HYDRATION STATUS, SALIVARY BIOMARKERS OF STRESS, BODY COMPOSITION, AND PERFORMANCE IN RESPONSE TO A 4-WEEK PRE-SEASON TRAINING CYCLE IN DIVISION 1 BASKETBALL PLAYERS: AN OBSERVATIONAL STUDY

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A thesis submitted to the faculty at 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).

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ABSTRACT

James David Ayscue: Hydration Status, Salivary Biomarkers of Stress, Body Composition, and Performance in Response to a 4-Week Pre-Season Training Cycle in Division 1 Basketball Players: An Observational Study
(Under the direction of Claudio L. Battaglini)

This observational study profiled various physiological parameters in a group of fourteen elite level Division-I male basketball players during four-weeks pre-season strength and conditioning training. Hydration status, hormonal changes (specifically testosterone and cortisol), body composition, training load, and performance (measured by a peak velocity test) variables were observed. The potential relationship between changes in hydration status, training volume and physical performance were observed. In addition, the relationship between individual player’s hormonal responses and performance showed weak but potentially clinically relevant associations mainly for testosterone. Overall, trends of change in study variables during the pre-season strength and conditioning training program were observed. More specifically, hydration and testosterone fluctuations may have been influenced by training load changes, in turn influencing the performance measure used in this study. Future investigations exploring these relationships are warranted, so that information may be provided to coaches to better gauge training load increases to maximize training response.
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CHAPTER I: INTRODUCTION

As a person exposes their body to an exercise program, certain physical and physiological responses occur. Whether the person experiencing the physical stress is an average person with no athletic background or an elite level athlete, the body’s processes will respond accordingly to the stress being exerted upon it. These processes involve but are not limited to changes in the cardiorespiratory, metabolic, muscular, endocrine, immune, and/or neurological systems. The changes seen in the abovementioned systems can be in response to acute exercise bouts or can be seen as more chronic changes over longer periods of time. The magnitude of the adjustments will vary depending on the duration, intensity, and type of exercise being performed as well as the prior level of training of the person performing the exercise. Some of the most impactful physiological changes seen in response to exercise are in relation to the upregulation or downregulation of certain hormones and cytokines. Due to the high levels of physical and psychological stress that elite level athletes are exposed to through increased volumes and intensities of their competition and training activities, their endocrine and neuroendocrine responses through hormonal and cytokine action are of great importance to study. Being able to understand hormonal responses associated with the training of elite level athletes at different volumes and intensities and their associations with changes in factors such as hydration status, body composition alterations, sleep, and fatigue could lead to vast improvements in the way in which athletes are monitored and trained. These improvements could include an ability of coaches to more accurately track their athlete’s physiological state and give coaches an idea of how physically ready their athletes are to perform at optimum levels of competition based on
hormonal changes associated with training volume, intensity, and performance markers specific to the sport.

Many previous studies have taken in depth looks at the responsiveness of certain hormones and cytokines to acute bouts of exercise and competition. Much of this research has focused around cortisol, testosterone, and the free testosterone to cortisol ratio. Conclusions from research of this matter have found that depending on the duration and intensity of the exercise or competition bout, cortisol levels will rise accordingly during activity and return to resting levels at a time dependent upon the duration and intensity of the physical activity and amount of rest post exercise (i.e. the longer the activity and/or the more intense the activity the more time it takes to return to resting levels) (Elloumi et al. 2003; Lac & Berthon, 2000; Passelergue & Lac, 1999). Testosterone has also been shown to have a decrease over the course of a competitive event which is followed by a rise over normal resting levels in the recovery days that follow the event (Elloumi et al. 2003; Lac & Berthon, 2000). This rise in testosterone over the recovery days coincided with an increased free testosterone to cortisol ratio above resting values. This is indicative of a more anabolic (protein synthesis) state in the days following competition as compared to the catabolic (protein degradation) state present during and immediately following competition due to a decreased free testosterone to cortisol ratio (Elloumi et al. 2003; Lac & Berthon, 2000; Passelergue & Lac, 1999). Other studies have chronicled the rise in cytokine activity, specifically IL-6 and TNF-alpha levels, immediately following athletic competition pointing towards a pro-inflammatory state in the competing athletes (Souglis et al. 2014). Conclusions drawn from the studies mentioned above are generally agreed upon and are very helpful for coaches and athletes when developing programs for recovery on the days following competition; however studies such as these do not provide much insight into the hormonal and cytokine activity seen in the training leading up to competition. Thus, the current
literature in the topic is scarce and empirical information on how to potentially use hormonal responses to training to assist coaches in modifying athletes pre-competitive training phases is lacking. Further research in this area is needed to give coaches the knowledge and allow them the opportunity to better optimize an athlete’s response to training which has the potential to lead to more optimum performances in competition.

Very few studies to date examined long-term approaches to monitoring hormonal activity throughout training cycles and competitive seasons in elite level athletes. One particular study that did take a longitudinal approach followed twenty-one collegiate football players throughout pre-season training and game competition taking measurements five times throughout the year. The testosterone to cortisol ratio was examined but the reasoning behind the up and down changes of the ratio seen throughout the study was inconclusive (Hoffman et al. 2005). A more recent study examined the hormone profile of elite level track and field athletes during preparation and pre-competition training cycles with the goal to identify biomarkers highly sensitivity to change throughout an extended period of training (Guilhem et al. 2015). Guilhem and colleagues (2015) evaluated hormonal and cytokine activity but found inconclusive results and were unable to draw substantial conclusions on the relationships between hormonal and cytokine changes to training volumes. Guilhem and colleagues (2015) study did include a psychological component of fatigue perception as a variable which could help provide insight on the mechanisms of perceived fatigue and its relationship to elite level athlete’s responses to training (Guilhem et al. 2015). One of the main reasons for the inconclusive results observed in Hoffman et al. 2005 and Guilhem et al. 2015 could be attributed to the frequency used for the assessment of the hormones. Both studies used monthly, weekly or a mixed monthly/weekly approach potentially missing day to day changes that could have painted a different picture on the relationship of changes in the hormonal responses to changes in weekly microcycles of
training. In order to continue to build the body of knowledge in this intriguing area of sports performance research, more frequent measurements taken throughout a mesocycle of training could potentially be very revealing. Furthermore, most studies performed up to this point in an elite athletic population tend to focus on a few select variables instead of using a wide spectrum approach that may be needed to possibly identify variables that are more sensitive to training responses and competition. In addition, no studies could be found that take into account chronic changes in hormonal responses over an extended training period involving both resistance and endurance type training in elite level athletes. Hormonal responses to resistance training have been researched in elite level weightlifters in which serum hormone concentrations where looked at in relation to training volume. However, no endurance based programming was included in the training regimen design for the study (Hakkinen et al. 1987). For many athletes, endurance and resistance training are important components of a training program as both areas of training play important roles in improving their athletic performance during competition.

Other relatively simple and important physiological markers previously shown to influence athletic performance such as hydration and body composition should be monitored as simple measurements to assist in the determination of the relationship between physiological changes and training volume and performance. Dehydration has been shown to be a fairly widespread problem across many populations of amateur and professional basketball players and it is well known that dehydration can have detrimental effects on athletic performance (Vukasinovic-Vesic et al, 2015; Minshull & James, 2013; Osterberg et al, 2009). Body composition has been shown to have significant changes throughout training cycles and competitive seasons within elite level athletic populations (Siders et al, 1991; Santos et al, 2014). Some of these changes are not always positive changes and could produce a detrimental effect on performance. Therefore, to get a full understanding of how athletes respond to physical stress
during a training program, variables spanning from hormonal and cytokines biomarkers to hydration markers to measures of body composition and their changes in response to varying training volumes could individually or collectively provide a better picture on an athlete’s readiness to perform. In addition, level of fatigue and duration of sleep have been correlated with changes in different hormones which in turn can significantly affect physiological changes due to training and performance variables in elite athletes (Spiegel et al. 1999). Therefore, it appears paramount that profiling lifestyle factors such as quality of sleep, levels of fatigue, as well as hormonal, hydration, and body composition alterations throughout a training cycle may help improve the current understanding on how the associations between these factors may influence athletes performance.

Coaches all over the world are in constant search for better methods that can more objectively track/monitor the effects of their training on the physiological changes aimed to promote optimum/maximum performance capacity. Today, some biological and psychological markers have been proposed as potential tools that can help coaches identify if athletes are unable to tolerate appropriately the training loads being imposed upon them. Furthermore, some of these markers could be used to determine if the training load can be increased to promote more profound physiological adaptations, which in turn could be translated into better performances during competition. To date, many different tools exist to help coaches monitor the progress of their athletes during training and competition. However, tools that can provide a more precise insight on the true readiness for the subsequent training sessions or competition continue to be a desirable and important topic of research in the world of sports performance.

**Purpose**

The purpose of this observational study is to profile the responses of hydration status, body composition, salivary biomarkers of stress, training loads, and performance changes in
Division 1 basketball players throughout a 4-week pre-season training cycle. A secondary purpose will evaluate the relationship between changes in physical fitness (Performance test), hydration status, body composition, salivary biomarkers of stress, and training loads.

**Definition of Terms**

*Training load:* Total training load is defined by the computation using a session-RPE score which uses the modified Borg RPE scale (a scale that is rated one to ten with one being no exertion and ten being maximal exertion) multiplied by the duration of the session in minutes. This multiplication gives the session-RPE score, which has been previously demonstrated to have a strong correlation with other accepted measurements of training load in athletes (Casamichana et al, 2013).

**Assumptions**

- It was assumed that all athletes accurately and honestly responded to the fatigue, sleep, and rate of perceived exertion questionnaires;
- It was also assumed that all athletes followed strictly and adhered to saliva collection procedures.

**Limitations**

- Limited number of participants based on the fact that the participants were drawn from a Division 1 college basketball team. Therefore this study may only be generalizable to Division 1 college basketball players;
- Training program was pre-set by the strength and conditioning staff and were not necessarily uniform for all athletes;
- Even though all measurements were planned to be assessed at specific times during the study, there were times, due to last minute coaches changes on the team training schedule, the assessment of certain variables were not collected;
• Fatigue questionnaire did not cover all possible aspects and reasoning behind potential causes of fatigue;

• Due to the observational nature of the study and the challenge of collecting the data without disturbing the daily routine of the team training program, the use of bioelectrical impedance as the method for the assessment of body composition may have produced inaccurate data, since athletes are often dehydrated due to the demands of the training regiment.

• Although all participating athletes were asked to maintained their normal dietary habits and not change drastically any aspect of their diet during the study, athletes do tend to alter their caloric intake and therefore these potential changes could influence the stress hormones analyses.

**Delimitation**

• All athletes recruited to participate in the study were confirmed members of a division 1 basketball program; any athlete who missed four or more of the planned training days will be excluded from data analyses.

**Research Questions**

RQ1. Do the observed variables of hormonal biomarkers, hydration status, or body composition show changes over the 4-week training program?

RQ2. Do weekly changes in training loads correlate with changes in hormonal biomarkers, changes in hydration variables, and/or body compositional changes observed in the 4-week training program?

RQ3. Do observed changes in hormonal biomarkers, hydration variables, and/or body compositional measures that show response to the weekly training loads correlate with changes in performance?
RQ4. How does the free testosterone to cortisol ratio change in response to the increasing training loads during the preseason 4-week training program?

**Research Hypotheses**

H1. There will be significant decreases in hydration status throughout the 4-week training program and these changes will inversely correlate with increasing weekly training loads.

H2. There will be significant changes to body fat percentage and lean muscle content throughout the 4-week training program and these changes will correlate with increasing weekly training loads.

H3. There will be significant increases in cortisol levels and decreases in testosterone levels throughout the 4-week training program.

H4. Increases in cortisol levels will positively correlate with increases in weekly training loads and decreases in testosterone levels will inversely correlate with increases in weekly training loads.

H5. There will be a significant inverse relationship between decreasing free testosterone to cortisol ratio and increasing weekly training loads.

H6. If significance is found between variables and increasing weekly training loads, these variables will show a significant relationship to decreased performance on the performance test.

**Significance of the Study**

Various factors across many aspects of a collegiate athlete’s life can have effects on athletic performance along with their training loads. Relatively few attempts have been made to generally observe elite-level collegiate athletes’ physiological responses to training cycles involving a combination of different resistance and aerobic/anaerobic conditioning training programs. While it is near impossible to observe and account for all possible factors that can
affect athletic performance, choosing certain factors that could play a more prominent role in
affecting performance in collegiate level athletes could prove helpful in informing coaches and
athletes on better ways to improve preparation for competition. The evaluation/profiling of
hydration status, body composition and, salivary biomarkers of stress during different training
periods may help shed light on how these relatively easy to collect markers of physiological
training response potentially affect performance. More specifically, how different training loads
may affect these factors cited above and how these potential changes influence performance.
CHAPTER II: REVIEW OF LITERATURE

This review of literature will cover previous research that deals with hormonal and cytokine responses to varying bouts of exercise and training most specifically in athletic populations. Much of the research reviewed here will focus on salivary samples of cortisol, testosterone, and the inter-relationship between what is known as the free testosterone to cortisol ratio and practical implications for what this relationship means for training status. In addition, research looking at cytokine responses in regards to exercise and training will be reviewed in the sections that follow. Studies that looked at the cytokines Interleukin-6 (IL-6) and Tumor Necrosis Factor Alpha (TNF-α) and their responses to the various training protocols will be the primary focus of the review. Research that takes a more generalized approach to monitoring athletes’ training status through the hormonal and cytokine biomarkers being studied will also be examined in an attempt to generally give an idea of research that has already been done in regards to tracking training status throughout longer periods of time. A small section on sleep debt and its effects on metabolic function will also be covered since the athletes partaking in this study will be asked questions every day of training on their previous night’s sleep. Hydration status variables as well as body composition data will be looked at as part of this review as well as indices of both hydration status and body composition will be tracked throughout this study. Studies that detail the effects of dehydration on athletic performance in basketball populations will be overviewed as well as research revolving around body composition changes throughout athletic training and competition. Session RPE scoring will be used as part of an assessment of
the training loads being attained during this study, research that looks at RPE based tracking will be reviewed in short at the end of this literature review.

**Acute Cortisol and Testosterone Responses to Exercise Bouts**

In the resistance training programming that will occur as a part of this study, the athletes being studied will perform a wide array of resistance exercises focused on increasing both maximal strength and power output. Beaven et al. (2011) conducted research and analysis on thirteen professional, male rugby players and assessed their salivary cortisol and free testosterone levels to eight total exercise bouts that occurred over a four week time period. Salivary samples were collected before exercise began, during the exercise bout, and after exercise was completed. There were four possible exercise combinations that the players could have completed: a power exercise followed by another power exercise (power-power), a power exercise followed by a strength exercise (power-strength), a strength exercise followed by a power exercise (strength-power), or a strength exercise followed by a strength exercise (strength-strength). Mid-point (during exercise) salivary samples showed that the players who began with strength training showed significantly higher free testosterone levels than those who completed power exercises. In addition, at the conclusion of the exercise bouts, all groups showed clear increases in free testosterone levels but the only significant increase relative to baseline was in the strength-power group. Also of note, the two groups that were combination groups (strength-power and power-strength) were the two groups that showed the largest increase in testosterone after the mid-way point of the exercise session. In regards to cortisol response, there was a significant difference with respect to the magnitude of response between the strength-power group and the power-power and power-strength groups respectively. However, total cortisol response showed non-significant increases in comparison to the resting day for all groups (Beaven et al, 2011).
An earlier study which investigated hormonal changes in elite level weightlifters found that during a six week resistance training period, the initial two weeks of training led to significant decreases in testosterone levels followed by a stabilization of these levels in the final four weeks of training. In addition, the final four weeks of the resistance training program led to significant decreases in cortisol levels (Hakkinen et al, 1987). Findings from the Beaven et al. (2011) and Hakkinen et al (1987) lead us to conclude that the type of resistance training that will be occurring with the elite level basketball athletes will be enough to induce a great enough free testosterone change and cortisol change in response to the exercises being performed. When it comes to cortisol, the training programs being completed by the elite level basketball players will be of a greater intensity and duration and thus will put more stress on the athletes than is being seen in the Beaven et al. (2011) study which showed non-significant cortisol changes.

The elite level basketball players will also be subjected to moderate to high intensity endurance training as part of their training programming and thus an understanding of the associated cortisol and testosterone responses to that is necessary. Hackney and Viru (1999) evaluated cortisol levels over a twenty-four hour period in response to high intensity and moderate intensity endurance exercise. Seventeen males who were regularly active in endurance exercise were recruited for the study that involved cortisol levels being assessed on a rest day, a day with moderate intensity exercise, and a day with high-intensity exercise each of which was spaced about one week apart. Blood samples were drawn before the exercise or resting session and immediately after the session which lasted an hour. Blood sampling continued on a regular basis between the exercise sessions (if it was an exercise day, not the rest day) and continued regularly after the second exercise session for the entirety of the twenty-four hour period. Sampling was not allowed after a certain time at night for ethical related reasons. Results from this study showed significant increases in cortisol levels over resting values immediately after
the first high intensity exercise session and these significant increases remained for two hours post-exercise. The second exercise session of that day (also high intensity) led to a significant increase in cortisol over resting state only in the sample drawn immediately following exercise. The same results held true for the moderate-intensity exercise days except for the significant increase over resting cortisol levels was only significant one hour post exercise after the first session. Regarding the night time levels that were allowed to be assessed, both moderate and high intensity cortisol responses showed significant decreases over resting values with the high intensity exercise groups showing lower cortisol levels than the moderate intensity group (Hackney & Viru, 1999).

Testosterone and its acute responses to submaximal and maximal aerobic exercise are of interest as well. Sgro et al (2014) demonstrated that total testosterone will show significant increases over baseline levels in response to both submaximal and maximal aerobic work and further showed that total testosterone levels will remain elevated for the following hour after both bouts of exercise. Submaximal aerobic exercise led to a higher level of total testosterone than did maximal. Free testosterone, which is the biologically active form of testosterone and will be analyzed in this review’s study through saliva, also increased significantly in response to both submaximal and maximal aerobic exercise and these levels also remained elevated for the following hour after the exercise bout (Sgro et al, 2014). Noting this study, it is shown that free testosterone will respond acutely to submaximal aerobic work which is what the basketball athletes being studied will participate in as part of their training program.

Results from the aforementioned study suggest that cortisol and testosterone will show certain longer term variations in response to an acute session of exercise and that these levels will remain elevated for a certain period of time following exercise. In addition, the night time suppression of cortisol associated with exercise bouts will be of interest in the current study as
hormonal levels will be measured at 8 AM following both rest days and days of varying exercise durations and intensities. While it may be of concern that two of the studies used blood sampling and the current study will be using saliva, Fryer et al (2014) showed that capillary, venous, and saliva levels of cortisol increased post-exercise, salivary cortisol just increased at a slower rate (Fryer et al, 2014). Since saliva samples will not be drawn immediately post exercise this will not affect the current studies results.

**Extended Cortisol, Testosterone, and Free Testosterone Responses to Exercise**

Many studies exist that look at an athlete's cortisol and testosterone response after a high stakes, high intensity athletic event. Some of these studies not only evaluate the hormonal levels of cortisol and testosterone immediately following the athletic event but also continue to monitor levels for many days following the event to get an idea of what is happening in the body during post-competition, recovery based days. Often times, the levels of cortisol, testosterone, and the free testosterone to cortisol ratio can give a lot of insight into how effectively and athlete is recovering and rebuilding muscles damaged following intense bouts of competition.

Passelergue and Lac (1999) investigated highly trained national and international level wrestlers and their salivary cortisol and testosterone responses during a two day competition and in the eight days that followed the conclusion of the last wrestling match. It is important to note that saliva samples were taken on only six of the eight post-competition days. Results of this study showed a significant steady state of higher cortisol levels over resting values after the first day of competition with a return to normal values by two hours post-competition on the second day. Testosterone levels were not significantly higher on either competition day over resting values but levels were significantly higher at the end of competition on the second day as compared to the first day. The free testosterone to cortisol ratio was significantly decreased on the two competition days as compared to resting values. When looking at the recovery days,
cortisol levels showed no significant differences but testosterone levels were significantly higher over resting values on all but one of the measured recovery days. The free testosterone to cortisol ratio was significantly increased over resting day values on day one and day three of recovery with over forty percent increases over resting levels (Passelergue & Lac, 1999).

Due to the common classification of testosterone as an anabolic agent and cortisol as a catabolic agent the free testosterone to cortisol ratio is often looked at as a way to indicate either protein synthesis (anabolic) or protein degradation (catabolic) in regards to musculature in the body. Studies have looked at this phenomenon and found evidence to back up the notion of an increased free testosterone to cortisol ratio as an indicator of muscle building and a decreased ratio to be indicative of muscle damage. Banfi et al (1993) found that the free testosterone to cortisol ratio could be used as an indicator for poor recovery and the potential of decreased performance in elite male speed skaters (Banfi et al, 1993). Another study published a couple years prior investigated male rowers training for the Olympics and found similar results in regards to a decreased free testosterone to cortisol ratio indicating the possibility of an incomplete recovery. It should be noted, that like the Banfi et al (1993) study, this study was comprised of a small sample size (six rowers) but still had findings of significance for the variables associated with the free testosterone to cortisol ratio changes (Vervoorn et al, 1991). From the Passelergue and Lac (1999) study it appears as though after undergoing the serious physical and mental stress of the wrestling competition, the body entered what would be seen as a catabolic state. In the post-competition days that followed there was more of a trend to an anabolic state which would logically make sense as these were days of decreased physical and mental stress on the body and it would be expected that the body would enter a healing and rebuilding phase.
A similar study conducted by Lac and Berthon (2000) was published a year later and it looked at the same types of hormonal responses in eight highly competitive, long distance runners of both genders (four males, four females) before, during, immediately following, and in the three post-competition days after a long distance relay type race. For the purpose of the present study the male values will be looked at. The first measurements occurred at 9 AM the day of the race and the race began at 10 AM. The males each ran, on average, a little under twenty-eight kilometers each at an average of nearly ninety-three percent of their maximal aerobic velocity thus indicating a high-intensity effort throughout the relays. Cortisol levels in the male racers increased throughout the race showing the highest values during the last two relays and remaining high in the evening values following the race. These values then showed significant decreases at both morning and evening levels as compared to the corresponding times on the race days. Male testosterone levels decreased steadily throughout the race and remained lowered at the evening measurement post-race. During the three recovery days that would follow, testosterone levels increased back to pre-race levels in the morning measurements and showed increases in post-competition evening levels in comparison to the testosterone levels measured on the post-race evening. The free testosterone to cortisol ratio showed significant decreases during the last half of the relays as compared to 9 AM race-day levels. Looking at the three post-competition days, the free testosterone to cortisol ratio was significantly higher in the morning as compared to pre-race levels and was significantly more higher in evening levels than the evening levels measured post-race (Lac & Berthon, 2000). Much like what was seen in regards to the Passelergue and Lac (1999) wrestling study, it appears as though the high intensity competition induced a catabolic state as indicated by the decreased free testosterone to cortisol ratio during and following the race while the post-competition days showed a much greater anabolic state as identified by the greatly increased free testosterone to cortisol ratio. This
reinforces the ideas that the body acts to repair itself through anabolic activity following intense athletically based activity and thus shows that there is a need for certain amounts of rest following these types of high intensity activity to allow for this period of restoration and repair. It should be noted that it appears during the post-competition days, the raised free testosterone to cortisol ratio is a function of a short term chronic lowered cortisol level as well as some increases in testosterone levels over resting levels (Lac & Berthon, 2000).

A later studied published in 2003 looked at the free testosterone to cortisol ratio in twenty Tunisian rugby players after a competitive, international level match and in the six post-match recovery days. Saliva samples were used to look at hormonal levels and they were taken two months before the competition to establish rest values, four times on the day of the competition, and twice a day (morning and evening; 8 AM and 8 PM) during the six recovery days. Results from the study showed a significant decrease in the free testosterone to cortisol levels immediately post-match and this drop appears to be based on a significant increase in cortisol levels immediately post-match with an associated slight drop in testosterone levels immediately following the match. The free testosterone to cortisol ratio showed significant increases over the post-match ratio in the morning values from the first day of recovery to the fourth and increases in the evening ratio from the second to the third days of recovery. These changes in the ratio can be attributed to the subsequent significant cortisol decreases over resting values in the morning measurements from the first to fourth day of recovery as well as significantly higher testosterone levels over resting values in all six post-competition days during both morning and evening measurements (Elloumi et al, 2003). Results from the Elloumi et al (2003) further show what previous studies in this literature review have highlighted; high level athletic and exercise activity lead to an increased catabolic state immediately following the activity followed by an anabolic recovering state that lasts at least up to three days and in some cases potentially longer.
This anabolic state appears to be a necessary function in regards to the body healing and
repairing itself after the high loads of stress applied to it through high level athletic activity.

Multiple studies have been conducted that have tracked the free testosterone to cortisol
ratio through training and competitive season that have longer durations than the studies
discussed earlier in this section. Martinez et al (2010) looked at the free testosterone to cortisol
ratio through serum samples in twelve international level basketball players throughout a season
that lasted approximately six months. Blood samples were drawn at four points throughout the
season: October (at the end of the first cycle of pre-season training), December (at the end of the
second cycle of training), March (at the conclusion of a multi-game tournament), and April (at
the end of the season). Results from the study showed decreases in the free testosterone to
cortisol ratio throughout the season but very few of these decreases were significant. Cortisol
showed significant increases over resting levels at two points during the season, at the conclusion
of the first cycle of pre-season training (October) and at the finale of a multi-day, multi-game
tournament towards the end of March. This marked increase of cortisol at the conclusion of the
tournament coincided with a significant decrease in the free testosterone to cortisol ratio at the
same time period (Martinez et al, 2010). Both of these findings are in line with what would
somewhat be expected in regards to hormonal responses to elevated training stresses. Cortisol
tends to rise in response to physical and/or mental stress and stress would be at higher levels
following intensified pre-season training as well as at the conclusion of a tournament in which
the volume of games being played is increased.

Hoffman et al (2005) looked at the testosterone to cortisol ratio in serum with twenty-one
Division III football athletes throughout the duration of a football season. The testosterone to
cortisol ratio looks at all levels of testosterone not just the free or biologically active form of
testosterone that has been looked at in many of the previous studies mentioned. Blood draws
were taken five times throughout the season: before the start of pre-season training camp, the last
day of training camp, and in weeks’ three, seven, and ten of the football season. Results from the
study showed the only significant decrease in testosterone to cortisol ratio occurred at the second
blood draw at the conclusion of pre-season training camp. Other conclusions from this study did
not show much significance in regards to the testosterone to cortisol ratio (Hoffman et al, 2005).
Pre-season training tends to be the most taxing and stressful time of training for athletes and as
was the case with the basketball players in the Martinez et al (2010) study, the football players in
the Hoffman et al (2005) study showed significant decreases in the associated ratio (either free
testosterone to cortisol or total testosterone to cortisol) at the conclusion of this pre-season
training period. This appears to make logical sense as these times of higher volume and intensity
training would correspond with the body being in a more catabolic state than at other times
during a season when coaches typically ramp down the training volume in anticipation of
keeping players fresh for competition. On the other end of the scale, Guilhem et al (2015) found
that international track and field athletes showed an increase in the free testosterone to cortisol
ratio during a time of lower volume training which was in place as a pre-competition training
program (Guilhem et al, 2015). This would indicate a more anabolic phase of protein synthesis
in the body which is what one would want to see as an athlete nears competition. These studies
taken together show that there are times when athletes experience both catabolic and anabolic
states and these are very dependent on the volume and types of training and stress that the body
is being subjected to.

While the two previous studies looked at athletes throughout training and competition
there are studies that look at other specific, high-level populations throughout longer term
training periods without competition involved. Passelergue and Lac (2012) studied fifteen elite
level junior wrestlers and their salivary hormonal responses to fifteen weeks of mixed aerobic
and resistance training programming. The program design itself consisted of more aerobic and muscular endurance focused exercises at the beginning and then progressed towards more maximal strength, power, and speed development exercises as the program proceeded on towards week fifteen. The free testosterone to cortisol ratio showed increases at week four of the training program over initial measurements taken before week one; however, this difference was not considered significant. The only decrease in the ratio observed that differed significantly from initial levels was with the week fifteen measurement. This week fifteen measurement also showed a significant decrease from the previous saliva sample taken during week twelve. These significant decreases in the free testosterone to cortisol ratio coincided with the only significant increase in cortisol levels found in the study. Week fifteen fell into the training period in which aerobic power, anaerobic power, and explosive strength were being programmed in. The stress and fatigue that accumulates due to these high intensity activities could have played a role in the suppression of the free testosterone to cortisol ratio and the subsequent increase in cortisol levels (Passelergue & Lac, 2012). The increase in cortisol and its potential association with build-up of fatigue as a response to increased intensity exercise and increased stress can also be seen in a study performed by Oliver et al (2015). They showed significant salivary cortisol increases over baseline in naval special warfare operators in response to a block of training that involved power development and strength endurance exercises such as Olympic lifts, high repetition fast paced strength circuits, and sprint work (Oliver et al, 2015). Looking at this evidence it becomes clearer that there is the possibility of cortisol increasing significantly in a more chronic state in response to high volume, high intensity exercise and this can push catabolism in the body if chronically elevated levels are allowed to subsist.
Pro-Inflammatory Cytokines IL-6 and TNF-α Responses to Exercise and Competition

The pro-inflammatory cytokines IL-6 and TNF-α are known to have large degrees of responsiveness to acute exercise. These acute increases are associated with varying intensities of exercise in which the body undergoes inflammatory responses as a result of the damage induced to the body because of the exercise bout. IL-6 has shown to have increases thirty minutes into a moderate to high intensity bout of treadmill running with increases of up to twenty-five times baseline levels shown at the conclusion of a two and a half hour treadmill run. In addition, IL-6 was found to remain elevated six times over baseline levels up to six hours post-treadmill run (Ostrowski et al, 1998). TNF-α and IL-6 levels were also found to be significantly elevated over pre-exercise levels in response to exhaustive exercise tests of varying type (running, swimming, or biking) in fifteen well trained male endurance athletes. TNF-α levels remained elevated twenty hours after the exercise test while IL-6 was only significantly elevated in two of the athletes (Weinstock et al, 1997). Inflammation post-exercise is to be expected and is not necessarily a bad thing in an acute sense so what these studies show in their results are normal physiological responses. Troubles tend to arise when inflammation becomes chronic and does not return to resting levels after a certain time period post-exercise.

IL-6 and TNF-α have also shown an ability to have large increases in response to single sessions of competitive sports. Souglis et al (2014) investigated acute responses of IL-6 and TNF-α following four male competitive sporting events: soccer, basketball, team handball, and volleyball. Results from the study showed that immediately post-match soccer players showed a 440% increase over pre-competition IL-6 levels and 240% increase over pre-competition TNF-α levels. Respectively, team handball showed 350% and approximately 120% increases, basketball 260% and approximately 120%, and volleyball 200% and 90%. For all athletes except soccer, cytokine levels returned to baseline by the morning following competition; soccer
athletes’ levels had returned to baseline by the second day following competition (Souglis et al, 2014). A similar study that only evaluated an elite, international level handball team’s IL-6 response to competition found similar large scale increases following a match with a subsequent return to baseline levels by twenty-four hours post-match (Chatzinikolaou et al, 2014). A comparable phenomenon occurred in thirteen male marathon runners. In these thirteen men, IL-6 levels significantly increased from pre-race to post-race. TNF-α also showed significant increase from pre-race to post-race, however, these increases were not to the same degree as IL-6 increases were (Bernecker et al, 2013). These studies together indicate that highly intense and/or long duration athletic activity have the potential to lead to large scale IL-6 and TNF-α increase and these increases could vary depending on the amount of movement and activity that is occurring as part of the sport or activity. For example, soccer, a sport in which there is more constant movement than volleyball had greater cytokine increases in its athletes than did the male volleyball players. These studies did however focus on athlete’s immunological responses to competitive settings which have other variables such as psychological and mental state that could play a role in responses seen so it is important to also evaluate athlete’s IL-6 and TNF-α responses to non-competitive training and mixed training and competition seasons.

Robson-Ansley et al (2007) looked at IL-6 response in male triathletes going through a four week period of training. Results from this study showed a significant elevation in IL-6 plasma levels following week one in response to an intensified training program. These levels remained elevated in a more chronic type state for week’s two and three and decreased slightly in the week four measurement but still not returning to baseline levels observed at the start of the training period (Robson-Ansley et al, 2007). A previous study by Robson-Ansley et al (2004) had drawn conclusions that linked chronically elevated IL-6 levels to greater feelings of fatigue and a decreased athletic performance (Robson-Ansley et al, 2004). It is important to note that
both studies were focusing on endurance based athletes and thus the responses seen are in response to aerobic type exercise and work. The elevated levels of IL-6 are of interest as it indicates that there is a point in which overtraining can occur and this could potentially lead to impaired training or competitive performance. It would be important to understand this concept in regards to the study to be performed in this paper. This study will also have a duration of four weeks and if chronic elevations of IL-6 are noticed it could be indication of an inapt training program which is over-taxing the athletes and thus sacrificing some of the quality of the training.

It has been shown in adolescent male basketball players who had cytokine levels analyzed before the beginning of the season, after a six week preparatory period, and after twenty weeks of competition and in-season training; that, TNF-α levels increased significantly by the end of the season over baseline pre-season levels and over measurements taken after the six week preparation training period before the start of competition. In addition, IL-6 levels showed significance increases over end of preparation phase levels but not over pre-season levels. These findings seemed to be indicative of a chronically elevated pro-inflammatory state caused by the micro-trauma muscle damage occurring as a result of training and competition (Brunelli et al, 2014). Another earlier study performed by Brunelli et al (2012) had found no significant increases in TNF-α or IL-6 levels over baseline in twelve young male athletes in response to pre-season preparatory phase training in the absence of competition (Brunelli et al, 2012). Through these two studies, it appears as though there may be a necessity of having competition and training in combination to possibly see a chronic state of elevated pro-inflammatory cytokine levels. As is the case with the present study to be performed, there will be no competition during this period so these findings could lend some explanatory functions as to there being a decreased likelihood of seeing chronically elevated cytokines. However, it is also of importance to note that the Brunelli et al (2012) study above that just observed
preparatory phase training only involved the adolescents in three training sessions per week of approximately ninety minutes and this is a lower volume than what will be seen in the basketball players to be studied (Brunelli et al, 2012). In addition, young teenage athletes may not be able to handle the same intensity of training as elite, collegiate athletes and thus may not experience the same degree of inflammatory responses.

**The Prevalence of Chronic Dehydration in Basketball Player Populations**

There is a common consensus among those who study and investigate athletic populations that dehydration in the body can be correlated to decreases in mental concentration, feelings of fatigue and exhaustion, and thus an overall decreased ability to have optimum athletic performance in training and competition. A study performed by Davis et al on eight collegiate athletes found that those in a dehydrated state had a greater decrease in sprint times over time than did those in a euhydrated (normally hydrated) state (Davis et al, 2015). Minshull and James showed similar findings in regards to a dehydrated state leading to a decreased ability to produce peak force over-time as well as a decreased rate of force production over time as compared to euhydrated athletes (Minshull & James, 2013). Some research has disputed these findings of dehydration having significant effects on decreasing athletic performance as shown in a 2010 study by Hayes and Morse. In their study, they showed in twelve college students that increasing states of dehydration had no significant effects on decreasing power and strength. However, it is important to note that the twelve students performing the study were not elite level athletes (Hayes & Morse, 2010). The ability to produce high levels of force and being able to produce that force quickly is a key component of athletic competition especially in elite level basketball players. If these abilities are compromised because of a dehydrated state, then basketball players’ performances on the court and in practice and preparation training will suffer and the players will not be able to perform up to their optimum levels. With research showing that
dehydration is rather prominent in amateur and professional basketball players around the country and the world, the importance of monitoring this during this study and aiding the players in becoming more aware of their hydration status becomes clear (Vukasinovic-Vesic et al, 2015; Osterberg et al, 2009).

**Body Composition Changes Across Seasons and Effects on Performance**

Bilsborough et al performed a study on ninety elite level Australian football players differing in levels of playing experience to investigate the effects of body composition on athletic performance. Findings from this study showed that athletes who had increased levels of fat free mass showed a greater ability to perform well in resistance training as well as increases in resisted squat jump performance (Bilsborough et al, 2015). Another study conducted by Maciejczyk et al looked at peak anaerobic power in thirty-six recreationally active males on a bike and found that males exhibiting higher body masses as a result of increased body compositional fat mass showed decreased peak anaerobic power values (Maciejczyk et al, 2015). Looking at these studies it appears as though there is enough evidence to suggest that poor body compositions (increased fat mass and subsequent decreased fat free [potentially lean] mass) can have detrimental effects on athletic performance which in and of itself shows body composition is something worth looking at when performing an observational study on athletes. However, the likelihood of seeing increasing fat mass during this study is most likely slim as a studies performed by Siders et al and Santos et al have shown that male collegiate basketball players tend to gain fat free mass during a season and have linked basketball associated training and competition to improved body compositional profiles (Santos et al, 2014; Siders et al, 1991). Looking at this data, the chances of performance decreasing in the athletes being observed in this study are probably low however being as this study is observational in nature it will be very
pertinent to track body composition as a means of looking for correlations to other variable changes seen.

**Effects of Sleep Deprivation and Sleep Debt Accumulation on Cortisol**

A lack of sleep, or sleep deprivation over an extended period of time can lead to the accumulation of sleep debt. In other words, the hours of missed sleep over multiple nights can become a summative debt of sleep that your body is not getting but most likely needs. In a non-athletic population, the accumulation of sleep debt led to significantly increased cortisol levels as compared to this same populations control condition. For this particular study, sleep debt was implemented as an independent variable and the participants were restricted to four hours of sleep per night for six straight nights. The control condition for the same participants was that they were allowed up to twelve hours of bed per night for six straight nights (Speigel et al, 1999). A more recent study found that sleep deprivation in servicemen caused a significant increase in cortisol levels as compared to a control condition (Song et al, 2015). Studies have looked into the effects of sleep deprivation on cortisol levels in response to athletic performance. One particular study looked at individuals performing peak aerobic bike tests after being subjected to sleep deprivation and found significant, yet not very convincing changes in cortisol levels in comparison to a control group. This study did only look at cortisol responses to an acute, single night of a sleep deprived state and not multiple nights of sleep deprivation (Mougin et al, 2001). It’s difficult to draw too many conclusions in the present as to any direct effects between sleep and cortisol response, however, there is evidence out there to suggest that chronic decreased sleep does indeed have an effect on resting cortisol levels and this could play a role in impacting the anabolic or catabolic state of the body if related back to the free testosterone to cortisol ratio. A chronically sleep deprived state leading to increases in resting cortisol levels could potentially
push protein towards degradation and thus have a negative impact on an athlete’s ability to train at a high level as well as their ability to recover effectively.

**RPE Monitoring as a Means of Tracking Training Load**

RPE, or the Rate of Perceived Exertion, is used quite frequently as a means of assessing an individual’s perception of the difficulty of an exercise or activity being performed. The standard RPE scale has ratings from six to twenty with six being no exertion and twenty being maximal/exhaustive exertion. This standard RPE scale was developed to be correlational to heart rate values if multiplying the RPE value times ten (i.e. and RPE of ten would correlate to a HR response of one hundred). Sometimes, a modified RPE scale, known as a Borg scale, will be used to allow for an easier understanding of the rating. This modified Borg RPE scale will be on a range from one to ten, with one being no exertion and ten being maximal/exhaustive exertion.

To track training loads using this modified RPE scale, the given RPE will be multiplied by the duration (in minutes) of the athletic activity to give a total training load value. This way of tracking RPE known as session-RPE has been previously verified in a study performed on rugby players looking at comparisons between training load, training strain, and total fatigue scores (Elloumi et al, 2012). The RPE score given will serve as the intensity marker for the exercise session and the duration will serve as the volume marker. A study conducted by Casamichana et al (2013) evaluated the relationships between different indicators of training loads in soccer players. This study compared session-RPE scores with a heart rate based load tracking and GPS based distance tracking. Results from the study showed that session-RPE had high correlation with GPS tracked total distance, frequency of high speed accelerations, as well as a player load which was calculated using a formula that took into account the volume of accelerations in multiple dimensions in each player. In addition, session-RPE had high correlations with the heart rate based tracking method used for this experiment. Conclusions from this study suggest
that session-RPE based tracking is a valid method for tracking and monitoring individual training load in athletes as it is highly correlated with other accepted measures for tracking training load (Casamichana et al, 2013).
CHAPTER III: METHODOLOGY

Participants

Fourteen elite-level, highly-trained Division 1 men’s college basketball players, from the University of North Carolina at Chapel Hill that participated in the 2016-17 initial 4 weeks of pre-season strength and conditioning training program, were recruited to participate in this study. Inclusion criteria for participation include:

* Ages between 18 and 24 years old;

* Free of injury that can preclude participation in any aspect of the study;

Exclusion Criteria for participation in the study include:

* Not pass a physical examination performed by the team sports physician;

* Be on any prescribed medication that can affect responses to exercise training.

Instrumentation

Players’ heights (cm) were taken using a wall mounted, tape measured ruler. Weight (kg) was measured on a Transcell Technology TI-500E electronic scale (Transcell Technology, Buffalo Grove, Illinois, USA). Resting heart rate was assessed using the palpation method using the radio artery during a 1 minute time period. Resting Blood pressure was measured manually by auscultation via a Diagnostix 700 aneroid sphygmomanometer (American Diagnostics Corporation, Hauppauge, New York, USA) and a Litmann stethoscope (3m, St. Paul, Minnesota, USA). Saliva samples will be analyzed using enzyme-linked immunosorbent assay (ELISA) kits (Salimetrics LLC, Carlsbad, California, USA). Hydration status was analyzed using a Misco refractometer (Misco, Solon, Ohio, USA). Body composition was assessed using the Bodystat®
QuadScan 4000 multi-frequency device and average velocity of bar speed was measured using a Tendo unit (Tendo Sports Machines, Irmo, South Carolina, USA). Eleiko bars and free weights Cemco brand (Cemco Strength Equipment, Inc, South El Monte, California, USA) were used as the main equipment for the training program (Eleiko Sports USA, Chicago, Illinois, USA). Samson racks were used to perform lifts on (Samson Equipment, Inc, Las Cruces, New Mexico, USA) as part of the performance test. Dietary evaluation will be performed using a dietary recall sheet as provided by the University of North Carolina sports nutrition staff (Chapel Hill, North Carolina, USA) and dietary data will be analyzed using Food Processor Nutritional Analysis software produced by ESHA Research (ESHA Research, Salem, Oregon, USA).

Overview of Study

This is a retrospective study using data collected during the 2016-17 4-week pre-season training of Division 1 basketball athletes from the University of North Carolina at Chapel Hill. The athletes underwent performance testing, hydration testing, body composition testing, and dietary tracking as part of this study. Salivary samples were also collected and frozen. Performance testing, hydration testing, and biomarker testing occurred twice a week while dietary tracking and body composition testing occurred once a week. The training program consisted of four days a week of training and varying weekly to create a dose-response. The earlier weeks in the study the athletes underwent four days of resistance training while the latter weeks in the study the athletes underwent five days of both resistance and aerobic/endurance training (three days resistance and three days aerobic). Below, on Table 1 is the study’s timeline of events:
### Table 3.1: Timeline of Events

<table>
<thead>
<tr>
<th>Approximate Dates:</th>
<th>Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid to Late August 2016</td>
<td>Athletes were introduced to study and signed informed consent form approved by the UNC biomedical IRB (IRB # 16-2189)</td>
</tr>
<tr>
<td>Late August to Early September 2016</td>
<td>Baseline Testing of Participants: Hydration testing, salivary biomarker testing, body composition testing, performance testing</td>
</tr>
<tr>
<td>Mid-September to Early October 2016</td>
<td>4 Week Training Program with testing as follows: hydration, salivary biomarker, and performance testing twice a week (early and late week) and body composition testing and dietary tracking once a week (late week)</td>
</tr>
<tr>
<td>Mid-October 2016</td>
<td>Post-training Testing: Hydration testing, salivary biomarker testing, body composition testing, performance testing</td>
</tr>
</tbody>
</table>

### Study Protocol

Recruitment: Basketball players were recruited from the men’s basketball team at The University of North Carolina at Chapel Hill and all have been previously cleared by a team physician for inclusion in Division I level physical activity. All athletes were brought in as a group for a meeting prior to baseline testing. During this meeting, all study procedures were explained in detail and all questions regarding any of the procedures were addressed. Athletes interested in participating in the study were then be asked to sign and date an informed consent form approved by the University Internal Review Board (IRB# 16-2189).

1. **Assessments**

   Prior to reporting for assessment throughout the study, all athletes were provided with pre-assessment guidelines (See Appendix I) and were asked to follow these guidelines strictly.
Before conducting any assessment, a research team member checked with the athletes to see if they followed the pre-assessment guidelines. In case the guidelines were not followed strictly, notes were taken and added to the athlete’s study files. Before any assessment, athletes were asked to fill out a sleep quality, fatigue, and muscle soreness questionnaires (See Appendix II).

Baseline and post-training testing were conducted in the following order to avoid the influence of one test to the following test following recommendations of the American College of Sports medicine: 1. As athletes arrived for testing, they were asked to provide a urine sample for the analyses of hydration status; 2. After a 5-10 minutes resting period, athletes resting vital signs were assessed (Resting Heart Rate and Blood Pressure); 3. Height and weight were then be assessed; 4. A salivary sample for the analyses of biomarkers was then collected; 5. After a warm-up period, all athletes’s underwent a performance test; 6. At the end of the assessment session, athletes were sent home with a dietary recall questionnaire for the evaluation of their currently dietary habits. Players were asked to bring back the questionnaire the following day. The results of the dietary questionnaire will be used for the assessment of dietary quality and quantity of caloric ingestion during the week. Below on Table 2 is the study assessment timeline of events:
Table 3.2: Assessment Timeline of Events

<table>
<thead>
<tr>
<th>Assessment to be Performed:</th>
<th>Timings of Assessment:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hydration Testing: Urinary Analysis</td>
<td>Baseline (week before training program begins), twice weekly throughout study (early week and late week), and post-training; hydration testing occurred in the morning prior to saliva samples being taken</td>
</tr>
<tr>
<td>2. Vital Signs</td>
<td>Resting heart rate and blood pressure were assessed before any assessment day</td>
</tr>
<tr>
<td>3. Body Weight/Body Height</td>
<td>Height was assessed at baseline only, Weight was assessed at baseline, once weekly late in the week, and at the conclusion of the study (post-training)</td>
</tr>
<tr>
<td>4. Body Composition</td>
<td>Bioelectrical Impedance (BIA) was performed to assess body composition baseline, once weekly late in the week, and post-training</td>
</tr>
<tr>
<td>5. Biomarker: Salivary Sampling</td>
<td>Saliva samples were collected at baseline, twice weekly during the training program (early week and late week), and post-training; samples were collected at 8 AM on days of sampling to control for circadian changes of hormones</td>
</tr>
</tbody>
</table>
6. Performance Testing
Performance testing occurred at baseline, twice weekly (early week and late week), and post-training; testing occurred prior to the resistance training sessions and involved a brief warm-up beforehand.

7. Dietary Tracking and Analysis
Athletes were given dietary recall sheet once per week, late in the week.

1. Resting Vital Signs
Resting heart rate was assessed using the palpation method using the radial artery during a 1 minute time period as is procedurally outlined in the ACSM’s Resource Manual for Guidelines for Exercise Testing and Prescription, 7th Edition (Swain & Brawner, 2012). Resting Blood pressure was measured manually by auscultation via a Diagnostix 700 aneroid sphygmomanometer (American Diagnostics Corporation, Hauppauge, NY USA) and a Litmann stethoscope (3m, St. Paul, MN USA).

2. Hydration Testing: Urinary Analysis
Urine samples were collected from the players twice a week on the same days on which the saliva samples were collected. A urine specific gravity test was performed to analyze the urine samples for hydration status. Athletes were given a cup and anti-bacterial wipe and were instructed to wipe the head of the penis with the wipe before urinating to minimize chances of contaminating the sample. Athletes were then asked to urinate a small amount before urinating into the cup. Athletes were instructed to urinate enough to fill up about a quarter of the sample cup they were given. One to two ounces of the
urine sample were pipetted out of the cup and used on the refractometer to determine the
density of the urine and thus hydration status.

3. Body Height/Body Weight

Athletes’ height was assessed using a wall-mounted, tape measured ruler following
procedures outlined in the CDC’s Anthropometry Procedures Manual (National Health
and Nutrition Examination Survey, 2007). Athletes’ mass was assessed using a
Transcell Technology TI-500E electronic scale (Transcell Technology, Buffalo Grove,
Illinois, USA).

4. Body Composition: Bioelectrical Impedance (BIA)

Bioelectrical impedance is a method of body composition assessment using low-level
electrical currency that travels through the body with the goal of measuring the
resistance to current flow that allows for the estimation of total body water (TBW).
Because free fat mass has relatively large water content (~ 73% water), by estimating
TBW, free fat mass and therefore percent body fat can both be predicted (Heyward,
2006). Since BIA relies on TBW for the determination of body composition, hydration
status can significantly influence the accuracy of determining body composition using
this method. Body resistance was measured according to standard procedures, on the
right side of the body, with athletes resting in a supine position. A Bodystat® QuadScan
4000 multi-frequency device that measures impedance at frequencies between 5 and 200
KHz (Douglas, Isle of Man, British Islands, UK) was used for the analyses. Hydration
levels including total body water, intracellular water and extracellular water were
computed by the Bodystat® QuadScan 4000 and recorded from the device. The validity
of BIA using DEXA as the reference method ranges from 0.84 to 0.96 in comparable
groups of women (Pineau, Guihard-Costa & Bocquet, 2007). The reliability of BIA ranges from 0.97 to 0.99 (ICCs) (Jackson et al., 1988).

5. Biomarkers

Saliva samples were collected using the Passive Drool Technique. This technique involves the athletes producing the saliva in their mouths and then tilting their heads slightly forward to allow the saliva to drip into the saliva collection tube. Athletes were instructed to not forcefully spit the saliva into the collection tube as this could lead to extra air bubbles being in the sample leading to a contamination of the sample. One milliliter of saliva was collected at 8 AM to control for the circadian cortisol changes and was collected twice per week: a beginning of week sample and an end of week sample. Before the saliva samples were collected, the athletes were asked to abide by a few pre-sampling guidelines. Athletes were required to not have brushed their teeth at least thirty minutes prior to the sample being collected.

In addition, athletes were asked to avoid food and drink with artificial colorings in the morning before the sample was collected and were required to rinse their mouths at least ten minutes prior to the sampling. Once the sample was collected, the samples were placed on ice as quickly as possible and transported back to the Applied Physiology Laboratory on campus at the University of North Carolina-Chapel Hill within an hour of being produced. Once the sample has been returned to the lab, it was centrifuged at -4°C Celsius at 3,000 RPM’s, pipetted out of the collection tubes into Cryo vials and stored at -80°C. Cortisol, free testosterone, TNF-α, and IL-6 levels will be assessed using ELISA kits and standard protocols for analysis will be used as outlined on the Salimetrics LLC website (www.salimetrics.com/assets/documents) (Salimetrics LLC, Carlsbad, California, USA).
6. Performance Testing

To monitor increases or decreases in performance for this study, athletes performed three squat jumps twice a week on the same days in which saliva and urine samples were collected. Athletes were instructed to hold a twenty-kilogram Eleiko bar on their backs and perform three repeat squat jumps squatting through their full range of motion. Average velocities of each jump were measured using a Tendo device ((Tendo Sports Machines, Irmo, South Carolina) which is attached around the end of the bar. Average velocities from all three jumps were recorded and the fastest value on each testing day will be used for performance analysis.

7. Dietary Tracking and Analysis

At the conclusion of each week, the athletes were required to fill out a dietary recall of the food they consumed in the week prior. The dietary recall consisted of broader food categories to ease the process for the athletes. Athletes were required to recall the type of food consumed, the way in which the food was prepared, and a general amount that was consumed. After this, each athletes’ dietary record were transferred to the Food Processor Nutritional Analysis software produced by ESHA Research (ESHA Research, Salem, Oregon, USA) which will break down the foods recorded into their various nutritional value as well as nutrient amounts.

2. Training Program

Athletes underwent a four-week training program that combined both resistance and aerobic based conditioning elements as part of the training program. All training sessions were administered and monitored by the men’s basketball strength and conditioning staff as well as members of the research team. All training sessions occurred during the afternoon at the
men’s basketball strength and conditioning weight room and at university owned and operated outdoor venues (i.e. turf fields, sand volleyball courts, outdoor education spaces). The resistance training sessions lasted approximately ninety minutes in duration while days of aerobic conditioning training, the sessions lasted approximately sixty minutes in duration. On days where both resistance and conditioning training occurred, the resistance training occurred first followed by the aerobic conditioning training. The schedule for the training program aimed to create a dose-response and consisted of the following:

Weeks 1-2: Four resistance training days (Monday, Tuesday, Thursday, & Friday); no aerobic conditioning days

Weeks 3-4: Three resistance training days (Monday, Tuesday, & Thursday); three aerobic/intermittent conditioning days (Monday, Wednesday, & Friday)

The resistance training and aerobic conditioning programs progressed in duration (volume) and intensity throughout the training program. Below on Table 3 is a detailed description of the intensity and volume of the resistance training:

Table 3.3: Resistance Training Progression

<table>
<thead>
<tr>
<th>Week</th>
<th>Intensity:</th>
<th>Volume:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70-75% of 1 RM with more power-based lifts (i.e. squats, Olympic lifts, presses); auxiliary lifts will have a lower intensity (40-60% of 1 RM)</td>
<td>90 minute sessions, 4 days a week; volume of power-based lifts will focus on lower sets and higher reps (3-4 sets of 6-10 reps); auxiliary lift volume will be 3-4 sets of 12-15 reps</td>
</tr>
<tr>
<td>2</td>
<td>75-80% of 1 RM max with power-based lifts; auxiliary lift intensity will remain the same</td>
<td>90 minute sessions, 4 days a week with higher set, lower rep volume on power-based lifts (6-8 sets, 4-6 reps); auxiliary lifts volume will remain the same</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>3</td>
<td>80-85% of 1 RM max with power-based lifts; auxiliary lift intensity will remain the same</td>
<td>90 minute sessions, 3 days a week with higher set, even lower rep volume than previous week working towards maximal strength on power-based lifts (6-8 sets, 2-4 reps); auxiliary lift volume will remain the same</td>
</tr>
<tr>
<td>4</td>
<td>85-90% of 1 RM max with power-based lifts; auxiliary lift intensity will remain the same</td>
<td>90 minutes sessions, 3 days a week with higher set, even lower rep volume than previous week working up towards 1 RM values (8-10 sets, 1-3 reps); auxiliary lift volume will remain the same</td>
</tr>
</tbody>
</table>

Resistance training programming was based on an Olympic-style and free weight philosophy.

More power-based and Olympic style lifts such as squats (and squat variations), cleans from
the hang, snatches from the hang, bench pressing, over-head pressing, and deadlifts (and
deadlift variations) were incorporated into the programming with the intent of building
maximal strength and maximal force production. Auxiliary lifts included variations of back
extensions, pull-ups, reverse hypers, rows, curls, and abdominal work and were used to build
up muscular endurance of the athletes. Below in Table 4 is a detailed description of the
intensity and volume of the aerobic/intermittent training:

**Table 3.4: Aerobic/Intermittent Training Progression**

<table>
<thead>
<tr>
<th>Week</th>
<th>Intensity:</th>
<th>Volume:</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>60-75% of maximal aerobic capacity and sprint work working towards 80-90% of maximal anaerobic capacity</td>
<td>60 minute sessions, 3 days a week with volumes of distance run ranging from 1000-2000 yards per session</td>
</tr>
<tr>
<td>4</td>
<td>70-85% of maximal aerobic capacity and sprint work working towards 85-95% of maximal anaerobic capacity</td>
<td>60 minute sessions, 3 days a week with volumes of distance run ranging from 1000-2000 yards per session</td>
</tr>
</tbody>
</table>

Aerobic training programming consisted of a mixture of runs ranging from 100-200 yards along with shorter duration, higher intensity sprint work that will involved straight line sprinting as well as change of direction, multi-directional movements. The mid-distance (100-200 yards) aerobic/intermittent running occured on flat surfaces such as turf or grass fields. The shorter distance, sprint and change of direction work occured on a variety of surfaces such as grass, turf, hardwood, and sand surfaces and involved some plyometric work as well as work on inclined and declined surfaces.
Statistical Analysis

This is an observational study with a single group where physical fitness, body composition, hydration status, and biomarker of stress data were collected throughout a 4-week pre-season strength and conditioning training program in Division I basketball players with the goal of observing potential alterations on these study parameters as a results of the training regimen. A secondary objective was to evaluate the relationship between changes in physical fitness, body composition, hydration status, biomarker alterations and total training loads. In the initial analysis of the data, study variables were examined independently of one another to observe changes that were out of the ordinary. Variables that showed unexpected changes were then be correlated with weekly training volume changes to evaluate the strength of the relationships. Variables that show stronger correlations with weekly training volumes were correlated with performance test variables to examine the strength of the relationships between the physiological variables and performance markers in an effort to see which variables, if any, had effects on performance of the athletes. For the evaluation of the relationships between study variables, simple regressions using percent change from baseline as well as from different time points of interest were calculated. All Statistical analysis were performed by computer-based statistical software (SPSS; version 21). The significance level for all statistical procedures was set a-priori at $\alpha < 0.05$. Total training load was computed using a session-RPE score which uses the modified Borg RPE scale (a scale that is rated one to ten with one being no exertion and ten being maximal exertion) multiplied by the duration of the session in minutes. This multiplication gave the session-RPE score, which has been previously demonstrated to have a strong correlation with other accepted measurements of training load in athletes (Casamichana et al, 2013). After each training session, total amount of time the session took in regards to the time in which exercise was being performed (i.e. the travel time between the weight room and the location for
aerobic conditioning will be excluded) along with each player’s reported RPE value was recorded. These session-RPE values were recorded on a spreadsheet for each player and were summed at the end of the week to compute a total weekly training load. Any of the athletes who missed four or more of the training days in the training program as a whole were excluded from data analysis. If an athlete missed fewer than four total days but happens to miss one of the sampling days, the mean from the previous sampling day and following sampling day was calculated and substituted in to the data set to maintain continuity with the data.
CHAPTER IV: RESULTS

The purpose of this observational study was to profile the responses of hydration status, body composition, salivary biomarkers of stress, training loads, and performance changes in Division 1 basketball players throughout a 4-week pre-season strength and conditioning training cycle. A secondary purpose evaluated the relationship between changes in physical fitness (performance test), hydration status, body composition, salivary biomarkers of stress, and training loads of variables that demonstrated trends of changes deemed to be of clinical relevance.

Subjects

Fourteen elite level collegiate Division-I basketball players were recruited as subjects for this study. One of the fourteen players (Subject 8) suffered an injury causing them to miss an extended period of time during the study thus leaving thirteen players as participants. These thirteen players completed seven total evaluation days in which saliva samples, urine samples, body compositional profiles, and performance data were collected. Four of the subjects were in their freshmen year as part of the basketball program and for the purposes of this study these four were divided out into a sub-group (freshmen). The remaining nine players were put into a sub-group as the upper-classmen. Thus, for all the data presented there are three groups shown: all players, freshmen players, and upper-classmen players.
Figure 4.1: Data Collection Timeline

<table>
<thead>
<tr>
<th>Player</th>
<th>Baseline</th>
<th>Early Week 2</th>
<th>Late Week 2</th>
<th>Early Week 3</th>
<th>Late Week 3</th>
<th>Early Week 4</th>
<th>Late Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.6</td>
<td>11.8</td>
<td>11.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.5</td>
<td>11.5</td>
<td>10.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12.6</td>
<td>13.2</td>
<td>13.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>18.3</td>
<td>13.8</td>
<td>no data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>13.3</td>
<td>14.8</td>
<td>16.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>9.9</td>
<td>9.8</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9.9</td>
<td>11.8</td>
<td>no data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>6.9</td>
<td>8.1</td>
<td>8.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>13.8</td>
<td>14.8</td>
<td>15.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>12.4</td>
<td>11.9</td>
<td>13.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>10.2</td>
<td>10</td>
<td>11.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>17.4</td>
<td>17</td>
<td>16.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>21.2</td>
<td>21</td>
<td>20.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*= volume was calculated on a weekly basis at the end of each week including week 1 (Baseline)

Body Composition

Below in Table 4.1 are results from the BIA body scan that was used to evaluate body composition. Three total BIA scans were completed during the study (at baseline, mid-point, and at the end of the study late on week 4). Data is presented as absolute changes for each player.

Table 4.1: Body Fat Percentages (body fat shown as percentage for each individual player at each time point)

<table>
<thead>
<tr>
<th>Player ID</th>
<th>Baseline</th>
<th>Mid-point</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.6</td>
<td>11.8</td>
<td>11.3</td>
</tr>
<tr>
<td>2</td>
<td>9.5</td>
<td>11.5</td>
<td>10.8</td>
</tr>
<tr>
<td>3</td>
<td>12.6</td>
<td>13.2</td>
<td>13.7</td>
</tr>
<tr>
<td>4</td>
<td>18.3</td>
<td>13.8</td>
<td>no data</td>
</tr>
<tr>
<td>5</td>
<td>13.3</td>
<td>14.8</td>
<td>16.7</td>
</tr>
<tr>
<td>6</td>
<td>9.9</td>
<td>9.8</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>9.9</td>
<td>11.8</td>
<td>no data</td>
</tr>
<tr>
<td>9</td>
<td>6.9</td>
<td>8.1</td>
<td>8.1</td>
</tr>
<tr>
<td>10</td>
<td>13.8</td>
<td>14.8</td>
<td>15.1</td>
</tr>
<tr>
<td>11</td>
<td>12.4</td>
<td>11.9</td>
<td>13.1</td>
</tr>
<tr>
<td>12</td>
<td>10.2</td>
<td>10</td>
<td>11.5</td>
</tr>
<tr>
<td>13</td>
<td>17.4</td>
<td>17</td>
<td>16.4</td>
</tr>
<tr>
<td>14</td>
<td>21.2</td>
<td>21</td>
<td>20.4</td>
</tr>
</tbody>
</table>

Hydration

For the purposes of this study hydration status was monitored using a refractometer to measure the specific gravity from player urine samples. Table’s 4.2 and 4.3 present the urine
specific gravity scores for players across seven time points: baseline and then twice weekly (early week and late week) for the following three weeks with the last measurement assessed late in week 4 (end of the study).

Table 4.2: Urine Specific Gravity Scores (means ± standard deviations with ranges in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Early Week 2</th>
<th>Late Week 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Players</td>
<td>1.021 ± .004</td>
<td>1.020 ± .006</td>
<td>1.020 ± .008</td>
</tr>
<tr>
<td></td>
<td>(1.015-1.030)</td>
<td>(1.008-1.028)</td>
<td>(1.005-1.030)</td>
</tr>
<tr>
<td>Freshmen</td>
<td>1.023 ± .004</td>
<td>1.022 ± .004</td>
<td>1.019 ± .010</td>
</tr>
<tr>
<td></td>
<td>(1.019-1.027)</td>
<td>(1.016-1.026)</td>
<td>(1.005-1.030)</td>
</tr>
<tr>
<td>Upper-Classmen</td>
<td>1.021 ± .005</td>
<td>1.020 ± .006</td>
<td>1.020 ± .008</td>
</tr>
<tr>
<td></td>
<td>(1.015-1.030)</td>
<td>(1.008-1.028)</td>
<td>(1.006-1.028)</td>
</tr>
</tbody>
</table>

Table 4.3: Urine Specific Gravity Scores cont. (means ± standard deviations with ranges in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Early Week 3</th>
<th>Late Week 3</th>
<th>Early Week 4</th>
<th>Late Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Players</td>
<td>1.017 ± .008</td>
<td>1.017 ± .005</td>
<td>1.021 ± .006</td>
<td>1.021 ± .007</td>
</tr>
<tr>
<td></td>
<td>(1.002-1.028)</td>
<td>(1.005-1.026)</td>
<td>(1.006-1.027)</td>
<td>(1.000-1.028)</td>
</tr>
<tr>
<td>Freshmen</td>
<td>1.013 ± .008</td>
<td>1.018 ± .003</td>
<td>1.022 ± .004</td>
<td>1.019 ± .003</td>
</tr>
<tr>
<td></td>
<td>(1.002-1.019)</td>
<td>(1.015-1.020)</td>
<td>(1.017-1.026)</td>
<td>(1.016-1.022)</td>
</tr>
<tr>
<td>Upper-Classmen</td>
<td>1.018 ± .007</td>
<td>1.017 ± .006</td>
<td>1.021 ± .007</td>
<td>1.021 ± .009</td>
</tr>
<tr>
<td></td>
<td>(1.006-1.028)</td>
<td>(1.005-1.026)</td>
<td>(1.006-1.027)</td>
<td>(1.000-1.028)</td>
</tr>
</tbody>
</table>

Training Load

For this study, training load was tracked using the session-RPE method in which total duration of training time is multiplied by a score given by each player on the modified Borg RPE scale which ranges from 1-10. This occurred each day that training occurred and weekly training loads for each player were determined. From this, group mean training loads were calculated and are represented below in Table 4.4.
Table 4.4: Weekly Session-RPE Scores (means ± standard deviations with ranges in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Players</td>
<td>4492.8 ± 443.2</td>
<td>3870.5 ± 372.2</td>
<td>4715.3 ± 474.9</td>
<td>3479.2 ± 360.5</td>
</tr>
<tr>
<td></td>
<td>(3655-5223)*</td>
<td>(3133-4314)</td>
<td>(3651-5337)</td>
<td>(2680-3950)</td>
</tr>
<tr>
<td>Freshmen</td>
<td>4330.4 ± 296.0</td>
<td>3920.1 ± 190.2</td>
<td>4779.8 ± 359.6</td>
<td>3499.4 ± 232.8</td>
</tr>
<tr>
<td></td>
<td>(4044-4683)</td>
<td>(3643-4060.5)</td>
<td>(4469-5299)</td>
<td>(3170-3670)</td>
</tr>
<tr>
<td>Upper-Classmen</td>
<td>4573.9 ± 498.5</td>
<td>3848.4 ± 438.9</td>
<td>4686.7 ± 535.5</td>
<td>3470.3 ± 417.5</td>
</tr>
<tr>
<td></td>
<td>(3655-5223)*</td>
<td>(3133-4314)</td>
<td>(3651-5337)</td>
<td>(2680-3950)</td>
</tr>
</tbody>
</table>

*Subject 13’s week 1 training load was exempt from mean calculations as he missed two days of activity with a minor injury and thus had a low weekly training load of 1349.

Table 4.5: Average Daily Session-RPE Scores Per Week (Weekly S-RPE Score/number of training days for that particular week)

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Players</td>
<td>1123.2</td>
<td>774.1</td>
<td>785.9</td>
<td>1159.7</td>
</tr>
<tr>
<td>Freshmen</td>
<td>1082.6</td>
<td>784.0</td>
<td>796.6</td>
<td>1166.5</td>
</tr>
<tr>
<td>Upper-Classmen</td>
<td>1143.5</td>
<td>769.7</td>
<td>781.1</td>
<td>1156.8</td>
</tr>
</tbody>
</table>

Performance Tests

Players completed weekly assessments of performance at baseline and then at the beginning of each of the following three weeks. These major performance test used was a measurement of peak velocity produced while holding a 20 kilogram bar on the back. It should be noted that Subject 14 due to a chronic lower extremity injury was not advised to participate in extraneous impact exercises and thus did not complete the performance tests. Therefore no performance test values from participant 14 are included in the following calculations. Below, Table 4.5 presents the peak velocity measurements.
Table 4.6: Peak Velocity Measurements (mean ± standard deviation with ranges in parentheses; measurements recorded in meters/second)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Early Week 2</th>
<th>Early Week 3</th>
<th>Early Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Players</td>
<td>2.99 ± .21</td>
<td>2.93 ± .32</td>
<td>3.05 ± .15</td>
<td>2.99 ± .20</td>
</tr>
<tr>
<td></td>
<td>(2.67-3.4)</td>
<td>(2.55-3.24)</td>
<td>(2.70-3.24)</td>
<td>(2.55-3.36)</td>
</tr>
<tr>
<td>Freshmen</td>
<td>2.81 ± .18</td>
<td>2.74 ± .13</td>
<td>2.91 ± .16</td>
<td>2.86 ± .25</td>
</tr>
<tr>
<td></td>
<td>(2.67-2.92)</td>
<td>(2.55-2.86)</td>
<td>(2.70-3.1)</td>
<td>(2.55-3.17)</td>
</tr>
<tr>
<td>Upper-Classmen</td>
<td>3.08 ± .19</td>
<td>3.02 ± .12</td>
<td>3.12 ± .08</td>
<td>3.05 ± .15</td>
</tr>
<tr>
<td></td>
<td>(2.75-3.4)</td>
<td>(2.86-3.24)</td>
<td>(2.98-3.24)</td>
<td>(2.86-3.36)</td>
</tr>
</tbody>
</table>

Salivary Hormones

Below in Table 4.6 are the group means for the saliva data on testosterone while Table 4.7 contains the group means for cortisol. Saliva was planned to be collected on all seven collection days as the case was with urine however on the early week 3 collection day saliva was unable to be collected.

Table 4.7: Free Testosterone Levels (measured in pg/mL; means ± standard deviations with ranges in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>All Players</th>
<th>Freshmen</th>
<th>Upper-Classmen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>118.11 ± 39.77</td>
<td>114.55 ± 17.37</td>
<td>119.69 ± 47.44</td>
</tr>
<tr>
<td></td>
<td>(53.66-208.63)</td>
<td>(91.00-132.44)</td>
<td>(53.66-208.63)</td>
</tr>
<tr>
<td>Early Week 2</td>
<td>117.89 ± 28.63</td>
<td>136.82 ± 16.04</td>
<td>109.48 ± 29.57</td>
</tr>
<tr>
<td></td>
<td>(80.32-162.57)</td>
<td>(124.84-160.48)</td>
<td>(80.32-162.57)</td>
</tr>
<tr>
<td>Late Week 2</td>
<td>115.11 ± 33.92</td>
<td>111.48 ± 26.54</td>
<td>111.692 ± 38.66</td>
</tr>
<tr>
<td></td>
<td>(70.99-201.99)</td>
<td>(84.33-145.85)</td>
<td>(70.99-201.99)</td>
</tr>
<tr>
<td>Late Week 3</td>
<td>121.56 ± 31.53</td>
<td>115.24 ± 37.33</td>
<td>123.93 ± 31.60</td>
</tr>
<tr>
<td></td>
<td>(77.65-192.39)</td>
<td>(77.65-152.31)</td>
<td>(81.52-192.39)</td>
</tr>
<tr>
<td>Early Week 4</td>
<td>121.31 ± 55.77</td>
<td>121.35 ± 14.94</td>
<td>121.29 ± 66.18</td>
</tr>
<tr>
<td></td>
<td>(61.86-275.53)</td>
<td>(107.81-137.38)</td>
<td>(61.86-275.53)</td>
</tr>
<tr>
<td>Late Week 4</td>
<td>118.74 ± 35.84</td>
<td>140.64 ± 26.44</td>
<td>111.46 ± 36.78</td>
</tr>
<tr>
<td></td>
<td>(71.10-178.04)</td>
<td>(122.57-170.96)</td>
<td>(71.10-178.04)</td>
</tr>
</tbody>
</table>
Table 4.8: Cortisol Levels (measured in µg/dL; means ± standard deviations with ranges in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>All Players</th>
<th>Freshmen</th>
<th>Upper-Classmen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.0726 ± 0.0304</td>
<td>0.09575 ± 0.0202</td>
<td>0.0643 ± 0.0293</td>
</tr>
<tr>
<td></td>
<td>(0.0192-0.1246)</td>
<td>(0.0765-0.1169)</td>
<td>(0.0192-0.1246)</td>
</tr>
<tr>
<td>Early Week 2</td>
<td>0.1160 ± 0.0304</td>
<td>0.1239 ± 0.0131</td>
<td>0.1133 ± 0.0345</td>
</tr>
<tr>
<td></td>
<td>(0.0558-0.1599)</td>
<td>(0.1089-0.1331)</td>
<td>(0.0558-0.1599)</td>
</tr>
<tr>
<td>Late Week 2</td>
<td>0.1423 ± 0.0805</td>
<td>0.2251 ± 0.1109</td>
<td>0.1147 ± 0.0491</td>
</tr>
<tr>
<td></td>
<td>(0.0424-0.1826)</td>
<td>(0.1133-0.3350)</td>
<td>(0.0424-0.1826)</td>
</tr>
<tr>
<td>Late Week 3</td>
<td>0.1330 ± 0.0551</td>
<td>0.1137 ± 0.0946</td>
<td>0.1402 ± 0.0395</td>
</tr>
<tr>
<td></td>
<td>(0.0268-0.2145)</td>
<td>(0.0268-0.2145)</td>
<td>(0.0863-0.1982)</td>
</tr>
<tr>
<td>Early Week 4</td>
<td>0.1227 ± 0.0859</td>
<td>0.1293 ± 0.0969</td>
<td>0.1211 ± 0.0901</td>
</tr>
<tr>
<td></td>
<td>(0.0382-0.2788)</td>
<td>(0.0680-0.1979)</td>
<td>(0.0382-0.2788)</td>
</tr>
<tr>
<td>Late Week 4</td>
<td>0.1662 ± 0.3118</td>
<td>0.2148 ± 0.2064</td>
<td>0.1500 ± 0.0587</td>
</tr>
<tr>
<td></td>
<td>(0.0415-0.4331)</td>
<td>(0.0415-0.4331)</td>
<td>(0.0652-0.2548)</td>
</tr>
</tbody>
</table>

Exploratory Analysis

Variables that appeared to potentially have noticeable change throughout the four-weeks training program were correlated with the performance measurement. Relationships between individual urine specific gravity scores and individual peak velocity produced on the performance test were evaluated. Each regression compared the early week score of each of the variables to the baseline value and correlated the delta scores (percent changes). Thus, the change percentage in urine specific gravity from week one to week two was regressed with the peak velocity change from week one to week two and so on for week’s three and four. No significant relationships were observed (p > .05) (R=.071, .234, and .321 respectively).

A correlation between the percent training load change seen between weeks two and three with the peak velocity performance test change seen between week’s three and four (with the underlying assumption that the volume change between week’s two and three would have more of an effect on the early week peak velocity test from week’s three and four) was explored. No significant relationship was observed (p > .05) (R=.05).
A simple regression between the percent change score from baseline to early week four between testosterone and peak velocity values was explored. No significant relationship was observed (p > .05) (R = .442).

A relationship between the percent change score from baseline to early week four cortisol values and peak velocity value was explored. No significant relationship was observed (p > .05) (R = .024).
CHAPTER V: DISCUSSION

Overview of Study

The purpose of this observational study was to profile and observe a wide spectrum of physiological and physical responses in elite level Division-I basketball players to a pre-season strength and conditioning training cycle. To our knowledge, no study to date has attempted to take this approach in profiling elite level collegiate basketball players in the way in which we have attempted to with the goal of observing potential relationships between changing training load and various physiological parameters along with associations to performance. Due to the nature of the study, there were certain aspects of the study that were out of control of the research team. The goal was to make observations of the changes in the selected variables in an unabated environment for the players. Thus, all attempts were made for the study to not interfere in any way with the pre-season strength and conditioning training regimen and allowed the coaches to dictate all player workouts whether that be in the weight room or on the court. This approach provides a great opportunity to observe the players in a true real world environment. Although this approach provides a realistic view of the day to day operations of a high level strength training program aimed to prepare elite players for a season of heavy competition, this approach limits the research team’s ability to collect data in a well controlled fashion due to the ever-changing schedule common to most collegiate athletic programs across the country. All things considered, the research team attempted to adjust scheduling on a moment’s notice to achieve as much consistency as was possible, thus creating some limitations that could have possibly altered the outcome of some of the variables included in the study. However, the
samples and data collected during this study have given the research team a rare opportunity to get an inside look at the physiologic responses of some of the most talented and gifted collegiate basketball players in the world. The biomarkers observed in this study along with the tracking of training loads and physical performance variables has brought to light certain trends seen within this group of players that will most certainly help guide future research.

**Body Composition**

After observing the body fat percentages seen throughout the study, a determination was made that very little change occurred for most players across the 4 weeks. Using a BIA to collect body composition parameters brings into account hydration status and its potential to skew or produce faulty results. Since the players often times were right on the line between euhydration and dehydration throughout the study, this could have played a role in producing some inaccuracies in the body fat percentage measurements. Due to these factors, it was the decision of the research team that it would be unwise to potentially attribute the small changes seen in body fat percentage to changing training volumes or attempt to relate these changes to other variables in the study. Perhaps if a method less sensitive to hydration status was to be used, more interpretable findings could be made about body composition changes throughout the study. However, the short period of 4-weeks of training and due to the logistic nature of the study, the BIA method was the most feasible way to attempt to monitor potential changes in body composition. Understandably so, body composition is not a variable that typically experiences noticeable changes over short periods of time so it is also likely that the study performed just did not have the length necessary to observe clinically relevant changes in body composition (Siders et al, 1991; Santos et al, 2014).
Hydration

It has been well documented that dehydration is a major problem and has a high prevalence in both amateur and professional basketball players (Osterberg et al, 2009; Vukasinovic-Vesic et al, 2015). Looking at the results of the urine specific gravity tests throughout this study it appears as though there was certain times during the study in which the group as a whole was certainly at least suffering from moderate dehydration [USG score above 1.020 (Osterberg et al, 2009)] based on their urine specific gravity scores. As demonstrated in Figure 5.1, the group of players as a whole trended towards a state of better hydration status from weeks one to three however then trended back towards a more dehydrated state by week four. Figures 5.2 and 5.3 demonstrate that the sub-groups of players (freshmen and upperclassmen) both experienced similar trends towards a more euhydrated state from weeks one to three followed by a decline back to dehydration during week four. The solid black line in the figures below indicates the 1.020 score in which any scores above this are defined in the literature as a marker of dehydration.

Collection Number Key for Hydration Graphs:
1 = Baseline
2 = Early Week 2
3 = Late Week 2
4 = Early Week 3
5 = Late Week 3
6 = Early Week 4
7 = Late Week 4
Perhaps a more understandable and usable way to interpret hydration status is to look at the total number of players at each collection day that could be deemed at least moderately dehydrated. Literature states that urine specific gravity scores above 1.020 can be indicative of dehydration ranging from three to five percent of body weight (Osterberg et al, 2009). Table 5.1 depicts this

**Table 5.1: Number of Dehydrated Players per Collection Point**

<table>
<thead>
<tr>
<th>Collection Point</th>
<th># of Dehydrated Players (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>7 (53.85%)</td>
</tr>
<tr>
<td>Early Week 2</td>
<td>7 (53.85%)</td>
</tr>
<tr>
<td>Late Week 2</td>
<td>6 (46.15%)</td>
</tr>
<tr>
<td>Early Week 3</td>
<td>5 (38.46%)</td>
</tr>
<tr>
<td>Late Week 3</td>
<td>2 (15.38%)</td>
</tr>
<tr>
<td>Early Week 4</td>
<td>7 (53.85%)</td>
</tr>
</tbody>
</table>
The reasoning behind the decreases in number of dehydrated players seen from baseline to week three of the study could be as simple as a greater recognition by the players of trying to hydrate. As urine was being collected, the players would ask questions about their hydration status and members of the research team would give them feedback. In addition, the strength and conditioning and athletic training staff associated with the players would look at the hydration scores and talk to certain players who were having higher scores demonstrating potential poor rehydration habits. This increased recognition of hydrations status could have led the players to take greater notice of their hydration habits as the study progressed. However, then this begs the question what caused this recognition to not cause the same effects into the last week of the study. Why then did the number of dehydrated athletes increase back to baseline levels during week four? To further investigate and explore these questions, a further look into the potential effects of the cumulative load of training and the awareness of players recognizing the influence of proper hydration of performance and recovery, should be explored in a prospective experiment.

**Hydration and Training Load**

The cumulative effects of an increasing training load could have played some role in the urine specific gravity score increases seen in week four of the study. Figure 5.4 depicts the weekly training load trends seen throughout the four weeks of the study.
In addition, evaluating the percent change in training volume from week to week is important for gauging potential relationships between the urine specific gravity scores and changing training volumes. In Table 5.2 below, the training load percent change per week is shown.

Table 5.2: Training Load Percent Change by Week

<table>
<thead>
<tr>
<th></th>
<th>Week 1 → Week 2</th>
<th>Week 2 → Week 3</th>
<th>Week 3 → Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Change over Previous Week</td>
<td>-13.8%</td>
<td>+21.8%</td>
<td>-26.2%</td>
</tr>
</tbody>
</table>

Understanding the timing of the urine sampling and the summation of the weekly training loads is paramount to being able to draw conclusions about training loads potential effects on hydration status. The week three urine samples which showed the least number of dehydrated athletes were taken coming of off week two of training load tracking and the week four urine samples (which showed the highest number of dehydrated athletes) were coming off of week
three of training load tracking. Studying the training load numbers, week two clearly has a lower average training load than week three with week three having the highest average training load of any of the weeks. The percent change in training loads from week two to week three was a 21.8% increase. Perhaps this acute increase in training load during week three was enough to cause some negative impact of the hydration status of the players. In addition, week three of the study had the most days of activity with six which included the players’ first official exhibition game of the year. Also of note is that the late week three urine collection time point (which showed the fewest number of dehydrated athletes throughout the entire study) came on the day following the only rest day during week three. After this, the players had four straight days of training leading up to the early week four collection date. It appears as though a combination of these factors may have played at least somewhat of a role in negating rehydration efforts.

**Hydration and Performance**

Concluding that there was at least some interaction between the training load and hydration status it is important also to look at possible effects of hydration status and the performance variable used during this study. Previous research has had mixed results in regards to dehydration’s acute effects on muscle strength and power production (Hayes & Morse, 2010; Minshull & James, 2013; Davis et al, 2015). Performance testing occurred early in the week in line with the baseline urine sampling and the following three early week urine samplings. Depicted in Figure 5.5 are the peak velocity changes throughout the four week training period.
Week three presented the highest team mean peak velocity throughout the four week training cycle with a subsequent decline in the team mean peak velocity the following week. While these changes were small, often times only tenths of a percentage, if they were to persist on they could raise some red flags for the strength and conditioning staff. It’s difficult to determine how much of a change in peak velocity development would affect athletic performance directly. Any time the basketball strength and conditioning staff sees decreases in peak velocity or other performance parameters, it is a cause at least for conversation into potential causes for that decrease. The week three peak velocities match up with the week three urine specific gravity scores which, if one recalls, had the fewest number of players above that 1.020 threshold indicating better overall team hydration status. How much of a role is hydration playing in peak velocity performance? The literature reviewed earlier in this paper had mixed opinions on this subject. Some research has shown that a dehydrated state leads to greater reductions in ability to produce peak force which seems to at least somewhat be supported by the
whole team trend seen here (Minshull & James, 2013). However, others have produced results indicating that hydration status has little to no real impact on muscular strength or the muscle’s ability to produce force (Hayes & Morse, 2010).

What we have found in this research seems to point that hydration played some type of role or had some type of connection to the performance of the athletes on the peak velocity test. The magnitude of this effect is hard to judge accurately but with the purpose of this study to observe and report this is one observation that shouldn’t be overlooked. Hydration status is a well-known and widely used marker for readiness to perform for athletes. For athletes who are dehydrated, the fix is simple: drink more water. Yet just because the fix is simple does not mean that athletes practice good hydration habits. Being dehydrated may not decrease athletic performance to the degree that some studies have shown, however dehydration will also surely not help athletic performance. Therefore, as simple as it seems, assumptions should not be made that all athletes are hydrating well. For four of the seven urine collection days of this study, half or over half of the players were in at least a moderate (if not worse) dehydrated state. They all know hydration is important, however what they lack may be the awareness of their hydration state. I firmly believe that this study brought more awareness to them earlier in the season than would normally be the case and perhaps that played a role in being able to perform and succeed the way they did this season.

**Training Load and Performance**

Looking further into the potential relationships seen during this study, more specifically during the time points that have been discussed previously in the hydration section (week two to week three), it is important to consider the possible affects training load may have on performance. As has been stated prior, the highest mean average for the team for the peak velocity test came at the early week three collection date. This date was preceded by a relatively
low total training load in week two. In addition, the peak velocity at the early week four collection date showed decreases from the week three velocity numbers and this was following the highest total training load in week three. It is not out of the question to believe that there could have been some connections between a greater amount of fatigue accumulating during week three of the training program and this fatigue causing some of the decreases in muscular performance during the peak velocity test of early week four. Week two’s lower training loads would have led to the players having less of accumulation of fatigue and would have given them the potential to perform better on the peak velocity test.

By the time this study commenced, sport specific practices were taking up approximately seventy-five to eighty percent of total training volume with weight training taking up the remainder. This is an important concept to understand as the main goal of the strength and conditioning staff at this point of the pre-season is no longer increasing muscular and power development, but instead trying to maintain what strength and power gains were achieved during summer and off-season lifting. As is shown above in Figure 5.5, power output (represented in this study by peak velocity), was variable throughout the study. While the changes may be statistically insignificant only varying by tenths of a meter/second every week, these types of changes (especially if they are decreases) could potentially make a difference in elite level athletic competition. When competing against elite-level competition, an athlete must be performing as close to one hundred percent as is possible. The extent to which the changes seen in this study in peak velocity relate to athletic performance and other physiological markers would need to be investigated further to understand the magnitude of the relationships if they exist at all.

For the strength and conditioning staff, it is a constant struggle to maintain strength and power in the face of large volumes of sport specific practices which can cause fatigue and tissue
breakdown. Being able to understand and visualize the effects that higher training volumes can have on future physical performance is paramount as this allows strength staff to react quickly in response to sudden increases in training volume and adjust weight training programming accordingly. The extent to which training volume of practices and weight training sessions directly affects physical performance for elite level collegiate athletes can not necessarily be determined in this study. However, the profiling and observation of the players in this study has opened up enough reason to potentially further investigate these effects that training volume can have on an athlete’s performance.

**Observed Hormonal Changes**

In the literature reviewed in preparation for this study there was fairly good congruency in agreement that after strenuous activity there would be acute increases in cortisol for up to one entire day post high effort physical activity. In addition, more chronic based physical activity would lead to significant resting cortisol increases over baseline values (Passelergue & Lac, 1999; Martinez et al, 2010). Similar research looking at testosterone level changes following high effort physical activity has shown a depression of testosterone levels immediately following the physical stress with returns to normal resting levels within one to two days after (Lac & Berthon, 2000). Below in Figures 5.5 and 5.6 are the free testosterone and cortisol responses

<table>
<thead>
<tr>
<th>Collection Number Key:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = Baseline</td>
</tr>
<tr>
<td>2 = Early Week 2</td>
</tr>
<tr>
<td>3 = Late Week 2</td>
</tr>
<tr>
<td>4 = Late Week 3</td>
</tr>
<tr>
<td>5 = Early Week 4</td>
</tr>
<tr>
<td>6 = Late Week 4</td>
</tr>
</tbody>
</table>
throughout the training program using the mean averages for all players.

The player’s hormonal responses had quite the range as can be seen in the descriptive statistics tables in chapter four of this document. Due to this, it may be unwise to generalize hormonal responses in the same way in which hydration responses were generalized for the team. Individuals, even being all in the same population of elite level athletes, will experience different physiological responses to training and hormones can respond very differently.
depending on the individual. While there are many ways in which the data from this study can be analyzed, the creation of a table in which total free testosterone and cortisol percent changes were throughout the entire study were matched up with the peak velocity percent changes from the same collection points. Table 5.3 below provides some observational insight into potential trends within certain athletes or for the athletes as a whole more so than just looking at mean averages of the hormones changing over time. Table 5.3 is a representation of the individual testosterone and cortisol percent changes from baseline values (in which baseline performance testing occurred as well) to the last day of performance testing which was saliva collection day 5.

**Table 5.3: Hormonal and Performance Percent Change Over the Course of the Study**
(numbers displayed are expressed as percentages of change from baseline collection to early week four testing which was the day of the last peak velocity test)

<table>
<thead>
<tr>
<th>Player</th>
<th>Testosterone</th>
<th>Cortisol</th>
<th>Peak Velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Player 1</td>
<td>+19.78%</td>
<td>Unreliable Sample</td>
<td>+9.69%</td>
</tr>
<tr>
<td>Player 2</td>
<td>-10.25%</td>
<td>+69.29%</td>
<td>-4.49%</td>
</tr>
<tr>
<td>Player 3</td>
<td>-10.22%</td>
<td>-20.52</td>
<td>-3.08%</td>
</tr>
<tr>
<td>Player 4</td>
<td>No post sample</td>
<td>No post sample</td>
<td>+5.09%</td>
</tr>
<tr>
<td>Player 5</td>
<td>+5.7%</td>
<td>+46.55%</td>
<td>+12.72%</td>
</tr>
<tr>
<td>Player 6</td>
<td>-16.40%</td>
<td>No Change</td>
<td>-4.8%</td>
</tr>
<tr>
<td>Player 7</td>
<td>No post sample</td>
<td>No post sample</td>
<td>-2.21%</td>
</tr>
<tr>
<td>Player 9</td>
<td>+32.07%</td>
<td>+290.48%</td>
<td>-2.93%</td>
</tr>
<tr>
<td>Player 10</td>
<td>-5.06%</td>
<td>+193.55%</td>
<td>+3.08%</td>
</tr>
<tr>
<td>Player 11</td>
<td>-15.43%</td>
<td>+112.77%</td>
<td>-4.36%</td>
</tr>
<tr>
<td>Player 12</td>
<td>-13.89%</td>
<td>-6.13%</td>
<td>-5.92%</td>
</tr>
<tr>
<td>Player 13</td>
<td>+15.27%</td>
<td>+130.21%</td>
<td>-1.18%</td>
</tr>
<tr>
<td>Player 14</td>
<td>-33.3%</td>
<td>-16.96%</td>
<td>No test performed</td>
</tr>
</tbody>
</table>

As was mentioned earlier, while some of the percent changes in peak velocity are rather small (under five percent), these can still have fairly big impacts on athletic performance especially at this elite level. Looking into table 5.3 a little more in depth, the players that cause the most concern are the ones who showed decreases in performance in response to the four week programming. Of the players listed here with data on both testosterone and cortisol, eight
players showed decreases. Of these eight, five players showed decreases in testosterone from baseline to week four. Being that testosterone is an anabolic agent that is a prime hormone for tissue build-up, this could tell part of the story for these five players. If they are experiencing a decrease in testosterone levels the body’s ability to build up tissue to replace and repair the damaged tissue caused from the micro traumas inherent in high level athletic participation could be compromised.

Four of the eight players to show decreased performance on the peak velocity test showed increases in cortisol over resting levels. Some of these increases being rather large percentages. When looking at the magnitude of the some of the increases, it is important to note that when these values were taken it was the day after 5 straight days of activity. There has been literature to support that cortisol responses can be rather large in response to activity and these responses increase as intensity and duration increases (Sgro et al, 2014; Beaven et al, 2011; Hackney & Viru, 1999). Since the collection of the week four samples came on the heels of the large week three training volume that involved more duration and arguably more intensity in it than some of the other weeks these increases can be understood. In addition the baseline (week one) samples were taken at the end of a weekend in which players had no rest so these values should resemble as good a resting cortisol level as is possible in this type of population.

Regarding the four players who showed increases in cortisol and decreases in performance, this could potentially be linked to cortisol actions as a fatiguing agent that favors catabolism (tissue breakdown) in muscle tissue. It is reasonable to make the argument that these increases in cortisol could cause muscle tissue to be in a greater broken down and damaged state than usual and this could contribute in some ways to performance decreases on the peak velocity test. Most concerning of all the players who showed decreases in performance from week one to week four are the two who showed both decreases in testosterone and increases in cortisol. As a
coach, these two athletes give me the most issue because their body is putting itself in a tough spot to be able to effectively rebuild damaged tissue and keep the body healthy as competition begins.

**Exploratory Analysis: Correlations**

No significant correlations were found between variables that had shown the most pronounced changes thought the study. However, although not significant, the relationship between percent changes of free testosterone throughout the study with the change in peak velocity resulted in a moderate to low R-value of .442. Reasoning behind the simple regressions showing no relationships may lie in the fact that this sample size was very small. Perhaps with a larger sample size a significant statistical relationship could be found. Another possibility comes in the fact that some of the changes, especially those of the peak velocity, were also very small. Although very small changes were observed for most of the variables included in this observational study, when it comes to a real world, elite athletic setting; considering such as small duration of the study, any small change could potentially be seen as clinically relevant.

**Conclusion/Future Research**

The purpose of this study was to observe and profile the physiological and performance responses to a four-weeks pre-season strength and conditioning training program. A wealth of data was collected from elite level collegiate basketball players in an environment where collecting data from this type of population is certainly not easy nor available to researchers in the area of human performance. As any study, many limitations may have precluded our ability to better interpret the data collected. Never the less, no one to our knowledge has ever been able to have access to collect biological samples of elite collegiate basketball players, which this study was able to. For future research, a more controlled research environment, a larger sample size, a more flexible data collection schedule are just a few of the recommendations that must be
taken into consideration when attempting to improve the quality of the current study. In conclusion, it can be speculated, that alterations in hydration and testosterone seen in this study, most likely due to changes in training loads throughout the 4-weeks of the pre-season strength and conditioning training, are variables that could be used in future research aimed to better inform coaches on the cumulative effects of training on the performance of their basketball athletes. However, due to some of the study limitations, a more controlled environment must be used to confirm or refute these initial findings of this current study.
Appendix 1.1: Pre-Assessment Guidelines

- Report to testing sessions in a normal hydrated condition (not intentionally under or over hydrating prior to).
- Avoid food and drink that contain artificial coloring at least an hour before saliva samples are produced.
- Do not brush teeth at least 30 minutes prior to saliva sample being produced.
- Rinse mouths 10 minutes prior to saliva sample being produced.
Appendix 1.2: Sleep & Fatigue Questionnaire

Q1) How many hours of sleep did you get last night?
   0-4  5  6  7  8  9  10+

Q2) How well did you sleep last night? (With 1 being not so well and 5 being extremely well)
   1  2  3  4  5

Q3) On a scale of 1-5, how physically sore is your body right now? (With 1 being no soreness and 5 being extreme soreness)
   1  2  3  4  5

Q4) On a scale of 1-5, how generally tired are you right now? (With 1 being not tired and 5 being extremely tired)
   1  2  3  4  5

Q5) On a scale of 1-5, how much anxiety and stress are you feeling right now based on friends, family and/or other personal relationships? (With 1 being no stress or anxiety and 5 being maximal stress and anxiety)
   1  2  3  4  5

Q6) On a scale of 1-5, how stressed do you feel in regards to school work and college classes right now? (With 1 being no stress and 5 being maximal stress and anxiety)
   1  2  3  4  5

Q7) On a scale of 1-5, do you feel any kind of sickness or general health problems right now? (With 1 being no sickness or health issues and 5 being severe sickness and health issues)
   1  2  3  4  5
REFERENCES


