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# CIRCADIAN RHYTHM VARIATION IN ENDOCRINE BIOMARKER RESPONSES TO HIGH-INTENSITY INTERVAL TRAINING IN COLLEGE AGED MALES

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CIRCADIAN RHYTHM VARIATION IN ENDOCRINE BIOMARKER RESPONSES TO HIGH-INTENSITY INTERVAL TRAINING IN COLLEGE AGED MALES

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## Abstract

The purposes of this study were to: 1) to examine the effects of time of day on salivary testosterone (sal-T) and salivary cortisol (sal-C) concentrations following acute bouts of high intensity interval training (HIIT) exercise performed in the morning and evening; and (2) to examine diurnal variations in the T:C ratio responses to HIIT exercise; and (3) to determine if there is a relationship between sal-T and sal-C responses to acute bouts of HIIT exercise and muscle mass. Ten men between the ages of 20-25 years who reside in the Norman or Oklahoma City Metro area were recruited for this study. Eligibility for participation was assessed using medical history and physical activity readiness questionnaire as well as completing the written informed consent and HIPAA agreement. Additionally, a dual energy x-ray absorptiometry (DXA) total body scan assessing bone-free lean body mass, fat mass, and percent body fat as well as a maximal graded exercise test to determine maximal oxygen uptake ($VO_{2peak}$) was performed prior to participation in the intervention sessions. This randomized cross-over design assessment was aimed to determine whether or not HIIT exercise performed in the morning or evening effects sal-T and sal-C concentrations as well as determining if a relationship exists between bone-free lean body mass and the percent changes in the sal-T and sal-C responses. The findings from this study were: significant increases were observed for the sal-T exercise response (PRE to IP); significant decreases were observed for the sal-C time of day response (evening); and significant increases were observed for the T:C ratio time of day response (evening). In conclusion, sal-T and sal-C responses to acute bouts of HIIT exercise were not affected by time of day and no significant relationships existed between bone-free lean body mass variables and percent changes in sal-T and sal-C in this study.

# CHAPTER 1: INTRODUCTION

## Introduction

The sleep and wake cycle within humans is pivotal in determining physiological and behavioral processes during a 24-hour (h) cycle and can be referred to as the circadian rhythm (Farud et al., 2018). Moreover, the circadian rhythms that humans exhibit have internalized over evolutionary time, leading to the synchronization of physiological and behavioral responses to one's external environment (Walker et al., 2020). The circadian clock is located within the suprachiasmatic nuclei (SCN) of the hypothalamus and coordinates rhythms for differing lifestyles and environmental factors (Ahmad et al., 2020). The SCN facilitates these rhythms with the help from melanopsin, photoreceptive ganglion cells located in the retina (Ahmad et al., 2020; Çakmur et al., 2018). Stimulation of the melanopsin cells by natural light will in turn stimulate the nerve pathway in the SCN via the optic nerve (Çakmur et al., 2018). To maintain this biological process, natural light is essential and allows the brain to be reset for a 24 h cycle. Yet, in Western society, natural light exposure has become less prominent due to everchanging lifestyles and societal obligations (Thomas et al., 2020). This decline in natural light exposure has led to a society living in a chronic state of circadian disruption, which is associated with increased risk for obesity and cardiovascular disease (Thomas et al., 2020). Although humans have adapted to the control of circadian rhythms, individuals in the Western society often make use of sleep medication and alarm clocks to align their sleep and wake times (Roennenberg et al., 2012). For example, many college students use alarms clocks to adhere to their daily schedule and experience sleep disturbances throughout the night (Zhang et al., 2019). This has led to college students regularly experiencing circadian disruptions and can be associated with feelings of stress, fatigue, and poor sleep quality (Zhang et al., 2019). In addition, humans also exhibit

daily oscillations for multiple hormones that have their own circadian profiles (Gnocchi & Bruscalupi, 2017). Testosterone and cortisol are two hormones under circadian control with both reaching peak serum concentrations in the early morning and subsiding as the day goes on (Gnocchi & Bruscalupi, 2017; González-Sales et al., 2015). It is well-known now that circadian rhythm and endocrine homeostasis are interconnected and deeply rooted in the behaviors humans perform each day (Gnocchi & Bruscalupi, 2017). Scientists have yet to fully understand why these two hormones are under circadian control. Nevertheless, disruptions to the circadian profiles of testosterone and cortisol can lead to mal-adaptations in endocrine homeostasis and many disorders, including cardiometabolic diseases and cancer (Gamble et al., 2014).

The endocrine system plays a vital role in the regulation of homeostasis and the adaptations that occur from exercise training (Dote-Montero et al., 2021; Hackney & Lane, 2015; Hayes et al., 2010). Chemical biomarkers are commonly assessed in the field of exercise science to investigate the responses of the endocrine system and monitor changes from an exercise intervention. To observe these changes, scientists typically collect biological samples in the form of serum or plasma. However, saliva has become more prominent in the monitoring of biomarkers due to its non-invasive methodology and time needed to collect sample (Hayes et al., 2016; Kilian et al., 2016). Furthermore, saliva has been shown to correlate well with serum values and represents the biologically active or "free" portion of the hormone under investigation (Hayes et al., 2016; Kilian et al., 2016). Two hormones that are commonly investigated are testosterone and cortisol in part to the anabolic and catabolic effects these hormones elicit on certain tissues. Testosterone is a androgenic steroid hormone that exhibits multiple physiological functions within the human body. In males, these functions include regulation of bone mass, fat distribution, and the growth and maintenance of skeletal muscle (Brownlee et al., 2005;

Campbell & Jialal, 2021). Testosterone's ability to promote protein synthesis in skeletal muscle allows for the anabolic nature of the hormone (Kraemer et al., 2020). Conversely, cortisol is the primary glucocorticoid steroid hormone found in the human body and is secreted from the adrenal glands in response to increased physical or psychological stress (Anderson & Wideman, 2017; Brownlee et al., 2005). Cortisol functions to maintain blood glucose levels during exercise by increasing lipid and amino acid mobilization in adipose tissue and skeletal muscle (Brownlee et al., 2005; Campbell & Jialal, 2021; Hayes et al., 2010). The assessment of these two hormones coupled together is used within exercise science due to the biological effects of these hormones during and following exercise and often is expressed as the T:C ratio (Hayes et al., 2010). It is postulated that the T:C ratio is indicative of an anabolic:catabolic state from the protein synthesis to protein degradation rates (Hayes et al., 2010). Although prolonged and chronic increases in cortisol promotes catabolic effects on skeletal muscle, intermittent increases have been shown to promote increases in skeletal muscle recovery and performance as well as sarcolemma repair (Kraemer et al., 2020).

High-intensity interval training (HIIT) is a method of exercise that has become popular in the 20$^{th}$ century due to the time needed to complete, minimal equipment required, and the rapid central and peripheral adaptations like that of continuous endurance exercise (Costigan et al., 2015; Dote-Montero et al., 2021; Li et al., 2022; Monje et al., 2020). When compared to moderate-intensity aerobic exercise, HIIT has been shown to produce greater improvements in maximal oxygen uptake ($VO_{2max}$), cardiac contractility, and endothelial function (Kemi et al., 2005). The adaptations that HIIT can produce have led researchers to believe there are implications for the possibility of preventing the onset of cardiovascular disease (Schoenfield & Dawes, 2009). HIIT consists of alternating high-intensity and low-intensity intervals, with the

high-intensity bouts performed above lactate threshold or close to an individual's $VO_{2max}$ (Schoenfeld & Dawes, 2009). The low-intensity bouts serve as a recovery period for the individual, allowing the body to buffer and clear lactic acid produced during the working intervals (Schoenfeld & Dawes, 2009). There is a plethora of recommended HIIT prescriptions for a variety of populations. For instance, athletic populations are recommended to perform 3-5 minute working intervals with a work-to-rest ratio (W:R) of 1:1 (Reuter & Hagerman, 2008). Whereas, for the general population, it is recommended to use a 1:2 or even 1:4 W:R ratio depending on the fitness level of the individual (Rozenek et al., 2007). HIIT is a very convenient and effective exercise method for populations with limited time due to their lifestyle. It is well-known that the lifestyle of a college student consists of early mornings and long nights, which could pose obstacles when deciding to exercise. Thus, it is relevant to investigate the physiological responses following a HIIT exercise session in the morning and late afternoon to help determine if there are time of day differences.

HIIT has been shown to produce similar health benefits as continuous endurance training and can be very beneficial for individuals lacking time in their day to exercise. College students experience a new lifestyle change and encounter many obstacles regarding exercise methods, mostly due to time constraints. Circadian rhythm has its unique role in determining hormone variation and can greatly affect activities for that given day. Salivary sampling provides a quick and non-invasive method to measure hormone concentration following an exercise intervention. Although HIIT is a popular exercise method today, further research needs to be done to examine the variations in hormone response following a HIIT exercise protocol in the morning versus evening. Therefore, the effects of HIIT in the morning versus evening could be used to determine different training strategies from the assessment of testosterone, cortisol, and the T:C ratio. This

study aims to examine the effect of HIIT on endocrine biomarkers during the morning and late afternoon in college males.

**Purpose**

The primary purpose of this study was to examine the effects of time of day on salivary testosterone (sal-T) and cortisol (sal-C) concentrations following acute bouts of HIIT performed in the morning and evening in college-aged men. The secondary purpose was to examine diurnal variations in the T:C ratio responses to HIIT.

**Research Questions**

1. Does time of day affect salivary testosterone and cortisol responses to an acute bout of HIIT exercise?

2. Does the pattern of the T:C ratio following an acute bout of HIIT exercise vary depending on the time of day (morning vs. evening)?

**Hypotheses**

1. It was expected that the salivary testosterone and cortisol responses to HIIT will be greater in the evening compared to the morning session.

2. It was expected that the evening salivary cortisol response will be greater than the salivary testosterone response, resulting in a lower T:C ratio compared to the corresponding responses to the morning acute bout of HIIT.

**Research Sub-question**

1. Is there a relationship between salivary testosterone and cortisol responses to acute bouts of HIIT and bone-free lean body mass (muscle mass)?

**Sub-hypothesis**

1. There would be a significant positive relationship between percent changes in salivary testosterone and cortisol and bone-free lean body mass.

**Significance of the Study**

Biorhythms dictate hormone concentrations at specific time points throughout the day, which may affect the activities within that 24 h cycle. Therefore, time of day for exercise can affect hormone concentration during and following a HIIT exercise session. Busy individuals like college students are limited on time to exercise, and HIIT can effectively combat this. Additionally, a prolonged increase in cortisol concentration with regard to the T:C ratio could potentially lead to protein catabolism and skeletal muscle degradation. Therefore, the findings from this study may help exercise scientists create effective exercise interventions using HIIT for college males aged 20-25 years that struggle to find time in their day to exercise.

**Delimitations**

1. The findings from this study apply to moderately active, healthy men between the ages of 20-25 years old.

2. Participants will be recruited from the University of Oklahoma.

3. All testing procedures will be performed at the Bone Density Research Lab in the Sarkey's Fitness Center in Norman, Oklahoma.

4. Participants will be limited to individuals who are not taking performance-enhancing drugs/supplements to improve muscle mass or cardiovascular function.

5. Participants will not have any known diseases affecting testosterone and cortisol production.

**Limitations**

1. Participants will volunteer to take part in this study and, therefore may not be representative of the general population.

2. Participants will not include sedentary individuals.

**Assumptions**

1. Participants will give an all-out effort for the test.

2. Participants will only consume water 60 minutes prior to all salivary sample collection.

**Operational Definitions**

1. Anabolism- The sequences of enzyme-catalyzed reactions by which relatively complex molecules are formed in living cells from nutrients with relatively simple structures (McCarthy & Esser, 2010).

2. Bone-free Lean Body Mass (BFLBM)- lean soft tissue, muscle + skin + organs.

3. Catabolism- The sequences of enzyme-catalyzed reactions by which relatively large molecules in living cells are broken down or degraded (McCarthy & Esser, 2010).

4. Cortisol (C) - Steroidal hormone part of the glucocorticoid family that responds to stress and stimulates substrate mobilization or degradation (Brownlee et al., 2005).

5. Circadian Rhythm- The physical, mental, and behavioral changes that follow a 24 h cycle (Brownlee et al., 2005).

6. Fat Mass (FM)- Absolute amount of fat expressed in kg.

7. High-intensity interval training (HIIT) - Alternating work and rest intervals at respective percentages of an individual's estimated maximal heart rate (ACSM, 2014).

8. Homeostasis- Any self-regulating process by which biological systems tend to maintain stability while adjusting to conditions that are optimal for survival (Brownlee et al., 2005).

9. Hormone assay- A blood, saliva, or plasma test to assess endocrine system status (Hayes et al., 2016).

10. Maximal oxygen uptake ($VO_{2max}$)- The maximal amount of oxygen that an individual can uptake and utilize during maximal exercise (Schoenfeld & Dawes, 2009)

10. Percent Body Fat (% BF)- (Fat mass/body weight)100.

11. Salivary sample- a saliva specimen used for research (Hayes et al., 2016).

12. Testosterone (T) - Androgenic steroidal hormone that stimulates protein synthesis rates and anabolism (Brownlee et al., 2005).

12. T:C ratio- The ratio between testosterone and cortisol suggests anabolic: catabolic processes (Cofré-Bolados et al., 2019).

# CHAPTER 2: REVIEW OF LITERATURE

The human body experiences changes in homeostasis when the biological clock or circadian rhythm is disrupted. Furthermore, metabolism can be referred to as the sum of all chemical processes that occur in the human body. Testosterone and cortisol are two hormones that exert anabolic and catabolic effects. There is a large body of research detailing the variations in the resting serum concentrations of these hormones throughout a 24 h cycle. Evidence shows that physical exercise leads to alterations in both  and cortisol levels. However, the effect of high-intensity interval training (HIIT) and time of day on salivary hormone concentrations should be further investigated. New insights could lead to limiting catabolic effects regarding the T:C ratio following a HIIT exercise session in college males.

The endocrine system has profound regulatory effects in response to physical exercise, which helps maintain various biological processes that occur in the human body (Hackney & Lane, 2015; Lee et al., 2017). It has been well documented that physical exercise disrupts homeostasis, leading to adaptations in a human's physiology (Fragala et al., 2011; Hackney & Lane, 2015; Viru & Viru, 2001). This is done via chemical messengers or hormones on target tissues that experience an increase in physiological demands from exercise (Fragala et al., 2011). The response of these hormones to physical exercise can be characterized by the rate of hormone responses, threshold intensity, threshold duration, and the training adaptations produced (Hackney & Lane, 2015). Furthermore, the maintenance of energy metabolism, fluid and electrolyte balance, and growth and development are among the key factors exercise scientists are interested in investigating. Numerous studies have investigated the hormonal response following resistance and endurance exercise protocols (Hackney & Lane, 2015; Kraemer & Ratamess, 2005; Kraemer et al., 2020). HIIT has emerged as a new method of exercise

associated with several health prevention benefits and in terms of the adaptations produced, which are similar to both resistance and endurance exercise (Wahl, 2013). For example, the acute hormonal response appears to be crucial for tissue growth and remodeling following a resistance exercise protocol, which is thought to be attributed to the high mechanical stimuli of the exercise (Kraemer & Ratamess, 2005). HIIT protocols, specifically Wingate-based training, appear to be similar to resistance exercise protocols when comparing the power output of the two modes of exercise (Wahl, 2013). Moreover, HIIT stimulates increased lactate production and lowers pH, which is crucial for endurance training adaptations and the acute hormonal response (Wahl, 2013). So, one can speculate that the high mechanical and metabolic stimuli associated with HIIT can lead to the acute hormonal response from the endocrine system and, therefore, the training adaptations produced.

The following literature review will provide more insight into the regulatory functions within the human body and how exercise may affect these functions. The following review of literature will cover circadian rhythm, cortisol responses to aerobic exercise and HIIT, testosterone responses to aerobic exercise and HIIT, and salivary hormone measurements.

**Circadian Rhythm**

Located in the hypothalamus, the master clock or suprachiasmatic nucleus (SCN) regulates all the biological clocks within the human body (Ahmad et al., 2020; Çakmur, 2018; Walker et al., 2020). Photoreceptor organisms like humans will experience circadian rhythms in response to the light-dark cycles and adjust sleep-wake cycles accordingly (Çakmur, 2018; Walker et al., 2020). The peripheral tissues will receive refinements coordinated by the SCN, which is regulated by clock genes and under autonomous control (Saner & Lee, 2020; Walker et al., 2020). These adaptations are vital for preserving life and ultimately dictate behavior for a 24

h cycle (Sieck, 2016). Human beings often display preferred activity due to their innate circadian rhythm and can be classified as Morning-type, Evening-type, or Neither-type (Natale & Cicogna, 2002). Recent research has focused on Morning and Evening-type individuals to investigate differences in hormone secretion and exercise performance (Bailey & Heitkemper, 2001; Rossi et al., 2015). Circadian rhythm disruptions can lead to maladaptations in homeostasis and may lead to cardiometabolic diseases throughout life (Poggiogalle et al., 2018).

Among the vast number of hormones in the human body, cortisol and testosterone are two that exhibit diurnal oscillations. Although circadian rhythmic hormone oscillations may affect resting hormone concentrations, these fluctuations in hormone secretion allow for peak values reached at specific time points throughout the day. For example, cortisol has been shown to peak between 7:00-8:00 a.m., and testosterone secretion reaches a peak between 7:00-10:00 a.m. (Gnocchi & Bruscalupi, 2017). It is thought that the rise and peak in cortisol and testosterone during these hours of the day may be due to the catabolic and anabolic nature of the two hormones. A study conducted by Hayes et al. (2012) investigated the diurnal variations of salivary cortisol and testosterone in eighteen healthy male college students. This study was two days long and consisted of saliva collection starting at 8:00 a.m. and every 60 minutes over a 12-hour waking period. Identical meals were given at 8:05 a.m., 1:05 p.m., and 6:05 p.m., and refraining from alcohol, caffeine, and exercise 24 hours before the investigation. The authors found significantly elevated salivary cortisol concentration between 8:00-9:00 a.m., but no difference was observed for salivary testosterone (Hayes et al., 2012).

Environmental factors or time cues provide information to clock genes that lead to alterations in the circadian rhythm (Kemler et al., 2020). Although light exposure is the most common environmental time cue responsible for influencing circadian rhythms, recent

discoveries indicate exercise is a valid cue and should be considered (Kemler et al., 2020). Teo et al. (2011) investigated circadian rhythms in exercise performance with regard to hormonal and muscular adaptation. The authors found that circadian rhythms have a major effect on physical performance during the early evening, which is postulated to be attributed to the peak in core body temperature observed at this time. For example, an increase in force production during early evening strength training exercises has been observed compared to those performed in the morning, even with an extended warmup (Teo et al., 2011). However, the diurnal fluctuations in hormone concentration do not appear to influence exercise performance (Teo et al., 2011). Although there is little evidence to provide on whether physical exercise influences the circadian profiles of cortisol and testosterone, some researchers have suggested that implementing time-of-day specific training could modify the resting hormone levels and concentration during exercise (Teo et al., 2011).

**Cortisol Response to Aerobic Exercise**

The hypothalamic-pituitary-adrenal axis (HPA) regulates the synthesis and secretion of glucocorticoids (Viru & Viru, 2001). Cortisol is a glucocorticoid steroid hormone that has rapidly increased in the blood under stressful conditions and can mobilize energy lipolysis in adipose tissue and proteolysis in skeletal muscle (Hackney & Lane, 2015; Mangine et al., 2018; Viru & Viru, 2001). Several factors are associated with how cortisol concentration will be altered during exercise, including the number of active receptors in the target tissue, plasma hormone concentration, and quantity of transport proteins (Thau et al., 2022). Cortisol, with its lipid-soluble properties, will bind to specific protein receptors in the target cell's cytoplasm, creating a cortisol-receptor complex (Thau et al., 2022). This cortisol-receptor complex will then be taken to the cell's nucleus, where gene transcription is affected (Thau et al., 2022). A decrease in

plasma volume during exercise will increase cortisol concentration due to the decreased capacity and affinity for binding transport proteins in the plasma. This then allows more "free" cortisol to bind receptors in the cytoplasm (Thau et al., 2022). However, plasma volume shifts due to exercise can be "corrected" to obtain the most accurate hormone assessment. Acute aerobic exercise can be a potent stimulus for cortisol secretion and has been shown to increase due to the intensity and duration of the exercise session (Hackney & Lane, 2015).

Hill et al. (2008) investigated the cortisol response in serum following a 30-minute aerobic exercise session at intensities of 40, 60, and 80% of $VO_{2max}$ in twelve moderately trained men on a cycle ergometer. Diet was controlled by at least 50% of daily caloric intake being carbohydrates and performing each experimental session in 4-hour post-prandial state. Circadian hormone fluctuations were controlled by standardizing the time of day for each exercise session. A 30-minute resting-control session was used and randomly assigned to a sub-sample of the subjects mimicking the experimental sessions. A significant increase in circulating cortisol was observed following the 60% and 80% exercise intensity sessions but not in the 40% exercise intensity session or in the resting-control session. Additionally, a significant difference was observed between the 60% and 80% exercise intensity sessions, with the highest intensity evoking the highest cortisol response. This study's findings further support that cortisol production depends on the intensity of the aerobic exercise session (Hill et al., 2008).

Hew-Butler et al. (2008) investigated cortisol responses in serum following a 60-minute steady-state exercise (SSE), high-intensity interval exercise to exhaustion (HIIE), and a 56 KM prolonged endurance exercise session in seven endurance-trained athletes. Diurnal variations in cortisol were controlled by performing the exercise sessions at the same time of day. Subjects were asked to consume a similar diet and to refrain from any vigorous activity 24-hours prior to

exercise sessions. The main finding from this study was that there was no significant increase in cortisol concentration following SSE or HIIE. However, a significant increase was observed following the prolonged endurance exercise session. Although exercise intensity is the primary catalyst for cortisol secretion during aerobic exercise, these findings further support that the duration of an exercise session is another contributing factor to cortisol secretion (Hew-Butler et al., 2008).

**Cortisol Response to HIIT**

HIIT has gained interest from exercise scientists in recent years and has been shown to improve cardiorespiratory health, endothelial function, substrate utilization, and hormonal responses (Dote-Montero et al., 2019). The cortisol response following an acute HIIT time of day-dependent exercise session remains unclear, though findings from previous studies provide a better understanding of the neuroendocrine response that can, in turn, lead to positive adaptations (Cofré-Bolados et al., 2019). Cofré-Bolados et al. (2019) investigated plasma cortisol responses following a 20-minute HIIT exercise bout in thirteen active young men (Cofré-Bolados et al., 2019). All conditions: a) 20-minute resting-control (CON); b) 20-minute high-intensity interval training (HIIT); c) 20-minute steady-state aerobic exercise (AEE) was performed at the same time of day (7:30 p.m.) to minimize diurnal variations. The authors found a significant increase from pre to 12 hours post following HIIT and CON. The increase of 12 hours post in CON can be partly attributed to the circadian oscillations cortisol exhibits during the morning. Therefore, it is not possible to rule out that the circadian oscillations could have been attributed to the significant increase in salivary cortisol 12 hours post following HIIT (Cofré-Bolados et al., 2019). Monje et al. (2020) examined the salivary cortisol response following an acute bout of HIIT in twenty endurance athletes with a 4:2 minute W:R ratio as the protocol and consisted of

ten total working bouts. Diet was controlled by providing a standardized diet for breakfast and lunch consisting of 125 grams of carbohydrates and 21 gram of protein on the day of the intervention. Diurnal variations in cortisol were controlled by performing the exercise sessions between 3:00-6:00 p.m. and performing the HIIT protocol in a 2-hour post-prandial state. The authors found a significant increase in salivary cortisol following the HIIT protocol, suggesting that the HIIT protocol used in this study can be an effective exercise method to elicit a salivary cortisol response. Another interesting finding was that T:C ratio following HIIT was stable, indicating that the anabolic:catabolic balance was not disturbed (Monje et al., 2020).

**Testosterone Response to Aerobic Exercise**

The function of testosterone has effects on many tissues throughout the body and is regulated by the hypothalamic-pituitary-gonadal axis (HPG) (Küüsma et al., 2016). Testosterone is an androgenic steroid hormone secreted by the testes in men and is responsible for skeletal muscle hypertrophy and increased expression of aerobic enzymes (Kilian et al., 2016). Once testosterone enters the target cell, it will bind to androgen receptors located in the nucleus or can be reduced to 5a-dihydrotestosterone (DHT) in the cytoplasm (McEwan et al., 2000). If bound to the androgen receptor, using chaperone protein complexes or heat shock proteins will cause a disassociation in the cytoplasm, ultimately leading to a conformational change in the receptor (McEwan et al., 2000). This results in the translocation of the receptor to the nucleus, where gene transcription can occur and consequently lead to protein synthesis (McEwan et al., 2000). Like cortisol, factors such as the number of receptors in the target tissue, plasma hormone concentration, and quantity of transport proteins will alter the testosterone concentration following exercise. Furthermore, the intensity of aerobic exercise appears to be the variable that induces the largest change in testosterone concentration following exercise.

Hough et al. (2011) investigated the effects of a 30-minute aerobic exercise on salivary and plasma testosterone elevations in ten recreationally active males using a cycle ergometer. Three endurance protocols were tested: a) Continuous cycle to fatigue at 75% W max (FAT); b) one minute 60%, one minute 90% W max for 30-minutes (60/90); c) one minute 55%, four minutes 80% W max for 30-minutes (55/80). Diet was controlled by a standardized breakfast tracked with a food diary 4-hours before the exercise sessions, and subjects remained fasted until the sessions were complete. Diurnal variations were controlled by performing the tests at the same time of day during the months May-August. The authors found significant increases in salivary and plasma testosterone increases from pre to post-exercise following the 60/90 and 55/80 protocols. However, the 55/80 protocol elicited the greatest increase and prolonged elevation in salivary and plasma testosterone compared with the other protocols (Hough et al., 2011). From these findings, the 55/80 protocol could be a useful HIIT protocol when researchers seek to examine salivary testosterone concentrations following an exercise session using a cycle ergometer in healthy, recreationally active males.

Another study conducted by Hackney et al. (2012) investigated the response of serum-free testosterone following high-intensity interval and steady-state exercise in fifteen endurance-trained males using a treadmill. A 45-minute resting-control session (CON), 42-47 minutes alternating 90-sec at 100-110% and 90-sec at 40% of $VO_{2max}$ interval exercise session (IE), and 45 minute at 60-65% of $VO_{2max}$ steady-state endurance exercise session (SSE) was used. Diet was controlled by a standardized 60% carbohydrate diet and required subjects to be in a fasted state prior to each session. Additionally, abstaining from sexual activity, exercise training, and excessive emotional stress 24-hours prior to each session was required. Diurnal variations were controlled by performing each session at the same time of day and corresponded with the

subject's typical exercising period. The authors found that free testosterone significantly increased from pre to post in the SSE and IE groups compared to the CON group. However, free testosterone in the SSE session 12-hours post-exercise was not suppressed like that of the IE session. Although free testosterone 12 post-exercise in the IE session was significantly suppressed, this session had the greatest increase immediately post-exercise compared to both the SSE and CON sessions (Hackney et al., 2012).

**Testosterone Response to HIIT**

It has been established that testosterone concentration increases in the body following physical exercise (Kraemer & Ratamess, 2005; Cofré-Bolados et al., 2019; Hackney & Lane, 2015). However, HIIT is still under question as to whether it provides enough stimulus to elicit a testosterone response post-exercise. Cofré-Bolados et al. (2019) investigated free testosterone response in the plasma following HIIT and continuous aerobic exercise (AEE) in 13 recreationally active college males. Three sessions consisting of a 20-minute resting-control (CON), 20-minute HIIT exercise, and 20 minutes AEE was used. The HIIT exercise protocol was performed in 15-sec intervals alternating between 110% and 40% VO2max and the AEE protocol was performed continuously at 70-75% $VO_{2max}$. Diet was controlled by a standardized 60% carbohydrate diet, and performing each session in a fasted state was required. Additionally, refraining from sexual activity and physical exercise 24 hours prior to each session was required. Time of day variations in free testosterone was controlled by performing each session at 7:30 p.m. on non-subsequent days. The authors found significant increases in free testosterone immediately post-exercise for both the HIIT and AEE sessions. However, the HIIT session produced the greatest increase in free testosterone immediately post-exercise compared to the CON and AEE sessions. Another interesting finding was that although free testosterone

increased following AEE and HIIT, the T:C ratio remained unchanged, suggesting that both exercise modalities did not provide a sufficient stimulus to affect anabolic processes (Cofré-Bolados et al., 2019).

**Salivary Hormone Measurements**

Salivary sampling provides a convenient and non-invasive method to obtain hormone concentration. This method of sampling has allowed for faster handling and process times and requires minimal expertise compared to blood sampling. Unlike serum or plasma sampling, saliva sampling procedures include approximately 1.8 mL of a saliva specimen using the drool method and placed into 2 mL cryovials for storage. Biological and technical errors can occur in salivary sampling ranging from gender, training status, age, mouth position, and sample storage. Furthermore, an enzyme-linked immunosorbent assay (ELISA) is the primary method to obtain salivary hormone concentration. While salivary sampling is rarely used in clinical settings, exercise scientists have adopted this new method which provides an accurate measurement like that of blood sampling (Hayes et al., 2016). The steroid hormones cortisol and testosterone represent the "free" or non-protein bound concentration when examining saliva concentrations, which are readily available to target tissues (Crewther et al., 2010). Additionally, Gozansky et al. (2005) found that salivary testosterone and cortisol are indicative of the biologically active cortisol found within the blood (Gozansky et al., 2005). Thus, sal-T and sal-C have been validated against blood concentrations and are widely used by researchers to obtain a reliable measurement following an exercise intervention.

**Summary**

The circadian rhythm provides organisms a sleep-wake cycle and time of day hormone secretion. The circadian oscillations in testosterone and cortisol have been shown to affect

exercise performance to some degree. Steps still need to be made to discover if exercising at a specific time of day will potentially alter the circadian profile of testosterone and cortisol or the T:C ratio. The endocrine system and SCN work side by side to provide the appropriate responses and adaptations to the environment imposed by daily behaviors and physical exercise. While aerobic exercise literature investigating hormonal responses is immense, HIIT has emerged in the past 20 years as a new and unique exercise method. HIIT has been shown to produce similar physiological benefits to continuous aerobic training and attracts many people with the amount of time needed to complete a single bout of HIIT. Saliva sampling has also emerged as a new and non-invasive method to obtain hormone concentration through ELISA techniques and correlates to blood and plasma concentrations. Determining time of day differences when examining sal-T, sal-C, and the T:C ratio while maintaining or improving the circadian profile of testosterone and cortisol is still unclear after an acute bout of HIIT exercise. Additional research is needed to investigate the biological rhythm of testosterone and cortisol circadian profiles.

# CHAPTER 3: METHODOLOGY

The primary purpose of this study was to examine the effects of time of day on salivary testosterone (T) and cortisol (C) concentrations following acute bouts of HIIT performed in the morning and evening in college-aged men. The secondary purpose was to examine diurnal variations in the T:C ratio responses to HIIT.

## Participants

Ten college males from the OU-Norman campus completed all study visits. A *priori* power analysis was performed with G*Power (v 3.1) to estimate the sample size for this study. Cofré-Bolados et al. (2019) examined plasma free testosterone and cortisol concentrations following an acute bout of HIIT. Based on their findings, the effect size for plasma cortisol is 1.54 (Cohen's d) and 1.06 (Cohen's d) for plasma free testosterone and will require 6 and 10 participants receptively for 80% power (Cofré-Bolados et al., 2019). Monje et al. (2020) examined salivary testosterone and cortisol concentration following an acute HIIT exercise session. Based on their findings, the effect size for salivary cortisol is 1.12 (Cohen's d) and 0.12 (Cohen's d) for salivary testosterone and required 9 and 532 participants, respectively, for 80% power (Monje et al., 2020). Due to feasibility, the sample size chosen for this study was 10 participants. Experimental procedures were approved by the Institutional Review Board at the University of Oklahoma. All participants provided written informed consent prior to completing a health history questionnaire, a Physical Activity Readiness Questionnaire (PAR-Q), and a physical activity questionnaire (IPAQ). Participant's height and weight were assessed using a wall stadiometer and a digital scale. Inclusion criteria for participants were: 1. males between the ages of 20-25 yr; 2. those who self-report an average of 150 minutes of moderate physical activity per week; and 3.those with no HIIT participation in the past 3 months. Participants were

excluded from the study based on the following criteria: 1. if there was a history of cardiovascular or pulmonary issues; 2. taking any prescription medication that affects concentrations of testosterone or cortisol; 3. taking any performance-enhancing drug or supplements, including anabolic steroids or blood doping techniques; 4. body mass over 300 lbs; 5. height over 6'4; 6. regularly consumed any form of tobacco in the past 3 months; and 7. any joint replacement or metal implants in the legs, hips, or spine. Participants were instructed to refrain from heavy exercise 24 hours prior to exercise visits and any alterations in their diet or sleep habits over the course of this study.

**Research Design**

This study incorporated a two factor within-subjects repeated measures design (time of day × exercise time point) to investigate changes in testosterone and cortisol responses to a single HIIT exercise session performed in the morning (8:30-10:00 a.m.) and evening (5:00-6:30 p.m.). The test order was randomly assigned. On the first visit, participants were asked to complete a HIPAA release form and a written informed consent. A Dual Energy X-ray Absorptiometry (DXA) total body scan was taken on this visit to determine body composition varibales. For the second visit, participants were asked to complete a maximal graded exercise test on the treadmill to establish $VO_{2peak}$. This determined the percentages used for the work and recovery intervals during the HIIT exercise sessions. The third visit served as the control day and salivary samples were taken in the morning (8:30-10:00 am) and evening (5:00-6:30 pm) to establish baseline values for the endocrine biomarkers under investigation. For the fourth and fifth visits, participants were asked to provide a saliva sample prior to each HIIT exercise session (PRE) followed by immediately post HIIT exercise session (IP). A flowchart of procedures is presented below.

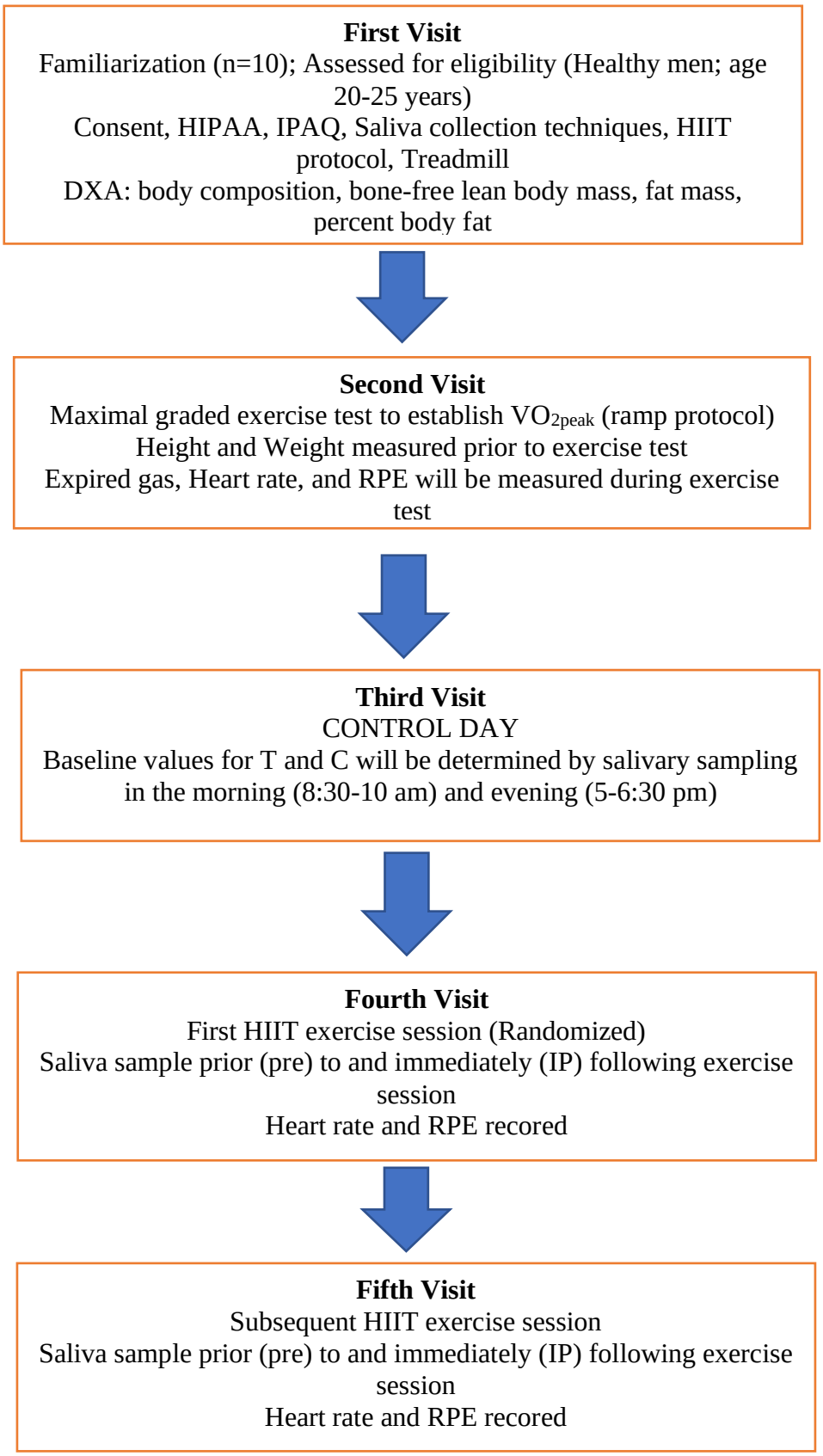



**Figure 1. Research Design**

**Familiarization**

Participants were familiarized with the treadmill, HIIT protocol, and saliva sampling procedures to better understand what will be expected from them. The purpose of this session was for the participants to become accustomed to each step of the respective procedures and to follow them in the correct order. During this session, the participants provided informed consent, were introduced to the treadmill, and a DXA total body scan was administered. The participants were informed that they will be required to provide a saliva sample before exercise and immediately post but at least understand the procedures for salivary sampling. This day helped to minimize researcher and experimenter technical errors throughout the study.

**Dual Energy X-ray Absorptiometry (DXA)**

A total body scan was performed to assess body composition, including bone-free lean body mass (BFLBM), fat mass (FM), and percent body fat using Dual Energy X-ray Absorptiometry (DXA; Lunar Prodigy, GE Medical System, Madison, WI). Scans were analyzed using the enCORE v 16 software (GE Healthcare, Madison, WI). For quality assurance, a standardized block with known densities was scanned prior to each day of testing. Participant's height and weight was assessed using a digital scale and wall stadiometer. Participants were then asked to remove their shoes, excess clothing, any metal accessories, and other attenuating materials. The scan took place once the participants were in the proper supine position on the DXA table with the head about 2-3 cm below the horizontal line at the top of the table. Participants legs, hips, and shoulders were aligned to the center of the table while their arms remaind close, but not touching, their body. Participants were asked to straighten their fingers as their thumbs hold and face the ceiling for the entirety of the scan. Participants legs were held close together using two Velcro straps located just below the knee joint and the other at the ankle

joint. The same trained technician conducted all scans. In the Bone Density Research Laboratory, the precision values, reported as root mean square coefficients of variation (RMS CV%), for the body composition variables, are: 2.5% for percent body fat, 1.39% for BFLBM, and 2.74% for FM.

**Maximal Aerobic Capacity Test**

The maximal aerobic exercise test was conducted on the second visit in order to establish working and recovery intervals for the HIIT protocol. The participants were advised to not perform any strenuous exercise activity 24 hours prior or the day of test**.** Participants were informed to not consume alcohol the day before or of visit. Lastly, participants were advised to eat something rich in carbohydrates and protein at least 2 hours before visit and to be adequately hydrated. The participants were given a 2 minute warmup at 2.5 mph before test began. The protocol used was the ACSM incremental speed test which began at 4 mph and increased 1 mph every minute (ACSM). Gas and flow-meter calibration preceeded all exercise testing and The PARVO Metabolic Cart was used to measure $VO_{2peak}$ for both relative and absolute values. The mask used was the Hans Rudolph and the treadmill used was the WOODWAY Pro. Variables including heart rate, RPE, $VO_{2peak}$, and time to exhaustion were recorded. Participants were advised to motion a thumbs down signal when they could not go any longer and a cool down phase was utilized until participants heart rate fell below 120 beats per minute.

**Salivary Sample Collection and Hormone Assays**

The saliva samples were collected from participants on the third visit to serve as a control day for baseline salivary hormone concentrations and prior to each HIIT exercise session (PRE) and immediately post HIIT exercise session (IP). The participants were informed that they could not eat, brush their teeth, or drink alcohol 24 hours prior to testing. Every participant used the

SalivaBio Collection Aid and perform the passive drool method for sample collection. Each sample was collected in a 1.8 mL SalivaBio cryovial and took approximately 30 minutes to collect. Participants were asked to provide a full vial of saliva, but if they could not, then what they could produce was taken for assessment. The saliva samples were stored in the Bone Density Research Lab and were used to determine testosterone and cortisol hormonal concentrations. Upon collection, samples were placed in a freezer at or below -80 C within four hours and may be stored up to 6 months. Samples were thawed to room temperature, vortexed, and then centrifuged for 15 minutes at approximately 3,000 RPM (1,500 x g) immediately before performing the assay at the Salivalab West in Carlsbad, California. Samples were tested for salivary testosterone using a high sensitivity enzyme immunoassay (Cat. No. 1-2402) and salivary cortisol (Cat. No. 1-3002). Samples test volume was 25 of saliva per determination. The salivary testosterone assay had a lower limit of sensitivity of 1 pg/ml, a standard curve range from 6.1-600 pg/ml, and an average intra-assay coefficient of varation of 4.60%, and an average inter-assay coefficient variation 9.85%. The salivary cortisol assay had a lower limit of sensitivity of 0.007 ug/dl, a standard curve range from 0.012-3.0 ug/dl, and an average intra-assay coefficient of variation of 4.60%, and an average inter-assay coefficient of variation of 6.00%. Both salivary testosterone and cortisol kits that were used are The Salimetrics® Salivary Cortisol Assay Kit (Cat. No. 1-3002) and The Salimetrics® Salivary Testosterone Assay Kit (Cat. No. 1-2402), without modifications to the manufacturer's protocol.

**HIIT Protocol**

One week following the familiarization day, the first HIIT session was completed and designed to induce testosterone and cortisol responses following exercise. A warmup of 5 minutes at 25% of $VO_{2peak}$ was used prior to the HIIT protocol. The HIIT protocol used the 1:2

W:R ratio and each participant completed 1 minute of running followed by 2 minutes of recovery, which is recommended for the general population (Rozenek et al., 2007). The working intervals were set at 81% of participants $VO_{2peak}$ and the recovery intervals were set at 40% of participant's $VO_{2peak}$ (Schoenfeld & Dawes, 2009). The total duration of the HIIT protocol was 20 minutes starting directly after the 5-minute warmup and consisted of seven working and seven recovery bouts. Heart rate was monitored by use of polar heart rate monitors. The participants reported their rating of perceived exertion (RPE) during the recovery periods and were instructed to rate the last completed working interval using the Borg scale. Once the participants completed the HIIT protocol, a 5-minute cool down at 25% $VO_{2peak}$ concluded the test.

**Data Analyses**

Data is presented as mean ± standard deviation (SD) in the tables and mean ± standard error (SE) in the figures. Dependent variables were checked for normality using the Shapiro-Wilks test. Only one variable was not normally distributed, PRE sal-C AM ($p = 0.038$), therefore all data were analyzed using parametric statistics. Paired sample t-tests were used to determine time of day differences for Final RPE, Final HR, control day sal-T and sal-C, and percent changes in sal-T and sal-C in response to the acute HIIT protocol. To determine the effects of time of day (morning vs. evening) on salivary hormone responses to acute bouts of HIIT exercise, two-way repeated-measure analysis of variance (ANOVA) (time of day × exercise time point) with Bonferroni post hoc tests was used. Pearson product-moment correlation coefficients (r) were used to determine relationships between body composition variables and percent changes in hormone responses. Statistical analysis was performed using SPSS v. 28 (SPSS Inc., Chicago, IL, USA) with the level of significance set at $p \leq 0.05$.

# CHAPTER 4: RESULTS & DISCUSSION

The purposes of this study were to: (1) examine the effects of time of day on salivary testosterone (T) and cortisol (C) concentrations following acute bouts of HIIT performed in the morning and evening in college-aged men; and (2) examine diurnal variations in the T:C ratio responses to HIIT. Relationships between body composition variables and percent changes in sal-T and sal-C also were investigated.

## Participant Characteristics

There were a total of 12 healthy college aged men who were enrolled in this study. Two participants were excluded for using prescription medication affecting the hormones under investigation or recent participation in HIIT exercise training. A total of 10 participants attended every testing visit and were used in the final data analysis. Descriptive characterisitcs of participants are shown in Table 1. Data are presented as mean ± standard deviation for age, height, weight, DXA measurements, and weekly metabolic equivalent of tasks.

**Table 1. Anthropometric and DXA Measurements (n=10) (mean ± SD)**

| Variables | Mean ± SD |
|---|---|
| Age (yr) | 23.15 ± 1.35 |
| Height (cm) | 176.95 ± 6.32 |
| Weight (kg) | 81.94 ± 11.19 |
| Percent Body Fat (%) | 20.48 ± 7.21 |
| BFLBM (kg) | 62.98 ± 10.92 |
| Fat Mass (kg) | 16.17 ± 6.05 |
| Fat-free Mass (kg) | 66.13 ± 11.31 |
| Arm BFLBM (kg) | 8.27 ± 1.87 |
| Leg BFLBM (kg) | 21.16 ± 3.86 |
| Trunk BFLBM (kg) | 29.79 ± 5.22 |
| IPAQ score (MET-min/week) | 2397.75 ± 2468.73 |

cm = centimeters: kg = kilograms: BFLBM = bone free lean body mass: IPAQ = International Physical Activity Questionnaire: yr = year

**Maximal Graded Exercise Test**

Data from 10 participants were used in the final data analyses for the maximal graded exercise test Table 2. Only one participant was able to reach their age predicted maximal heart rate and no participants were able to achieve a plateau in $VO_2$. However, the maximal RPE recordings showed near maximal working capacity (>17 RPE) for participants and all but one participant were able to achieve a respiratory exchange ratio (RER) of >1.10. The fitness levels of the participants were deemed to be Fair according to ACSM criteria for $VO_{2max}$ (ACSM, 2010). The working and recovery intervals were determined by using a percentage (81% for

Work Speed, 40% for Recovery Speed) of participants last mile per hour performed during the maximal exercise test.

**Table 2. Physiological Responses to the Maximal Graded Exercise Test (n=10) (mean ± SD)**

| Variables | Mean ± SD |
|---|---|
| $VO_2$peak (L/min) | 3.50 ± 0.72 |
| $VO_2$peak (mL/kg/min) | 42.64 ± 6.13 |
| Maximal RPE | 18.10 ± 1.97 |
| Maximal HR (bpm) | 189.70 ± 9.32 |
| Maximal Speed (mph) | 9.20 ± 0.92 |
| Work Speed (mph) | 7.46 ± 0.74 |
| Recovery Speed (mph) | 3.68 ± 0.37 |

$VO_2$peak = maximal oxygen consumption that did not reach a steady state: L = liters: kg = kilograms: RPE = ratings of perceived exertion: mph = miles per hour: HR = heart rate: bpm = beats per minute

**Physiological Responses to HIIT Sessions**

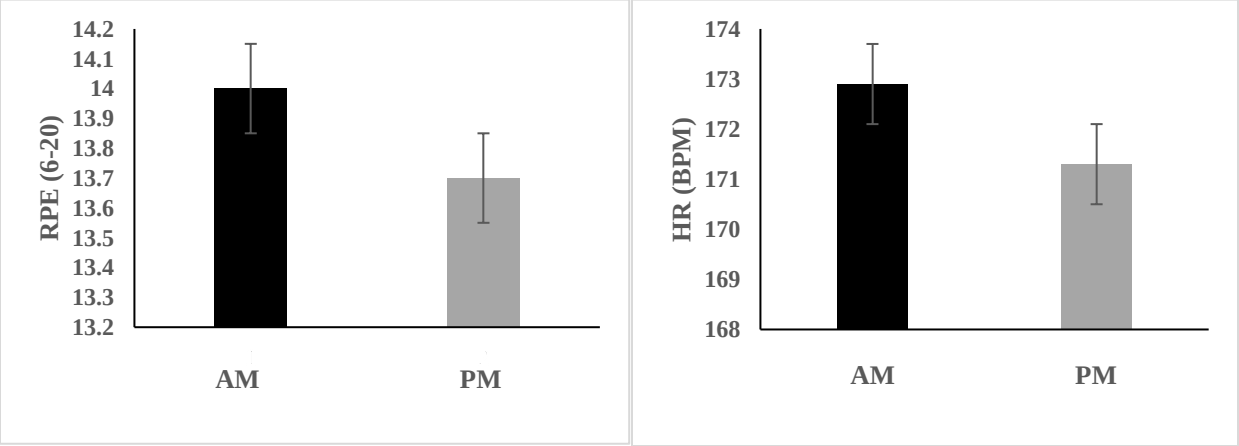
Data from 10 participants were included in the physiological responses to the HIIT sessions and are presented as mean ± standard deviation for RPE and HR in Table 3. Data are presented as mean ± standard error for Final RPE in the morning and evening, and Final HR in the morning and evening in Figure 2. No significant differences were observed between the morning and evening Final RPE ($p = 0.664$) or the morning and evening Final HR ($p = 0.494$). The paired samples t-test showed a Cohen's (*d*) effect size of 0.142 for Final RPE and 0.226 for Final HR. This indicated that there was a small effect size for Final RPE and a medium effect size for Final HR between the morning and evening.

**Table 3. Ratings of Perceived Exertion and Heart Rate at the end of Morning (AM) and Evening (PM) HIIT sessions (n=10) (mean ± SD)**

| Variables | Mean ± SD |
|---|---|
| Final RPE AM | 14.00 ± 1.94 |
| Final RPE PM | 13.70 ± 2.41 |
| Final HR AM (bpm) | 172.90 ± 9.72 |
| Final HR PM (bpm) | 171.30 ± 13.18 |

bpm = beats per minute: RPE = rating of perceived exertion: AM = ante meridiem: PM = post meridiem.

**Figure 2. Final Ratings of Perceived Exertion and Final Heart Rate for the Morning (AM) and Evening (PM) HIIT Sessions (n=10) (mean ± SE)**




RPE = rating of perceived exertion: HR = heart rate: AM = ante peridiem: PM = post peridiem: BPM = beats per minute

**Hormonal Responses Acute HIIT Session**

Data from 9 participants were used in the repeated measures data analyses for hormone responses, as the pre-test saliva sample for one participant was determined by the company to be

insufficient volume to assay. Salivary testosterone (sal-T) and cortisol (sal-C) were assayed using The Salimetrics® Salivary Cortisol Assay Kit (Cat. No. 1-3002) and The Salimetrics® Salivary Testosterone Assay Kit (Cat. No. 1-2402), without modifications to the manufacturer's protocol.

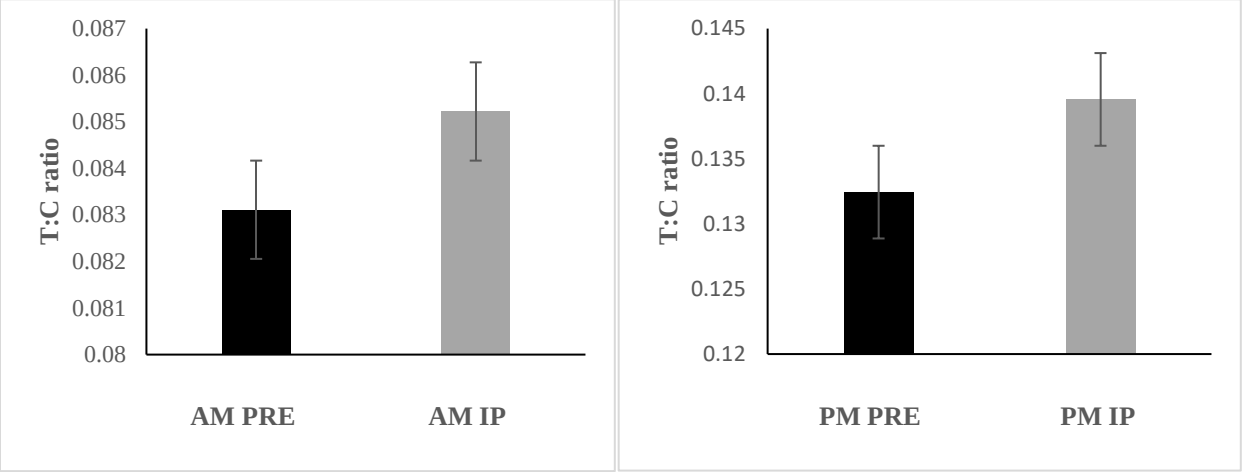
Mean sal-T and sal-C data PRE and IP exercise in the morning and evening are presented as mean ± standard deviation in Table 4. The T:C ratio PRE to IP exercise is presented in Figure 3 for both the morning and evening sessions. No significant differences were observed in sal-T for time of day main effect ($p = 0.179$) or time × exercise interaction effect ($p = 0.223$); however, significant increases in sal-T were observed PRE to IP for both the morning and evening exercise sessions ($p = 0.041$). There was a significant time of day main effect for sal-C ($p = 0.029$) with the evening session showing lower sal-C concentrations for both PRE and IP time points compared to the morning session. There was no significant exercise main effect ($p = 0.503$) or time of day × exercise interaction effect ($p = 0.696$) for sal-C. No significant differences were observed in the T:C ratio PRE to IP exercise for both the morning and evening exercise sessions ($p = 0.773$). However, significant differences were observed in the T:C ratio for the exercise time of day ($p = 0.013$).

**Table 4. Salivary Hormone Responses to Acute HIIT Performed in the Morning versus Evening (n=9) (means ± SD)**

| | AM | | PM | | Time of Day Effect | Exercise Effect | Time × Exercise Effect |
|---|---|---|---|---|---|---|---|
| Variable | PRE | IP | PRE | IP | (p) | (p) | (p) |
| Sal-T (pg/mL) | 274.34 ± 91.39 | 292.16 ± 87.65 | 233.83 ± 70.50 | 281.47 ± 93.78 | 0.179 | 0.041* | 0.223 |
| Sal-C (µg/dL) | 0.463 ± 0.348 | 0.491 ± 0.380 | 0.210 ± 0.110 | 0.297 ± 0.244 | 0.029* | 0.503 | 0.696 |
| T:C ratio | 0.083 ± 0.044 | 0.085 ± 0.043 | 0.132 ± 0.056 | 0.140 ± 0.073 | 0.013* | 0.773 | 0.742 |

SD = standard deviation: AM = ante peridiem: PM = post peridiem: PRE = pre-exercise: IP = immediately post exercise: Sal-T = salivary testosterone: Sal-C = salivary cortisol: T:C ratio = testosterone:cortisol ratio: p = p-value:

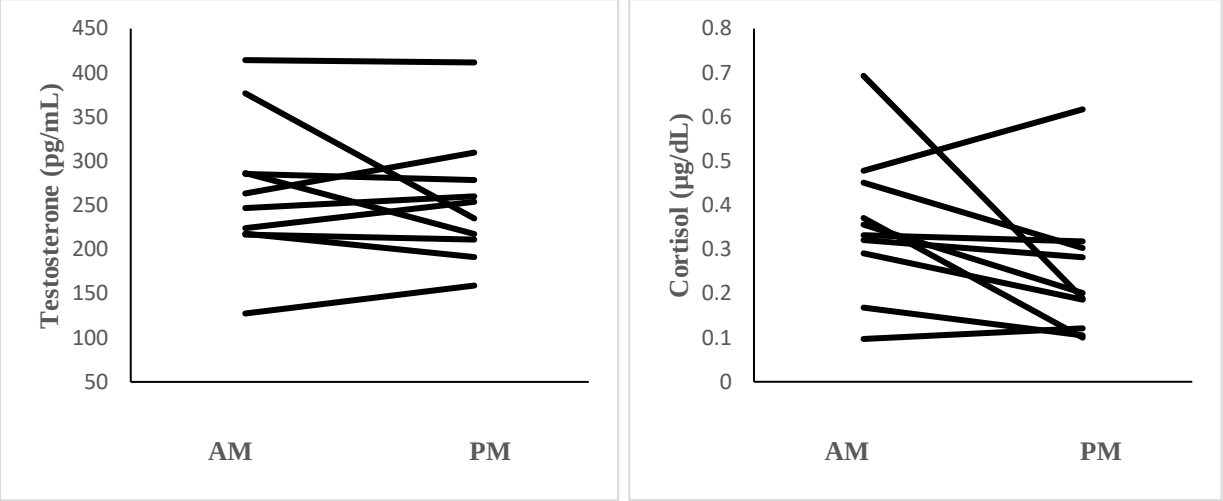
**Figure 3. T:C ratio PRE to IP for the Morning (AM) and Evening (PM) Exercise Sessions (n=9) (mean ± SE)**




T:C = Testosterone/Cortisol ratio: AM = ante peridiem: PM = post peridiem: PRE = pre-exercise: IP = immediately post-exercise

Individual sal-T and sal-C data for the control day are presented in Figure 4. This day served to establish baseline morning and evening sal-T and sal-C values for each participant. No significant differences were observed between the morning and evening sal-T concentration ($p = 0.476$) or sal-C concentration ($p = 0.073$). However, significant diferences were observed in the baseline T:C ratio ($p = 0.019$). The paired samples t-tests showed a Cohen's (*d*) effect size of 0.235 for sal-T, 0.643 for sal-C, and -0.899 for the T:C ratio. This indicated that there was a medium effect size for sal-T, large effect size for sal-C, and a very large effect size for the T:C ratio between the morning and evening.

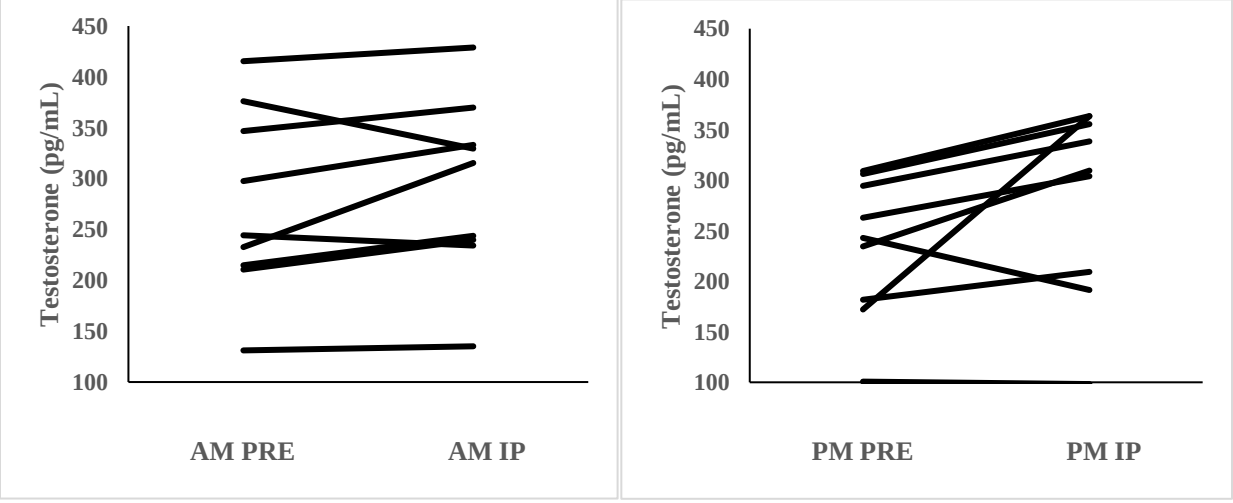
**Figure 4. Individual Salivary Testosterone and Cortisol Concentrations for Control Day Morning and Evening Time Points (n=10)**




AM = ante peridiem: PM = post peridiem

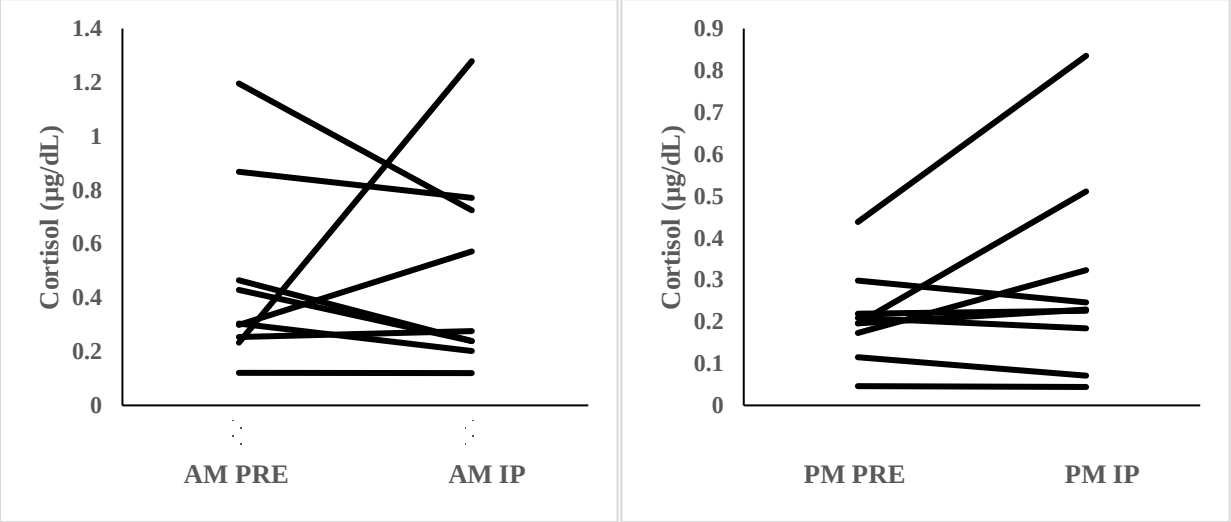
Indiviudal sal-T and sal-C PRE and IP exercise responses are presented in Figure 5 and Figure 6. These samples were collected prior to exercise and immediately post exercise once the participant finished the HIIT exercise bout.

**Figure 5. Individual Salivary Testosterone Responses to Acute HIIT Exercise Performed in the Morning and Evening (n=9)**




AM = ante peridiem: PM = post peridiem: PRE = pre exercise: IP = immediately post exercise

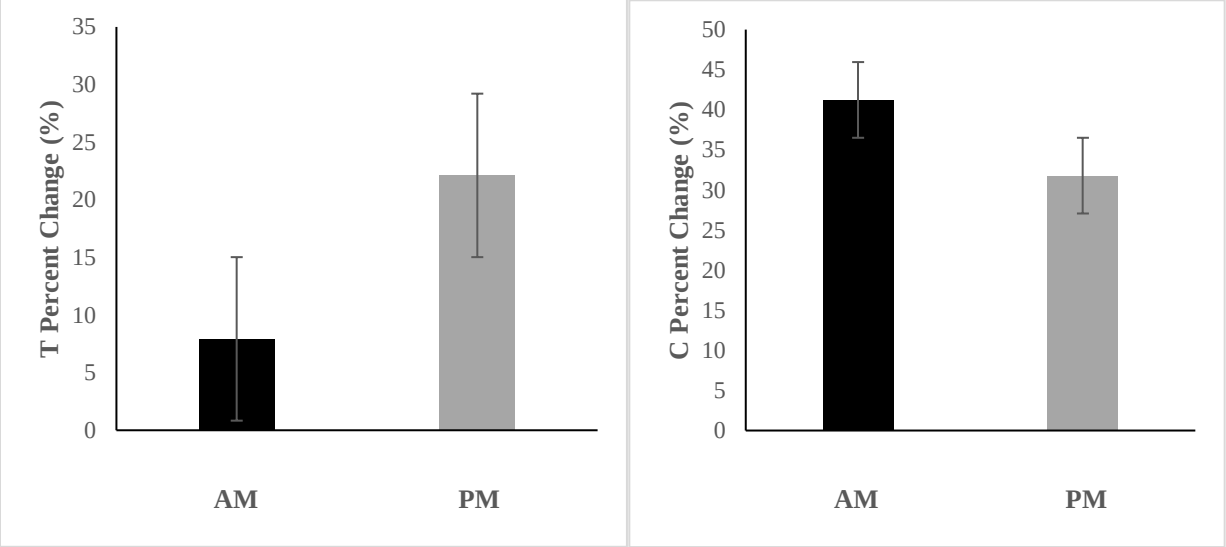
**Figure 6. Individual Salivary Cortisol Responses to Acute HIIT Exercise Performed in the Morning and Evening (n=9)**




AM = ante peridiem: PM = post peridiem: PRE = pre exercise: IP = immediately post exercise

Percent changes in sal-T and sal-C from PRE to IP for acute HIIT sessions performed in the morning and evening exercise are shown in Figure 7. No significant differences were observed in percent change sal-T between the morning and evening ($p = 0.269$) or in percent change sal-C between the morning and evening ($p = 0.854$). The paired samples t-test showed a Cohen's (*d*) effect size of 0.349 for percent change in sal-T and 0.184 for percent change in sal-C. This indicated that there was a medium effect size for percent change in sal-T and a small effect size for percent change in sal-C from pre to IP exercise for the morning and evening.

**Figure 7. Percent Change in Salivary Testosterone and Cortisol in Response to Acute HIIT Performed in the Morning versus Evening (n=9) (mean ± SE)**




T = Testosterone: C = Cortisol: AM = anter peridiem: PM = post peridiem Percent change = ((IP – Pre/Pre) × 100)

Relationships between percent changes from PRE to IP in sal-T and sal-C for the morning and evening and Total BFLBM and Leg BLFBM are presented in Table 5. No significant relationships were observed between percent change in sal-T and sal-C morning and evening time points and Total BLFBM or Leg BFLBM

**Table 5. Correlations (r) between Percent Changes in Salivary Testosterone and Salivary Cortisol and Bone-Free Lean Body Mass Variables (n=9) (means ± SD)**

| **Variables** | **Total BFLBM** | | **Leg BFLBM** | |
|---|---|---|---|---|
| | **r (p)** | **r (p)** | **r (p)** | **r (p)** |
| | **AM** | **PM** | **AM** | **PM** |
| **Sal-T** | **-0.197 (0.611)** | **0.587 (0.096)** | **-0.195 (0.615)** | **0.482 (0.189)** |
| **Sal-C** | **-0.285 (0.457)** | **0.134 (0.731)** | **-0.314 (0.411)** | **0.037 (0.925)** |

AM = anter peridiem: PM = post peridiem Percent change = ((IP – Pre/Pre) × 100) BFLBM = bone-free lean body mass: Sal-T = salivary testosterone: sal-C = salivary cortisol:

## Discussion

The primary purpose of this study was to investigate endocrine biomarker responses following acute bouts of HIIT exercise in the morning and evening in college aged males. The secondary purpose of this study was to evaluate the diurnal variations in the T:C response to the exercise bouts.

The unique findings from this study were: 1) there were no significant differences observed between the morning and evening sal-T baseline concentrations or morning and evening sal-C baseline concentrations; 2) there were no significant differences observed in sal-T for exercise time of day; 3) there were no significant differences observed in sal-C PRE to IP exercise for both exercise time points.

The confirmatory findings from this study were: 1) there was no significant differences observed between the morning and evening RPE or morning and evening HR; 2) there were significant increases in the T:C ratio for the baseline evening data compared to the morning; 3) there were significant increases in sal-T from PRE to IP exercise for both exercise time points; 4) there were significant decreases observed in sal-C for the evening exercise time of day; 5) there were significant increases in the T:C ratio for the evening exercise time of day; 6) there were no significant differences observed in the T:C ratio PRE to IP exercise for both exercise time points.

### Testosterone

While numerous studies have shown that physical exercise can lead to an increase in T, many of these studies did not control for time of day differences with regards to the circadian profile of this hormone. It has been established that T follows a circadian rhythm, which reaches its peak concentration during the morning hours and subsiding as the day goes on (Gnocchi & Bruscalupi, 2017; González-Sales et al., 2015). To control for the circadian oscillations T

exhibits, baseline salivary samples were taken from participants in the morning and evening for this study and served as the control day. No significant differences were observed in sal-T control values between the morning and evening. Although no differences were observed in this study, it is important to note that this is line with Hayes et al. (2012) who found no difference in sal-T when examining time of day differences in healthy young men (Hayes et al., 2012). This may have been attributed to the amount and times at which these control samples were taken. All control samples were taken between the hours of 8:30-10:00 am and 5:00-6:30 pm on the same day and only one sample was taken to establish a baseline value for sal-T. While it may seem like this methodology for determining a baseline sal-T value is valid, it is important to note that in protracted exercise interventions with no control group, the sal-T response following exercise may be a product of circadian fluctuations. This study employed acute HIIT exercise that lasted approximately 30 minutes in duration, which may have been longer in duration than most participants were used to. It is thought that time-matching may be more useful to control for circadian varaiations in sal-T when obtaining baseline values without a control group. For example, it may have been more beneficial to obtain PRE and IP baseline values like that of the HIIT exercise sessions to determine if any alterations in sal-T were exercise induced. Furthermore, participants were allowed to come in between specificed hours for the control day, it may have been more useful to deem one time point across all participants to determine the truest sal-T concentration.

Testosterone plays a pivotal role in one's physiology following physical exercise due to its anabolic effect it has on skeletal muscle (Lane et al., 2015). This study examined sal-T following acute bouts of HIIT performed in the morning versus evening and found significant increases from PRE to IP exercise for both exercise time points. This is line with Cofré-Bolados

et al. (2019) who found a significant increase in plasma T from pre to immediately post following an acute bout of HIIT exercise in recreationally active college aged males (Cofré-Bolados et al., 2019). Another study conducted by Hough et al. (2011) who investigated hormonal responses following HIIT protocols found signficiant increases in plasma and sal-T pre to post-exercise for two different HIIT protocols. Kilian et al. (2016) also examined biomarker response following a single bout of HIIT exercise in young athletes and found signficiant increases in sal-T pre to immediately post and 30 minutes post-exercise. The increases in sal-T PRE to IP exercise observed in this study can be attributed to various factors. For example, the testosterone response following exercise is typically intensity and duration dependent. This study used a HIIT protocol that required working intervals at 81% of $VO_{2peak}$ with the total duration of exercise lasting 31 minutes. The age of the participants may have influenced the sal-T increase PRE to IP exercise as young men have been shown to have a more robust total testosterone response following heavy resistance exercise compared to their older counterparts (Raichy et al., 2020). Although changes in metabolic clearance rates and plasma volume shifts could not be analyzed or recorded for this study, these variables may have played a role in the sal-T increase PRE to IP exercise (Hackney & Lane, 2015) . Furthermore, no significant differences were observed in sal-T for exercise time of day.

**Cortisol**

Similar to testosterone, cortisol has been shown to undergo circadian oscillations beginning in the morning and leveling out as the day goes on. This increase in cortisol upon awakening has been observed across many studies (Adebero et al., 2020; Hayes et al., 2012; Teo et al., 2011; Zar et al., 2021). Time of day differences in cortisol could pose obstacles for exercise performance and recovery as cortisol promotes catabolic processes on skeletal muscle.

This study found no significant differences when examining baseline sal-C between the morning and evening. While there were no differences for baseline sal-C between the morning and evening, it should be noted that several factors including individual living habits, social activity, and work/school schedule may have contributed to these results (Hayes et al., 2016). For example, this study did not control for the amount of social activity between participants during the control day. Most participants were involved in classes or organziations around campus which may have led to increases in sal-C throughout the day. Furthermore, the living status of each individual was not recorded as some participants either lived with roomates or alone. This may have attributed to these results in part to the psychological stressors of living with roomates or the potential of financial hardships. In addition, the dietary status of each participant was not tracked and each participant was instructed to consume their normal diet during this day. Differences in macronutrient choice between participants may have led to these conflicting results as the hormonal response may have been altered due to specific nutrient timing (Hayes et al., 2016).

While cortisol does promote degradation in skeletal muscle, this endocrine response is necessary during physical exercise (Hackney & Lane, 2015; Hew-Butler et al., 2008; Mangine et al., 2018). Factors including activating energy production pathways and mobilizing energy substrates gives cortisol its key role in response to exercise. Typically, cortisol release will begin at less than one minute into exercise by stimulation of the HPA axis. If exercise continues, cytokines like interleukin-6 will be released from skeletal muscle which affects the release of cortisol and may lead to substrate mobilization. This study examined sal-C following acute bouts of HIIT exercise and found no significant differences from PRE to IP exercise for the morning or evening sessions. Hackney & Lane (2015) determined that the endocrine response tends to be

proportional to the intensity and duration of an exercise bout, although not always linear. The HIIT protocol employed in this study may not have been a strong enough stimulus to elicit a sal-C response PRE to IP exercise. This could have been due to participants individual training status as most participants were resistance trained but not aerobically trained. It is recommended to perform exercise at 60-70% of $VO_{2max}$ to observe a cortisol response, however, a large inter-individual variability exists and this may have contributed to the findings of this study. Cofré-Bolados et al. (2019) found no significant differences pre to immediately post exercise for plasma cortisol in recreationally active college males, however, found significant increases in plasma cortisol 12 hours post exercise. The authors attributed the increase in plasma cortisol 12 hours post exercise to the circadian oscillations cortisol undergoes. In addition, significant decreases were observed when examining sal-C for exercise time of day, as the evening sessions showed lower PRE and IP sal-C concentrations compared to the morning. This is line with previous literature showing that sal-C does undergo circadian oscillations and time of day should be controlled when investigating sal-C (Hill et al., 2008; Monje et al., 2020; Zurek et al., 2022).

**T:C Ratio**

The T:C ratio is thought to be indicative of what physiological processes are dominating following an exercise intervention due to the anabolic and catabolic effects it has on skeletal muscle (Hayes et al., 2016). There were significant differences observed in the baseline T:C ratio as the evening data indicated a more favored ratio for testosterone. This is in line with Zar et al. (2021) who investigated responses in the T:C ratio following acute bouts of HIIT exercise in the morning versus afternoon in active young men and found significant increases for the pre afternoon time point. Implications for these results suggest that the T:C ratio during the evening may be greater compared to the morning, which may be useful for exercise scientists when it

comes to choosing time of day for exercise sessions in order to optimize anabolic processes and potentially performance (Teo et al., 2011).

This study examined the T:C ratio following acute bouts of HIIT exercise performed in the morning versus evening and found no significant differences from PRE to IP exercise for the morning or evening sessions. Kilian et al. (2016) investigated responses in the salivary T:C ratio following a single session of HIIT and HVT in young athletes and found no significant differences pre to post exercise. Another study conducted by Zurek et al. (2022) found no significant differences in the salivary T:C ratio following acute bouts of sprint interval training in endurance, strength, and non-trained men. The researchers in these studies stated to minimize circadian variation in hormonal response, the participants performed the HIIT exercise at the same time of day. In contrast, this present study found significant differences in the salivary T:C ratio for exercise time of day, with the evening session showing a ratio that favored testosterone. Although the T:C ratio is a useful indicator of an individual's physiological status following an exercise intervention, it is important to note that it should not be used to monitor exercise performance but rather used to help determine if an individual is recovering appropriately (Rowell et al., 2018).

With regards to the relationship in percent changes of sal-T and sal-C to BFLBM variables there were no significant differences observed for all variables measured for the morning and evening values. These included Total BFLBM, Arm BFLBM, Leg BFLBM and Trunk BLFBM. While this study employed HIIT exercise performed on a treadmill and requires the greatest amount of muscle mass used, a significant positive relationship was not observed for this study. There were limitations that may have attributed to these findings. For example, biological errors from salivary collection techniques may have occurred as some participants

samples contained external residue. Dietary status, training regimens, and emotional strain outside of the testing environment were not recorded. Technical errors including differences in ambient temperature and improper equipment handling may have occurred during the baseline and exercise sessions. These limitations have been acknowledged by the authors and will be noted in an applied setting regarding the use of HIIT exercise in healthy college aged males.

# CHAPTER V: CONCLUSIONS

The primary purposes of this study was to investigate biomarker responses following acute bouts of HIIT exercise in the morning versus evening as well as the T:C ratio in healthy college aged males. In addition, percent changes in sal-T and sal-C for the morning and evening were assessed in relation to DXA body composition variables to investigate if a potential relationship exists.

## Research Questions

Research Question 1*:* Does time of day affect salivary testosterone and cortisol responses to an acute bout of HIIT exercise?

There was no significant time of day effect observed in sal-T. However, there was a significant exercise effect observed for sal-T. A significant time of day effect was observed for sal-C but no significant exercise effect was observed.

Hypothesis 1**:** It was hypothesized that the salivary testosterone and cortisol responses to HIIT will be greater in the evening compared to the morning session.

The findings of this study did not support this research hypothesis as sal-T showed no difference for time of day. It is important to note there was a significant difference observed in sal-C for a time of day effect with the evening showing lower sal-C values compared to the morning.

Research Question 2: Does the pattern of the T:C ratio following an acute bout of HIIT exercise vary depending on the time of day?

There was a signficiant time of day effect observed in the T:C ratio. This difference showed a ratio that favored sal-T for the evening session compared to the morning. No

significant exercise effect or time × exercise effect was observed in the T:C ratio for the morning or evening.

Hypothesis 2***:*** It was hypothesized that the evening salivary cortisol response will be greater than the salivary testosterone response, resulting in a lower T:C ratio compared to the corresponding responses to the morning acute bout of HIIT.

The findings of this study did not support research hypothesis 2 as the T:C ratio showed a significant increase for the evening session in response to HIIT exercise.

Research Question 3: Is there a relationship between salivary testosterone and cortisol responses to acute bouts of HIIT and bone-free lean body mass?

There were no significant relationships observed between sal-T AM and PM & sal-C AM and PM and Total BFLBM, Arm BFLBM, Leg BFLBM and Trunk BFLBM.

Hyopthesis 3***:*** It was hypothesized that there will be a significant positive relationship between percent changes in salivary testosterone and cortisol and bone-free lean body mass.

The findigs of this study did not support research hypothesis 3 as no significant relationships were observed between percent changes in sal-T and sal-C and BFLBM variables for the morning or evening

## Practical Significance

The practical takeaways for this present study suggest that acute bouts of HIIT exercise in college aged males provides enough stimulus to elicit biomarker responses and circadian hormonal oscillations should be controlled when investigating sal-T and sal-C. This is seen from significant increases in sal-T PRE to IP exercise for both exercise time points, significant differences in the evening T:C ratio compared to the morning, and significant decresases in evening sal-C compared to the morning. These findings may help exercise scientists develop

HIIT exercise protocols for college aged males lacking time in their day to exercise. In addition, the circadian variations observed in this study further the debate on time of day differences in biomarker response.

## Suggestions for Future Research

Future studies should include additional time points assessed for the control day. For example, if the exercise intervention has a pre and post time point, the control day should also have a pre and post time point. However, a control group would be the most beneficial along with the experimental group. In addition, a dietary log, emotional status, and training regimens outside of the testing environment should be recorded in order to better understand if the changes in hormone concentration following an intervention is exercise-induced. Finally, future research should explore gender differences for HIIT exercise and time of day variations in sal-T, sal-C and the T:C ratio, as well as employing a training protocol as opposed to the acute bouts of HIIT exercise used in this study.

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# Appendices

## Appendix A: IRB Approval Letter

The UNIVERSITY of OKLAHOMA

**Institutional Review Board for the Protection of Human Subjects**

**Initial Submission – Board Approval**

| | | | |
|---|---|---|---|
| **Date:** | October 28, 2022 | **IRB #**: | 15178 |
| **To**: | Debra A Bemben, PhD | **Meeting Date**: | 10/24/2022 |
| | | **Approval Date**: | 10/27/2022 |
| | | **Expiration Date**: | 09/30/2023 |
| **Study Title**: | Circadian Rhythm Variation in Endocrine Biomarker Responses to High-Intensity Interval Training in College Aged Males | | |
| **Study Status**: | Active - Open \| CR Req | | |

The University of Oklahoma Health Sciences Center's Institutional Review Board (IRB) reviewed the above-referenced research study at its regularly scheduled meeting and requested specific changes to the submission. On behalf of the IRB, I have verified that the specific changes requested by the convened IRB have been made and I grant final approval for this study.

Approval for this research is limited to the activities described in the approved protocol and application. In accordance with this approval, specific conditions for the conduct of this research are listed below, and informed consent from participants must be obtained as indicated.

**Risk/Benefit Assessment:** Research not involving greater than minimal risk.

**Informed Consent Determination:**
Informed consent and research privacy authorization must be obtained using the currently approved, stamped forms. You must retain all original, signed forms.

**Continuing Review Determination:**
As part of this approval, annual continuing review is required. You must promptly submit a Continuing Review/Final Closure Report Form and appropriate supporting documents to the IRB upon notification; approximately 60 days prior to the expiration date indicated above.

**Principal Investigator Responsibilities:**

- Conduct the research study in a manner consistent with the requirements of the IRB and federal regulations at 45 CFR 46 and/or 21 CFR 50 and 56.
- Request approval from the IRB prior to implementing any/all modifications.
- Promptly report to the IRB any harm experienced by a participant that is both unanticipated and related per IRB Policy.
- Maintain accurate and complete study records for evaluation by the HRPP quality improvement program and if applicable, inspection by regulatory agencies and/or the study sponsor.

**The following are also required if applicable to this research study:**

- You may *not begin your study* until the contract through Office of Research Administration (ORA) is finalized and signed as per OUHSC Institutional policy.
- If this study involves external sites requiring a reliance agreement for OUHSC to serve as IRB of record, submit a modification to add each non-OU site and non-OU collaborator to the application after a reliance agreement has been finalized.

# Appendix B: Screening Checklist

**Screening Checklist**

**Circadian Rhythm Variation in Endocrine Biomarker Responses to High-Intensity Interval Training in College Aged Males**

Name:_____ Date:_____

Age (years):_____ Height (cm):_____ Body Weight (kg):_____

**Does the participant meet the inclusion criteria for the study?**

| | YES | NO |
|---|---|---|
| Males between the ages of 20 and 25 years. | | |
| Those who self-report an average of 150 minutes of moderate physical activity per week. | | |
| Those with no HIIT participation in the past 3 months. | | |

**Does the participant have any exclusion criteria?**

| | YES | NO |
|---|---|---|
| History of cardiovascular or pulmonary dysfunction. | | |
| Consuming any prescription medication that affects concentrations of Testosterone and Cortisol. | | |
| Body mass over 300 pounds. | | |
| Height over 6 ft 4 in. | | |
| Taking any performance enhancing drugs or supplement: Blood doping techniques, Anabolic steroids | | |
| Joint replacement or metal implants in the legs, hips, or spine. | | |
| Sedentary or vigorously active | | |
| Previous surgery or injury preventing them from participation in exercise | | |
| Regularly consumed any form of tobacco in the past 3 months. | | |

**STOP HERE**

Is the subject qualified for the study (circle one)? YES NO

Principal Investigator approval
Dr. Debra Bemben

Signature: _____ Date: _____

# Appendix C: Informed Consent



**Consent Form to Participate in a Research Study**
**University of Oklahoma Health Sciences Center (OUHSC)**
**University of Oklahoma - Norman Campus**

**Study Title:** Circadian Rhythm Variation in Endocrine Biomarker Responses to High-Intensity Interval Training in College Aged Males

**Principal Investigator: Debra Bemben, PhD**
**Phone Number: (405) 306-3194**

## *KEY INFORMATION ABOUT THE RESEARCH STUDY*

You are being asked to participate in a research study. Research studies are voluntary and include only people who choose to take part. This consent form begins with a 'Key Information' section to provide important information to help you decide whether or not to participate in this study. More detailed information is provided after the key information. Please take your time, discuss this with family and friends, and ask the investigator and study team any questions you may have.

**WHY HAVE I BEEN ASKED TO PARTICIPATE IN THIS STUDY?**
You are being asked to participate in this research study because you are a recreationally active male between the ages of 20-25 years who does not perform high-intensity interval training on a regular basis.

**WHY IS THIS STUDY BEING DONE AND HOW LONG WILL IT LAST?**
The purpose of this study is to investigate time of day differences on salivary endocrine biomarkers in response to acute bouts of high-intensity interval training performed in the morning vs. evening. This study will potentially allow researchers to better understand salivary biomarker responses to high-intensity interval training at different times of day. In addition, this study will also examine the relationship between the percent changes in biomarker responses and body composition variables.

This study will span about 2-3 weeks for each participant and require approximately 9 hours total.

**WHAT WILL I BE ASKED TO DO IN THIS STUDY?**
If you decide to participate in this study, you will be asked to visit the Bone Density Research Laboratory (Rm 4 SJ Sarkeys Complex) for 5 test sessions. The first visit will take approximately 1.5 hours and consist of consenting process, questionnaires, and DXA total body scan. The second visit will take approximately 45 minutes and a maximal graded exercise test will be performed to establish maximal aerobic capacity. The third visit will take approximately 1.5 hours and you will provide a saliva sample in the morning (8:30-10:00 a.m.) and late afternoon (5:00-6:30 p.m.) to determine baseline values for endocrine biomarkers, which will serve as the control day. The fourth and fifth visits will be randomized and take approximately 2 hours for each session in which you will perform the HIIT exercise sessions. In addition, saliva samples will be collected prior (pre) to the HIIT session and immediately (30P) following exercise.

**WHY MIGHT I WANT TO PARTICIPATE IN THIS STUDY?**
If you agree to take part in this study, there will not be direct medical benefit to you. We hope that the information learned from this study will benefit other individuals who wish to use HIIT as a training method in the future.

**WHY MIGHT I NOT WANT TO PARTICIPATE IN THIS STUDY?**
The researchers do not know all of the side effects that could happen. For a complete description of known risks, refer to the Detailed Information section of the consent form.

**WHAT OTHER OPTIONS ARE THERE?**
You may choose not to participate in this study.

**HOW WILL PARTICIPATING IN THE STUDY AFFECT ME FINANCIALLY?**
There is no additional cost to you if you participate in this study.

## *DETAILED INFORMATION ABOUT THE RESEARCH STUDY*

**HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?**
About 15 males between the ages of 20-25 will take part in this study.

**WHAT IS INVOLVED IN THE STUDY?**
If you take part in this study, 5 visits will be needed. The first visit consisting of the following tests in order to determine history relating to physical activity level, health history, and a DXA total body scan. This visit will last about 1.5 hours.

- Informed consent and HIPAA – must sign and date an informed consent form (this document) stating that you understand all procedures and your rights as a participant.
- Health Status Questionnaire – you may be excluded from the study if any answer on this questionnaire indicates you may not be eligible for this study.
- Physical Activity Readiness Questionnaire- will determine if you are healthy enough to engage in exercise.
- International Physical Activity Questionnaire – will measure how much physical activity you do on a regular basis to determine whether you meet the physical activity requirement for the study.
- Anthropometric Measurements - Height (cm) and weight (kg).
- Dual Energy X-ray Absorptiometry (DXA) - total body scan will take place and bone-free lean body mass, fat mass, and percent body fat will be recorded.
- You will be familiarized with the treadmill and the methods for providing saliva samples.

The second visit will consist of performing a maximal graded treadmill exercise test to establish maximal aerobic capacity. This visit will last about 45 minutes

- Aerobic Capacity - You will complete a graded exercise test where you begin by walking on the treadmill, as time continues you will begin to move faster for as long as you can tolerate. You will be wearing a heart rate monitor and facemask so researchers can collect your expired breaths.

The third visit will serve as the control day where you will provide a saliva sample in the morning and late afternoon to determine baseline endocrine biomarker values. This visit will last about 2 hours and occur at least 24 hours after the maximal exercise test.

The fourth and fifth visits will be performed in random order and will consist of evaluating your endocrine biomarkers prior and following the HIIT exercise session performed in the morning and late afternoon. Each session will take about 2 hours.

- High-Intensity Interval Training (HIIT) sessions- Participants will perform the HIIT exercise sessions in the morning (between 8:30 and 10 am) and evening (between 5 and 6:30 pm). The HIIT exercise involves performing bouts of high intensity running on a treadmill for 1 minute, separated by 2 minutes of low intensity running. There will be a total of 7 work:recovery bouts.
- Saliva sampling- Participants will provide a saliva sample pre and 30 after each HIIT exercise session.
- Ratings of perceived exertion – Participants will be asked to give their perception of how hard the exercise felt at the end of each work bout during the HIIT sessions.

**WHAT ARE THE RISKS OF THE STUDY?**
While in the study, you are at risk for these side effects; however, there may also be unforeseeable risk with participation. You should discuss these with the researcher prior to providing your consent.

Risks and side effects related to physical performance testing include acute and delayed muscle soreness, musculoskeletal injury, discomfort during exercise, feeling tired, lightheaded, or faintness. Researchers will make sure that you have eaten food and are hydrated prior to exercise to help minimize these symptoms.

Radiation Risks
Risks and side effects related to having a DXA scan include radiation exposure from which is a type of x-ray procedure. This procedure is for research only and not needed for your medical care. The amount of additional radiation to which you will be exposed is approximately 1% of the amount of radiation to which we are exposed annually from background sources such as the Earth and Sun. The risk from radiation exposure increases over your lifetime as you receive additional exposure to radiation.

**TO WHAT EXTENT WILL MY INFORMATION BE KEPT CONFIDENTIAL?**
Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations outside the OUHSC that may inspect and/or copy your research records for quality assurance and data analysis. These organizations may include the US Food & Drug Administration and other regulatory agencies. The OUHSC Human Research Participant Program office, the OUHSC Institutional Review Board, OUHSC Office of Compliance, and other University administrative offices may also inspect and/or copy your research records for these purposes.

Storing and Sharing Your Information:
Any personal information that could identify you will be removed from your samples. Your samples will not be used for any future research studies.

**CAN I WITHDRAW FROM THE STUDY?**
You can stop participating in this study at any time. However, if you decide to stop participating in the study, we encourage you to talk to the researcher and your regular doctor first. There may be

circumstances under which your participation may be terminated by the investigator without your consent.

**WHAT ARE THE COSTS?**
There is no cost to you for participating in this study.

**WHAT IF I AM INJURED OR BECOME ILL WHILE PARTICIPATING IN THIS STUDY?**
In the case of injury or illness resulting from this study, emergency medical treatment is available. However, you or your insurance company will be expected to pay the usual charge for this treatment. Complications arising as a result of the natural progression of an underlying or pre-existing condition will be billed to you or your insurance. Please check with the investigator or with your insurance company if you have questions. No other funds have been set aside by the University of Oklahoma Norman or Health Sciences Center campuses to compensate you in the event of injury.

**WHAT ARE MY RIGHTS AS A PARTICIPANT?**
Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled.

If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. However, at certain times during the treatment, it may be harmful for you to withdraw, so please be sure to discuss leaving the study with the principal investigator or your regular doctor. You may discontinue your participation at any time without penalty or loss of benefits to which you are otherwise entitled.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished. You consent to this temporary restriction.

You can receive more information regarding these rights in the Privacy Notice for Research Participants, located on the OUHSC Office of Human Research Participant Protection (HRPP) website at *https://compliance.ouhsc.edu/HRPP/Participant/Privacy-Notice*.

If you have any questions and requests, please contact the HRPP Office at 405-271-2045.

**WHOM DO I CALL IF I HAVE QUESTIONS, SUGGESTIONS, OR CONCERNS?**
If you have questions, concerns, or complaints about the study or have a research-related injury, contact Dr. Debra Bemben at 405-306-3194 or dbemben@ou.edu.

If you cannot reach the Investigator or wish to speak to someone other than the investigator and for questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection, at 405-271-2045.

**SIGNATURE:**
By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

| | | |
|---|---|---|
| **PARTICIPANT SIGNATURE (age ≥20)** | **Printed Name** | **Date** |
| **SIGNATURE OF PERSON OBTAINING CONSENT** | **Printed Name** | **Date** |

# Appendix D: HIPAA

**University of Oklahoma Health Sciences CenterResearch Privacy Form 1**
**PHI Research Authorization**

**AUTHORIZATION TO USE or SHARE**
**HEALTH INFORMATION[1] THAT IDENTIFIES YOU FOR RESEARCH**
*An Informed Consent Document for Research Participation may also be required.*
*Form 2 must be used for research involving psychotherapy notes.*

Title of Research Project: **Circadian Rhythm Variation in Endocrine**

**Biomarker Responses to High-Intensity Interval Training in College Aged Males**

Leader of Research Team: **Debra Bemben, Ph.D.**

Address: **1401 Asp Avenue, Norman, OK, 73071**

Phone Number**: 405-306-3194**

If you decide to sign this document, University of Oklahoma Health Sciences Center (OUHSC) researchers may use or share information that identifies you (protected health information) for their research. Protected health information will be called PHI in this document.

**PHI To Be Used or Shared**. Federal law requires that researchers get your permission (authorization) to use or share your PHI. If you give permission, the researchers may use or share with the people identified in this Authorization any PHI related to this research from your medical records and from any test results. Information used or shared may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form; medical records and charts; name, address, telephone number, date of birth, race, government-issued identification numbers, and nothing else.

**Purposes for Using or Sharing PHI.** If you give permission, the researchers may use your PHI to investigate the changes on time of day salivary endocrine biomarkers, body composition variables, and aerobic capacity in college aged males in response to acute bouts of high-intensity interval training performed in the morning vs. evening.

**Other Use and Sharing of PHI**. If you give permission, the researchers may also use your PHI to develop new procedures or commercial products. They may share your PHI with other researchers, the research sponsor and its agents, the OUHSC Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Food and Drug Administration (FDA) and the Department of Health and Human Services (HHS), and when required by law. The researchers may also share your PHI with no one else.

**Confidentiality**. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information

[1] **Protected Health Information includes all identifiable information relating to any aspect of an individual's health whether past, present or future, created or maintained by a Covered Entity.**

confidential, but confidentiality is not guaranteed. The law does not require everyone receiving the information covered by this document to keep it confidential, so they could release it to others, and federal law may no longer protect it.

**YOU UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING A COMMUNICABLE OR NONCOMMUNICABLE DISEASE.**

**Voluntary Choice**. The choice to give OUHSC researchers permission to use or share your PHI for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OUHSC researchers to use or share your PHI if you want to participate in the research and, if you cancel your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care unrelated to this study from OUHSC.

**Canceling Permission**. If you give the OUHSC researchers permission to use or share your PHI, you have a right to cancel your permission whenever you want. However, canceling your permission will not apply to information that the researchers have already used, relied on, or shared or to information necessary to maintain the reliability or integrity of this research.

**End of Permission.** Unless you cancel it, permission for OUHSC researchers to use or share your PHI for their research will never end.

**Contacting OUHSC**: You may find out if your PHI has been shared, get a copy of your PHI, or cancel your permission at any time by writing to:

| | | |
|---|---|---|
| Privacy Official<br>University of Oklahoma Health Sciences Center<br>PO Box 26901<br>Oklahoma City, OK 73190 | or | Privacy Board<br>University of Oklahoma Health Sciences Center<br>PO Box 26901<br>Oklahoma City, OK 73190 |
| If you have questions, call: (405) 271-2511 | or | (405) 271-2045. |

**Access to Information.** You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study is completely finished. You consent to this temporary restriction.

**Giving Permission**. By signing this form, you give OUHSC and OUHSC's researchers led by the Research Team Leader permission to share your PHI for the research project listed at the top of this form.

**University of Oklahoma Health Sciences CenterResearch Privacy Form 1**
**PHI Research Authorization**

**Patient/Participant Name (Print): _____**

_____ _____
Signature of Patient-Participant Date
or Parent if Participant is a minor

*Or*

_____ _____
Signature of Legal Representative** Date

**If signed by a Legal Representative of the Patient-Participant, provide a description of the relationship to the Patient-Participant and the authority to act as Legal Representative:

_____
OUHSC may ask you to produce evidence of your relationship.

***A signed copy of this form must be given to the Patient-Participant or the Legal Representative at the time this signed form is provided to the researcher or his representative.***

## Appendix E: PAR-Q

# 2022 PAR-Q+

### The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

## GENERAL HEALTH QUESTIONS

| Please read the 7 questions below carefully and answer each one honestly: check YES or NO. | YES | NO |
|---|---|---|
| 1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐? | ☐ | ☐ |
| 2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity? | ☐ | ☐ |
| 3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise). | ☐ | ☐ |
| 4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE:** _____ | ☐ | ☐ |
| 5) Are you currently taking prescribed medications for a chronic medical condition? **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE:** _____ | ☐ | ☐ |
| 6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? Please answer **NO** if you had a problem in the past, but it **does not limit your current ability** to be physically active. **PLEASE LIST CONDITION(S) HERE:** _____ | ☐ | ☐ |
| 7) Has your doctor ever said that you should only do medically supervised physical activity? | ☐ | ☐ |

**If you answered NO to all of the questions above, you are cleared for physical activity.**
**Please sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.**

- Start becoming much more physically active – start slowly and build up gradually.
- Follow Global Physical Activity Guidelines for your age (https://www.who.int/publications/i/item/9789240015128).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 yr and NOT accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

**PARTICIPANT DECLARATION**

If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for its records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

**If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.**

**Delay becoming more active if:**

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- Your health changes - answer the questions on Pages 2 and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.



 

01-11-2021

# 2022 PAR-Q+

## FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

**1. Do you have Arthritis, Osteoporosis, or Back Problems?**

If the above condition(s) is/are present, answer questions 1a-1c  If **NO** ☐ go to question 2

| | | |
|---|---|---|
| 1a. | Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) | YES ☐ NO ☐ |
| 1b. | Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)? | YES ☐ NO ☐ |
| 1c. | Have you had steroid injections or taken steroid tablets regularly for more than 3 months? | YES ☐ NO ☐ |

**2. Do you currently have Cancer of any kind?**

If the above condition(s) is/are present, answer questions 2a-2b  If **NO** ☐ go to question 3

| | | |
|---|---|---|
| 2a. | Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and/or neck? | YES ☐ NO ☐ |
| 2b. | Are you currently receiving cancer therapy (such as chemotheraphy or radiotherapy)? | YES ☐ NO ☐ |

**3. Do you have a Heart or Cardiovascular Condition? This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm**

If the above condition(s) is/are present, answer questions 3a-3d  If **NO** ☐ go to question 4

| | | |
|---|---|---|
| 3a. | Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) | YES ☐ NO ☐ |
| 3b. | Do you have an irregular heart beat that requires medical management? (e.g., atrial fibrillation, premature ventricular contraction) | YES ☐ NO ☐ |
| 3c. | Do you have chronic heart failure? | YES ☐ NO ☐ |
| 3d. | Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months? | YES ☐ NO ☐ |

**4. Do you currently have High Blood Pressure?**

If the above condition(s) is/are present, answer questions 4a-4b  If **NO** ☐ go to question 5

| | | |
|---|---|---|
| 4a. | Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) | YES ☐ NO ☐ |
| 4b. | Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer **YES** if you do not know your resting blood pressure) | YES ☐ NO ☐ |

**5. Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes**

If the above condition(s) is/are present, answer questions 5a-5e  If **NO** ☐ go to question 6

| | | |
|---|---|---|
| 5a. | Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies? | YES ☐ NO ☐ |
| 5b. | Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness. | YES ☐ NO ☐ |
| 5c. | Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet? | YES ☐ NO ☐ |
| 5d. | Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease, or liver problems)? | YES ☐ NO ☐ |
| 5e. | Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future? | YES ☐ NO ☐ |


IRB NUMBER: 15178
IRB APPROVAL DATE: 10/27/2022

 

01-11-2021

# 2022 PAR-Q+

**6.** **Do you have any Mental Health Problems or Learning Difficulties?** This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome

If the above condition(s) is/are present, answer questions 6a-6b If **NO** ☐ go to question 7

| | | |
|---|---|---|
| 6a. | Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) | YES ☐ NO ☐ |
| 6b. | Do you have Down Syndrome **AND** back problems affecting nerves or muscles? | YES ☐ NO ☐ |

**7.** **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*

If the above condition(s) is/are present, answer questions 7a-7d If **NO** ☐ go to question 8

| | | |
|---|---|---|
| 7a. | Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) | YES ☐ NO ☐ |
| 7b. | Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy? | YES ☐ NO ☐ |
| 7c. | If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week? | YES ☐ NO ☐ |
| 7d. | Has your doctor ever said you have high blood pressure in the blood vessels of your lungs? | YES ☐ NO ☐ |

**8.** **Do you have a Spinal Cord Injury?** *This includes Tetraplegia and Paraplegia*

If the above condition(s) is/are present, answer questions 8a-8c If **NO** ☐ go to question 9

| | | |
|---|---|---|
| 8a. | Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) | YES ☐ NO ☐ |
| 8b. | Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting? | YES ☐ NO ☐ |
| 8c. | Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)? | YES ☐ NO ☐ |

**9.** **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*

If the above condition(s) is/are present, answer questions 9a-9c If **NO** ☐ go to question 10

| | | |
|---|---|---|
| 9a. | Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) | YES ☐ NO ☐ |
| 9b. | Do you have any impairment in walking or mobility? | YES ☐ NO ☐ |
| 9c. | Have you experienced a stroke or impairment in nerves or muscles in the past 6 months? | YES ☐ NO ☐ |

**10.** **Do you have any other medical condition not listed above or do you have two or more medical conditions?**

If you have other medical conditions, answer questions 10a-10c If **NO** ☐ read the Page 4 recommendations

| | | |
|---|---|---|
| 10a. | Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months? | YES ☐ NO ☐ |
| 10b. | Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)? | YES ☐ NO ☐ |
| 10c. | Do you currently live with two or more medical conditions? | YES ☐ NO ☐ |

**PLEASE LIST YOUR MEDICAL CONDITION(S) AND ANY RELATED MEDICATIONS HERE:** _____

_____

**GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.**



 3/ 4

01-11-2021

# 2022 PAR-Q+

**If you answered NO to all of the FOLLOW-UP questions (pgs. 2-3) about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:**

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually - 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:

You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program - the **ePARmed-X+ at www.eparmedx.com** and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

**Delay becoming more active if:**

- You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ **at www.eparmedx.com** before becoming more physically active.
- Your health changes - talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

## PARTICIPANT DECLARATION

- All persons who have completed the PAR-Q+ please read and sign the declaration below.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.

*I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that the community/fitness center may retain a copy of this form for records. In these instances, it will maintain the confidentiality of the same, complying with applicable law.*

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

**For more information, please contact**

**www.eparmedx.com**
**Email: eparmedx@gmail.com**

**Citation for PAR-Q+**
Warburton DER, Jamnik VK, Bredin SSD, and Gledhill N on behalf of the PAR-Q+ Collaboration. The Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) and Electronic Physical Activity Readiness Medical Examination (ePARmed-X+). Health & Fitness Journal of Canada 4(2):3-23, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+ Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or the BC Ministry of Health Services.

**Key References**

1. Jamnik VK, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the effectiveness of clearance for physical activity participation: background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance: Consensus Document. APNM 36(S1):S266-s298, 2011.
3. Chisholm DM, Collis ML, Kulak LL, Davenport W, and Gruber N. Physical activity readiness. British Columbia Medical Journal. 1975;17:375-378.
4. Thomas S, Reading J, and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). Canadian Journal of Sport Science 1992;17:4 338-345.

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# Appendix F: Health Status Questionnaire

***Bone Density Research Laboratory***
***OU Department of Health and Exercise Science***
***Health Status Questionnaire***

Complete each question accurately. All information provided is confidential.

**Part 1. Information about the individual**

1._____
Date

2. _____
Legal name Ethnicity

3._____
Mailing address

_____
Home phone Business/cell phone

4.Gender (circle one): Female Male

5. Year of birth: _____ Age _____

6. Number of hours worked per week:
NA (retired) Less than 20 20-40 41-60 Over 60

If not retired, more than 25% of time spent on job (circle all that apply)

Sitting at desk Lifting or carrying loads Standing Walking Driving

**Part 2. Medical history**

7. Circle any who died of heart attack before age 50:
Father Mother Brother Sister Grandparent

8.Date of: Last medical physical exam: _____Last physical fitness test: _____
Year Year

9. Circle operations you have had:
Back Heart Kidney Eyes Joint Neck Ears Hernia Lung Other _____ **NONE**


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10. Please circle any of the following for which you have been diagnosed or treated by a health professional:

| | | |
|---|---|---|
| Alcoholism | Diabetes | Kidney problem |
| Anemia, sickle cell | Emphysema | Mental illness |
| Anemia, other | Epilepsy | Neck strain |
| Asthma | Eye problems | Obesity |
| Back strain | Gout | Osteoporosis |
| Bleeding trait | Hearing loss | Phlebitis |
| Bronchitis, chronic | Heart problems | Rheumatoid arthritis |
| Cancer | High blood pressure | Stroke |
| Cirrhosis, liver | Hypoglycemia | Thyroid problem |
| Concussion | Hyperlipidemia | Ulcer |
| Congenital defect | Infectious mononucleosis | Other _____ **NONE** |

11. Circle all medicine taken in last 6 months:

Asthma (list type) _____High-blood-pressure medication (list type) _____

| | | |
|---|---|---|
| Blood thinner | Epilepsy medication | Thyroid |
| Corticosteroids | Estrogen | Diuretic |
| Depression | Heart-rhythm medication | Digitalis |
| Diabetic pill | Insulin | Nitroglycerin |
| | | Other _____ **NONE** |

12. Any of these health symptoms that occurs frequently is the basis for medical attention. Circle the number indicating how often you have each of the following:

**1 = Practically never 2 = Infrequently 3 = Sometimes 4 = Fairly often 5 = Very often**

a. Cough up blood
1 2 3 4 5

b. Abdominal pain
1 2 3 4 5

c. Low back pain
1 2 3 4 5

d. Leg pain
1 2 3 4 5

e. Arm or shoulder pain
1 2 3 4 5

f. Chest pain
1 2 3 4 5

g. Swollen joints
1 2 3 4 5

h. Feel faint
1 2 3 4 5

I. Dizziness
1 2 3 4 5

j. Breathless with slight exertion
1 2 3 4 5

**Part 3. Health-related behavior**

13. Do you now smoke? Yes No

14. If you are a smoker, indicate number smoked per day:

Cigarettes: 40 or more 20-39 10-19 1-9
Cigars or pipes only: 5 or more or any inhaled Less than 5, none inhaled

15. Weight now: _____lb. One year ago: _____lb. Age 21 (if applicable): _____lb.

16. Do you engage in exercise or hard physical labor at least three times a week? YES NO


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# Appendix G: IPAQ

## INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (October 2002)

## LONG LAST 7 DAYS SELF-ADMINISTERED FORMAT

### FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

***Background on IPAQ***

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

***Using IPAQ***

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

***Translation from English and Cultural Adaptation***

Translation from English is encouraged to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

***Further Developments of IPAQ***

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

***More Information***

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective.* Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

# INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

***PART 1: JOB-RELATED PHYSICAL ACTIVITY***

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

***PART 2: TRANSPORTATION PHYSICAL ACTIVITY***

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

***PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

***PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY***

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

***PART 5: TIME SPENT SITTING***

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

27. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekend day**?

_____ **hours per day**
_____ **minutes per day**

**This is the end of the questionnaire, thank you for participating.**

# Appendix H: Mass Email Recruitment Script

Mass e-mail script

We are looking for healthy men between the ages of 20-25 years old to participate in our study titled, "Circadian Rhythm Variation in Endocrine Biomarker Responses to High-Intensity Interval Training in College Aged Males". Potential participants must be recreationally active, non-smokers, weigh less than 300 lbs. and be less than 6 feet and 4 inches tall. In addition, potential participants should not be taking any performance enhancing drugs or supplements that may affect muscle mass. Participants will be excluded if they have artificial knee/hip joints or other metal implants in the spine or hips. Men with recent surgeries, or physical disabilities preventing them from performing maximal aerobic exercise testing and vigorous aerobic exercise will also be excluded from this study.

This study requires 5 visits for a total time of 9 hours (visit 1 = 1.5 hours; visit 2 = 45 minutes; visit 3 = 2 hours; visits 4&5 = 2 hours each). This study requires exposure to a small amount of radiation by DXA meant to assess your bone-free lean body mass, fat mass, and percent body fat. Participants will also perform a maximal aerobic exercise test to establish maximal aerobic capacity. Participants will perform 2 high intensity interval training sessions and saliva samples for measurement of endocrine biomarkers will be obtained before and after each session.

There are possible risks involved with participation, including risks associated with radiation exposure and maximal aerobic exercise testing. There is a possibility of mild soreness due to the maximal aerobic test and aerobic exercise sessions. Information regarding the results of the tests will be provided at the end of the study upon your request. You will not be paid for your time or participation, but you will receive a gift card after completing the 5 visits.

If you are interested in this study, please contact Cy Shuler via email (cy.shuler.1@ou.edu).
Principal Investigator: Dr. Debra Bemben
The University of Oklahoma is an equal opportunity institution.


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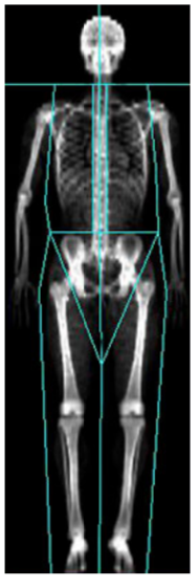
**Appendix I: Recruitment Flyer**

# Want Body Composition and Physical Performance Testing?

**Ciradian Rhythm Variation in Endocrine Biomarker Responses to High-Intensity Interval Training in College Aged Males**

**To Participate**

- Males aged 20-25 years
- Be physically active
- No HIIT participation in the past 3 months
- Nonsmokers
- No metal implants in the hip or spine
- Not taking medications or performance enhancing drugs known to affect muscle mass



**Required Testing**

- 5 visits over 2 weeks
- About 9 hours total time
- Non-invasive saliva sampling
- DXA total body scan
- Aerobic capacity testing

Possible risks are associated with radiation exposure and vigorous exercise.

*Tests will take place at SJ Sarkeys Complex Bone Density Research Lab, University of Oklahoma Norman Campus*

If you are eligible and interested, please contact:

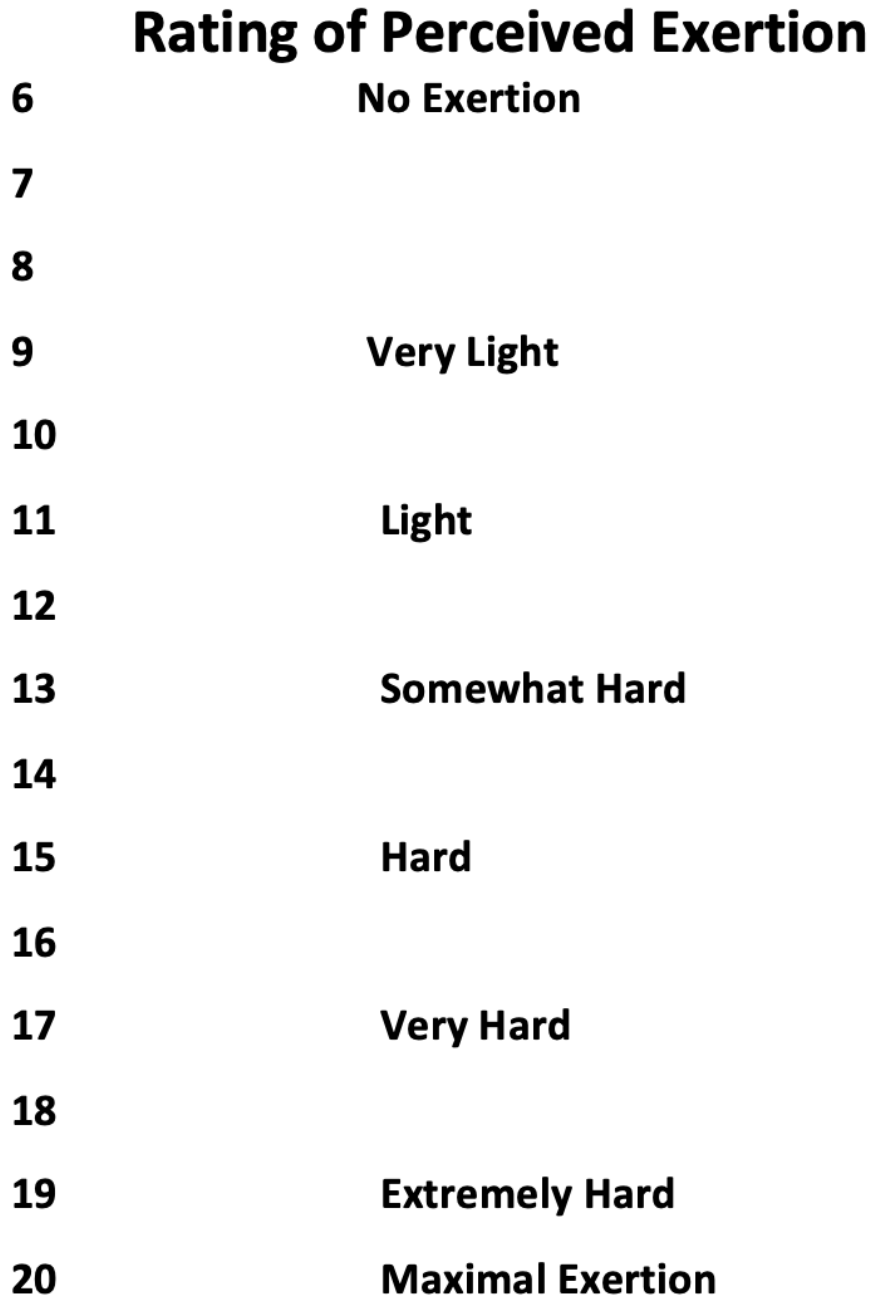
Cy Shuler at 405-837-5678, cy.shuler.1@ou.edu

Department of Health and Exercise Science

**The University of Oklahoma is an equal opportunity institution.**

Cy Shuler
405-837-5678
cy.shuler.1@ou.edu

**Appendix J: Ratings of Perceived Exertion Chart**



# Appendix K: Salivary Sampling Techniques



SALIMETRICS®

SalivaBio
Exclusively from Salimetrics

## Collection Methods: Passive Drool with the Saliva Collection Aid

**Introduction:** Whole saliva is the gold standard when collecting oral fluid for biological testing. It avoids localized secretions of specific salivary glands providing a more consistent specimen. Whole saliva can be easily evaluated for volume collected and for salivary flow rate. Free from being compromised by absorbing materials used with other collection methods, whole saliva can be assayed for all salivary analytes of interest.

The Saliva Collection Aid (SCA) is an ideal collection tool for collecting whole saliva (passive drool). Its ease of use reduces participant burden and improves compliance for collecting whole saliva.


No. 61/524,096
Patent Pending

**The Saliva Collection Aid offers the following benefits:**

- ✓ Simple and easy to use, one time use
- ✓ Individually packaged in a clean, foil pouch *(non-sterile)*
- ✓ Ready-to-go instructions
- ✓ Comfortable, no-mess collection
- ✓ Universal fit with external threaded cryovials
- ✓ Vented design reduces sample foaming
- ✓ Collection directly into cryovials right in the field, reducing freezer storage space
- ✓ Use for participants 3 years of age and older*
- ✓ Constructed of polypropylene
- ✓ Eliminates time and material needed to transfer specimen to storage vials in the lab

*NOTE: Sample collection with passive drool is designed for saliva donors who are able to follow simple instructions. For children younger than approximately 4 years of age, there may be wide ranging individual differences in their capability of collecting whole saliva. Thus, we encourage pilot study.

**Passive Drool Cautions:**

1. Use only as directed; Use each Saliva Collection Aid only once.
2. Do not use this device for children under the age of three (3), or without adult supervision
3. Do not disassemble or pull apart device; discard if disassembled.

**Materials Needed:**

- ☐ Cryovials (Salimetrics Item No. 5002.01-06)
- ☐ Saliva Collection Aid (Item No. 5016.02)
- ☐ Bar-coded labels (Item No. 5007.00)
- ☐ 2" swab storage tubes boxes (Item No. 5023.05)

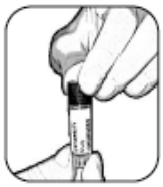
Salimetrics LLC, 101 Innovation Boulevard, Suite 302, State College, PA 16803 USA
Tel: 800.790.2258 Fax: 814.234.1608 www.salimetrics.com

## Instructions for Use:

**Step 1:**
Open foil pouch and remove the Saliva Collection Aid (SCA).

**Step 2:**
Place ribbed-end of the SCA securely into a pre-labeled collection vial *(see Caution 3 above)*.

**Step 3:**
Allow saliva to pool in mouth. Then, with head tilted forward, gently guide saliva through the SCA into the vial. Fill to the required volume.*



**Step 4:**
Remove and discard SCA. Attach cap to collection vial and tighten.

*NOTE: Reserve a small amount of air space in the vial to accommodate liquid expansion during freezing.

**Sample Handling and Processing** *(As described in the Saliva Collection Handbook)*:

- Immediately after collection, freeze samples at or below -20°C. If freezing is not possible, refrigerate immediately at 4°C and maintain at this temperature for no longer than necessary (ideally less than 2 hours) before freezing at or below -20°C (temperature of a regular household freezer).
- Samples stored for more than 4 months should be frozen at -80°C.
- Freeze-thaw cycles should be minimized for some analytes. It is critical that storage conditions are researched prior to initiation of sample collection.
- It is recommended that tubes be organized into cryostorage boxes (9x9 grids, 81 tubes) before storing or shipping.

## How to Reference this SalivaBio Device in Your Research (Recommended)

*"Whole saliva samples were collected with SalivaBio's 2 mL cryovials and the Saliva Collection Aid (exclusively from Salimetrics, State College, PA), a collection device specifically designed to improve volume collection and increase participant compliance, and validated for use with salivary [Analytes]."*

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**References are available online at;** http://salimetrics.com/collection-system/passive-drool

Developed in collaboration with the **Center for Interdisciplinary Salivary Bioscience Research** at the **Johns Hopkins University School of Nursing**

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