), 382-391.

Stewart, J. M., Gewitz, M. H., Weldon, A., Arlievsky, N., Li, K., & Munoz, J. (1999). Orthostatic intolerance in adolescent chronic fatigue syndrome. *Pediatrics, 103*(1), 116-121.

Strahler, K., Ehrlenspiel, F., Heene, M., & Brand, R. (2010). Competitive anxiety and cortisol awakening response in the week leading up to a competition. *Psychology of Sport and Exercise, 11*(2), 148-154.

Tanskanen, M. M., Kyrolainen, H., Uusitalo, A. L., Huovinen, J., Nissila, J., Kinnunen, H., . . . Hakkinen, K. (2011). Serum sex hormone-binding globulin and cortisol concentrations are associated with overreaching during strenuous military training. *Journal of Strength and Conditioning Research, 25*(3), 787-797.

Therrien, F., Drapeau, V., Lupien, S. J., Beaulieu, S., Doré, J., Tremblay, A., & Richard, D. (2008). Awakening cortisol response in relation to psychosocial profiles and eating behaviors. *Physiology & behavior, 93*(1), 282-288.

Traustadottir, T., Bosch, P. R., & Matt, K. S. (2005). The HPA axis response to stress in women: effects of aging and fitness. *Psychoneuroendocrinology, 30*(4), 392-402.

Urhausen, A., & Kindermann, W. (2002). Diagnosis of overtraining: what tools do we have? *Sports Medicine, 32*(2), 95-102.

Uusitalo, A. L., Uusitalo, A. J., & Rusko, H. K. (2000). Heart rate and blood pressure variability during heavy training and overtraining in the female athlete. *International Journal of Sports Medicine, 21*(1), 45-53.

van Lieshout, J. J., Wieling, W., Karemaker, J. M., & Eckberg, D. L. (1991). The vasovagal response. *Clinical Science (London), 81*(5), 575-586.

Viru, A., Karelson, K., & Smirnova, T. (1992). Stability and variability in hormonal responses to prolonged exercise. *International Journal of Sports Medicine, 13*(3), 230-235.

Viru, A., & Viru, M. (2000). Nature of training effects. *Exercise and Sport Science*, 67-95.

Viru, A., Viru, M., Karelson, K., Janson, T., Siim, K., Fischer, K., & Hackney, A. C. (2007). Adrenergic effects on adrenocortical cortisol response to incremental exercise to exhaustion. *European Journal of Applied Physiology, 100*(2), 241-245.

Wahbeh, H., Kishiyama, S. S., Zajdel, D., & Oken, B. S. (2008). Salivary cortisol awakening response in mild Alzheimer disease, caregivers, and noncaregivers. *Alzheimer Disease & Associated Disorders, 22*(2), 181-183.

Wallace, L., Slattery, K., & Coutts, A. J. (2014). A comparison of methods for quantifying training load: relationships between modelled and actual training responses. *European Journal of Applied Physiology, 114*(1), 11-20.

Wallace, L. K., Slattery, K. M., & Coutts, A. J. (2009). The ecological validity and application of the session-RPE method for quantifying training loads in swimming. *The Journal of Strength & Conditioning Research, 23*(1), 33-38.

Wichers, M. C., Kenis, G., Koek, G. H., Robaeys, G., Nicolson, N. A., & Maes, M. (2007). Interferon-α-induced depressive symptoms are related to changes in the cytokine network but not to cortisol. *Journal of Psychosomatic Research, 62*(2), 207-214.

Wolfram, M., Bellingrath, S., & Kudielka, B. M. (2011). The cortisol awakening response (CAR) across the female menstrual cycle. *Psychoneuroendocrinology, 36*(6), 905-912.

Wust, S., Federenko, I., Hellhammer, D. H., & Kirschbaum, C. (2000). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology, 25*(7), 707-720.

Wust, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N., & Kirschbaum, C. (2000). The cortisol awakening response-normal values and confounds. *Noise and Health, 2*(7), 79.

Wyller, V. B., Barbieri, R., Thaulow, E., & Saul, J. P. (2008). Enhanced vagal withdrawal during mild orthostatic stress in adolescents with chronic fatigue. *Annals of Noninvasive Electrocardiology, 13*(1), 67-73.

Wyller, V. B., Fagermoen, E., Sulheim, D., Winger, A., Skovlund, E., & Saul, J. P. (2014). Orthostatic responses in adolescent chronic fatigue syndrome: contributions from expectancies as well as gravity. *BioPsychoSocial Medicine, 8*(1), 22.

Wyller, V. B., Saul, J. P., Amlie, J. P., & Thaulow, E. (2007). Sympathetic predominance of cardiovascular regulation during mild orthostatic stress in adolescents with chronic fatigue. *Clinical Physiology and Functional Imaging, 27*(4), 231-238.