Analysis of the Immunological and Neuro-Endocrine Responses to Resistance Training in Division-I Football Players

Simon J. Haake

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

Chapel Hill
2013

Approved by:

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

E. D. Ryan, Ph.D.

E. Sobolewski, M.S.

ABSTRACT

SIMON J. HAAKE: Analysis of the Immunological and Neuro-Endocrine Responses to Resistance Training in Division-I Football Players
(Under the direction of Anthony C. Hackney, Ph.D., D.Sc.)

Twenty Division-I American Football athletes (age = 19.1 ± 1.1 y) participated in a 6-week off-season strength and conditioning program. Athletes resistance trained for 6 weeks at 85-100% of their 1-repetition maximum (RM). Evaluations were performed at Week 1, Week 4, and Week 6 of training. At Week 6, resting measures of both IL-6 and cortisol were elevated above Week 1 baseline measures ($p<0.05$). Body weight increased Weeks 2-5 as well as 1-RM on the three main lifts investigated (Bench = +4.8 ± 4.2%; Squat = +2.1 ± 3.1%; Clean = +2.0 ± 3.3%). REST-Q questionnaire showed small, significant decreases in four perceived affective categories. Correlation coefficients revealed significant relationships of IL-6 and cortisol at Weeks 1 and Week 6. It appears that the training utilized in the study was strenuous enough to produce a positive physical response and increases in biomarkers, but did not cause overtraining.

ACKNOWLEDGEMENTS

Throughout the process of implementing and writing my thesis I was helped by a multitude of people. Essential to the completion of my thesis was my advisor, Dr. Anthony Hackney, who made this all possible and guided me to the finish. Without Dr. Hackney I would not have been able to get my idea off the ground. Also my entire committee, including Dr. Eric Ryan and Eric Sobolewski, provided crucial insight and support throughout the process. I also want to acknowledge my classmates who were there to bounce ideas off and communicate with about both my thesis and everything else important in the process of graduate school. The process of completing my masters was made possible by the whole EXSS department who were extraordinary in their support.

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CHAPTER I

BASIS FOR STUDY

Introduction

A high level of player performance in Division-I athletics is the main goal for strength and conditioning coaches. By optimizing a combination of training modality, duration, intensity, and volume these strength and conditioning coaches attempt to bring their athletes into games, throughout the competitive season, in a non-fatigued, high-performance, healthy state. In the off-season, strength and conditioning coaches play a large part in the physical preparation of the athlete for competition. The achievement and maintenance of a healthy state is a combination of many physiological factors working together at an optimal functional state. Two critical physiological systems that are part of the health status of the athlete are the immunological and neuro-endocrine systems.

In current contemporary times, strength coaches train athletes throughout the year at, or near, their maximal capability using a process called overreaching. In the overreaching process, one or more of the training factors (modality, duration, intensity, and/or volume) are increased beyond what the athlete is accustomed to in order to elicit a super-compensatory response (Mackinnon, 2000; Selye, 1950). This super-compensatory

response is characterized by increases in strength, speed, or sport performance beyond previous measures. Regrettably, overreaching, when prolonged for several days to weeks, can possibly lead to a state called “Overtraining”; a rapid deterioration in performance that does not respond to an ‘appropriate’ rest or regeneration period (Lehmann et al., 1993). An athlete who is ‘overtrained’ may display a myriad of physiological symptoms including fatigue, depression, lack of interest in training, and sleeplessness. Also, overtrained athletes often experience drops in performance in their sport. Research findings suggest these symptoms can largely be attributed to the changes in immunological and neuro-endocrine status of the athlete; i.e., these systems become compromised (Smith, 2000).

It is well documented that exercise, specifically intense or prolonged bouts, can lead to the production of and subsequent elevation in pro-inflammatory cytokines; components within the immune system. These cytokines, such as IL-1a, IL-1b, and IL-6, are part of an acute pro-inflammatory response to physiological stressors such as is presented during exercise training. Generally, these pro-inflammatory cytokines are offset by production of anti-inflammatory cytokines (IL-10) and cytokine inhibitors (IL-1ra, sTNF-r1). That is, the anti-inflammatory cytokines mitigate the effects of the pro-inflammatory cytokines. During periods of overreaching, though, pro-inflammatory cytokines may reach excessively elevated levels which in turn can promote symptoms of fatigue. When fatigue is left unmanaged by either a reduction in training volume or intensity, it has been proposed that overreaching leads to an overtrained state (Smith, 2000). Recently Dr. Lucille Smith has developed the ‘Cytokine Hypothesis’ to explain overtraining in athlete, specifically proposing that excessive production and/or

heightened sensitivity in tissues to the specific cytokine IL-6 is the principle factor leading to the overtrained state (Robson, 2003). The relationship between IL-6 and hormonal influences on performance have yet to be investigated with competitive American collegiate football players involved in resistance-strength training.

Research suggests that the neuro-endocrine system is the second focal point in determining whether an athlete is overtrained. During prolonged and/or intense exercise, the endocrine system releases hormones that moderate the metabolic response to exercise, specifically glucocorticoids which are fundamental to this process. The glucocorticoid hormones also play an important role on the functioning of the immune system during exercise. Cortisol, the major steroid glucocorticoid hormone released in response to physical and psychological stress, has such a role as it is a strong inhibitory factor of the immune response. The cortisol-glucocorticoid response, in turn, is also mediated by the pro-inflammatory cytokines, specifically IL-6 (Steensberg et al., 2003). This response of IL-6 is primarily from contracting muscles used during exercise and does not seem to be via adipocyte IL-6 release (Pedersen et al., 2004; Steensberg et al., 2000).

Purpose

The primary purpose of this study is to evaluate aspects of the immunological and neuro-endocrine system of Division-IA American football athletes participating in resistance training for a six week period. The cytokine IL-6 will be used to assess the immunological status and the hormone cortisol response to assess the neuro-endocrine status. Both of these biomarkers will be measured in saliva due to it being a non-invasive

means of collecting a biological sampling and thus not interfering with the athletes (i.e., subjects) exercise training.

Hypothesis

1. There will be a significant elevation of salivary IL-6 and cortisol concentrations over baseline by the end of the 6-week training period.
2. There will be significant correlations between salivary IL-6 and cortisol concentrations at each of the 6 time points within the training period.

Significance

The results of this study will help elucidate the response of the IL-6 cytokine to typical resistance training protocols used by strength and conditioning coaches of Division-I American football teams. Elevated levels of IL-6 have been shown to exist in athletes displaying overtraining symptoms. Understanding the cytokine response (and those factors affecting it such as cortisol) to resistance training in athletes has the potential to help strength and conditioning coaches more properly manage the exercise training protocols used with their athletes.

Definition of terms

Cortisol – A glucocorticoid hormone secreted from the zona fasciculata of the adrenal cortex. Cortisol release is stimulated by adrenocorticotropin hormone (ACTH) from the anterior pituitary (Mastorakos et al., 2005).

Corticotropin releasing hormone (CRH) – a hormone secreted by the paraventricular nucleus (PVN) of the hypothalamus in response to stress (Mastorakos et al., 2005).

Hypothalamic-pituitary-adrenocortical (HPA) axis – a complex feedback system which makes up a major part of the neuroendocrine system and reacts to stress as well as regulating many of the body's processes including the immune system.

Interleukin-6 (IL-6) – a cytokine released by most tissue cells within the body. Responsible for stimulation of acute phase proteins and a mediator of fever. IL-6 release is stimulated by infection, psychological and physical stress, and muscular contractions.

Delimitations

1. Subjects are healthy, resistance-trained males between 18-25 years of age.

2. Subjects report to each trial 2 hours post-prandial, and maintain and control their diet preceding each of the sampling sessions.

3. Subjects participate in all training sessions throughout the 6-week study period.

4. Each of the sampling sessions are conducted at the same time of day (within each subject) to account for circadian rhythms.

5. Psychological stress is controlled for at the start of the study as each subject demonstrates normal scores on the Recovery-Stress Questionnaire (REST-Q) before proceeding with study.

Limitations

1. Salivary measures will be taken in place of serum measures for both biological markers (IL-6 and cortisol). Salivary measures have been shown to be accurate for cortisol measures, but may not account for minute changes in IL-6 concentrations.

2. The results can only be generalized to the sample studied: healthy, highly resistance trained adult males between 18 and 25 years of age participating in standard football training practices.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The review of literature is organized first to present cytokine function and immunology, organized by relevance to exercise and sport performance. Second, the review will present cytokine response to exercise, specifically looking at IL-6 response in healthy subjects. Studies will be organized based on exercise modality, intensity, volume, and duration. Third the review will present the 'cytokine hypothesis' and overtraining in terms of interleukin-6 response to training. Lastly, the review will review cortisol's response to training and the relationship between the cytokine IL-6 and the neuro-endocrine system.

Background

Cytokines are cell-signaling glycoproteins that mediate communication between and within immune and nonimmune cells, organs and organ systems throughout the body (Moldoveanu et al., 2001). In response to exercise, both pro- and anti-inflammatory cytokines are released based on the intensity, mode, and duration of the exercise.

Cytokines bind to surface receptors on target tissues in the body, causing a desired enzymatic response based on the stimulus provided, such as infection or trauma (Corwin, 2000). This response may occur locally (autocrine/paracrine action) or on distant tissues (endocrine action), similar to classic hormones (Corwin, 2000). Interleukins are a specific subset of cytokines that communicate between various white blood cell populations, generating a variety of responses including the release of acute phase proteins from the liver (Dinarello, 1999). These acute phase proteins help to mediate the physiological response to the stressor in an attempt to promote homeostasis.

During bouts of prolonged running, cytokines have been shown to reach concentrations similar to trauma and infection (Febbraio & Pedersen, 2005). This acute phase response has been hypothesized to relate to the amount of damage being done to tissues within the body through the exercise stimulus (Bruunsgard et al., 1997; Ostrowski et al., 1998), though some studies refute this idea (Jonsdottir et al., 2000; Ostrowski et al., 2000). The cytokine response, specifically the cytokine IL-6 has been shown to specifically be released through a muscular contraction-based mechanism (Steensberg et al., 2002). While most cytokines are released through an immune system mechanism, from macrophages and white blood cells, IL-6 is found to increase in contracting limbs (muscle) based on the intensity and duration of contraction (Penkowa et al., 2003; Steensberg et al., 2002). This contraction based response precedes the production of other acute phase cytokines that have both pro- and anti-inflammatory effects. Of note, IL-6 has also been shown to be released from the brain but not adipose tissue during exercise (Febbraio & Pedersen, 2005).

The cytokine IL-6 plays a complicated role in the physiological reaction to stress. IL-6 is a key cytokine in the response to stress and trauma in humans, acting both locally during physical stress and systemically during infection and trauma (Fischer, 2006). Receptors for IL-6 are found in numerous tissues throughout the body, stimulating T-cell activation, anti-body formation, and the release of acute phase proteins from the liver (Corwin, 2000; Moldoveanu et al., 2001; Pedersen & Febbraio, 2008). Acute phase proteins are largely responsible for the inflammatory response to infection, trauma, and strenuous exercise, helping to both target foreign pathogens and repair tissues (Gabay & Kushner, 1999). IL-6 has been shown to precede and largely affect the C-reactive protein (CRP) and response to anaerobic exercise; CRP is a major acute phase protein that impacts the inflammatory state in many disease populations (Gabay & Kushner, 1999; Meyer et al., 2001). IL-6 has also been shown to have anti-inflammatory mechanisms, stimulating the release of cortisol, which acts to counter the secretion of more IL-6 as well as stimulate the release of IL-1 receptor antagonist (Corwin, 2000; Fischer, 2006; Steensberg et al., 2002).

During strenuous exercise, the IL-6 response has shown to simulate the response regularly seen in infection or trauma states (Meyer et al., 2001; Niemen et al., 2003; Ostrowski et al., 1998). The rapid increase in IL-6 mRNA during exercise is detectable within 30 minutes of start and by the end of strenuous exercise up to a 100-fold increase can be found (Pedersen & Febbraio, 2008). This release is sensitive to the exercise intensity, mode, and duration, which determine the magnitude of response (Pedersen & Febbraio, 2008). Mode has been determined to either not have a significant effect or have no effect on the IL-6 response, which has shown no difference between resistance or

aerobic training when matched for intensity and duration (Fischer, 2006; Mendham et al., 2010). Intensity of exercise is important due to discoveries that type 2 fibers may be dependently responsible for much of the IL-6 release accounting for 51% of the variance in IL-6 concentrations post-exercise, showing a greater release during fatiguing bouts of exercise where type 2 fibers are serially recruited to maintain force (Febbraio & Pedersen, 2005). Duration has been deemed the most important factor in the magnitude of IL-6 response with numerous studies showing a linear relationship between time and response (Fischer, 2006).

The response of interleukin-6 to exercise may have dual immune/metabolism objectives. During exercise the appearance of IL-6 in the blood marks an increase in glucose appearance and uptake and increases lipolysis as seen in healthy individuals (Fischer, 2006). Also, IL-6 increases adrenocorticotropic hormone (ACTH) in a cortisol-releasing hormone (CRH) dependent manner (Fischer, 2006). Increased cortisol also plays a role in lipolysis and hepatic glucose uptake during exercise and has been shown to play an interactive role with IL-6 and catecholamines during exercise (Papanicolaou et al., 1996). Higher plasma IL-6 concentrations are also responsible for increases in glucagon and growth hormone, both of which may play a role in increasing lipolysis (Galton & Bray, 1967). IL-6 also plays an exercise-induced immunological role post-exercise relating to the release of several pro- and anti-inflammatory proteins. Exercise induced elevations in IL-6 increase the release of IL-1receptor antagonist (IL-1ra), cortisol, IL-10, and CRP (Steensberg et al., 2003). Both IL-1ra and IL-10 have shown anti-inflammatory mechanisms through their reduction of inflammatory cytokines IL-1 and TNF-alpha, IL-6, IL-8, and IL-1beta (Corwin, 2000; Niemen et al., 2004). Thus the

response of IL-6 to exercise has a marked role in both the immune and metabolic physiology post-exercise.

IL-6 Response to Exercise

The response of interleukin-6 to exercise has been investigated exhaustively and a full review is beyond the scope of this chapter, thus only key studies pertaining to IL-6 and anaerobic related exercise will be discussed.

Meyer et. al. (2001) compared a single bout of anaerobic exercise on a cycle ergometer, 60 seconds (SMT) vs. repeated anaerobic bouts 60 seconds plus 8 repetitions for 10 seconds (AN-TS) for changes in interleukin-6, IL-8, CRP, and cortisol in healthy male subjects. Twelve trained, male volunteers performed both exercise bouts in a randomized order, separated by at least 24 hours. Blood samples were taken at rest before exercise, 15 minutes post-exercise, 2 hours post, and 24 hours post. Results showed a significant elevation in IL-6 15 minutes post-exercise for both AN-TS and SMT, with AN-TS being significantly elevated (mean increase of 15 pg/ml) in comparison to SMT (4 pg/ml) and a control day with no exercise (Co-Day) (2 pg/ml). SMT showed an elevation at 15 minutes post as compared to Co-Day. CRP was significantly elevated for AN-TS at 24 hours post with no significant changes for SMT or Co-Day. Cortisol was significantly elevated in both SMT and AN-TS at 15 minutes post, with AN-TS being significantly elevated over SMT and Co-Day. There was no significant change in IL-8 values over the course of the study. These findings indicate a significant elevation in IL-6

post-exercise with anaerobic bouts of exercise lasting less than 30 minutes in duration and a correlating increase in CRP at 24 hours post-exercise.

Steensberg et. al. (2002) investigated the IL-6 and TNF-alpha response to 180 minutes of two-legged knee-extension exercise in healthy, male subjects. Maximal knee extension was determined for the 6 subjects and on a separate day they performed 180 minutes of knee-extension in one leg at 55% of their maximal workload. Biopsy samples were taken pre- and post-exercise and blood samples were taken from the femoral artery at 30, 60, 90, 120, and 180 minutes during exercise. IL-6 and TNF-alpha were analyzed over the course of the exercise with IL-6 significantly increasing at 30minutes during exercise (~10fold mRNA increase) and peaking at 180minutes (~100fold mRNA increase). There was a slight, but non-significant increase in TNF-alpha over the exercise bout. The results of this study show that IL-6 was produced in the contracting muscles during the two-legged knee-extension exercise, demonstrating the importance of muscular contraction to the release of IL-6.

Mendham et. al. (2010) compared moderate- and low-intensity resistance training to intensity-matched aerobic training exercise for responses of IL-6, CRP, Creatine Kinase (CK), and cortisol in sedentary male subjects. Subjects randomly performed the four exercise bouts; low-intensity resistance, mod-intensity resistance, low-intensity aerobic, and mod-intensity aerobic exercise each lasting 40 minutes. Exercise bouts were performed after a familiarization period with a 7 day recovery period between sessions. Blood samples were taken pre-, immediately-post, 3 hours post, and 24 hours post. Immediately post-exercise, IL-6 concentrations for both moderate intensity exercise bouts were elevated above baseline and were significantly elevated above their respective low-

intensity pairs. There was no significant difference between moderate-intensity resistance training (0.74±0.27 pg/ml) and moderate-intensity aerobic training (0.90±0.13 pg/ml) at any time-point post-exercise. Both low-intensity exercise bouts did not significantly elevate any investigated marker at any time-point. The results of this study suggest that the intensity of exercise is the central mediating factor for IL-6 production in exercise bouts lasting 40 minutes in sedentary individuals.

Niemen et. al. (2003) investigated the effects of carbohydrate supplementation on subjects performing 2 hours of resistance training for IL-6 response. Subjects were randomized to carbohydrate groups (CHO) or placebo groups (Pla). Both exercise groups performed 10 exercises, 4 sets each, 10 repetitions, with 2- to 3-minutes rest intervals between sets. Blood samples were taken pre- and post-exercise for each group. Both groups saw a significant increase in mRNA IL-6 concentrations post-exercise, but a non-significant difference between groups (CHO: ~80 fold increase, Pla: ~80 fold increase). The results of this study suggest that resistance training increases IL-6 production but carbohydrate supplementation does not affect modest increases in post-exercise IL-6 production during resistance training.

The studies reviewed offer support for the increase in IL-6 post-exercise in response to resistance training. A limitation with these studies is that they do not show responses for highly trained athletes and do not analyze change over time for baseline measures of IL-6.

IL-6 and the 'Cytokine Hypothesis'

The existence of overtraining in athletics is a center of concern for both coaches and trainers. Overtraining, or underperformance, has been linked to the occurrence of excessive elevations of pro-inflammatory cytokines and symptoms of fatigue, stress, and depression (Robson, 2003; Smith, 2000). Current theories propose that trauma to muscular or connective tissue is responsible for chronic elevations in inflammatory markers. Repeated high-intensity, high-volume training without proper rest is theorized to produce a chronic systemic immune response that causes underperformance in athletes (Smith, 2000). Cytokines, which serve to coordinate the response of neutrophils and monocytes during stress and trauma, are elevated in order to help deal with damaged tissues during exercise. Chronic elevation of cytokines, such as IL-6, leads to elevated baseline measures and increases in cytokine receptors such as soluble IL-6 receptors to handle increases in cytokine numbers (Smith, 2000). On subsequent exposure to stress, IL-6 has been shown to be elevated beyond normal levels when subjects are in a state of underperformance (Robson, 2003). Because of the development of overtraining-underperformance in individuals with increased cytokines researchers have developed the 'cytokine hypothesis', relating chronic systemic elevation of cytokines as well as the development of intolerance to cytokines such as IL-6 to overtraining and underperformance in athletes (Robson, 2003; Smith, 2000).

Role of Cortisol

The glucocorticoid cortisol is another marker frequently used to profile training intensity and stress in athletes (Hackney, 2006). As a catabolic hormone, cortisol promotes the breakdown of substrates to provide energy during physical and psychological stress. Cortisol also has a role on the immune system through mediating inflammation, with a connection to the IL-6 response to exercise. The inflammatory cytokine IL-6 signals through the hypothalamus for the release of corticotropin releasing hormone (CRH), which functions to increase production of cortisol in the adrenal gland (Smith, 2000). Cortisol blocks production of IL-6 mRNA, limiting the production of this inflammatory cytokine (Swolin-Eide & Ohlsson, 1998). Cortisol also functions to increase lipolysis during exercise, which negatively influences the release of IL-6 (Pedersen et al., 2001). Both cortisol and IL-6 exhibit moderating factors on each other, helping to regulate both metabolic and inflammatory processes. The feedback system between IL-6 and cortisol was shown to be evident when recombinant human IL-6 is infused into the bloodstream in healthy human subjects, with a subsequent increase in cortisol (Van Hall et al., 2003).

The release of cortisol also has a negative feedback role on CRH, helping to mediate the glucocorticoid response (Luger et al., 1987). Chronic elevation of IL-6 is known to occur in highly trained athletes exposed to training stress (Pedersen, 2007). Elevated levels of IL-6 feed-forward to stimulate the cortisol response through the hypothalamus, which promotes a catabolic environment (Mastorakos et al., 2005).

The role of cortisol in overtraining is also evident through multiple mechanisms. Cortisol reduces the incorporation of amino acids into myosin-heavy chain type II muscle fibers, limiting growth of fast twitch fibers (Goldberg, 1969). Also, chronically elevated cortisol levels have shown to increase muscular fatigue, muscular soreness, and decreased muscular performance due to what is known as "glucocorticoid myopathy" (Hooper et. al., 1993; Lehmann et al., 1992; Lehmann et al., 1993). Due to these factors, along with its interaction with IL-6 and the immune system, cortisol has been named as one of the central factors in explaining performance decrements with overtrained athletes.

Summary

In summary, IL-6 is an important cytokine that modulates the response of several other pro- and anti-inflammatory cytokines and hormones (Corwin, 2000; Moldoveanu et al., 2001; Pederson et al., 1998). The response of IL-6 to exercise is dependent in large part to the duration of exercise as well as the intensity (Pedersen & Febbraio, 2008). The effect of exercise on the production of IL-6 has been well documented in many populations excluding highly-trained resistance-based athletes. Along with having an immune-regulating effect, IL-6 also plays a part in exercise metabolism through the promotion and release of cortisol, glucagon and growth hormone (Fischer, 2006; Galton, 1967). Repeated exposure to cytokine activity, such as in disease or chronic inflammation, is associated with elevated resting levels of IL-6 (Gabay & Kushner, 1999). The chronic elevation of IL-6 has been noted in many disease-state individuals as

well as individuals with underperformance syndrome or overtraining (Jankord & Jemiolo, 2004; Peterson et al., 2004; Robson, 2003; Smith, 2000).

The relationship between cortisol and IL-6 is an important factor to examine with athletes during training. Due to the catabolic nature of cortisol, regulation of this hormone during training can play a role in the ability to recover from workouts and athletic performance.

A limitation within the aforementioned literature is a lack of comparison to resistance-trained athletes based studies. Also, it appears no cross-sectional data has been used to analyze IL-6 concentrations over time through a standard training period with athletes. While repeated high-volume exercise has been shown to elicit underperformance syndrome (overtraining), the association of IL-6 to this underperformance has not been investigated in full with athletes participating in resistance training programs.

CHAPTER III

METHODOLOGY

Subjects

Moderate to highly strength-trained male subjects (ages 18-21) were recruited from the University of North Carolina-Chapel Hill Division I varsity football team for this study. All participating athletes who were deemed to be in a healthy, non-diseased or injured state were allowed to participate. Subjects must have had full participation in team activities for a minimum of 3 days a week for 3 months prior to the study as well as having had at least two years history of resistance training consisting of at least one training session per week to be eligible. Those excluded from the study included: subjects with injuries preventing full participation in lifting and conditioning sessions; subjects with immunological irregularities; subjects taking any medication reported to affect the inflammatory response; or subjects with any smoking background. All subjects were required to provide written informed consent prior to commencing testing procedures. Subjects and coaches were provided with full disclosure of the risks and purposes of the study before consent was obtained.

Protocol

Each subject was asked to report to the Athletic Training Room within Kenan Stadium at UNC-Chapel Hill on six separate occasions. Anthropometric data (age, height, weight, body fat percentage via skinfolds) was collected on the first training day at the beginning of the first training week. Body weight was tracked weekly with the salivary measures of IL-6 and cortisol. Subjects were to refrain from physical activity or exercise for 48 hours prior to the initial sampling-training day; all athletes were returning from a 3 day weekend off from football activities. Subjects were also told to refrain from alcohol or caffeine consumption for 24 hours prior to each sample day.

Salivary samples were taken on the first day of training, at the time of day that each subject was scheduled to arrive for weight room training for the duration of the study (offensive players at 2:15 pm and defensive players at 3:45 pm), this time remained consistent over the course of the study. Subjects were told to refrain from eating or drinking for at least 1 hour prior to sampling. Each subject was asked to rinse out their mouth with water thoroughly for 30 seconds prior to providing a saliva sample. Saliva samples were taken via passive drool sampling into a collection tube for the IL-6 and cortisol analysis. A minimum of 1.0 mL was required. Subsequent samples were taken before the first training activities on the first day of each week (Monday) for six weeks at the same time of day (±15 minutes) for each subject (6 total samples).

Actual Assessment Day	Day 1	Day 8	Day 15	Day 22	Day 29	Day 36
Measures Assessed	Anthropometric, IL-6, Cortisol	BW, IL-6, Cortisol	BW, IL-6, Cortisol	BW, IL-6, Cortisol	BW, IL-6, Cortisol	BW, IL-6, Cortisol
Representative Term Used In Text	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

Figure 1. Overview of experimental protocol; BW = body weight.

Exercise Protocol

Training load, intensity, and volume were tracked for the six week period and cataloged to ensure a similar training stimulus between athletes for the study period. Training load was calculated as total weight lifted divided by body weight. Throughout the six week period training load was controlled by the principle investigator to stay within ± 10% of baseline load as typical of UNC-CH Football team's traditional training style. Lifting maxes were catalogued for the bench press, back squat, and Olympic-style clean from prior to participation in the study to completion of the study (pre- and post-).

Subject Arrived		REST-Q Administered Saliva Sample Container Given		REST-Q Collected Saliva Sample Collected		Team Warm-Up Weight Training Session Team Conditioning*		Subject Released
	5min		5min		2min	(45min-1hr Total)		

Figure 2. Overview of typical experimental trial (typical training day).

Each training session consisted of a 10-15 minutes warm-up comprised of three separate stations, each lasting 3-5 minutes. One station contained a core/abdominal exercise (e.g., sit-ups, crunches, plate sit-up, Russian twists, and see-saw abs) for between 40-75 total repetitions. These exercises were interchanged over the 6 week period. Another station consisted of both dynamic and static stretching. A third station featured shoulder exercises for repetitions of 10 with two 4.5 kg plates (lateral raise, front raise, rear deltoid raise). The warm-up series remained constant over the 6 week training period.

Bench Press*	Back Squat*	Power Clean*
Incline Bench Press	Front Squat	Hang Clean
Dumbbell Bench Press	Barbell Lunges	Barbell Shrugs
Incline Dumbbell Press	Romanian Deadlift	Pull Ups
Front Press	Calf Raises	Barbell Row
Dumbbell Overhead Press	Deadlift	Barbell Curl
Close-Grip Bench Press	Push Ups	Dumbbell Curl

Table 1. List of exercises used throughout the 6-week study period. *Indicates main lift.

After the warm-up, training consisted of whole body and body part isolated resistance training exercises including back squat, bench press, power clean, hang clean, incline bench, lunges, and several assistance exercises (see Table 1 for list). Each training session began with one of the main lifts (bench, power clean, or squat), working up to a near-maximum intensity. Supplementary exercises were completed after completion of

the main lift. Training sessions lasted between 30-45 minutes total (45 minutes to 1 hour total including warm-up).

Progressive intensity was used for each exercise during the training session to achieve maximal intensity by the final set. The relative intensity of each assistance exercise remained at near maximum as determined by the strength and conditioning coach for each athlete through the training period. In cases where athletes were able to increase intensity, the strength coach would add weight to satisfy the criteria of near-maximum intensity. This criteria was judged by the strength coach to be the heaviest that the athlete could go in each lift for a given day based on correct technique and completing all repetitions. After the training session was completed, athletes were released.

Training volume increased gradually over the course of the training period through manipulation of total sets completed per exercise and increased number of exercises completed. This was done to account for increases in training capacity and/or strength over the course of the training period. Manipulations were controlled by the strength coach to ensure that a proper training stimulus was being applied to each athlete. Relative intensity (training stimulus as percentage of maximum capability), total training load, and training load for main lifts (bench, squat, and clean) were catalogued for a sample of athletes to ensure an appropriate training stimulus was being applied.

Beginning on the second week of training, conditioning runs were completed twice a week on Tuesdays and Thursdays. These conditioning sessions were completed immediately post-weight room training session and consisted of 100 yard runs (at a set pace; 16 seconds for skill players, 18 seconds for big-skill players, and 20 seconds for

Offensive-Line/Defensive-Line players) and 300 yard shuttles (at a set pace; 55 seconds, 60 seconds, 65 seconds respectively) on Tuesdays and Thursdays correspondingly. Each week volume was increased to create a higher physiological demand on the athlete. On the fifth and sixth weeks of training, the 300 yard shuttles were replaced with a speed/agility circuit lasting 45 minutes. The circuit consisted of 4 separate drills completed for a total of 8 minutes, with 2 minutes rest between drills.

Instrumentation

The height (cm) and body mass (kg) of each subject were determined by the lead researcher using a stadiometer (Perspectives Enterprises, Portage, MI) and a mechanical scale (Detecto, Webb City, MO). Skinfolds were measured in triplicate at select sites (abdomen, chest, and thigh) using skinfold calipers (Skyndex, Fayetteville, AR) and body fat percentage was calculated using the Jackson-Pollock equation (Jackson et al., 1978). All weight training equipment was supplied by Hammer Strength (Lifetime Fitness, Schiller Park, IL). Psychological analysis performed using the Recovery-Stress Questionnaire for Athletes (REST-Q) (Kellmann, 1999).

Saliva Specimen Procedures

Collection and storage

Prior to collection of saliva samples, subjects were asked to rinse their mouths with water, spit, and then allow saliva to accumulate in the pool of their mouth. If saliva secretion needed to be stimulated, subjects were asked to chew on paraffin film. Accumulated saliva samples (minimum of 1.0 ml necessary) were collected from the

subjects' mouths directly into a 1.8 ml collection tube. No more than 15 minutes past the desired time point (2:15 pm/3:45 pm) was allowed to pass before saliva is collected. Collected samples were stored in ice for 24 hours and transported to a freezer (-80° C) for later analysis.

Biochemical Analysis

The stored saliva samples were assessed for IL-6 and cortisol concentrations. Stored saliva samples were allowed to thaw and were then centrifuged at 3000 x *g* at 4 degrees Celsius. The resulting supernated saliva specimens were assayed for IL-6 and cortisol levels using expanded range high sensitivity enzyme immunoassay (ELISA) kits (Salimetrics, State College, PA, USA).

REST-Q Analysis

The Recovery-Stress Questionnaire for Athletes (REST-Q) was used to monitor psychological markers over the course of the training study. A 12 item questionnaire was administered to the athletes each sampling day as soon as the athlete entered the locker room prior to their involvement in exercise training for that day (either 2:15 pm or 3:45 pm). Athletes were given five minutes to review and answer each of the 12 questions.

Design-Data Analysis

This study design is descriptive in nature. No experimental manipulations were utilized to alter the normal, required activities of the subjects.

Data analysis was performed using a computer based statistical software program (SPSS version 20.0, IBM Technologies, Inc., Armock, NY, USA). Mean and standard deviations were computed for all anthropometric measurements (age, height, mass, and body fat %)

Two separate one-way, within subjects - repeated measures analysis of variance (ANOVA) were used to determine if significant changes occurred in saliva IL-6 and cortisol over the study period (Week 1 – Week 6). If either ANOVA analyses revealed significant F-ratios, Tukey post-hoc tests were used to determine which means were significantly different within each specific measurement. The significance level was set a priori at $\alpha \leq 0.05$.

A one-way, within subjects – repeated measures ANOVA was used to determine if there was a significant change in body weight (kg) after the six week training period. If ANOVA analyses revealed significant F-ratios, Tukey post-hoc tests were used to determine which means were significantly different within each specific measurement. The significance level was set a priori at $\alpha \leq 0.05$.

Separate one-way, within subjects - repeated measures analysis of variance were used for each question in the RESTQ (12 total questions) to determine if significant changes occurred in psychological parameters analyzed through the questionnaire. Mean substitution was used in case of missing responses. If either ANOVA analyses revealed significant F-ratios, Tukey post-hoc tests were used to determine which means were significantly different within each specific measurement. The significance level was set a priori at $\alpha \leq 0.05$.

Four separate one way, within subjects – repeated measures ANOVA were used for each affective category within the REST-Q. Question scores were combined for each affective category and means analyzed. If any ANOVA analysis revealed significant F-ratios, Tukey post-hoc tests were used to determine which means were significantly different within each specific measurement. The significance level was set a priori at $\alpha \leq 0.05$.

Three separate paired-samples t-tests were used to determine if significant changes occurred in bench press max, back squat max, and power clean max from pre- to post- completion of the study. The significance level was set a priori at $\alpha \leq 0.05$.

Pearson product-moment correlations were also used to assess the relationships between saliva IL-6 and cortisol concentrations as part of an exploratory analysis.

CHAPTER IV

RESULTS

Subject Characteristics

Twenty collegiate football athletes from the UNC-Chapel Hill team participated in this investigation. The physical characteristics of the subjects (n = 20), expressed as mean ± standard deviation (SD) were as follows: age (yrs) = 19.1 ± 1.1; height (cm) = 185.4 ± 6.7; mass (kg) = 102.0 ± 22.2; body fat (%) = 14.7 ± 7.6. Weekly body weight (kg) displayed below in Table 2.

Weekly Body Weight

(kg)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Body Weight	102.0 ± 22.2	102.5 ± 22.3*	102.8 ± 22.1*	103.0 ± 22.4*	103.3 ± 22.4*	102.8 ± 22.2

Table 2. Mean values of weekly body weight (kg) ± SD. *Indicates significant change from baseline.

Weight Training and Lifting Maxes

All subjects were training for a minimum of four days per week for 60 minutes or more in the previous six months before they began the research study. The subjects in the

study had been trained in the methods used throughout the study for a minimum of six months prior to involvement in the study, with experience in all lifting and running schemes utilized during the research study.

Each of the subjects had completed max testing on all major lifts used in the study (bench press, back squat, and power clean) prior to involvement. Max tests were performed as typical of National Strength and Conditioning Association (NSCA) Guidelines (Fry & Kraemer, 1994). Maxes achieved during the six week study period were also catalogued. The results of both pre- and post- study maxes are displayed below in Table 3.

Lift	Pre-Study Max (kg)	Post-Study Max (kg)	% Change
Bench Press	121.6 ± 36.3	127.4 ± 35.9*	+4.8 ± 4.2%
Back Squat	187.2 ± 30.2	190.9 ± 28.1*	+2.1 ± 3.1%
Power Clean	116.8 ± 14.6	119.2 ± 14.5*	+2.0 ± 3.3%

Table 3. Mean values of maximum performances on main lifts performed during the study period for subjects. Values are expressed as mean ± standard deviation (SD). (n=20). *Significant differences from respective pre-trial ($p < 0.05$).

Cortisol Analysis

The mean (±SD) salivary cortisol responses analyzed over the six week study period are displayed below in Table 4.

	Week 1 (*ug/d*L)	**Week 4 (*ug/d*L)**	**Week 6 (*ug/d*L)**
Salivary Cortisol	0.093 ± 0.089	0.133 ± 0.124	0.193 ± 0.179*

Table 4. Mean salivary cortisol concentrations for each respective sampling trial (n=20). *Significant differences from Week 1 trial ($p < 0.05$).

For financial reasons, analysis of cortisol samples was reduced to the Week 1, 4, and 6 sampling trials; providing a baseline, middle, and end point to the study period. Though not statistically significant, there was an upward trend from Week 1 to Week 4 ($p = 0.236$), and Week 4 to Week 6 ($p = 0.230$), however the mean difference between Week 1 and Week 6 was significant, showing a ~108% increase from baseline ($p = 0.004$).

Interleukin-6 Analysis

The mean (±SD) salivary IL-6 responses analyzed over the six week study period are displayed below in Table 5.

	Week 1 (*pg/m*L)	**Week 4 (*pg/mL*)**	**Week 6 (*pg/m*L)**
Salivary IL-6	1.42 ± 1.77	4.19 ± 8.27	5.60 ± 12.57

Table 5. Mean salivary IL-6 concentrations for each respective sampling trial (n=20). *Significant differences from Week 1 trial ($p < 0.05$).

There were no significant differences between means for salivary IL-6 over the course of the study ($p = 0.170$). However, due to the large amount of variability in the responses, the data were transformed into log to the base 10 values and reanalyzed. Log base 10 values showed a significant increase in IL-6 values from Week 1 to Week 6 ($p = 0.0013$).

Cortisol and IL-6 Correlational Analysis

The correlation coefficients for each case analysis between cortisol and IL-6 are displayed below in Table 6.

Trial	***Pearson Product Moment Correlation Coefficients***		
	Week 1 Cortisol	**Week 4 Cortisol**	**Week 6 Cortisol**
Week 1 IL-6	r = 0.6404*	r = -0.1037	r = 0.6817
Week 4 IL-6	r = 0.4860	r = 0.0946	r = 0.6845
Week 6 IL-6	r = 0.2462	r = -0.1412	r = 0.6805*

Table 6. Correlation coefficients for each case analysis between cortisol and IL-6 (n=20). *Significant correlation between cortisol and IL-6 ($p < 0.05$).

There were pertinent significant correlations between the Week 1 Cortisol and IL-6 values ($r = 0.6404$) as well as the Week 6 Cortisol and IL-6 values ($r = 0.4495$). Week 4 values did not correlate.

REST-Q Analyses

Individual REST-Q Question Scores: The mean score (scoring range 0-6) for each question was analyzed using within-subjects ANOVA to examine individual changes in aspects of stress perceived by the athletes. Between weeks there were minute, though

non-significant, changes in mean scores. By Weeks 4 and 5 several scores reached significant low points as compared with baseline scores (Questions 1, 2, 4, 8, and 12).

Sum of REST-Q Responses: The sums for all questions were analyzed using within-subjects ANOVA to examine the overall stress levels of all athletes over the six week period. There was a slight drop in scores by Week 5; however, there were no significant changes between weeks for the sum of scores.

REST-Q Affective Category Analysis: The mean (±SD) combined REST-Q scores for each affective category (4 total; anger, depression, fatigue, vigor). Scores for each category were combined for each week in the six week period and are displayed below in Table 8.

Affective Category	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**
Anger	1.56±1.2	1.38±1.5	1.05±1.2*	1.36±1.7	0.83±1.3*	1.15±1.4*
Depression	1.67±1.6	1.81±1.4	1.32±1.3*	1.46±1.6	1.62±1.8	1.83±1.9
Fatigue	1.85±1.3	1.91±1.6	1.46±1.4*	1.54±1.5	1.44±1.4*	1.56±1.4
Vigor (Motivation)	1.43±1.4	1.10±1.2*	1.32±1.5	1.23±1.5	1.06±1.3	0.92±1.1*

Table 8. Mean REST-Q Score for each affective category (4 total) for each sampling trial (n=20). Values are expressed as mean ± standard deviation (SD). *Significant difference from respective Week 1 baseline measure ($p < 0.05$).

Affective Category (Anger): The mean response score for anger questions (Q1, Q2) reached a statistically significant decrease from baseline (Week 1) levels by Week 3, Week 5, and Week 6. A significant decrease in the combined means for this affective category suggests a decrease in feelings of anger over the course of the study period.

Affective Category (Depression): The mean response score for depression questions (Q3, Q7, Q8) reached a statistically significant decrease from baseline (Week 1) levels by Week 3. A significant decrease in the combined means for this affective category suggests a decrease in feelings of depression over the course of the study period.

Affective Category (Fatigue): The mean response score for fatigue questions (Q4, Q5, Q9, Q12) reached a statistically significant decrease from baseline (Week 1) levels by Week 3 and Week 5. A significant decrease in the combined means for this affective category suggests a decrease in feelings of fatigue over the course of the study.

Affective Category (Vigor): The mean response score for vigor questions (Q6, Q10, Q11) reached a statistically significant decrease from baseline (Week 1) levels by Week 2 and Week 6. A significant decrease in the combined means for this affective category suggests an increase in feelings of vigor and motivation over the course of the study.

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Question 1	1.4±1.1	1.2±1.5	1.1±1.3	1.2±1.7	0.7±1.3*	1.2±1.5
Question 2	1.7±1.4	1.6±1.5	1.0±1.1*	1.5±1.8	0.9±1.3*	1.2±1.3
Question 3	2.1±1.9	2.4±1.4	1.6±1.3	2.1±1.8	2.2±1.8	2.3±2.0
Question 4	2.0±1.3	1.7±1.4	1.2±1.1*	1.1±1.3*	1.3±1.2*	1.4±1.3
Question 5	2.1±1.2	1.7±1.2	1.7±1.5	2.3±1.7	1.8±1.4	2.0±1.5
Question 6	1.9±1.3	1.2±1.2*	1.8±1.7	1.6±1.6	1.4±1.4	1.1±1.2*
Question 7	1.7±1.4	1.9±1.5	1.7±1.4	1.7±1.7	1.7±1.8	1.9±1.9
Question 8	1.3±1.4	1.2±1.2	0.6±1.0*	0.6±1.0*	0.9±1.4	1.3±1.7
Question 9	1.7±1.4	3.1±1.8*	2.0±1.4	2.1±1.4	1.8±1.5	2.1±1.8
Question 10	1.9±1.6	1.5±1.5	1.6±1.7	1.5±1.8	1.4±1.5	1.3±1.3
Question 11	0.5±0.5	0.6±0.7	0.6±0.8	0.6±0.8	0.4±0.7	0.4±0.7
Question 12	1.6±1.5	1.1±1.2	0.9±1.3	0.7±1.1*	0.8±1.5	0.8±1.1
SUM of Responses	18.8±12.8	18.7±12.3	15.7±10.8	16.8±12.4	15.4±11.0	16.8±11.8

Table 7. Mean REST-Q Response Scores, both sum and individual, for the six week study period. Scores displayed as Mean ± SD. *Significant difference from baseline (Week 1) response score ($p < 0.05$).

CHAPTER V

DISCUSSION

Introduction

The primary purpose of this study was to investigate the resting levels of immunological and endocrine biomarkers to typical off-season training in collegiate football athletes by tracking the markers IL-6 (immunological) and cortisol (endocrine) over a six-week period. These biomarkers were investigated to provide insight about the physiological reaction to strenuous resistance training while also looking at psychological parameters through use of a recovery-stress questionnaire. The hypothesized outcome was that there would be a significant increase in both biomarkers IL-6 and cortisol by the end of the six week study period as compared to baseline measures. This was expected due to the production of both IL-6 and cortisol in response to exercise, with increases in baseline measures expected during the strenuous training periods.

The discussion in this chapter is organized into several sections. First, the physical characteristics of the subjects are described to outline a typical collegiate football athlete. Second, both the subjects' IL-6 and cortisol responses are discussed, analyzing how they compared with other related exercise studies. Third, the subjects' REST-Q responses are

discussed along with their relationship to the biomarkers investigated. Fourth, the relationship of each variable will be discussed in regards to overtraining. Finally, limitations and conclusions of the present study are discussed.

Athlete Characteristics

As highlighted in Table 3, the exercise prescription produced significant positive changes in all three main lifts used throughout the study (bench press, back squat, and power clean). All of these values were found to be in agreement with a range for typical strength levels for Division I American football players as shown in Table 9 (Fry & Kraemer, 1994; Hoffman & Kang, 2003; Ware et al., 1995). Performances in each of the three main lifts were consistent with high level collegiate football players across the NCAA. The individual increases in strength levels for each main lift are typical of strength and conditioning programs utilizing a progressive overload methodology combined with proper rest and recovery methods (Burke et. al., 2001, Kraemer & Ratamess, 2004). In typical cases of overtraining, performance decrements are expected, which were not observed in the present study as can be seen in Table 3.

Bench Press Max	**Back Squat Max**	**Power Clean Max**	**Study**
127.4 ± 35.9	190.9 ± 28.1	119.2 ± 14.5	Current Study
136.9 ± 25.8	185.2 ± 35.7	118.1 ± 17.7	*Fry & Kraemer, 1994*
124.7 ± 21.0	163.3 ± 30.0	N/A	*Hoffman & Kang, 2003*
124.3 ± 18.3	179.2 ± 35.5	N/A	*Ware et al., 1995*

Table 9. Comparison of current study strength maxes (kg) to collected literature. Maxes expressed as mean ± standard deviation.

Anthropometric measures were standard for those typical of American football athletes (Fry & Kraemer, 1994; Hoffman & Kang, 2003). The increase in body weight over the course of the study suggests that proper recovery and nutrition tactics were being utilized by the athletes in order to increase lean tissue mass; however, no post-study body fat percentage numbers were collected. However, the increase in max training values suggests that increases in body weight may have been associated with increases in muscle mass over the 6-week period. As typical of this strength and conditioning program during off-season resistance training, gains in muscular strength and size are the main goal for weight room activities.

Cortisol Response

All baseline cortisol salivary values taken Week 1 were within the normal expected range of values (Salimetrics, USA). Since the athletes were returning from a break from training of ≥ 48 hours it was expected that they would show low levels for cortisol considering its nature as a stress-activated hormone. By Week 4, cortisol values increased approximately 43% above these baseline measures, though not statistically significant. The Week 6 analysis provided a significant positive increase in salivary cortisol with a ~108% increase over baseline measures.

Several studies have shown significant increases in salivary cortisol in response to high intensity (75%) resistance exercise training both immediately post as well as hours after the exercise bout (McGuigan et al., 2004; Paccotti et al., 2005). Low intensity resistance exercise (30%) however did not result in any significant increases (McGuigan et al., 2003). The current study took salivary measures at baseline at least 48 hours after

the most recent exercise bout, providing a resting measure. Taken at rest each week, these increased values suggest a rise in weekly resting inflammatory or stress status among the study participants since during periods of normal resistance training studies have shown no significant increases in resting levels of cortisol (Kraemer & Ratamess, 2005).

While there was a significant rise in the resting cortisol level over the 6-week period, there were no associated drops in performance or body weight to associate the change in hormonal status to the athletes having reached an overtrained state. Similar studies show varying responses of resting levels of cortisol during periods of intense resistance overtraining with some increasing, decreasing, or not significantly changing even with evident decrements in physical performance (Hakkinen et al., 2000; Hakkinen & Pakarinen, 1991; Hakkinen et al., 1987; Hooper et al., 1993; Potteiger et al., 1995).

IL-6 Response

All baseline salivary IL-6 values taken Week 1 were within the normal expected range of values (Rananto et al., 1999). Over the course of the study resting IL-6 values increased, reaching statistical significance by Week 6; an increase over baseline of ~294%. By the Week 6 sampling date mean subject concentration for IL-6 reached 5.60 *pg/mL*.; above the normal baseline ranges typical for training athletes (Nieman et al., 2001; Robson-Ansley et al., 2006). Week 4 IL-6 concentrations were increasing from Week 1 but the level was not significantly different from either Week 1 or Week 6 values.

The increase in IL-6 concentrations with training athletes has been associated with feelings of fatigue, stress, and worsening of athletic performance (Robson-Ansley et al., 2004). For example, levels as low as 5 *pg/mL* were reported to affect athlete perceived exertion during activity (Robson-Ansley et al., 2006). Current cytokine theories for overtraining suggest that the overproduction, and/or hyper sensitization, of IL-6 during extended periods of intense training is responsible for drops in performance and increased feelings of fatigue and stress (Robson-Ansley et al., 2006; Smith, 2000). The current study would suggest that while IL-6 may increase during periods of intense resistance training, but this level of increase may not be associated with corresponding drops in athletic performance or increased feelings of fatigue, anger, depression, or decreased vigor.

Increases in IL-6 have been reported up to ~100 fold over baseline immediately post-exercise (e.g., marathon running; Pedersen et al., 2001), but little research has been done investigating resting levels of IL-6 during periods of intense resistance training. Elevated post-exercise IL-6 values have typically been shown to return to baseline by 24 hours post exercise (MacIntyre et al., 2001; Toft et al., 2002). The current study allowed at least 48 hours between the preceding exercise session and resting salivary sampling; nonetheless, there was a persistent elevation in the IL-6 levels of the subjects. A central tenant within the cytokine hypothesis, as stated by Smith, is that a rise in inflammation due to physiological stress is cause for increasing levels of IL-6 at rest and in response to exercise (Smith, 2000). IL-6 levels increase dramatically with intense and/or prolonged exercise as seen with athletes demonstrating classical signs of overtraining. This was obviously not the case in the present study.

Another possible reason for the elevation in resting IL-6 is the athletes reaching a state of muscle glycogen depletion. Studies have shown that glycogen depletion is associated with an increased release of IL-6 (Miki et al., 1999; Steensberg et al., 2000). Glycogen depletion has also been associated with a reduction in body weight, exercise performance, and an increase in central and peripheral fatigue when glycogen levels reach near depletion (Costill et al., 1988). However, Costill et al. showed that highly trained athletes tend to have greater levels of stored glycogen, showing an improved ability to handle glycogen depleting tasks without a reduction in exercise performance. Within the current study it is possible that the football athletes had large stores of muscle glycogen that were not depleted significantly to impact their main lift performance.

The levels of IL-6 and cortisol correlated significantly at both Week 1 and Week 6, but not Week 4. This would suggest the immune system and endocrine system appeared to be in congruence at baseline, and by the end of the study matched up again. That is, the correlation implies that a level of intercommunication between the two systems as related to the degree of inflammation within the athletes, a fact that has been studied and confirmed previously (Pedersen et al., 2004; Steensberg et al., 2003).

REST-Q

REST-Q data provided a quantitative report for the athletes' feelings of fatigue, anger, depression and vigor. The 12 total questions were broken down into these four affective categories to investigate specific psychological parameters that the athletes felt over the period of training. All four affective categories showed significant decreases, at

different times, over the course of the 6-week period. A decrease in values within the REST-Q signifies a decrease in negative feelings for each affective category, suggesting that the athletes in the current study felt less fatigue, anger, depression, and higher vigor as training progressed through the 6-week period.

In several studies, increased levels of cortisol during intensive training correlated well with increases in REST-Q scores (Steinacker et al., 1999; Steinacker et al., 2000). REST-Q scores also have correlated well with increases in training intensity, providing feedback for how athletes perceive changes in their training (Kellmann & Kallus, 2001; Kellmann & Altenburg, 2001). The current study showed no relationship between increases in both salivary cortisol and IL-6 and corresponding REST-Q response scores over the 6-week period. Also, even though resistance training intensity was maintained at a very high level (>80% maximum) there was no corresponding increase in REST-Q scores; i.e., in fact the opposite tended to occur. It is possible that the training stimulus was great enough to produce and physiological response adaptations but not strenuous to the point that the athletes felt affective feelings of fatigue, anger, depression or a drop in vigor (i.e., classical signs of overtraining).

Overtraining

The variables measured in the current study have all been associated with the occurrence of overtraining in sport. Both cortisol and IL-6 have been used as biomarkers to investigate the hormonal and immunological reactions to strenuous training and their role in increasing feelings of fatigue and stress in athletes along with drops in exercise

and sport performance. The recovery-stress questionnaire used in the current study has also been shown to associate well with intensive training and a decline in physical performance (Kellmann et al., 2001). Another good indication of the overtraining status of an athlete is a significant drop in body weight and performance on resistance training tasks (Stone et al., 1991). Within the current study all these variables were investigated with collegiate football athletes performing their typical training program in the spring off-season exercise program, and were found not to change to indicate that overtraining occurred.

A major aim of this study was to investigate the combined responses of cortisol and IL-6 of football athletes during their resistance training regimen. It was expected, based on previous studies, that significant baseline increases in each of the biomarkers would impair performance on physical tasks such as weight lifting due to increased sensation of fatigue, illness, and stress (Smith, 2000). However, by the end of the 6-week period there were significant increases in each of the three main lift 1-repetition maxes (performance improved) and body weight increase. It is important to note that the athletes studied had all trained a minimum of two years prior to participation in the research study, therefore improvements in main lifts over a 6-week period is unlikely to be due to a lack of prior exposure to resistance training methods. Furthermore, the athletes did not have greater levels of anger, fatigue or depression. Thus, the athletes showed no overt signs of being overtrained, but the biomarkers were significantly elevated. Therefore, the current training regimen was effective, caused positive adaptations and stimulated immunological-endocrine responses; but did not provoke enough of a immunological-

endocrine response to induce the developing of overtraining symptoms within the 6 week time frame.

Limitations

There are potential limitations in this investigation which may have impacted the results and potentially limit the reliability and validity of the findings. First, it was expected that athletes adhered to experimental compliance procedures, including; training history, diet (2 hours post prandial, and no alcohol, NSAIDs, or caffeine in the previous 24 hours), acute training (no strenuous activity in the 48 hours prior to sampling), and stress states (forthright answers in the REST-Q questionnaire). Inaccuracies in the information and/or procedures may have introduced systematic error into the study and confounded outcomes.

The collection of salivary samples may have also introduced error into the study. Athletes were instructed to have been at least 2 hours post prandial, however, there is the possibility that eating or drinking substances were present in the saliva, interfering with the assay results. The subjects' mouths were not inspected prior to saliva sampling to determine if this could have been a problem. The researcher tried to control all of these factors through communication with the subjects about adherence to the sampling protocol; nonetheless, errors and oversights may still have occurred.

Summary

The present study is one of the only studies investigating the response of cortisol and IL-6 to typical off-season training in Division-I collegiate football players. This study provides valuable insight into the immunological and endocrine responses to resistance training and their relation to physical performance on typical exercise tasks. Collectively, the results suggest that salivary measures of cortisol and IL-6 provide important information regarding the physical status of the athlete, but baseline increases do not necessarily indicate a state of overtraining in a 6-week period of intense resistance training.

Utilization of the REST-Q on the current subjects provided another assessment tool for coaches to manage training intensity and volume with athletes. Within the current study, REST-Q scores did not correlate with either biomarker to indicate a decline in physical or psychological state. The resistance training program utilized was successful in creating a positive physiological response without creating symptoms of overtraining typically seen with increases in cortisol and/or IL-6.

CHAPTER VI

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Despite the limitations of this study, there are many insights that can be gained from the findings. Salivary cortisol and IL-6 measures both provided consistent data regarding the physical status of the athletes throughout the study period. It was found that a resistance training program that maintained lifting intensity >80% of 1-RM caused significant increases in both biomarkers by the end of the 6-week period, but were not associated with increased perceived feelings of fatigue, anger, depression, or a drop in vigor. Also, the resistance training program created positive changes in body weight and maximum performance on the bench press, back squat, and power clean in the athletes studied. The physical and psychological variables investigated in the current study provide support for the resistance training program utilized in increasing performance on lifting without risk for overtraining in a 6-week period.

Also, the current study findings support (to some extent) the use of both the REST-Q questionnaire and salivary sampling for both cortisol and IL-6. The findings of this study suggest that these tools provide accurate data regarding the psychological and physical status of athletes during a typical training period. Both data collection tools are

also easy to administer without need for a physician or phlebotomist to be present. However, with salivary sampling there is a possibility for error and controlling for adherence to the sampling protocol is crucial when drawing conclusions. Monitoring levels of cortisol, IL-6, and REST-Q scores may all provide valuable insight into the stress of typical resistance training programs implemented by strength and conditioning coaches and may help lead to better implementation of training strategies to maximize physical performance.

Conclusions

Research hypothesis #1: *There will be a significant elevation of salivary IL-6 and cortisol concentrations over baseline by the end of the 6-week training period.* This hypothesis was accepted since both biomarkers increased significantly by Week 6 over baseline.

Research hypothesis #2: *There will be significant correlations between salivary IL-6 and cortisol concentrations at each of the 6 time points within the training period.* This hypothesis was rejected for the Week 4 sampling time point, but accepted for the Week 6 sampling time point. Cortisol and IL-6 concentrations correlated significantly on Week 1 and Week 6, but not Week 4.

APPENDICES

A. Informed consent form

B. Data collection sheets

C. REST-Q form

D. Assay information

E. Sample Training Program

APPENDIX A

Informed Consent

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

Consent Form Version Date: 1/2/2013
IRB Study # 12-2498
Title of Study: Analysis of the Immunological and Neuro-Endocrine Responses to Resistance Training in Division-I Football Players
Principal Investigator: Simon Haake
Principal Investigator Department: Exercise and Sport Science
Principal Investigator Phone number: 919-219-9062
Principal Investigator Email Address: sjhaake@unc.edu
Co-Investigators: Eric D. Ryan
Eric Sobolewski

Faculty Advisor: Anthony C. Hackney
Faculty Advisor Contact Information: ach@email.unc.edu

What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary. You may refuse to join, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. 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 evaluate aspects of the immunological and neuro-endocrine system of Division-IA American football athletes participating in resistance training for a six week period. The cytokine IL-6 will be used to assess the immunological status and the hormonal cortisol response to assess the neuro-endocrine status. Both of these biomarkers will be measured in saliva due to it being a non-invasive means of collecting a biological sampling and not interfering with the athletes' (i.e., subjects) exercise training.

Are there any reasons you should not be in this study?
You should not be in this research study if you have a disease or condition that would affect your ability to complete all the training. Also, if you have any immunological irregularity that may impact your response to training.

How many people will take part in this study?
There will be approximately 40 people in this research study.

How long will your part in this study last?
Your participation will last 10 minutes for each session, with a total of seven sessions over a 6-week period. Total time of your participation will be 70 minutes.

What will happen if you take part in the study?
If you choose to participate in this research study you will perform the following:

Visit the UNC Football Locker room on 7 different occasions over a 6-week period to provide salivary samples and have body weight assessed. You will also respond to a REST-Q, a rest and recovery questionnaire and have your height and body fat % (skinfold) analyzed on two separate occasions (at the beginning and end of the study).

On the first visit, you will complete this form along with the REST-Q (Recovery-Stress Questionnaire for Athletes). You will also have your height, weight, and body fat % assessed. The body fat % will be assessed using a skinfold caliper on 3 body sites (chest, abdomen, thigh). You will also be asked to provide a salivary sample. The salivary samples will be collected by passive drool into a sterile test tube. Approximately 1ml of saliva will be collected.

On subsequent visits, you will have your body weight assessed and you will provide a salivary sample. Sampling procedure will be consistent for each visit. This procedure will be repeated on the first training day of each week for each study week (7 total samples).

On the final visit, you will complete the REST-Q as well as having your weight and body fat % assessed. A final salivary sample will be taken. At this point your involvement in the study will be complete.

Skinfold measurements will be taken on 3-sites. You will be asked to remove shirt to expose both abdomen and chest for skinfold caliper measurements. Also, you will be asked to wear compression shorts for thigh caliper measurement.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study.

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

There is little risk in providing salivary samples. To be involved in this research study you will be expected to take part, fully, in all mandatory training sessions associated with participation in team strength and conditioning activities. You may miss up to 2 total sessions for the 6 week period without being dropped from the study. Participation in regular strength and conditioning practices is associated with risk for injury and soreness.

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?

Upon agreement to participate in this research study, you will be given a unique research ID number which will be used throughout the duration of the study and on all study documents to avoid using any identifying information or your name. A form will be created listing the research identification numbers with the corresponding names of participants and this document will be filed and kept in a locked cabinet in the Principle Investigator's office at UNC Kenan Stadium Football Facility. Data from study documents will be transferred to a designated research computer with password protection access will only be granted to members of the research team. All identifiable hard-copy files will be shredded and disposed of. You will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, 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?

There is very little risk in providing salivary samples. However, there is inherent risk for injury in participation in strength and conditioning practices.

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

Will you receive anything for being in this study?
No.

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, standing on the team, or academic 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 (including payments), 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 B

DATA COLLECTION SHEET

Subject Name _____ Subject ID _____

Informed Consent

1. Inform participant of the experimental protocol

2. Make certain that the subject is aware of the possible risks

3. Sign informed consent

Participant Compliance Questions

1. Did subject refrain from strenuous physical activity for 24h prior to sampling/training?

Yes No

2. Did the subject report to the lab at least 1h post-prandial?

Yes No

3. Did the subject take NSAIDs, consume alcohol, or caffeine 8 hours prior to sampling/training?

Yes No

Physical Characteristics

1. Age _____ yrs

2. Height _____ cm

3. Mass _____ kg

4. *Percent Body Fat* _____ %

Skinfolds:

a. Chest (diagonal fold midway between upper armpit % nipple) _____mm

b. Abdominal (vertical fold; 1 inch to right of navel) _____mm

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

APPENDIX C

Rest-Recovery Questionnaire for Athletes (REST-Q)

Answer all the questions with respect to your feeling and/or status over the **last week.**

1. I feel like I have been in a bad mood.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

2. I feel like I have been angry with people.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

3. I feel like I have been under a lot of pressure.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

4. I feel like I have not been able to concentrate well.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

5. I feel fatigued or tired.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

6. My sleep at night has not been sound and restful.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

7. I feel overwhelmed with all I have to do.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

8. I have been feeling “down”.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

9. I have been experiencing muscle soreness and/or pain.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

10. I have been feeling sick or ill (for example; colds, flu, sore throat).

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

11. I don’t feel like I want to go to practice and train for football.

0	1	2	3	4	5	6

Not at all			Somewhat			Very Strongly

12. I have not enjoyed eating and my appetite is not good.

0	1	2	3	4	5	6
Not at all			Somewhat			Very Strongly

APPENDIX D

ASSAY INFORMATION

Salivary Cortisol Assay Procedures

1. Bring all reagents to room temperature and mix before use.
2. Prepare 1X wash buffer (and reconstitute stop solution, if appropriate).
3. Bring plate to room temperature and prepare for use with NSB wells.
4. Prepare tube with 24 mL of assay diluent for conjugate dilution, which will be made later.
5. Pipette 25 µL of standards, controls, and unknowns into appropriate wells.
6. Pipette 25 µL of assay diluent into zero and NSB wells.
7. Make final 1:1600 dilution of conjugate (15 µL into 24 mL assay diluent), mix, and immediately pipette 200 µL into each well.
8. Mix plate for 5 minute at 500 rpm. Incubate for an additional 55 minutes at room temperature.
9. Wash plate 4 times with 1X wash buffer. Blot.
10. Add 200 µL TMB solution to each well.
11. Mix plate for 5 minutes at 500 rpm. Incubate in dark at room temperature for 25 additional minutes.
12. Add 50 µL stop solution to each well. Mix for 3 minutes at 500 rpm.
13. Wipe plate bottom clean and read within 10 minutes of adding soap.

Calculations

1. Compute the average optical density (OD) for all duplicate wells.

2. Subtract the average OD for the NSB wells from the average OD of the zero, standards, controls, and unknowns.

3. Calculate the percent bound (B/Bo) for each standard, control, and unknown by dividing the average OD (B) by the average OD for the zero (Bo).

4. Determine the concentrations of the controls and unknowns by interpolation.

APPENDIX E

SAMPLE TRAINING PROGRAM

NAME: [redacted]
WEEK 2 (OFF SEASON)

		Set 1 RP x WT	Set 2 RP x WT	Set 3 RP x WT	Set 4 RP x WT	Set 5 RP x WT
	MONDAY 1/14					
1	POWER CLEAN	5 x 135	3 x 176	3 x 220	3 x 240	3 x 265
2	SHRUG	15 x 308	15 x 308	15 x 308		
3	PULLUP	10 x BW	10 x BW	10 x BW		
4	ROW	10 x 132	10 x 132	10 x 132		
5	STR. BAR CURL	10 x 108	8 x 108	6 x 108		
6	DB HAMMER CURL	10 x 40s	10 x 40s	10 x 40s		
7	4 WAY NECK	10 x 45 lb				
	TUESDAY 1/15					
1	FRONT SQUAT	5 x 135	5 x 185	5 x 225		
2	RDL / CALF	10 x 132	10 x 132			
3	BENCH	5 x 135	4 x 185	4 x 225	4 x 245	4 x 265 (1 help)
4	DB INCLINE	6 x 70	6 x 75	6 x 80		
5	~~UPRIGHT ROW/LAT.~~	10 x ___	10 x ___	10 x ___		
6	ROW TO PRESS	10 x BB 10	10 x ___	10 x ___		
7	PUSHUP	15 x ✓	15 x ___			
	THURSDAY 1/17					
1	HANG CLEAN	5 x 135	3 x 176	3 x 220	3 x 240	3 x 265
2	SHRUG	15 x 308 →	15 x ___	15 x ___		
3	PULLUP	10 x BW	10 x ___	10 x ___		
4	ROW	10 x 135 →	10 x ___	10 x ___		
5	EZ BAR CURL	10 x 88	10 x 88	10 x 88		
6	DB CURL	10 x 40	8 x 50s	6 x 60s		
7	4 WAY NECK	10 x 45 lb				
	FRIDAY 1/18					
1	SQUAT	5 x 135	4 x 225	4 x 275	4 x 315	4 x 365
2	LUNGE	12 x 135	12 x ___			
3	INCLINE	10 x 135	8 x 185	6 x 205	6 x 225	
4	FRONT PRESS	6 x 135 →	6 x ___	6 x ___		
5	CLOSE GRIP	5 x 135	5 x 185	5 x 205	5 x 225	

12 x 100's Tue

~~Fri~~ No shuttles

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