CIRCADIAN RHYTHM AND EXERCISES: HOW TIME OF DAY EFFECTS PHYSIOLOGICAL VARIABLES RESPONSIBLE FOR EXERCISE PERFORMANCE

by

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Throughout my undergraduate career in UTA, I have had the pleasure of meeting many professors that propelled, inspired, and pushed me to go above and beyond what has been academically asked. Though I had some ideas on what I desired to study under the senior project, nothing truly materialized throughout the years of my degree. In 2020, while most of my peers were confined to their rooms, rather than experiencing the full college experience the lecture halls, inspiration was lacking. Stress from current events, financials, and the inability to physically meet peers and professors affected different factors of my life, especially in sleep. Ironically enough, nights of sleep disruption encouraged me to naturally research physiological mechanics of sleep, and sequentially the circadian sleep cycle.

Reaching out to professors was handled smoothly as Dr. Judy Wilson readily received me under her research wing and became my mentor for the remainder of my senior year. From her, I learned many of my lab technical skills regarding protocols and etiquette in an exercise lab setting. In addition, I would also like to acknowledge my teacher assistances who aided in operating instruments and calibrating machines for proper readings for the experiment. Finally, a thank you to my family for lifting me up during the hard time in college and God for directing me to this path in life and revealing my general purpose in life.

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ABSTRACT

CIRCADIAN RHYTHM AND EXERCISES: HOW TIME OF DAY EFFECTS PHYSIOLOGICAL VARIABLES RESPONSIBLE FOR EXERCISE PERFORMANCE

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In contemporary physiology, the circadian rhythm influences more than the neurotransmitters melatonin and cortisol. Monitored from hypothalamic pacemakers and suprachiasmatic nuclei (SCN), metabolic and physiological structures are under a biomolecular schedule. Likewise, peripheral tissues are coordinated through the hormonal, autonomic, and behavior stimuli that direct the metabolic processes that occur in the skeletomuscular system. The purpose of the current study was to determine whether the time of day could significantly affect the metabolic response to an anaerobic capacity test. Subjects had weight, height, and body fat percentage (BF%) recorded before the Wingate

Anaerobic Test. Resting blood lactic levels were documented prior to exercise and the accumulation of blood lactate was documented 5 minutes post-exercise protocol. Fatigue index, peak and mean power were recorded post-test. There was no significant difference in baseline blood lactate prior to exercise protocol peak power, mean power, fatigue index, and blood lactate post protocol between the morning and evening periods ($p \geq .05$). It was concluded that time of day did not have a significant influence on exercise performance in the physiological variables measured.

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CHAPTER 1

INTRODUCTION

1.1 What is the Circadian Rhythm

One might have heard of the phrase "circadian rhythm" at one point when discussing the activity of sleeping. Colloquially, people understand this sleep-wake cycle as a biological mechanic responsible for sleeping and the accompanying phases of eye movements accustomed to it. Melatonin and the other hormones that regularly circulate throughout the body are chiefly controlled through the endocrine system. Before recent development, physiologists understood that these hormones were produced through the pineal gland and segments of the ocular system (1). Although this assertion is still correct and agreed upon by most human physiologists, recent studies have included other important factors that control more than the sleep-wake cycle. The entire endocrine system, according to research, follows similar mechanisms of cycles described in the production of melatonin and serotonin. These daily and periodic changes throughout the endocrine system operate in the hypothalamic pacemaker, dubbed by researchers as the "mammalian internal clock" (2).

The region of the forebrain that contains the "pacemaker" is officially known as the suprachiasmatic nucleus (SCN) and is responsible for all circadian rhythms that occur throughout the body (2). The nuclei influence the daily, monthly, yearly, and generational cycles in a mammal's endocrine circulation in a predictable manner (3). What differentiates the penial gland, and this nucleus is that though absences of direct sunlight have been shown to disrupt the daily sleep-wake cycle, a heavily reduced effect of the internal clock has been observed. This suggests that the region is autoregulated and can anticipate biological challenges throughout the 24-hour cycles (2). Questions arise between research on how exactly these nuclei can spread this influence across anatomical gaps.

1.2 Genetic Circadian Expression

The paired nuclei, situated above the optic chiasm, control the endodontic and metabolic rhythms via anatomical connections, specifically the neuroendocrine and autonomic pathways. However, scientists began discovering the amplitude of cells to adopt and follow a dynamic circadian rhythm like those located in the forebrain. Over the past two decades, physiologists began investigating the prominence of cellular gene expression in the functionality of the circadian cycle for every single neuron, according to experiments done on rodents and later fruit flies (4). An autoregulated, deferred, negative feedback loop occurs between two gene families: Period (Per1, Per2, Per3) and Cryptochrome (Cry1, Cry2). These gene transcriptions become activated through the introduction of "Clock" and bmal1 proteins. In the protein-to-protein mRNA interactions, Per and Cry fluctuate within the SCN throughout the 24-hour circadian cycles, increasing protein levels and peaking during the daytime period. Messenger RNA (mRNA) then begins to decline due to negative feedback action, as seen in the concentration of Cry proteins (5). Of course, there is much more to learn in this field of genetics, especially in the interaction of DNA and its structures.

1.3 Peripheral Tissues

As mentioned previously, circadian "clocks" have been observed in peripheral tissues, operating their physiological functions within a 24-hour cycle. Since the discovery of the circadian pacemakers at the turn of the century, physiologists and endocrinologists believed that the SCN was the chief operator of cycle expression in the peripheral tissues, as it does to endocrine, autonomic, and behavior efferent signals. However, near the turn of the century, research resulted in a study that investigated the circadian gene expression in mammalian tissues via serum shocking cell cultures (6). Researchers concluded that despite the initial separation of mammalian tissues from organ systems, the introduction of an anaphylactic shock caused fibroblasts to continue exhibiting circadian cycles in gene expression. This groundbreaking study opened the realm of chronobiology, as other research soon observed functional and fluctuating oscillators in transgenic rats when peripheral tissues were separated and propagated in culture dishes (7). Findings suggest that each organ system exhibits intrinsic circadian clocks independent of the SCN, though the nuclei can influence their "clock speed" when appropriate. Though the peripheral cells are just as functional as those observed in the central nervous system, the peripheral tissues have "clocks" that are more likely to diminish in rhythmicity and are described as "less competent" when compared to the hypothalamic nuclei (7).

1.4 Circadian Rhythm and General Health

For all-encompassing factors in human physiology, it's only natural that health and circadian cycles share a symbiotic relationship, especially in maintaining optimal metabolic functions. The inverse, likewise, is also true. Disruption in circadian rhythms has been observed from endocrine secretion rates to cardiovascular function, mood, and metabolic activities (8). Researchers have studied the general health of shift workers that frequently vary in working hours, i.e., day and night shifts and discovered that those with varied schedules present an increased rate of metabolic disease, gastrointestinal (GI) disturbances, and hypertension (9). The negative effects of a disrupted circadian rhythm continue from there, as more studies assert that obesity, diabetes mellitus, premature mortality, and loss of concentration have all been associated with shifting circadian cycles. As the field of medicine continues research on the circadian cycles in patients, more branches in human physiology are emerging (10).

1.5 Entrainment Signals and the Circadian Cycles

In the field of chronobiology, entrainment signals are a phenomenon that matches events to organism's external oscillation. Though sleep patterns form the bulk of a person's individual circadian cycle, research suggests that everyday activities can form the physiological pattern seen in previously discussed studies. For example, eating can elicit the regulation of insulin levels in the liver and alter the temporal metabolic activities of the organ, especially after fasting (11). Psychologically, emotions such as anger, irritability, and aggression are regularly experienced in different parts of the day. These variations in mood are known as a person's chronotype (i.e., a morning or evening person) (12). In the scope of exercise physiology, strong evidence exists that exercises appear to be a chief entrainment signal for mammalian circadian clocks. In addition to the archetypal light/night cycles in sunlight exposure through the photoreceptors in the SCN, physiological stress caused by exercise induces a phasic shift in circadian cycles, changing the sleep-wake schedule among humans. These external signals are known by professionals as "Zeitgebers", which allow an organism to properly adapt to their environment (13). Theoretically, if one were to train themselves to exercise at specific parts of the day, circadian clocks throughout the body would consequently adapt to the change in stressor, and physiological factors such as metabolic substrates and cardiovascular function will adapt respectively.

1.6 Skeletomuscular Functionality within Circadian Cycles

As mentioned, circadian clocks have been observed on peripheral tissues outside the central nervous system, including the musculoskeletal system. The musculoskeletal system makes up most of the body mass and is responsible for voluntary movement, posture, breathing, blood flow, through venous return, and metabolic responses. Peripheral tissues were thought to be controlled primarily through the SCN concerning circadian cycles, as believed before the works of Schibler et al. in 1998, but now are known to host their own molecular clock. This leaves scientists to examine the metabolic activities of skeletal muscles, as numerous studies have been conducted regarding the metabolic cycles in the liver (14). As expected, the researchers observed the same circadian-dependent oxidation of carbohydrates and fatty acids, officially seen in a higher respiratory quotient of rodents during specific periods of the day, which denotes carbohydrate usage in physical activity (15). Furthermore, glucose intake forms muscle glycogen through gluconeogenesis in the skeletal muscles. Distinct from other peripheral tissues, skeletal muscle activity, seen through a voluntary contraction, appeared to be able to affect the organ system's circadian rhythmicity (16). This powerful ability to alter the system’s circadian clock seems to strictly influence the muscle's rhythmicity and does not vary the SCN circadian clock. Review articles concerning the performances of skeletal muscles have shown that performances variables (i.e., torque, strength, and power) become higher during afternoon hours when compared to morning hours (17).

A reoccurring theme in these articles are subjects sampled from older athletic populations outside the college 18–22-year-old range and their prior experiences in exercises, which could determine a person's propensity in exercising at specific time schedules. Also, routine exercise bouts at specific times creates an anticipatory effect in the circadian clock genes. The protein responsible for antioxidation in responsive to reactive oxygen species created during exercise, Ucp3, increases chronologically before the exercise bout starts. This reaction, according to researchers, leads to improved performances during the same time in scheduled training (18). Each person exhibits their own preferences in exercising at a specific time, sharing this characteristic with one's sleep-wake cycle. Understanding how a person may respond to exercise at different hours of the day can optimize performance and reduce the chances of injury (19).

1.7 The Known and Unknown

In sports competitions, the smallest edge can make a difference in the components of sports performances (i.e., speed, muscular strength, short term output) and could be the difference in which a team, nation, or individual reach first place. Thus, much research has been conducted in chronobiology to maximize training gains, improve performance, lower chances of injuries, and heighten flexibility. The major components of sports performances have been observed to vary in a "sinusoidal" fashion, increasing and decreasing at differences throughout the 24-hour cycle and peaking during the evening hours (20). This pattern of performances coincides with core temperature as well, fluctuating throughout the day and peaking near evening hours. It is presently unknown if the time of day could influence physiological adaptation (i.e., muscle strength, hypertrophy, endurance, etc.) in a set training protocol where the time of exercises does not change and is strictly followed

(20). Moreover, different age groups respond to changes in exercises, demonstrating how athletes of a particular age, for example, those at or above the age of 50 years. seem to display a proclivity to morning hours, when compared to much younger athletes.

For skeletal muscles to produce voluntary movement, the tissues must utilize metabolic substrates to complete the contraction. Through excision of rats' livers and skeletal muscles post swimming exercise protocol, investigators were able to document changes in muscle and liver glycogen stores through the 24-hour cycle. The glycogen content examined from the excised tissues were higher among rats who swam during the morning than in the late evening hours (21). The significance of this study and others like it is that those metabolic substrates responsible for producing adenosine triphosphate (ATP) and other cellular activity can fluctuate through the waking hours. This supports the assertion of sports performances being predicated on variables like metabolic subtracts and how the time of day plays a role. Another consideration in exercise performance is how training routines can change the innate propensity for exercise at certain hours. As formerly mentioned, having an individual train at specific periods throughout their daily routine will eventually increase their anaerobic peak power at a regularly scheduled training time. There are more questions to solve concerning chronobiology and exercise sciences.

1.8 The Purpose and Hypothesis

Given the scope of this project, there are several questions about performances that should be investigated, especially in the field of exercise science. Investigating time-of-day of sessions and how it could affect performances for activities involving anaerobic power will be invaluable in the world of sports and athletic training. The purpose of this research study was to measure any significant differences in exercise performances in a

Wingate Anaerobic Test (WAnT) performed at different times of the day. Several variables are involved in the measurement of power in the field of human physiology, but for the purpose of this trial, the paper's focus shifts towards anaerobic peak power, mean power, an individual's fatigue index, and blood lactate concentrations.

Peak power is defined as the largest output of production of work over a given amount of time and accounts for the sum of strength, velocity, and neuromuscular force production possible, expressed in wattage (W). Mean power, following similar logic, is the expression of power over time. Using these variables, a fatigue index is formed, which is the measurement of the anaerobic power of an individual. Simply put, a fatigue index illustrates the rate of time to exhaustion when performing strenuous physical activity. Performance variables were documented for absolute (W) and relative values (W/kg). Blood lactate concertation is an important assessment of glycolytic activity occurring within the skeletal muscle system. Measured in mmol/L, levels fluctuate throughout rest and physical activity, only peaking at the maximum rate of exertion (22). It was hypothesized that blood lactate concertation would not be significantly different, while peak/mean power and fatigue index would be significantly different at different times of the day. In theory, it was thought that force production would occur later in the afternoon/evening when the core the body temperature was the highest (23).

CHAPTER 2

METHODOLOGY

2.1 Subject Population

Volunteers for the study (n = 5) were all male and of traditional college-age (22.2 ± 2.95 yrs.). Prior to exercise, each subject had their height (172.4 ± 8.1 cm) and weight (87.3 ± 20.3) measured. Skinfolds were measured to assess body fat percentage. Body fat percentages were determined using the 3-site skinfold methodology for men (22.1 ± 6.9 %).

2.2 Skinfold

To perform the skinfold, a fold of skin was produced between my thumb and forefinger, about 1 cm above each anatomical landmark. Skinfold large calipers by Beta Technologies, Inc (Cambridge, MD) were applied to the fold and the spring handles released fully. When the pointer on the dial steadied, a measurement in millimeters was read and recorded. The measurements were all taken on the right side and were repeated a second time to form an avenger of each site. Chest or pectoral, skinfolds were produced via a diagonal fold, with at least one-half the distance between the anterior axillary line and the nipple. Abdominal folds were measured by a vertical fold, at 2 cm to the right of the umbilicus. Thigh skinfolds were measured from a vertical fold, on the anterior midline of the thigh, midway between the proximal border of the patella and the inguinal fold.

Figure 2.1: Lange Calipers for Skinfold

<u>2.3 Wingate Anaerobic Test Protocol</u>

Before arrival, subjects were advised to avoid heavy eating, strenuous exercises, alcohol, and caffeine, before the exercise protocol. Subjects were instructed to enter the kinesiology lab on two different separate occasions on different days: 8-10 a.m. and 4-6 p.m. To ensure proper rest between each protocol session, at least 48 hours were given for complete recovery. Preliminary tests and demonstrations were done to familiarize the subject with the Wingate Anaerobic Test (WAnT) protocol and calibrated settings were adjusted for subjects on the cycle ergometer by Lode for Life (Groningen, The Netherlands). A 2-minute warmup of cycling was done to increase muscle temperature, and blood flow and prepare working joints for the upcoming workload. During warmup, subjects pedaled above 60 rpm.

Figure 2.2: Warm-up Segment of the Wingate Anaerobic Test

At 1:55 minutes of the warmup, command "start," subjects pedaled as fast as possible against the resistance and were encouraged to do so for 30 seconds. Throughout the 30 seconds, the ergometer applied resistance according to the weight of the subject in kilograms. The resistance, or test weight, was measured as 0.075 * kgs of body weight. The primary investigator monitored the subject to ensure proper form. Verbal encouragement was given to the subject to boost motivation against the resistance. After the 30-second protocol, resistance was lifted, and the subject was encouraged to pedal for 2-3 minutes to cool off and avoid fainting or injuries.

Figure 2.3: Wingate Anerobic Test Protocol

2.4 Blood Lactate

Blood lactate concertation at rest was measured at the start of each session after body fat percentages were evaluated. Subjects were redirected to a metabolic room to ensure sanitary procedures were followed. Each subject's fingers were cleaned and sterilized with alcohol wipes to avoid infection. A medical lancet was used to prick the finger to obtain a blood sample via capillary action. The blood samples were placed on a blood lactate strip and placed inside the Nova Biomedical Accusport Lactate Analyzer. (Waltham, Ma). The lactate level of the blood was then determined using the instrument. The subject was then given a piece of gauze to stem the bleeding and a band-aid as necessary. All equipment used was disposed of in the proper biohazard waste containers.

Figure 2.4: Blood Lactate Stripes and Nova Biomedical Analyzer

After WAnT protocol and cool off, subjects were redirected back into the metabolic lab and had their blood lactate read 5 minutes post protocol. Peak power, mean power, and fatigue index was documented through Software Lode for Life which was connected to the ergometer.


Figure 2.5: Blood Lactate Concentration Reading

2.5 Statistical Analyses

Performance and blood lactate concentrations variables in time-of-day variations were analyzed by one-way analysis of variance (ANOVA) with t-test repeated (paired) measures according to the time of day. All analyses were accomplished using Microsoft Excel version 2108 for Microsoft 365 MSO. The statistical significance was decided as $p \leq 0.05$.

CHAPTER 3

RESULT

3.1 WAnT Performance Results

The three measured performance variables from the WAnT at opposing hours of the day were presented in average values of participants. Presented at Table 2, the average peak and mean power values are shown in their absolute values (W) as well as fatigue indexes. For absolute peak power production, average morning values (733.2 ± 226.6 W) were slightly higher than evening values (689.8 ± 187.1 W), as seen in Figure 3.1.

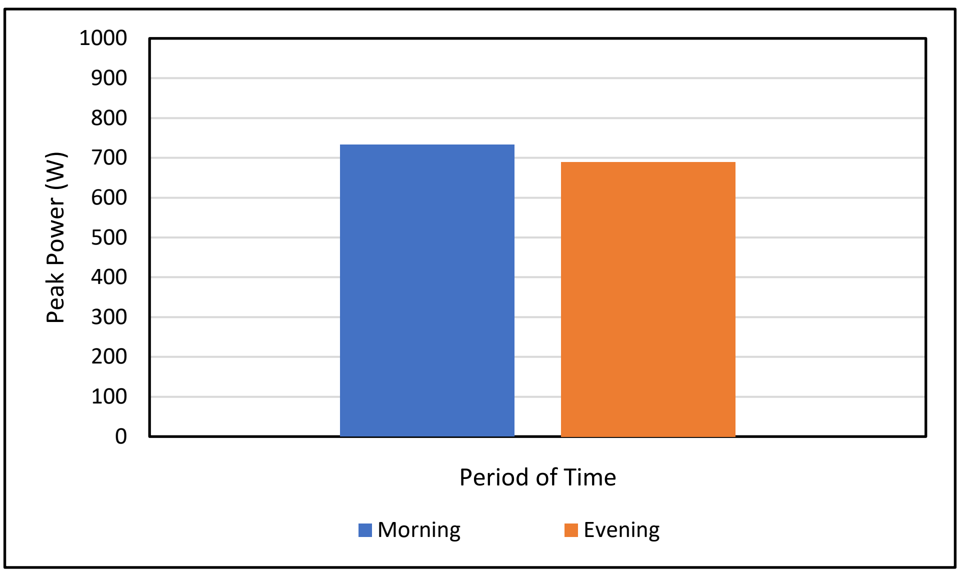

Figure 3.1: Absolute Peak Power Produced

For absolute mean power production, morning values average morning (491 ± 101.4 W) and evening (492.3 ± 101.7 W). Values were nearly undistinguishable and varied little, as shown in Figure 3.2.

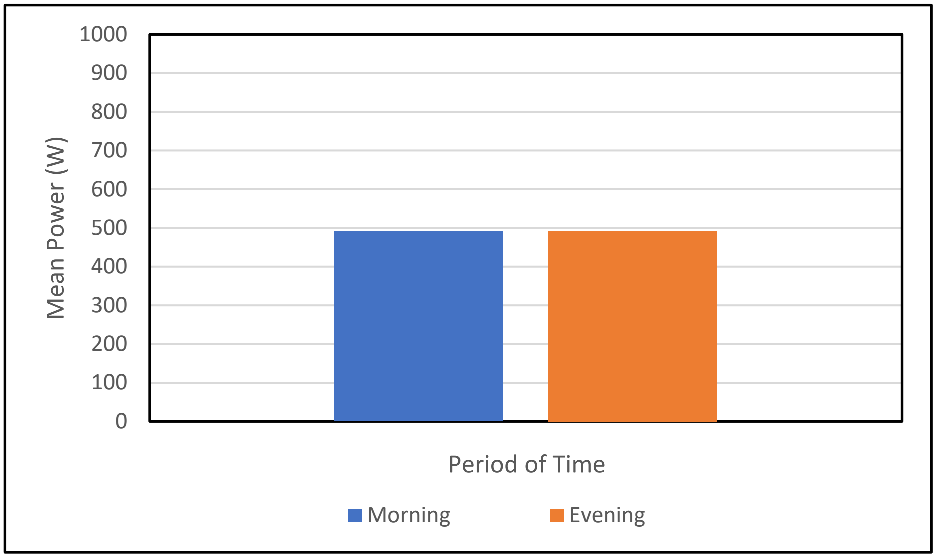

Figure 3.2: Absolute Mean Power Produced

Fatigue indexes followed similar result, exhibiting meager differences in average morning (64.1 ± 24.2 %) and evening values (61.9 ± 18.8 %), presented in Figure 3.3.

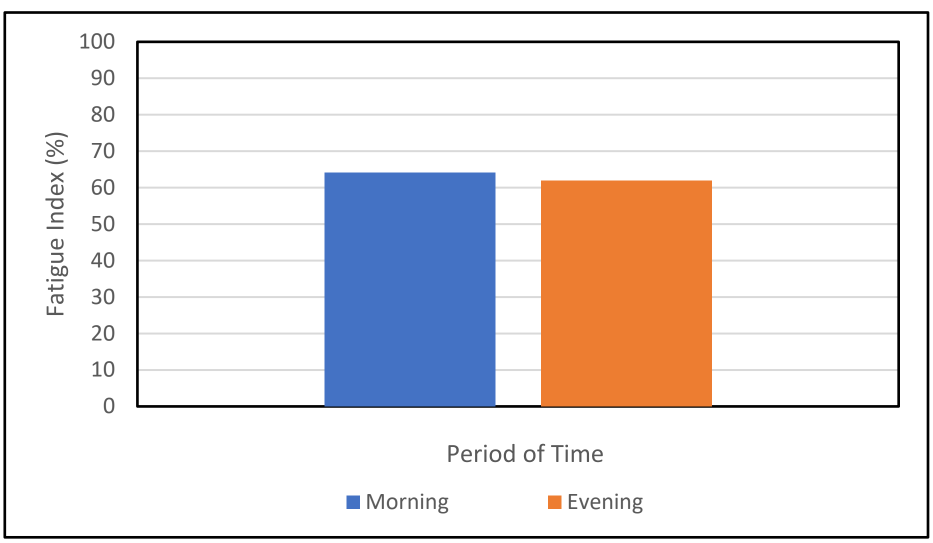

Figure 3.3: Fatigue Indexes throughout the WAnT

Blood lactate concertation before and after the protocol during the morning and evening sessions are given in Table 1. Resting blood lactate concentration for morning (1.66 ± 0.6) and evening sessions (1.74 ± 0.5) shared little observable difference, as seen in Figure 3.4.

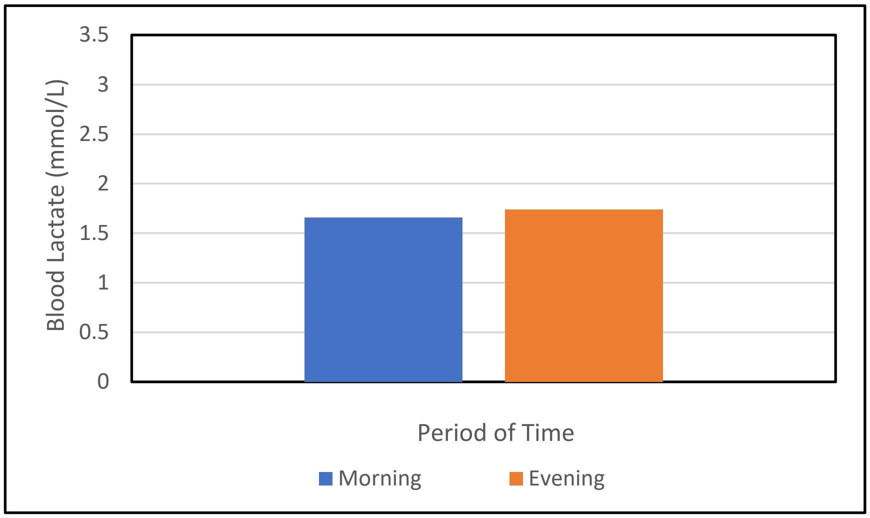

Figure 3.4: Resting Blood Lactate

Blood lactate concentration post protocol showed slightly varied results, with lower morning (8.9 ± 1.4 mmol/L) and higher evening sessions (1.74 ± 0.5 mmol/L), values seen in Figure 3.5.

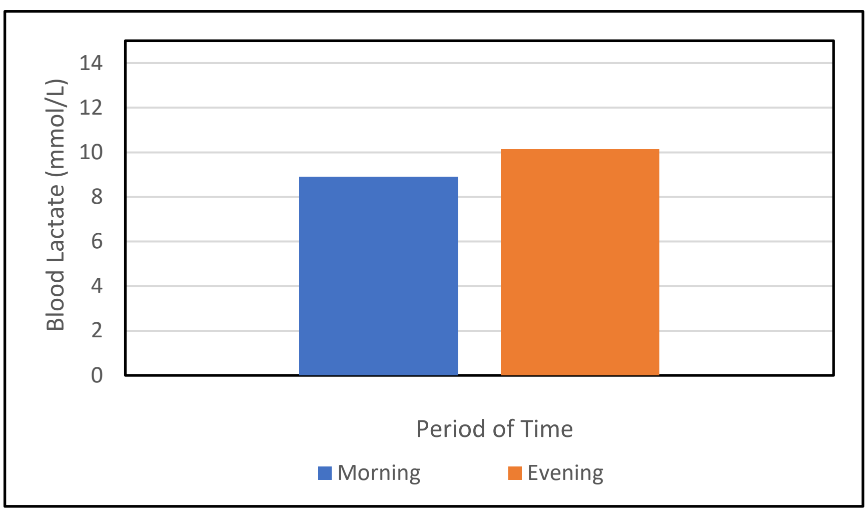

Figure 3.5: Post Exercise Blood Lactate

ANOVA statistical analysis between hours showed no significant difference in absolute peak power, mean power, fatigue indexes, resting and post protocol blood lactate concentration. ($p > 0.05$).

	8-10 a.m.	4-6 p.m.	F ratio	*p*
Peak Power (W)	733.2 (226.6)	689.8 (187.1)	0.1090	0.75
Mean Power (W)	491.0 (101.4)	492.3 (101.7)	0.0004	0.98
Fatigue Index (%)	64.1 (24.2)	61.9 (18.8)	0.0263	0.88
Resting Blood Lactate Concentration (mmol/L)	1.66 (0.6)	1.74 (0.5)	.0278	0.87
Post-Protocol Blood Lactate Concentration (mmol/L)	8.9 (1.4)	10.12 (5.1)	1.1525	0.31

Table 3.1: Absolute Values of Exercise Performance

Software by Lode also calculated the relative values based on weight of subjects of anerobic power produced from the WAnT, which are presented in Table 3. In the relative peak power, average morning values (8.4 ± 3.1 W/kg) were slightly higher than evening values (7.9 ± 0.6 W/kg). In the inverse, relative mean power showed no discernable difference between morning (5.7 ± 0.8) and evening session (5.7 ± 0.8). Similar to absolute values, ANOVA statistical analysis between hours showed no significant difference in relative peak power and mean power, as seen in Table 2 ($p > 0.05$).

	8-10 a.m.	4-6 p.m.	F ratio	*p*
Peak Power (W/kg)	8.4 (3.1)	7.9 (0.6)	0.3724	0.56
Mean Power (W/kg)	5.7 (0.8)	5.7 (0.8)	0.0008	0.97

Table 3.2: Relative Values of Anaerobic Power Production

CHAPTER 4

DISCUSSION

The following experiments were performed at two opposing periods of the day, morning, and evening hours with at least 48 hours of resting between protocols to avoid muscular fatigue or delayed onset muscle soreness. The rationale for conducting the test at different timetables was to coincide with research suggesting that there was a fluctuating core temperature of humans and its correlation to exercise performance (24). The lack of significant differences between peak and mean power in the morning and afternoon was surprising, as studies have documented observable differences in power production (25, 26, 27). However, studies have disputed the notion of differences in anaerobic performance concerning the time of day (28). The psychological state could be a factor in performance in the WAnT and could influence an individual's anaerobic performances. Mental factors, such as verbal motivation and psychological drive, or the lack thereof, may have a larger impact in short-term exercise testing than expected and has already been investigated in anaerobic, short-term exercise performance (29). Likewise, variation of test weight protocol could affect the peak power and mean power. The two-minute warm-up applied before the protocol would most likely be a factor in the similarities of performances, especially as studies have documented the case of performance improvement with increasing levels of core body temperature, a consequence of the warmup (30). If anything, it signifies the importance of properly warming up before an exercise bout.

Other confounding factors may include the training schedule of subjects. Though the population chosen were all college-aged male students of similar builds, their training schedules were not evaluated during the assessment process. When engaging in training routines at different hours of the day, measurable performances begin to broaden out, markable differences in exercise performances lowers. As a result, individuals would be able to perform similarly throughout the day, further reducing diurnal exercise performance differences (31). To mitigate this factor, questionnaires could be developed to assess the training schedule of potential subjects and sort out those whose training schedules are too broad or too narrow.

A finding of no significant differences between resting and post-exercise blood lactate concentrations was expected. For example, due to the duration of the WAnT, the fast glycolytic metabolize pathways were simply unable to engage their fullest physiological potential to engage the anaerobic glycolytic pathway and release blood lactate as a byproduct (32). Though physiologists are aware of the anaerobic enzymes responsible for the production and clearance of blood lactate and that they are positively affected by core temperature, the warm-up, protocol, and diurnal variation were not sufficient to elicit a noticeable difference in anaerobic glycolytic metabolism (33). Studies that introduce other modes of exercise such as cycling (34) and incremental rowing protocol (35) boasted similar results of little to no changes in blood lactate concertation. However, other researchers have investigated aerobic metabolic pathways, and have seen a notable difference in blood lactate concertation when subjects were prescribed longer training programs rather than shorter lengths (36).

CHAPTER 5

CONCLUSION

This study has shown that time of day does not significantly affect the anaerobic power or capacity. As seen in both relative and absolute mean power and peak power, the WAnT was unable to elicit notable changes in performances based on time of day. The fatigue indexes derived from the protocol performances also failed to show a significant difference. Resting and post-protocol blood lactate concentration resulted in no significant difference in time-of-day variation for short-term anaerobic exercise. The limitations faced in the experiment were a smaller sample population, and future endeavors should focus more on larger population from samples exhibiting proficiency in the WAnT protocol. Future recommended investigations could apply training programs to detect differences in adaption and examine for other physiological variables, such as muscular activation through an EMG and recruitment.

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BIOGRAPHICAL INFORMATION

Chidozie Nwankwo was born on December 27, 1999. He graduated at the age of 18 from St. Paul’s Preparatory Academy, then continued to the University of Texas at Arlington (UTA). At UTA, he is a member of the Honors College and the Society of Kinesiology Scholars. He is pursuing an Honors Bachelor of Science in Exercise Science. Chidozie has become interested in physical therapy and is looking forward to entering a doctorate program for physical therapy around the DFW area after graduation. He hopes to continue his knowledge of human anatomy and physiology before, during, and after receiving his Ph.D.