DISSERTATION

COUNTERMEASURES TO THE CARIDOMETABOLIC IMPAIRMENTS ASSOCIATED WITH SLEEP AND CIRCADAIN DISRUPTION

Submitted by

Sophie Lynn Seward

Department of Health and Exercise Science

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Doctoral Committee:

Advisor: Josiane L. Broussard

Jennifer M. Blankenship
Michelle T. Foster
Kerrie L. Moreau

ABSTRACT

COUNTERMEASURES TO THE CARIDOMETABOLIC IMPAIRMENTS ASSOCIATED WITH SLEEP AND CIRCADAIN DISRUPTION

Sleep and circadian disruption are widespread and have both been recognized as independent risk factors for cardiometabolic diseases, including cardiovascular disease and type 2 diabetes. Circadian misalignment, defined as a mismatch between the endogenous circadian system and external behavioral/environmental factors such as sleep, energy intake, activity and light, is one mechanism suggested to increase the risk of cardiometabolic disease risk during combined sleep and circadian disruption. In some populations, such as night shift workers, sleep and circadian disruption are often unavoidable and lead to an increased risk for cardiovascular disease and type 2 diabetes. Therefore, strategies are needed to mitigate the negative impact of sleep and circadian disruption on cardiometabolic disease risk.

The following dissertation is comprised of a series of experiments with the overall aims to (1) examine the acute cardiometabolic impairments associated with sleep and circadian disruption; and (2) investigate a potential countermeasure to mitigate cardiometabolic impairments when sleep and circadian disruption are unavoidable.

Several study designs were employed to address the aims of this dissertation. In the first investigation, a quasi-randomized crossover study was conducted in a free-living setting to compare blood pressure in adults during a 24h period during both a day shift versus a night shift. Because elevated blood pressure is an important CVD risk factor, throughout this study blood pressure was measured every 30 minutes to assess

24h, waking, and sleeping levels. In the second study, a 2-week consecutive design experiment was conducted in healthy adults to examine the impact of altered meal timing on cardiometabolic health. During Week 1, participants consumed energy over a 13h period with meals individually anchored to habitual wake time. In week 2, participants matched their food intake from week 1 but restricted intake to an 8h period. At the end of each week, participants were admitted to the laboratory for an in-patient overnight stay where hourly blood samples were collected and assayed for circulating factors including glucose, insulin, and lipid species. This alteration in meal timing was then employed in another study where participants underwent a simulated nightshift work protocol. In this study, participants underwent a 6d randomized crossover inpatient study with at least 1 month between conditions. In one condition, participants ate during the biological night as typically done during night shift work (circadian misalignment). In the other condition, participants consolidated meals to the biological daytime (circadian misalignment + time restricted eating), while still providing the same sleep opportunity and diet. Consolidating food intake to the biological daytime, particularly in night shift work, was hypothesized to reduce the risk of cardiometabolic disease by reducing the mismatch between the endogenous circadian system and external behaviors.

Major themes emerging from these studies are that as little as one night of shift work in a free-living setting is sufficient to induce multiple CVD risk factors including increased BP and reduced sleep duration in healthy adults. Furthermore, data presented throughout this dissertation suggest daytime eating during sleep and circadian disruption, within a highly controlled laboratory setting, improves important markers of cardiometabolic health including blood pressure.

Data presented here indicates that meal timing interventions may be a potential countermeasure to improve cardiometabolic health in conditions of circadian disruption. However, more research is needed in a free-living shift work population. Additionally, future research should explore how other countermeasures may improve cardiometabolic health when sleep and circadian disruption are unavoidable. Overall, work presented throughout this dissertation underscores the urgent need for effective strategies to mitigate cardiometabolic risks associated with sleep and circadian disruption, especially in vulnerable populations such as night shift workers.

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"If you want to be successful, surround yourself with people who are more successful than you."

-Coach Tim Hebert

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SYNOPSIS

This thesis comprises a compilation of one book chapter and three original research articles at different stages of preparation, submission, and publication. This format adheres to the Colorado State University Graduate School guidelines. The introduction in Chapter 1 includes a copy of the book chapter published in the Encyclopedia of Sleep and Circadian Rhythms (2nd ed.), entitled “Continuous Glucose Monitoring in Sleep and Circadian Research.” Chapter 2, entitled “Acute Night Shift Work is Associated with Increased Blood Pressure and Reduced Sleep Duration in Healthy Adults,” is an original research article currently under review by the American Journal of Physiology: Physiological Reports. Chapter 3 has been prepared for submission to Obesity and is presented in its final form, excluding collaborator contributions. Chapter 4 provides a general summary and discussion of the future directions of this research. Additionally, an original research article with ongoing data collection is included in Supplement 1. The candidate is the first author on all included articles.

PREFACE

The average human spends over 24 years of life asleep, though the exact function of sleep remains a mystery. Sleep is not a behavior unique to humans. In fact, sleep-like behavior can be found across most species including organisms without centralized nervous systems like the box jellyfish (1). Sleep is crucial for optimal cognitive and physical performance, health, and well-being, and unrelenting total sleep deprivation is fatal when implemented in rodents (2). This widespread presence and critical importance of sleep across diverse species provide compelling evidence that sleep is a fundamental component of biology and life.

To regulate sleep, the human body relies on an internal timekeeper known as the circadian rhythm. This circadian rhythm signals when to sleep and wake, aligning our physiology and behavior to the 24h rotation of the earth. This system includes the suprachiasmatic nucleus located in the hypothalamus, which acts as the central clock, and peripheral clocks that are present in nearly all tissues and organs (3). At the cellular level, these rhythms are generated by transcriptional-translation feedback loops. Light is the most robust external timekeeper or zeitgeber that keeps our circadian rhythm aligned with the 24h rotation of the earth. When the retinas are exposed to light, a signal is transmitted to the brain via the optic nerve. This signal then reaches the hypothalamus and the pineal gland, which suppresses the secretion of melatonin, a hormone that promotes sleep. In addition to light, other potential zeitgebers such as meal timing and exercise may provide time cues that act on peripheral clocks throughout the body. The synchronization of these signals is important for maintaining

cardiometabolic health. Disruptions in the timing of light exposure, energy intake, or physical activity can negatively impact overall health and well-being.

Circadian alignment, as discussed in this dissertation, refers to the match between the endogenous circadian system and external behavioral/environmental factors such as sleep, energy intake, activity and light. In contrast, circadian misalignment occurs when behavioral and environmental factors are incompatible with the endogenous circadian system, which is linked to negative health ramifications, including cardiometabolic diseases. Evidence suggests that the mistiming of behaviors such as sleep, light exposure, energy intake, and physical activity may increase the risk of cardiometabolic diseases. One example of acute circadian misalignment is jet lag, which occurs traveling across time zones. Jet lag consists of changes in external cues such as light-dark cycles and meal times, which can disrupt the body's endogenous circadian rhythms, cause acute sleep disturbances (4) and deteriorations in glycemic control (5).

People who work evening, night, or rotating shifts—referred to as "shift work"—have gained recent attention in the sleep and circadian field because they experience repeated bouts of circadian misalignment and are at an elevated risk for cardiometabolic diseases. In fact, people who participate in shift work are 44% more likely to develop type 2 diabetes (T2D) (6) and 40% more likely to develop cardiovascular disease (CVD) (7) compared to people who work day shifts. Shift work is prevalent in the United States, constituting a quarter of the United States workers (8) and includes professions such as police, paramedics, firefighters, military personnel, pilots, doctors and nurses, and truck drivers.

This dissertation will focus on night shift work. Night shift work is defined by the International Labor Organization as any work that is performed for at least 7 consecutive hours between 2400 to 0500 (9). Due to work schedule demands, people who work night shifts conduct work operations during the night when the internal circadian system promotes restfulness. These workers then sleep during the day when the circadian system promotes wakefulness. It is hypothesized that the mismatch between the timing of external behaviors (e.g., sleep, light exposure, energy intake, and physical activity) and the endogenous circadian system may contribute to the elevated risk for cardiometabolic diseases among people who work during the night compared to people who work during the day. However, circadian misalignment may not be the only factor that contributes to the elevated risk for cardiometabolic diseases amongst night shift workers compared to day shift workers. Daytime sleep, which is common in night shift workers, is associated with insufficient sleep duration and quality. For example, a study conducted in rotating shift workers compared sleep duration and quality using polysomnography following a night shift compared to an afternoon shift and found that participants slept 2 hours less and had significant reductions in Stage 2 and REM sleep compared to sleep following an afternoon shift (10). As a result, it is thought that both the repeated bouts of insufficient sleep and circadian misalignment amongst night shift workers contribute to the increased cardiometabolic risk.

Insufficient sleep and circadian misalignment are interrelated, and both contribute to increased cardiometabolic risk. For example, circadian misalignment, such as in night shift work, leads to insufficient sleep. Conversely, insufficient sleep can also cause circadian misalignment. For example, a person may need to wake up early for a flight.

In order to get to the airport on time, they may curtail their sleep in the morning, causing them to be awake during the biological nighttime when melatonin levels are elevated. Data from an in-patient study indicates that such scenarios result in circadian misalignment and compromise insulin sensitivity, which is linked to an heightened risk of obesity, diabetes, and metabolic syndrome (11). Given that 35% of adults receive less than the recommended amount of sleep (12), chronic and acute circadian misalignment is likely more widespread than commonly recognized. Throughout this dissertation the term “sleep and circadian disruption” will be used to refer to the combined effects of insufficient sleep and circadian misalignment.

Small, acute changes in sleep and circadian disruption have a significant impact on cardiometabolic health. Recent epidemiological evidence suggests that for every hour that an adults sleeps less than seven hours, there is a 11% increased risk of CVD (13). Further, the transition from Standard Time to Daylight Savings Time, which reduces sleep opportunity by an hour, serves as a clear example of the consequences of sleep and circadian disruption on CVD risk. The acute sleep loss associated with Daylight Savings Time is linked to a notable spike in myocardial infarctions and strokes immediately following the shift (14). Evidence from studies indicate a 5% to 17% increase in hospitalizations the week following the shift to Daylight Savings Time (15). Conversely, following the transition from Daylight Savings Time to Standard Time, which allows for an extra hour of sleep, evidence suggests the rate of myocardial infarctions declines.

Both sleep and circadian disruption are increasingly recognized as risk factors for cardiometabolic diseases, including T2D and CVD. This dissertation focuses on both

the acute and chronic impact of sleep and circadian disruption on cardiometabolic health. Chapter 1 will begin by reviewing literature on the impact of sleep and circadian disruption on glucose homeostasis and insulin sensitivity in highly controlled inpatient research settings. Additionally, it will discuss how incorporating wearable sensors, like continuous glucose monitors, offers a novel opportunity to understand behaviors and interventions in a free-living setting.

Chapter 2 will shift focus to the cardiovascular impairments associated with acute sleep and circadian disruption. This chapter will add a cardiovascular perspective complementary to the metabolic focus of Chapter 1. Specifically, in this chapter, we explore the acute impact of shift work on CVD risk factors including 24h blood pressure and sleep duration in a free-living setting among rotating shift workers. This chapter demonstrates how as little as one night of shift work is associated with increased blood pressure and reduced sleep duration in healthy adults, highlighting how sleep and circadian disruption are intertwined and likely both contribute to the CVD risk associated with shift work.

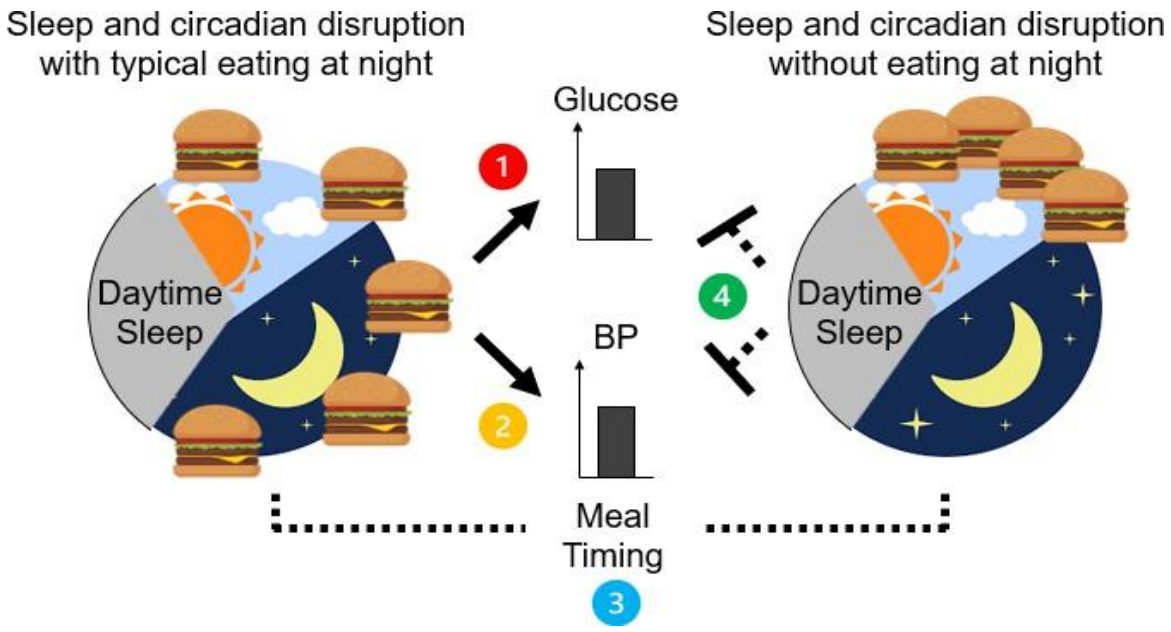
After the cardiometabolic consequences of sleep and circadian disruption are discussed in Chapters 1 and 2, the natural question arises: What solutions exist? Chapter 3 delves into behavioral alterations, such as meal timing, that can have significant implications on cardiometabolic health. Data from previous studies have found that glucose tolerance varies across the 24h period, with higher blood glucose response to an identical oral challenge in the evening compared to the morning (16). Early time-restricted eating (eTRE) is a dietary strategy that restricts energy intake to a 6-10h period beginning in the morning. It has been suggested that eTRE improves

cardiometabolic health by aligning meal timing with circadian rhythms that promote metabolism, independent of weight loss (17). In this chapter, we explore how shortened energy intake for one week influences temporal changes in circulating factors related to cardiometabolic health.

Finally, in the Supplement, we integrate findings from Chapters 2-3, which address cardiometabolic impairments associated with sleep and circadian disruption, with Chapter 4's exploration of eTRE as a dietary strategy to enhance cardiometabolic parameters. Leveraging my NIH-funded F31 project (Visualization of Dissertation), we investigate whether implementing eTRE in a healthy cohort of individuals experiencing acute sleep and circadian disruption can mitigate these cardiometabolic impairments. Specifically, this chapter explores if altering meal timing to align with the endogenous circadian system, when sleep timing cannot be adjusted (i.e. in a shift work population), can mitigate the cardiometabolic impairments associated with sleep and circadian disruption. This chapter aims to assess whether avoiding food intake during the biological night can reduce the impact of sleep and circadian disruption on cardiometabolic factors in a simulated night shift work setting.

In conclusion, sleep is a universal biological process regulated by our circadian rhythm to the 24h day and is essential for optimal health and well-being. Chapters 1 and 2 of this dissertation investigate how disruptions in sleep and circadian rhythms contribute to cardiometabolic risk. Chapter 3 and the Supplement propose behavioral interventions, such as meal timing strategies, to mitigate the risks associated with sleep and circadian disruption. Ultimately, this research aims to examine the acute impairments associated with sleep and circadian disruption, and to develop potential

strategies for mitigating the cardiometabolic effects of sleep and circadian disruption in vulnerable populations such as night shift workers.



Visualization of Dissertation – Numbered circles delineate dissertation chapters aimed at addressing the current gap in knowledge.

Sleep and circadian disruption are associated with heightened risk for T2D (red circle; Chapter 1) and CVD (yellow circle; Chapter 2). During sleep and circadian disruption, there is a mismatch between the endogenous circadian system and external behavioral/environmental factors such as sleep, energy intake, activity and light, which likely contribute to the cardiometabolic risk associated with sleep and circadian disruption. In populations, such as night shift workers, sleep and circadian disruption are often unavoidable. A strategy is needed to mitigate the impact of sleep and circadian disruption on cardiometabolic disease risk. Our lab and others have investigated the

impact of consolidating meal timing on circulating factors related to cardiometabolic health (blue circle; Chapter 3). This strategy, particularly in shift works, has been hypothesized to reduce the risk of cardiometabolic disease in shift workers by lessening the mismatch between the endogenous circadian system and external behavior. Evidence from our lab suggests daytime eating during sleep and circadian disruption in a highly controlled laboratory setting improves important markers of cardiometabolic health including glucose and BP (green circle; Supplement).

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CHAPTER 1 – CONTINUOUS GLUCOSE MONITORING IN SLEEP AND CIRCADIAN RESEARCH[1]

SLEEP AND CIRCADIAN DISRUPTION IN METABOLIC HEALTH

The prevalence of type 2 diabetes mellitus (T2DM) is increasing at an alarming rate in the United States and worldwide. By current estimates, more than 34 million people in the United States have been diagnosed with T2DM, representing 11% of the population (1). In 2017, diabetes-related health expenditures in the United States were $327 billion and the annual cost of diabetes is predicted to nearly double by 2030 (2). Thus, reducing the risk of T2DM, as well as the burden of disease management, is critically important.

Traditional risk factors for T2DM include the presence of obesity (body mass index or BMI > 30 mg/m^2), older age (≥ 45 years old), family history of diabetes, high blood pressure, elevated cholesterol and triglycerides, and physical inactivity. More recently, however, sleep and circadian disruption have emerged as important risk factors for the development of T2DM. In fact, in a meta-analysis that examined 1,061,555 people from 36 different studies, sleep and circadian disruption were associated with an increased risk for diabetes with similar odds ratios for developing diabetes as physical inactivity (3).

Insufficient sleep is common in the United States, in which average reported nightly sleep duration in adults has progressively declined over the last

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[1] Seward S, Blankenship J & Broussard J. Continuous glucose monitoring in sleep and circadian research (2022). In Taft, G., Di Piazza, L., & Carolyn, G. (Eds.), Encyclopedia of Sleep and Circadian Rhythms (2nd ed.). Elsevier.

100 years from~8.8h to 6.8h (4–6). A large segment of the United States population is impacted by insufficient sleep, with 1 in 3 people reporting <6.5h of sleep on weeknights (7). The Sleep Research Society and American Academy of Sleep Medicine recommend at least 7h of sleep per night for adults, supported by numerous studies in which findings demonstrate an increased risk for all-cause mortality with sleep duration less than 7h (8–12). Given the clear links between insufficient sleep and disease risk, as well as the prevalence of individuals who do not meet the minimum sleep duration guidelines, the Center of Disease Control and Prevention declared insufficient sleep a public health epidemic (13).

Along with insufficient sleep, circadian disruption is recognized as an independent risk factors for T2DM (14, 15). Circadian misalignment is defined as inappropriately timed sleep and wake associated behaviors, and is commonly experienced by people who work evening, night, or rotating shifts (i.e., “nonstandard” work hours). Shift workers represent 1 in 5 United States employees (16) and are 44% more likely to develop diabetes compared to people who work standard day shifts (15). Collectively, these data underscore the importance of sleep and circadian biology in the regulation of metabolism and cardiometabolic disease risk.

DIURNAL REGULATION OF GLUCOSE HOMEOSTASIS AND INSULIN SENSITIVITY

Glucose homeostasis is highly regulated. For an individual weighing 70 kg, approximately 4 g of glucose circulates in the blood between meals (17). Numerous organ systems work together in synchrony to maintain glucose in a very narrow range to ensure organs receive a steady source of fuel. Uncontrolled

hyperglycemia can result in glucotoxicity, organ and tissue damage, and eventual exhaustion of the insulin producing beta cells in the pancreas. In contrast, hypoglycemia can lead to coma and ultimately death. Thus, the consequences of dysregulated glucose are significant.

Several recent reviews in this area have described the exquisite physiological control that occurs to maintain glucose homeostasis (18, 19).

Glucose regulation is known to vary across the 24h cycle with diurnal fluctuations in glucose tolerance first characterized in the 1970s (20–22). In these initial studies, oral glucose tolerance tests (OGTTs) were performed at several different times during the day (i.e., morning, afternoon, and evening) and collectively demonstrated that in healthy volunteers, glucose tolerance is highest in the morning compared to the evening. Later investigations using frequent blood sampling, intravenous delivery of glucose via constant glucose infusion or glucose tolerance tests, and enteral nutrition all confirmed diurnal fluctuations in glucose tolerance with better glucose disposal in the morning in healthy participants (23). Decreased glucose tolerance in the later part of the day is likely due to reduced glucose utilization, decreased insulin sensitivity, and reduced insulin secretion without any evidence of variations in glucose production or insulin clearance across the day.

Alternations of the normal daily profiles of glucose tolerance have been identified in normal aging, obesity and diabetes. Evidence suggests that in participants with obesity and/or diabetes, diurnal glucose regulation is absent (24–26) or inverted compared to normal participants, such that insulin sensitivity

and glucose tolerance are higher in the evening compared to the morning (22, 27, 28). Together, these studies support the idea that glucose tolerance and insulin sensitivity possess diurnal fluctuations in healthy participants, which may be disturbed in people with metabolic disease.

IMPACT OF SLEEP AND CIRCADIAN DISRUPTION ON GLUCOSE HOMEOSTASIS AND INSULIN SENSITIVITY

Seminal work by Dr. Eve Van Cauter and colleagues at the University of Chicago initially demonstrated that insufficient sleep (4h/night for 6 nights) impairs glucose tolerance by 40% (29). These findings launched numerous investigations that have consistently found and reported impairments in insulin sensitivity and glucose homeostasis induced by sleep and circadian disruption (14, 30). Furthermore, sleep and circadian disruption impair lipid homeostasis (31–33) and lead to metabolic alterations in peripheral metabolic tissues including adipose and muscle (32, 34–36). Even a single night of total sleep deprivation in the dog model is sufficient to impair insulin sensitivity to a similar degree as a long-term high fat diet (37). Finally, the degree of insulin sensitivity impairment induced by insufficient sleep in healthy individuals is comparable to the magnitude of insulin resistance in people with obesity and T2DM compared with lean controls (38, 39). It is also unclear how much recovery sleep is needed after a bout of insufficient sleep to fully restore insulin sensitivity (40, 41).

The link between sleep and circadian disruption and impaired glucose homeostasis is well established in these highly controlled inpatient research settings using small, homogenous study populations. However, significant burdens (e.g., staff time, costs of research, subject burden) as well as drawbacks

(applicability to the general population) are present in such studies. An important next step for the fields of sleep, circadian and metabolic research will be to examine the impact and potential countermeasures to these behaviors in larger, more heterogenous populations in free- living environments. However, conducting studies in ecologically relevant environments (e.g., workplace, home, travel, etc.) is difficult due to the challenges of assessing physiological variables outside of a controlled setting. Wearable devices, on the other hand, offer opportunities to overcome these obstacles and move studies out of the lab and into real world environments.

WEARABLE TECHNOLOGY IN SLEEP AND CIRCADIAN RESEARCH

Sleep and circadian researchers have historically relied heavily on actigraphy to estimate sleep parameters in free-living environments. Given recent developments in wearable biosensors, the evaluation of metabolic parameters in real world settings is more feasible. Specifically, continuous glucose monitors (CGMs), have emerged as powerful tools which can assess the impact of real-living behaviors on glucose fluctuation. CGMs are small biosensors that frequently measure interstitial glucose concentrations for extended periods of time. As depicted in Figure 1.1, these devices consist of a small wire sensor inserted into the subcutaneous adipose tissue of the upper arm or abdominal area with a thin needle applicator. Glucose data are analyzed by the sensor in real time and data are subsequently stored either on the sensor itself, or in a cloud-based platform. With frequently sampled data from CGMs, temporal and dynamic changes in glucose in large-scall free-living populations can be

investigated. For example, CGMs have been used in conjunction with accelerometers to understand the glycemic impact of reducing sedentary time in healthy individuals and those with T2DM (42–46).

While these large streams of data are powerful, there are important considerations to be made to ensure the usefulness and interpretability of CGM data. In the following sections, we will discuss the evolution and development of CGMs, technical underpinnings of CGM technology, and important considerations in the design and implementation of CGM in clinical sleep and circadian research.

EVOLUTION OF CGMS

CGM devices were initially approved by the FDA for the frequent self-monitoring of glucose in individuals with type 1 diabetes (T1D). Prior to the CGM, the only method available for self-monitoring blood glucose was to obtain venous blood from capillaries in the finger (i.e., finger sticks) (47). Although original CGMs represented a major advancement in T1D management, they had multiple components and were rather cumbersome. Users were required to calibrate the device 2-4 times daily with capillary glucose measurements (i.e., finger sticks and glucometers). Additionally, sensors were only approved for use up to 3 days at a time. Since their inception in 1999, there have been significant advances in CGM technology. In 2017, Abbott released the Freestyle Libre Pro CGM which was approved to extend the wear period to 14 days. As sensor technology improves, wear duration are expected to continue to increase, as evidenced by the recent release of the Eversense CGM from Ascentia Diabetes, which is approved for use up to 90 days. Another major improvement in CGM technology has been the

elimination of manual finger stick calibrations. Devices are now pre-calibrated and do not require any input from users to produce reliable and accurate measurements.

Technical principles of measuring glucose with continuous glucose monitors

As described above, glucose values obtained from self-monitored glucose methods are typically assessed from venous blood from capillaries in the finger. In contrast, CVMs measure glucose concentrations in the interstitial fluid of the subcutaneous fat. While the technology is slightly different between manufactures, CGMs estimate glucose concentrations using the glucose oxidase method (48). Glucose oxidase is an enzyme which catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide is then oxidized within the CGM sensor, producing electrons. The resulting electron flow is linearly proportional to the glucose concentration and thus provides a corresponding glucose value. This method is also commonly used for the enzymatic determination of glucose in solution and is employed by gold-standard technology used to assess glucose from plasma samples collected intravenously (e.g., YSI Biochemistry Analyzer).

Glucose in the interstitial fluid is tightly correlated with blood glucose, however, there are important differences to note. The absolute values of glucose concentrations are slightly lower in the interstitial fluid compared to venous glucose (49, 50). Additionally, the rate of glucose appearance and disappearance in the interstitial fluid differs from that observed in blood glucose. Glucose appears in interstitial fluid via passive diffusion from blood vessels, and therefore

changes in interstitial glucose levels are typically delayed by 5-20 minutes compared to changes in blood glucose, depending on a number of variables including meal timing and exercise (both duration and intensity) (50–53). For example, 1h of moderate intensity aerobic exercise results in changes in blood glucose that appear just over 10 min later in interstitial glucose (54). Similarly, changes in interstitial glucose following a meal can be observed 10 min after changes in blood glucose (54). Methods have been proposed to mathematically correct for the physiological lag times observed between the time glucose appears in the blood to its appearance in the interstitial fluid (52). However, the transport of glucose from the blood to the interstitium itself may be impaired by disease states (55–57), thus any mathematical corrections may mask relevant impairments in glucose transport.

OUTPUTTING DATA FROM CGMS

Depending on the model, CGMs can be programmed to display data in real time to the wearer or can record data to be downloaded at a later date. The decision to display data in real time is dependent on the application and research question. In clinical populations, glucose values in real time are critical for glucose control and management. For example, following behaviors such as meals or exercise, an individual with T1D can adjust insulin administration to minimize dramatic swings in glycemia. CGMs are highly beneficial in these situations, as they can also be programmed to alert patients of low glucose values before the onset of hypoglycemia.

In research applications, investigators may elect to download stored data

from the CGM at the completion of the study so that participant behavior is not influenced by glucose concentrations displayed on the CGM reader. Alternatively, researchers may leverage data displayed in real time to enhance behavioral interventions, particularly in individuals with metabolic disease. For example, Allen and colleagues used CGM data collected in individuals with T2DM as a tool for dietary and exercise education (58). Because data were collected from 3 consecutive days, participants had multiple opportunities to associate lifestyle behaviors with daily fluctuations in glucose levels.

Further, individuals with T1D who regularly use CGMs are more likely to reach clinically relevant HbA1c targets (<7%), have reduced hospitalization rates, and few emergency room visits (59, 60). Preliminary results also suggest individuals with prediabetes and T2DM who use CGMs have improved HbA1c values, better weight loss, and are more likely to make healthier lifestyle choices (59–61).

CAPTURING BEHAVIORAL CONTRIBUTIONS TO GLYCEMIC CONTROL WITH CGMS

One of the major challenges to interpreting CGM data collected in free-living settings is the number of factors that can potentially influence glucose levels. Meal timing and composition, physical activity, sleep and disease states are examples of factors that have a direct impact on glucose and can complicate the interpretation of CGM data. Depending on the research question, these factors should be considered in the design of the study and controlled or monitored with additional wearable technology and subjective reporting, as

discussed below.

Accelerometry represents perhaps one of the most readily available methods that could be implemented in conjunction with CGM to increase the interpretability of CGM data. Accelerometers can be used to measure sleep timing and duration, physical activity, and sedentary behavior. These devices are generally very small and have a low subject burden. Although there is inherent error associated with measuring human behaviors using exclusively acceleration signals, the added contextual information gained is highly valuable. CGM traces overlaid with continuously measured physical activity and light exposure from other devices can dramatically improve the interpretation of CGM data.

Energy intake is another major factor that can influence glucose levels. In the free-living environment, it is common for individuals to eat across the majority of a 24h period (62), which can complicate the interpretation of CGM data. For example, uncontrolled eating across a 24h period results in frequent fluctuations in glucose that make it difficult to discern interpretation of CGM data (Figure 1.2., top row). In contrast, controlling the timing of eating as well as diet composition can aid with the interpretation of CGM data (Figure 1.2., bottom row). Provision of standardized meals may not be feasible, of course, but there are some alternatives that can be employed to maintain a level of experimental control at a lower cost. One option is to standardize the composition and timing of a single meal or beverage over multiple days. This approach allows researchers to investigate changes in response to a standardized meal over time and under different conditions. Another alternative

is to instruct participants to choose a standardized meal from fast food restaurants. While it may seem unorthodox, fast food restaurants often have strict measures in place to ensure food is prepared consistently and matches FDA menu labeling requirements. Providing research participants gift cards to specific restaurants may be another alternative to reduce research staff burden and cost while maintaining some level of dietary control.

Finally, subjective reports on other behaviors known to affect glucose control in the free-living environment such as meal timing and composition, physical activity/exercise, and sleep/wake timing, can aid in data interpretation and help identify and resolve errors in objective monitoring. Ultimately, the greater level of detail described on self-reported logs will provide a more comprehensive understanding of the potential contributing factors to different glucose patterns.

INTERPRETING CGM DATA IN THE ABSENCE OF INSULIN

It is important to recognized that CGMs do not provide information on the various hormonal factors that contribute to changes in glucose metabolism such as insulin and glucagon. Insulin, the primary glucose lowering hormone, is secreted in response to a meal and plays an integral role in glucose metabolism. Further, the insulin response to glucose is an important indicator of metabolic health and disease risk (63). Without information on insulin levels, therefore, we cannot make conclusions regarding insulin sensitivity using CGM data alone. Rather, we can only report on glucose homeostasis. Moreover, since blood glucose is so highly regulated, there may be changes in insulin levels required to maintain euglycemia (31, 64–69) that will go undetected by CGM (or any glucose

measure for that matter). For example, insufficient sleep is consistently associated with reductions in insulin sensitivity without changes in fasting blood glucose (31, 64–69). It is therefore important to recognize the limitations of interpreting glucose data when no measures of insulin are available.

ANALYTICAL CONSIDERATIONS FOR CGM RESEARCH

Although collecting large amounts of data with CGM is relatively straightforward, choosing an approach to analyze generated data can pose significant challenges. When worn continuously, CGMs can produce as many as 288 timepoints per day (24h × 12 readings per hour). In this section, we will review factors to consider when analyzing the large datasets generated by CGM.

The first essential step of CGM data analysis is to establish a screening process of CGM data from aberrant glucose values. Although automated approaches can filter out glucose values above and below a set threshold, visually inspecting data for non- physiological changes over time is equally important. Erroneous glucose fluctuations can be caused by miscalibration, sensor noise, or physical pressure at the sensor insertion site, called compression artifact. Such compression artifacts are typically caused by lying on the sensor itself and commonly result in a temporary but precipitous drop in glucose levels reported. As expected, compression artifacts are more common during the sleep period and have been reported to last from 30-70 min (70). This is a recognized issue in CGM research and potential solutions are currently under development (71). One method to minimize the occurrence of compression artifacts would be to discuss sleep habits with participants and avoid CGM

placement on the side on which individuals typically sleep. Integrating other wearable devices to detect sleep can also help with data cleaning and removal of compression artifacts.

Following artifact removal, the next challenge is to summarize the CGM data in a meaningful way. There are currently no gold standard approaches to analyze CGM data and many investigators average glucose values over 24h periods to provide a measure of daily glycemic control. The Advanced Technologies & Treatments for Diabetes (ATTD) Congress recently convened an expert panel of researchers, physicians and individuals with diabetes to develop guidelines for summarizing CGM data (72). As highlighted by the AATD Congress, numerous metrics can be used to quantify glycemic control. In addition to mean 24h glucose concentrations, duration of hyperglycemia, as defined as time above a user-defined threshold glucose value is also acceptable. Thus, metrics and normative glucose values should be determined based on population (73).

An additional approach to examine continuous glucose data is to divide the 24h period into meaningful segments. Comparisons between waking/sleep and feeding/fasting glucose levels may yield more meaningful results than a single mean 24h measure. In one recent study, daily glucose concentrations were reduced during a physical activity intervention, which persisted during the sleep period (74). Researchers are therefore encouraged to segment 24h CGM data by behavior, if possible, to provide a comprehensive assessment of glycemic control in response to different behaviors and perturbations.

THE FUTURE OF CGM AND CONCLUDING REMARKS

CGMs have seen dramatic improvements in the technology and usability in the last two decades since their emergence and are now considered the standard of care for successful glucose management in diabetes (47). Furthermore, CGMs are being marketed to metabolically healthy individuals as a tool to monitor glucose levels over time and in response to different behaviors in a free-living setting (75). Finally, CGMs are commonly implemented in intervention studies to provide biofeedback to participants and potentially reinforce behaviors that improve glucose control.

As wearable technology and personal health monitoring become standard in everyday life, methodological approaches to integrate data from multiple wearable devices are needed to maximize the potential information gained. Applying rigorous methods to wearable sensors in free-living behaviors and interventions on metabolic health. Sleep and circadian physiologists are well positioned to apply their perspectives of time-based analyses to make important contributions to the interpretations of CGM data collected in free-living populations.

FIGURES

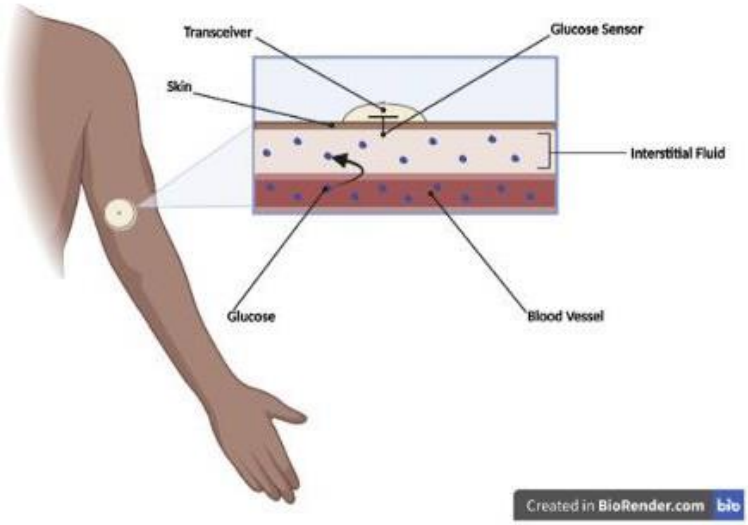

Figure 1.1. Continuous glucose monitor (CGM) placement. CGMs are inserted with a small catheter that contains a subcutaneous sensor and can be inserted in multiple locations including the abdomen, lower back, and back of the upper arm (as depicted). Glucose molecules (depicted as blue circles) passively diffuse from the blood to the interstitial fluid where they are detected by the CGM.

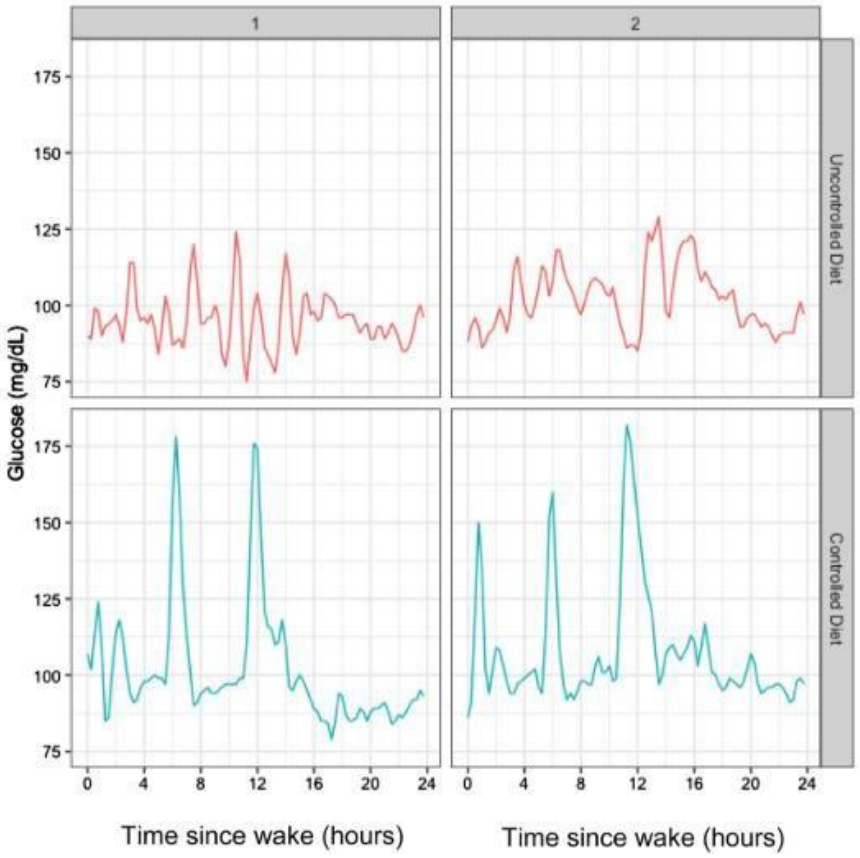

Figure 1.2. Effects of dietary control on continuous glucose monitor (CGM) output. Interstitial glucose concentrations are displayed from CGM from on healthy participant over 4 different recording days. During 2 days of free-living conditions (top row, red line), glucose concentrations are highly variable and meal-related peaks are difficult to discern. Following the implementation of a controlled diet with scheduled mealtimes (bottom row, blue line) clear meal-related glucose excursions emerge, which may improve the interpretability of the data.

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CHAPTER 2 – ACUTE NIGHT SHIFT WORK IS ASSOCIATED WITH INCREASED BLOOD PRESSURE AND REDUCED SLEEP DURATION IN HEALTHY ADULTS[2]

OVERVIEW

INTRODUCTION: Shift workers have a 40% higher risk for cardiovascular disease (CVD) compared to people who work day shifts. However, the acute impact of shift work on CVD risk factors in free-living settings remains unclear. We therefore investigated the impact of acute night shift work on factors related to cardiovascular health including blood pressure (BP) and sleep duration.

METHODS: Twenty-four rotating shift workers (19F, 23±4y, BMI: 23±3kg/m^2; mean±SD) participated in a quasi-randomized crossover study. Assessments were conducted over the course of one day shift and one night shift in a free-living setting. BP was measured every 30 minutes by an ambulatory monitor. Sleep and wake times were recorded. Mixed effects models were conducted to examine changes in variables between conditions.

RESULTS: Acute night shift work was associated with significantly higher 24h systolic (107±1 v 104±1 mmHg; $p<0.0001$) and diastolic (67±1 v 64±1 mmHg; $p<0.0001$) BP, as well as blunted dipping patterns in systolic BP (8±1 v 12±1%; $p=0.032$), as compared to day shift work. Sleep duration was significantly shorter during the night shift as compared to the day shift (4h04±19min v 8h22±18min; $p<0.0001$).

CONCLUSION: As little as one night of shift work in a free-living setting is sufficient to induce multiple CVD risk factors including increased BP and reduced sleep duration in healthy adults. It is critical to identify strategies to prevent or attenuate the negative

[2] Seward S, Kishman E, & Broussard J. Impact of nightshift work on 24h ambulatory blood pressure (under review at American Journal of Physiology: Physiological Reports).

impact of shift work on CVD risk in a large portion of the working population.

INTRODUCTION

Over one quarter of the US workforce engages in evening, night, or rotating shifts (i.e. shift work) to meet the requirements of modern society (1). People who conduct shift work have a 40% higher risk for developing cardiovascular disease (CVD) compared to people who work day shifts (2–5). Furthermore, the duration of exposure to shift work is related to CVD risk. For example, in a prospective study in female nurses with varying years of shift work history, nurses who conducted 10 years of shift work were twice as likely to develop CVD compared to nurses who spent <5 years performing shift work (3).

Aside from age, elevated BP is the leading CVD risk factor (6). Furthermore, BP alterations at specific times of the day are independently associated with elevated CVD risk. For example, during a normal night of sleep in healthy adults, there is a 10-20% dip in BP compared to waking values (referred to as “BP dipping”). Blunting of this BP dip (defined as a <10% reduction from pre-sleep to sleeping BP) is indicative of elevated CVD risk even in normotensive individuals (8, 9, 10). Furthermore, in healthy adults, there is a 10-20 mmHg increase in BP within the first 2 hours of waking as compared to the final 2 hours of sleep, typically referred to as the morning BP surge (10). An exaggerated morning BP surge (defined as >20 mmHg SBP) has also been associated with an increased risk for CVD (11–13). Together, these findings highlight the importance of assessing BP profiles over the entire 24h period to uncover important and clinically relevant changes in BP across the day and night during both wake and sleep.

Findings from controlled in-laboratory studies in healthy adults with no prior history of shift work reveal that just two nights of simulated night shift work leads to

increases in mean 24h BP as well as blunted BP dipping (14). Consistent with these findings, results from a controlled in-laboratory study in chronic shift workers found that simulated night shift work increases BP even in people with a history of shift work (15) highlighting elevated BP and loss of BP dipping as a potential mechanisms by which shift work contributes to the risk of CVD in this population. However, the impact of shift work on the waking BP surge has not been elucidated.

Despite the finding that simulated night shift work in healthy adults acutely increases BP and blunts BP dipping in controlled inpatient settings, studies on the impact of shift work on BP patterns in a free-living environment are limited. In one outpatient study, participants who conducted rotating shift work had higher 24h BP as well as blunted BP dipping during night shift work as compared to a non-work day (16). However, this study was conducted in emergency medical services workers, in whom job stress and BP are presumably higher during work days as compared to non-work days, regardless of whether the shift occurred at night. Furthermore, there was a large range of age and body mass index (BMI) in this study, which can have significant impacts on BP.

Similarly, results from another study conducted in young, healthy medical residents demonstrated that BP was higher during a 24h shift compared to an 8h day shift (17). Finally, in young, healthy female nurses, evening and night shift work was associated with higher 24h BP and sleep BP as compared to day work (18). However, in all of these studies, information on shift work history was not provided and therefore, the timeline of BP impairments induced by shift work exposure remains unclear.

To assess the timeline of BP impairments associated with shift work exposure, one study examined 24h ambulatory BP in newly-hired transit workers in a free-living setting. After six months of shift work participants experienced a significant blunting of sleep BP dipping as compared to baseline values (19). However, there were no restrictions to age or BMI in this study, thus the population was highly heterogenous.

Taken together, research on the impact of shift work on CVD risk factors in free-living settings suggests that shift work is consistently associated with significant increases in BP and blunted BP dipping. However, nearly all prior free-living studies have been conducted in heterogenous populations with varying history of shift work exposure and varying degrees of job stress. Thus, disentangling the impact of night shift work on BP patterns in free-living individuals has proven challenging.

The objective of this study, therefore, was to investigate the impact of acute night shift work in a free-living setting, in a homogenous group of young, healthy adults exposed to intermittent night shift work for less than 2 years. We hypothesized that a single night shift would be associated with increases in CVD risk factors such as increased 24h BP, blunted BP dipping, and an increased BP surge upon waking compared to day shift work.

METHODS

Participants

Healthy adults 18-35 years old and with a BMI of 18.5-29.9 kg/m^2 were recruited for this study. Participants were intermittent rotating shift workers (<2 years of rotating shift work exposure), defined as working at least one day shift and one night shift per month. Participants were excluded if they had any clinically significant medical or

surgical conditions within the last year; were diagnosed with diabetes or CVD, taking hypertensive medication, or pregnant as determined by urine hCG test. All participants provided written informed consent to participate, and all procedures were reviewed and approved by the Colorado State University Institutional Review Board.

Experimental protocol

Participants underwent a quasi-randomized crossover study conducted under two free-living conditions consisting of approximately 24 hours starting approximately 20 hours prior to wake time and ending approximately 4 hours after wake. An example study protocol is shown in Figure 2.1. Day shift and night shift conditions were conducted approximately three months apart (110±25d). The day shift was defined as a shift that commenced at or before 09:00. The night shift was defined as a shift that commenced at or after 19:00. Total sleep duration, sleep onset, and sleep offset (i.e wake time) were recorded and verified by sleep logs and call-ins or texts to a time stamped recorder.

Blood pressure monitoring

Blood pressure assessments were collected every 30 minutes throughout the study with an ambulatory OnTrak 90227 monitor (Spacelabs^{TM}, Snoqualmie, WA) to assess systolic BP (SBP), diastolic BP (DBP), and heart rate (HR). Participants were instructed to remain still during ambulatory BP measurements to reduce monitor errors and ensure accurate readings. In the event of an error message, the ambulatory BP monitor repeated the measurement approximately 2 minutes later. Participants were

instructed to refrain from wearing the ambulatory BP monitor while driving, showering, or exercising to mitigate safety concerns and potential monitor errors. Participants were instructed to discontinue the use of the ambulatory BP monitor if the cuff induced discomfort to the extent that it hindered their ability to sleep. Participants were instructed to resume ambulatory BP monitoring upon wake if they removed it during sleep. Prior to analyses, the ambulatory BP data underwent visual inspection to ensure completeness. The expected number of ambulatory BP observations were calculated by multiplying the total wear time by 2, given that BP measurements were taken every 30 minutes. The actual number of ambulatory BP observations were recorded. The ratio of actual observations to expected observations were calculated and expressed as a percentage. Further, total ambulatory BP monitor wear time was calculated as the duration between the first record of an BP measurement and the last record of BP measurement in each condition. To minimize discomfort, the ambulatory BP monitor automatically inflated to 130 mmHg; however, if SBP was not detected, the cuff automatically increased pressure in increments of 30 mmHg until SBP was detected. Participants were blinded to the ambulatory BP results during the study.

Blood pressure parameters

Twenty-four-hour BP was defined as the mean BP during the entire collection period. Wake and sleep BP were defined as the mean BP during self-reported wake or sleep periods based on sleep logs and call-ins. The BP dipping during sleep was calculated as the percent change from waking BP to sleeping BP as previously described (20). Different definitions and thresholds have been used to define the blood

pressure surge that occurs after waking up calculated by subtracting the lowest sleeping blood pressure from the waking blood pressure (10). Most reports use the phrase "morning BP surge" however, in participants who perform shift work, "morning" is likely not the same as “waking” and therefore may not be appropriate. For our purposes, we use the term "waking surge" calculated as the absolute difference between the mean BP from the 2 hours before to the 2 hours after waking up. This has also been referred to as the “prewaking surge” in some studies (10).

Statistical analyses

Statistical analyses were conducted using SAS (version 9.4; Cary, NC) with a significance threshold of $\alpha = 0.05$. To examine differences between SBP, DBP, and HR during the 24h period, wake periods, and sleep periods, mixed-effect linear models with a random intercept for participant ID were used. The differences between BP dipping, waking BP surge, sleep duration, sleep onset, and sleep offset were also examined between day shift and night shift using a mixed-effects linear model. Mixed-effects linear models were performed to use all available data.

Time since wake was determined based on self-reported wake time. BP and HR were aggregated into 30-minute intervals based on time since wake. Mixed-effect linear models with a condition by time (time since wake) interaction were used to examine whether BP and HR were different over the 24h period between night shift and day shift conditions. SBP, DBP, and HR were dependent variables. All models were adjusted for ambulatory BP monitor wear time. Results are presented as least-squares

mean±standard error of the mean (SEM), except for baseline characteristic data, which are displayed as raw mean±standard deviation (SD).

RESULTS

Participants

A total of twenty-seven adults were assessed for eligibility, twenty-five were enrolled, and one participant dropped out of the study due to arm numbness due to the ambulatory BP monitor. Twenty-four participants completed at least one condition of the study (23±4 y; 23±3 kg/m^2, mean±SD; Table 2.1). Of those 24, 83% were female (n=20), 8% Asian (n=2), 4% American Indian (n=1), 4% Black/African American (n=1), 75% White (n=18), and 8% two or more races (n=2). Twenty participants completed both conditions. Participants had performed intermittent night shift work for 173 ± 32 days (mean±SEM); range 0 to 491 days; at least one night shift per month. In this quasi-randomized study, 16 participants completed the day shift condition first whereas 8 participants completed the night shift condition first.

Over 90% of scheduled BP measurements (every 30 minutes) were successfully collected across both conditions. Since data collection ended shortly after waking up, the total wear time for the ambulatory BP monitor was approximately 2.5 hours shorter during night shift work as compared to day shift work (15h28±49min v 18h6±45min; p=0.026).

Shift work and systolic and diastolic blood pressure

Night shift work was associated with significantly higher mean 24h SBP (night: 107±1 mmHg v day: 104±1 mmHg; p<0.0001) and DBP (night: 67±1 mmHg v day: 64±1

mmHg; p<0.0001) as compared to day shift work (p<0.0001 for both; Table 2.2). There were no differences in mean waking SBP between conditions (Table 2.2). However, mean sleeping SBP was higher during night shift work as compared to day shift work (night: 100±2 mmHg v day: 96±1 mmHg; p=0.0004; Table 2.2). There were no differences in waking or sleeping DBP between conditions (Table 2.2).

There was a significant condition by time interaction for 24h SBP and DBP (p<0.0001 for both), indicating that BP profiles were different between night shift work and day shift work. SBP and DBP were significantly different at multiple timepoints between conditions (Figure 2.2a and 2.2b).

Shift work and heart rate

Twenty-four-hour heart rate (HR) was not different between conditions (Table 2.2). In contrast, waking HR was lower during night shift work compared to day shift work (night: 69±2 bpm v day: 73 ±2 bpm; p<0.0001; Table 2.2). Sleeping HR was not different between conditions (Table 2.2). Finally, HR profiles were not different between conditions (p=0.183; Figure 2.2c).

Shift work and blood pressure dipping

Night shift work was associated with a significantly blunted SBP dip during sleep as compared to day shift work (night: 8±1 % v day: 12±1 %; p<0.0001; Table 2.2), which is below the clinical cut off for blunted dipping associated with increased CVD risk. There were no differences in DBP dipping between conditions.

Night shift work was associated with a smaller waking SBP surge compared to day shift work (night: 6±2 mmHg v day: 12±1 mmHg p=0.013; Table 2.2). In contrast,

there were no differences in the waking DBP surge between conditions.

Shift work and sleep duration and timing

Night shift work was associated with a significantly shorter sleep duration as compared to day shift work (night: 4h04±19min v day: 8h22±18min; $p<0.0001$; Table 2.2), as well as significantly later sleep onset (night: 7:16a±15min v day: 10:55p±14 min; $p<0.0001$) and sleep offset (night: 11:23a±21 min v day: 7:17a±20 min; $p<0.0001$; Table 2.2)

DISCUSSION

The purpose of this study was to examine the impact of acute night shift work on CVD risk factors in otherwise healthy participants with a relatively short history of intermittent night shift work exposure. We found that acute night shift work was associated with increased mean 24h SBP and DBP, as well as blunted SBP dipping as compared to day shift work. Further, we found that acute night shift work was associated with a significant reduction of over 4 hours in self-reported total sleep duration compared to day shift work. These findings in a homogenous and otherwise healthy population are consistent with previous studies and support the idea that acute night shift work is associated with increased CVD risk.

In our hands, night shift work was associated with a 3 mmHg increase in mean 24h SBP and DBP compared to day shift work. These results are consistent with a 4 mmHg increase in both SBP and DBP found in young, healthy medical residents during a 24h work shift as compared to a 8h day shift work (17), and a 1 mmHg increase in both SBP and DBP during simulated night shift work compared to a simulated day shift work in chronic shift workers (15). Despite an increase, SBP and DBP remained within

the normal range (<130/80 mmHg). However, results from prior research indicate that progressive increases in BP increase the risk of CVD even in healthy, normotensive adults (21). Therefore, the increase in BP induced by acute night shift work in this study represents a clinically significant increase and supports the link between chronic night shift work and CVD risk (2–5).

We also demonstrated that acute night shift work blunts SBP dipping as compared to day shift work. Notably, we observed an 8% SBP dip during night shift work compared to 12% during day shift work. Results from epidemiological studies suggest that a BP dip of <10% is associated with future CVD development (9, 22), highlighting the clinical relevance of BP alterations induced by night shift work. Our results of blunted BP dipping are consistent with some (14, 18, 19), but not all (17, 23, 24), prior studies of shift work. Methodological discrepancies, such as the differences in total sleep duration or potential napping during night shift work, may explain some of these inconsistencies. For example, in one outpatient study conducted in emergency medical services workers, participants demonstrated blunted BP dipping during a night shift as compared to a non-work day (16). However, in a subset of participants in this study who napped for >60 min during the night shift, BP dipping was in the normal range of 10-20% (16), suggesting that insufficient sleep is a likely mechanism underpinning blunted BP dipping in night shift workers.

We also observed a significantly lower waking BP surge during night shift work as compared to day shift work. To our knowledge, this is the first study to report the waking BP surge during simulated or free-living night shift work. A reduction in waking BP surge is contrary to our original hypothesis though not unexpected based on the blunted BP dip, which may limit waking BP surge potential. Previous studies that have

demonstrated a link between higher waking BP surge and increased risk of CVD and stroke were conducted in people who slept at night and woke up in the morning (11–13). In a previous longitudinal study, however, blunted BP dipping pattern was associated with a smaller morning BP surge and heightened CVD risk (25). Thus, the link between elevated waking BP surge and increased CVD likely depends on the population and whether a robust BP dipping pattern is present.

In our hands, acute night shift work was also associated with a 4 hour reduction in self-reported sleep duration, which is consistent with data from previous studies that report short sleep duration during night shift work as compared to day or afternoon shift work (17, 26). Insufficient sleep, defined as less than 7 hours of time in bed per night, is independently associated with increased 24h BP and CVD risk (27–31). For example, results from a randomized crossover study conducted in women showed that insufficient sleep (achieve by reducing sleep by 1.5 hours per night) for 6 weeks was associated with increased 24h SBP compared to habitual sleep (31). Furthermore, results from another study in healthy adults demonstrated that participants who were exposed to repeated bouts of insufficient sleep (4h of sleep/night for three nights followed by recovery sleep of 8h, repeated four times in succession) had blunted BP dipping and increased 24h BP as compared to participants who were provided sufficient sleep (32). Finally, previous research indicates that for every hour of sleep less than 7h per night, the risk of coronary heart disease increases by 11% (33). Altogether these results suggest that insufficient sleep is likely an important mechanism by which night shift work increases BP and the risk for CVD.

Limitations

This study also has several limitations. First, we did not restricte exercise or caffeine in our study, both of which could impact BP and sleep. Second, frequent BP assessments associated with ambulatory monitoring has been associated with discomfort as well as sleep disruptions (34–36). Although BP assessments were conducted in both conditions, they may be more disruptive to sleep during the night shift condition in which sleep occurred later in the morning at a time that sleep is already reported to be disrupted (37, 38). Third, we assessed sleep duration by self-report and did not assess sleep objectively. However, previous studies have demonstrated a strong correlation between self-reported sleep duration and sleep duration assessed by actigraphy (39, 40). Fourth, our study design was quasi-randomized due to work schedules that dictated the order of conditions and therefore order effect may have implications on the findings of this study findings. Additionally, our study included young, healthy participants and therefore results may not be generalizable to all shift working populations. Finally, participants were primarily female who were premenopausal and therefore fluctuations in hormones that may impact BP such as estrogen may contribute to our findings.

Future Directions

Night shift work is largely unavoidable in modern society. Thus, the identification, development, and testing of strategies to improve cardiometabolic health and subsequently lower CVD risk in a sizeable portion of the population are warranted. Countermeasures to attenuate the negative effects of shift work may include interventions known to improve cardiometabolic health in other contexts. Indeed,

increasing sleep duration may be a viable countermeasure to reduce BP in night shift workers, given that napping for as little as one hour during night shift work has been shown to restore BP dipping patterns (16). Furthermore, sleep extension by 1h per night for 6 weeks in participants with elevated BP and who chronically slept less than 7h was associated with reduced SBP and DBP as compared to baseline levels (41).

Finally, future research using an “ecolabical” approach may provide valuable insights that are more translatable and involve less participant and staff burden (42). Such an approach involves the combination of rigorous control typically associated with an inpatient study in conjunction with free-living conditions to provide an understanding of physiology in real life. Successful ecolabical approaches may include the implementation of short but highly controlled inpatient assessment periods at baseline and following a free-living intervention, or the use of monitoring devices such as wearables to assess behavior and continuous glucose and/or BP monitors as done in the current study to assess physiologically relevant changes in response to an intervention.

TABLES

Table 2.1. Baseline participant characteristics	
n (completed at least 1 condition)	24
Female	19
Age, y	23 ± 4
Weight, kg	66 ± 11
Height, cm	167 ± 9
BMI, kg/m^2	23 ± 3

Table 2.1. Baseline participant characteristics. Data are presented as mean ± SD. BMI, body mass index.

Table 2.2. BP and sleep characteristics

	Day shift (n=24)	Night shift (n=20)	P value
24h SBP (mmHg)	104 ± 1	107 ± 1	<0.0001*
24h DBP (mmHg)	64 ± 1	67 ± 1	<0.0001*
24h HR (mmHg)	68 ± 2	67 ± 2	0.156
Waking SBP (mmHg)	110 ± 1	109 ± 1	0.481
Waking DBP (mmHg)	69 ± 1	70 ± 1	0.073
Waking HR (bpm)	73 ± 2	69 ± 2	<0.0001*
Sleeping SBP (mmHg)	96 ± 1	100 ± 2	0.0004*
Sleeping DBP (mmHg)	56 ± 1	56 ± 1	0.800
Sleeping HR (bpm)	62 ± 2	62 ± 2	0.499
Sleeping SBP dip (%)	12 ± 1	8 ± 1	0.032*
Sleeping DBP dip (%)	19 ± 1	19 ± 2	0.888
Waking SBP surge (mmHg)	12 ± 1	6 ± 2	0.013*
Waking DBP surge (mmHg)	11 ± 1	8 ± 1	0.194
Sleep duration (h:min)	8:22 ± 0:18	4:04 ± 0:19	<0.0001*
Sleep time onset (h:min)	22:55 ± 0:14	7:16 ± 0:15	<0.0001*
Sleep time offset (h:min)	7:17 ± 0:20	11:23 ± 0:21	<0.0001*

Table 2.2. BP and sleep characteristics. Data are presented as least square mean ± SEM. Least square means were analyzed by a linear mixed model. * *P* value ≤ 0.05. Systolic blood pressure (SBP); diastolic blood pressure (DBP); Heart rate (HR); hour (h); beats per minute (bpm).

FIGURES

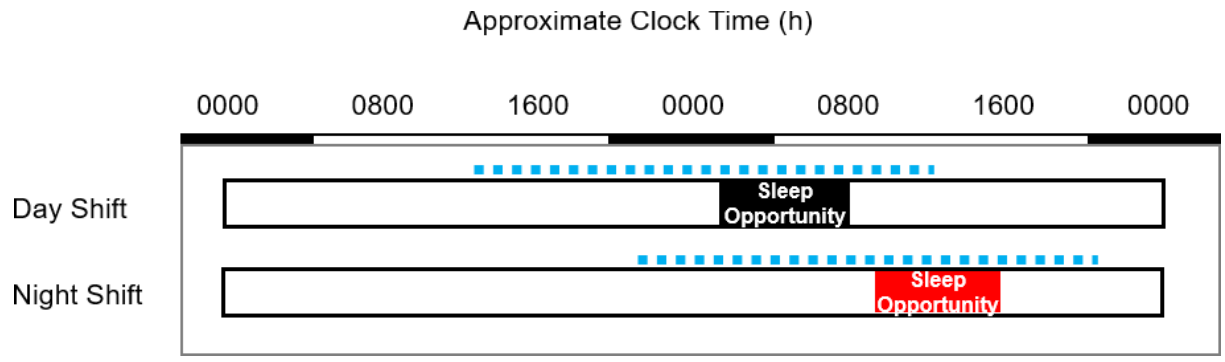

Figure 2.1. Overview of experimental protocol. Data collection was conducted in a quasi-randomized crossover order under free-living conditions for two work shifts including a day shift (top; A) and a night shift (bottom; B). SBP, DBP and HR were recorded every 30 minutes as represented by the blue dotted line. The gray rectangle indicates the mean self-reported sleep period during day shift ± SEM. The red rectangle indicates the mean self-reported sleep period during night shift ± SEM.

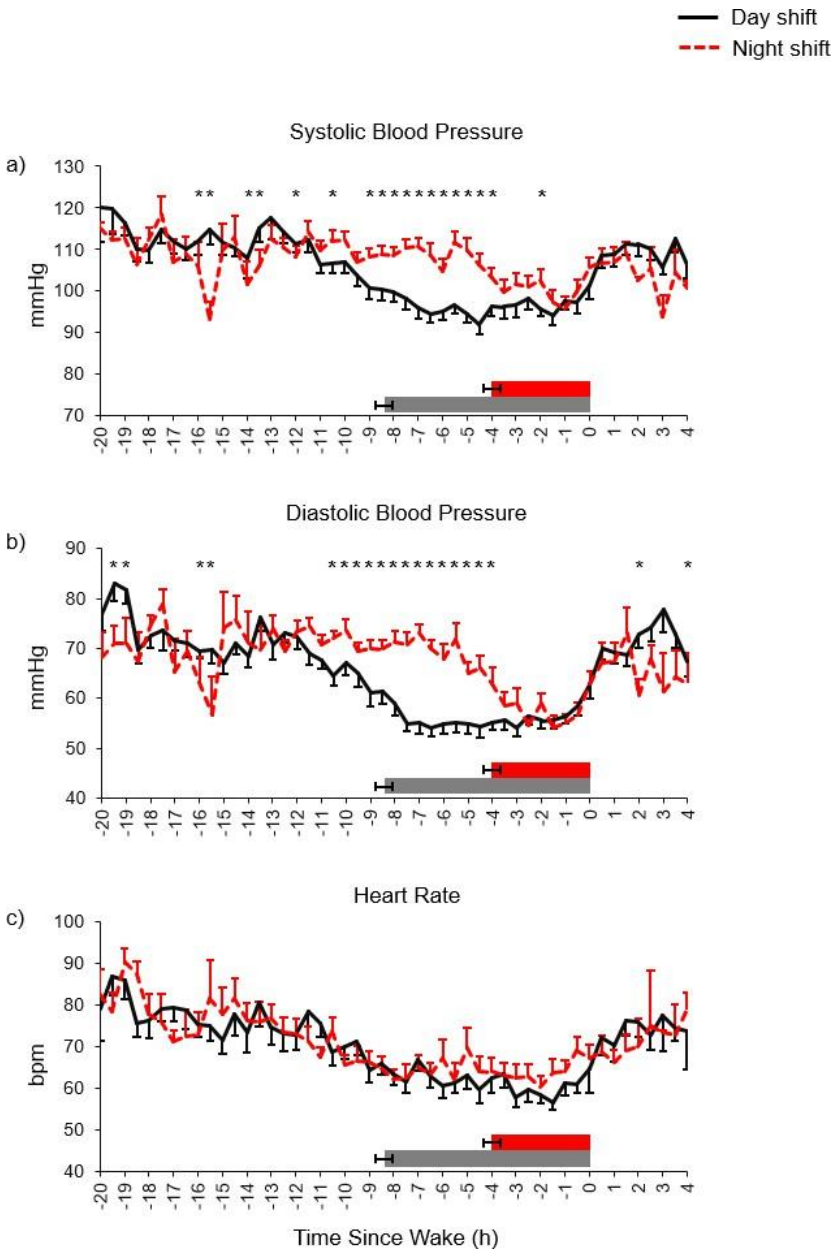

Figure 2.2. Systolic blood pressure (left; A), diastolic blood pressure (middle; B) and heart rate (right; C) readings assessed every 30 minutes during each condition and analyzed by mixed-effects model. Data are presented as least square means ± SEM. The gray rectangle indicates the mean self-reported sleep period during day shift ± SEM. The red rectangle indicates the mean self-reported sleep period during night shift ± SEM. **P* value < 0.05.

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CHAPTER 3 – EARLY TIME-RESTRICTED EATING REDUCES CIRCULATING GLUCOSE, INSULIN, AND BIOACTIVE LIPIDS IN HEALTHY ADULTS[3]

OVERVIEW

INTRODUCTION: Altering the timing of behaviors, such as meal timing, has significant implications for cardiometabolic health. Isocaloric early time-restricted eating (eTRE) is a dietary strategy that restricts energy intake to a 6-10h period beginning in the morning and may be associated with cardiometabolic improvements. However, the temporal changes in circulating hormones and metabolites in response to a shortened energy intake period are unknown. We therefore investigated the impact of 1 week of isocaloric 8h eTRE on cardiometabolic-related outcomes such as circulating levels of glucose, insulin, and in healthy men and women.

METHODS: Thirteen healthy adults (8F, 26.9±3.9 years, BMI: 23.3±2.1 kg/m2; data are mean±SD) participated in a 2-week protocol using a consecutive design. During Week 1, energy was consumed over a 13h period with meals individually anchored to habitual wake time with breakfast, lunch, dinner, and snack consumed at +1h, +6h, +11h, +14h after wake. In week 2, participants were instructed to match food intake from week 1 but restrict intake to an 8h period (meals at +1h, +5h, and +9h after wake). A continuous glucose monitor was worn throughout the study to assess interstitial glucose concentrations (FreeStyle Libre Pro; Abbott, Alameda, CA, USA). At the end of each week, participants were admitted to the laboratory for an in-patient overnight stay. Blood samples were collected hourly overnight beginning 4 hours before habitual bedtime for

[3] **Seward S**, Rynders C, Berry K, Panda S, Rice J, Bergman B, & Broussard J. Early time-restricted eating alters circulating bioactive lipids, glucose and insulin in healthy adults (*in prep to Obesity*).

14 hours and assayed for circulating glucose, insulin, and glycerol levels. Individual species of circulating plasma TAG, 1,2-DAG, ceramide, and acylcarnitine were measured by quantitative lipidomics. On the morning following each overnight stay an oral glucose tolerance test was performed to estimate insulin sensitivity. Mixed effects models were conducted to examine changes in variables between conditions.

RESULTS: One week of isocaloric eTRE to 8h was associated with a significantly lower circulating plasma glucose, insulin, and glycerol ($p<0.05$ for all) compared to habitual eating without changes to or insulin sensitivity. eTRE also was associated with lower lipid species including triglycerides and 1,2-DAGs.

CONCLUSION: Acute eTRE improves markers of circulating hormones and metabolites that may have implications for cardiometabolic health. This study sheds light on the fundamental biology of acute isocaloric eTRE in healthy, lean men and women.

INTRODUCTION

More than half of adults in the United States have one or more cardiometabolic diseases including obesity, type 2 diabetes, and cardiovascular diseases (1–3). Recent attention has focused on the impact of the timing of lifestyle behaviors on the development and progression of these cardiometabolic diseases. Specifically, behaviors such as meal and sleep timing, when occurring at inappropriate times of the day, are increasingly recognized as contributors to the prevalence of cardiometabolic disease in the United States (4, 5). Meal timing based on alignment with circadian rhythms may be particularly important for cardiometabolic health. Understanding the impact of meal timing on cardiometabolic health as significant implications; therefore, this study investigates how altering meal timing impacts factors related to cardiometabolic health.

Consuming energy over the majority of clock hours, particularly late at night, is

increasingly prevalent and has been linked to increased cardiometabolic health risks. According to data from the National Health and Nutrition Examination Survey, the percentage of Americans eating 3 meals a day has dropped from 75% and 60%, while the proportion consuming more than half of their daily energy intake as snacks has doubled over the past 40 years (6). Additionally, the median daily eating period has increased to approximately 14h, with only 10-15% of adults maintaining a daily eating period of 12h or less (7, 8). Eating during specific times of the day, such as late at night, can be particularly problematic. Late-night eating, defined as consuming food within 2h of bedtime, is associated with increased risks for cardiometabolic diseases. One study involving 110 young adults over a week found that the timing of food intake relative to melatonin onset, which marks the biological night, was significantly correlated with higher body fat percentage and body mass index (9). These findings highlight the detrimental effects of consuming energy over the majority of clock hours, especially late-night eating, on cardiometabolic health.

Recently, efforts have focused on altering meal timing to mitigate the cardiometabolic disease risks in a large portion of the United States population. One promising intervention that has gained attention is time-restricted eating (TRE), a dietary strategy that restricts energy intake to a 6-10h period. Studies in rodent models have demonstrated the TRE can reverse glucose intolerance and hyperinsulinemia induced by high fat feeding diets (10, 11), and even extend lifespan by 35% (12). Human studies on TRE, while less extensive, suggest potential cardiometabolic benefits, including modest weight loss and improvements in markers such as fasting glucose, insulin, and triglycerides (13), decreased blood pressure (14, 15), as well as improved glucose tolerance and insulin sensitivity (16, 17). However, there are inconsistencies in these

effects across studies that may stem from methodological discrepancies, such as whether the study design allowed late-night eating (14, 15, 18).

To address these inconsistencies, early time-restricted eating (eTRE) has emerged as a focused variation of TRE, aiming to align the eating window with the biological day and eliminate late-night eating. This approach may offer distinct advantages for cardiometabolic health by promoting eating at times that align with circadian rhythms in metabolism (19). In humans, data suggest that eating in the morning may be optimal because insulin sensitivity is higher in the morning compared to the evening (20, 21). Moreover, while many studies have evaluated TRE in the context of weight loss, the independent impact of TRE on cardiometabolic parameters remains less explored. For example, data from a study in individuals with prediabetes demonstrates that isocaloric eTRE improves morning insulin sensitivity and blood pressure without weight loss (22), highlighting the potential benefits of TRE beyond weight loss. However, temporal changes in metabolic responses to eTRE during the night are still poorly understood, despite the known importance of nocturnal hormones and metabolites in cardiometabolic health. Furthermore, despite the importance of circulating lipids in cardiometabolic health and disease (23), to our knowledge, lipidomics have not been investigated during TRE.

This study aims to fill these gaps by investigating the cardiometabolic effects of a 1 week, isocaloric eTRE intervention in a controlled, consecutive study design. Participants were compared between habitual eating over a 13h period and an 8h eTRE period starting 1h after waking. During an inpatient stay, participants underwent hourly assessments of circulating hormones and metabolites (e.g., glucose, insulin, lactate, free-fatty acids, triglycerides, glycerol, and bioactive lipids) throughout the biological

nighttime. By focusing on homogenous young, lean, healthy adults, this pilot study sought to probe fundamental biological responses to changes in meal timing, providing insights into the temporal dynamics of eTRE on cardiometabolic health.

METHODS

Participants

Healthy women and men between 18 and 65 years of age were recruited from the community through flyers and digital advertisements. Participants had a body mass index (BMI) between 18.5 and 29.9 and were weight stable (<5 lb change in the past 6 months). Participants were required to have access to a smart phone or computer with a camera to receive mealtime reminders and report sleep/wake and meal timing, as well as provide photographs of all energy intake.

Exclusion criteria included any diagnosed medical conditions, sleep disorders (as assessed by a score on the Sleep Disorder Questionnaire [SDQ]), night/rotating shift work in the past 6 months, travel outside of mountain standard time (MST) in the past 3 weeks, living below 5,200 feet within the last 3 months, depressed mood (as assessed by a score on the Beck Depression Inventory (BDI) of >13) or anxious mood (as assessed by a score on the Beck Anxiety Inventory (BAI) of >10), use of any prescription or over-the-counter (OTC) medications (except oral contraceptives) or supplements, use of tobacco products, substantial consumption of alcohol (males >14 drinks/week or > 5 drinks/day; females >7 drinks/week or >3 drinks/day) or caffeine (>400 mg per day) or abnormal, clinically significant findings during medical screening. The Institutional Review Board of Colorado State University approved the protocol and all participants provided written informed consent.

Experimental protocol

Participants underwent a 2-week protocol using a consecutive design (Figure 3.2a). During week 1 of the protocol, participants consumed energy over a 13h period with meals individually anchored to habitual wake time with breakfast, lunch, dinner, and snack consumed at +1h, +6h, +11h, +14h after wake. Week 1 was the habitual eating condition. Automated text reminders were sent to participants daily, 30 minutes prior to scheduled mealtimes. Participants also recorded all energy consumption using paper-based food diaries and captured digital time-stamped photographs of all meals, which were sent to study staff using Google Voice. Week 2 was the eTRE condition, in which, participants were instructed to match energy intake using the paper-based food diaries and digital records from week 1 but restrict intake to an 8h period with meals scheduled at +1h, +5h, and +9h after wake.

At the end of each week, participants were admitted to the laboratory in the evening based on hour since wake 11 for an inpatient overnight stay. Exercise was proscribed for 24 hours prior to the inpatient stay to avoid any acute impact of exercise on outcome measures.

In the laboratory, participants were permitted sedentary activities during wakefulness such as reading, watching TV, or use of personal electronic devices. Personal electronics were removed prior to lights out. Participants were provided an 8h sleep opportunity and lights out occurred at +16 hour since awake. Upon waking in the laboratory participants remained in dim light conditions (<8 lux at the angle of gaze confirmed with a digital light meter [Model YF-170; Yu Fong Electronics, Taipei, Taiwan]). Room temperature was kept constant between 21-23°C.

Body weight and resting vitals

Upon admission after week 1 and week 2, body weight was measured in kilograms (kg) using a digital scale (Health O meter ® Professional 500KL Digital Scale, McCook, IL, USA). In a subset of 8 participants, resting systolic and diastolic blood pressure (BP) and heart rate (HR) were measured 5 minutes after waking in the laboratory with a digital cuff (Omron Healthcare Inc, BP786N, Lake Forest, IL, USA).

Continuous glucose monitoring

Participants wore a continuous glucose monitor (CGM; FreeStyle Libre Pro; Abbott, Alameda, CA, USA) to assess interstitial glucose levels for the duration of the protocol. Briefly, the CGM probe was inserted by trained staff in the subcutaneous fat on the left or right posterior side of the iliac crest as previously described (24, 25). CGM measurements were obtained every 15 min and were blinded to participants. CGM data were downloaded by research staff upon admission for each inpatient stay. Due to device failure in 1 participant, CGM data are not available for week 2 for that participant. Interstitial glucose values were averaged across week 1 and week 2 and values and grouped by 24h, sleep, and wake. Interstitial glucose values of ≤45 mg/dL were excluded from the final analyses.

Overnight blood sampling

Upon admission, an indwelling intravenous catheter was inserted in the vein of the antecubital fossa and hourly blood samples were collected based on hour since wake12 – 24 (13 samples total). During scheduled wake, blood samples were collected from participants in a semi-recumbent or seated position. During scheduled sleep, blood

samples were collected through a 12-foot tube extended through a port in the wall to an adjacent antechamber to allow blood sampling with minimal disturbance to sleep. Whole blood was collected in blood collection tubes coated with ethylenediaminetetraacetic acid (EDTA) to prevent clotting (Vacuette® K3E K3EDTA Blood Collection Tube; Greiner Bio-One North America Inc., NC, USA) and immediately centrifuged at 4°C. Plasma was separated and stored at -80°C for later analyses. “Evening” profiles were defined as hour since wake 14–15. "Nocturnal” profiles were defined as hour since wake16–24. Hourly circulating factors were assessed from 14-24.

Assays

Plasma was assayed for glucose and lactate using a 2900 YSI biochemistry analyzer (YSI 2900 Series Biochemistry Analyzer; YSI Inc, Yellow Springs, Ohio, USA). Plasma insulin concentrations were measured by chemiluminescence assays using the Immulite immunochemistry system (Diagnostic Products, Los Angeles, CA). Free fatty acids, glycerol, and triglycerides (TAG) were analyzed using a colorimetric assay (Wako Chemicals, Richmond, VA, USA). Saliva was assayed for melatonin using direct double-antibody RIAs previously validated by gas chromatography-mass spectroscopy(26) at the Endocrinology Laboratory at Colorado State University Foothills Campus.

Lipidomics

A subset of plasma samples from hour since wake timepoints 13, 15, 17, 20, 22, 24 were subjected to additional lipid species analysis (Figure 3.2b). Specially, the lipid species analyses measured concentrations of triglycerides (TAG), diacylglycerides (DAG), sphingolipid, and ceramide, utilizing liquid chromatography/electrospray

ionization (LC/ESI) on an Agilent 1100 High-Performance Liquid Chromatography (HPLC) system connected to an API 2000 triple quadrupole mass spectrometer (Sciex, Framingham, MA) 16. Chromatographic separation of DAG isomers was achieved using a hydrophilic interaction chromatography column with dimensions 2.1 μm, 3 × 100 mm. Concentration determination involved comparing unknown ratios with deuterated DAG and TAG, as well as C12:0 sphingolipid internal standard, and referencing standard curves that encompassed the majority of TAG, DAG, and sphingolipid species run with each sample set 17.(27).

Insulin sensitivity

Upon waking in the laboratory, a 3-hour, 75g oral glucose tolerance test (OGTT) was administered. Blood samples were collected at T = -15, -5, 30, 60, 90, 120, 150, and 180 min and assayed for glucose, insulin, concentrations. Estimates of whole-body insulin sensitivity were derived from the Matsuda Insulin Sensitivity Index (10,000/square root of [fasting glucose x fasting insulin] x [mean glucose x mean insulin during OGTT]) as previously described (28, 29).

Statistical Analyses

Statistical analyses were performed in SAS (version 9.4; Cary, NC) using a significance threshold of $\alpha = 0.05$. Data from all thirteen participants were included in the analyses, despite one participant only completing week 1.

In the laboratory portion of the study, measurements for fasting eating duration, weight, body mass index, activity, glucose, insulin, lactate, FFA, TAG, glycerol, FGF21, VEGF, SBP, DBP, and HR were measured immediately upon waking (hour since

wake=24) and compared between week 1 to week 2 using a mixed-effect linear model.

To assess differences circulating factors (i.e. glucose, insulin, lactate, FFA, TAG, glycerol, FGF21, and VEGF) between the evening and nocturnal periods in week 1 and week 2, mixed-effect linear models with a random intercept for participant ID were used. The evening period was defined as hour since wake 12-15, and the nocturnal period was defined as hours since wake 16-24.

Hourly comparisons of circulating factors between week 1 and week 2 were performed using a mixed-effect linear model based on time since wake. Time since wake was determined by the time participants were woken in the laboratory. Mixed-effect linear models with a condition-by-time (time since wake) interaction were ultized to compare glucose, insulin, lactate, FFA, TAG, glycerol, FGF21, and VEGF and various lipid species (i.e. TAG, AC, 1,2 DAG, 1,3 DAG, Cer, dhCer, GluCer, LacCer) across hourly intervals between week 1 and week 2. All models were adjusted for body mass index.

Insulin sensitivity was calculated with the Matsuda Index for each participant at the end of week 1 and week 2. Insulin sensitivity between week 1 and week 2 were assessed using a mixed-effect linear model.

During the outpatient portion of the study, interstitial glucose and activity were collected for days 1-6 in both week 1 and week 2. These measurements were averaged across the days of each respective week to create 24h profiles for week 1 and week 2. Interstitial glucose and activity (i.e. time sitting/lying, time stepping, step count, sit to stand transitions, and energy expenditure) between week 1 and week 2 were assessed using linear mixed models to compare 24h and night parameters based on self-reported sleep time during the outpatient phase. Add a sentence about how we measured

glucose variability in interstitial glucose comparing week 1 and week 2.

Results are presented as least-squares mean ± standard error of the mean (SEM), except for baseline characteristic data, which are displayed as raw mean ± standard deviation (SD).

RESULTS

Participants

A total of 272 adults were assessed for eligibility, 28 qualified for the study, and 13 were enrolled, and one participant dropped out of the study due to a scheduling conflict. A study flow chart is shown in Figure 3.1. Thirteen healthy lean individuals completed at least one week of the study (27 ± 4 y, 23.3 ± 2.1 kg/m^2; Table 3.1). Of those 13, 62% were female (n=8), 8% American Indian (n=1), 8% Hispanic or Latino, 77% White (n=10), and 8% two or more races (n=1).

Participants completed a 2-week protocol using a consecutive design. During week 1, participants consumed food over a 13h period whereas in week 2, participants consumed food over an 8h period (Habitual: 12.90 ± 0.04 v eTRE: 8.13 ± 0.13, respectively; p=<0.0001; Table 3.2).

Body weight and resting vitals

Body weight remained stable throughout the protocol (Habitual: 70.3 ± 3.1 v eTRE: 70.1 ± 3.2 kg, respectively; p=0.508; Table 3.2). There were no differences in mean resting systolic blood pressure, diastolic blood pressure, or heart rate between week 1 and week 2 (Table 3.2).

Fasting parameters

There were no differences in mean activity or fasting glucose, insulin, lactate, FFA, TAG, glycerol, FGF21, VEGF, SBP, DBP, or HR between week 1 and week 2 (Table 3.1).

CGM

eTRE was associated with significantly lower 24h interstitial glucose levels (Habitual eating: 82 ± 0 v eTRE: 78 ± 0 mg/dL, respectively, p=0.0002; Figure 3.3) Compared to habitual eating, interstitial glucose was significantly decreased during the sleep (Habitual eating: 74 ± 0 v eTRE: 66 ± 0 mg/dL, respectively, p=0.0008; Figure 3.3) and wake (Habitual eating: 85 ± 0 v eTRE: 83 ± 0 mg/dL, respectively, p=0.0130; Figure 3.3) periods during eTRE.

Evening and Nocturnal Circulating Plasma

During the evening period, eTRE was associated with significantly lower mean glucose (Habitual: 94.14 ± 4.93 v eTRE: 85.56 ± 3.12 mg/dL, respectively; p=0.014; Table 3.3) and insulin (Habitual: 12.61 ± 3.18 v eTRE: 6.18 ± 1.57 mU/L, respectively; p=0.005; Table 3.3) as compared to habitual eating. In contrast, eTRE was associated with significantly higher mean FFA during the evening period (Habitual: 0.29 ± 0.05 v eTRE: 0.41 ± 0.07 mmol/L, respectively; p=0.004; Table 3.3) as compared to habitual eating. There were no differences in evening lactate, TAG, glycerol, FGF21, or VEGF between conditions.

During the nocturnal period, eTRE was associated with significantly reduced insulin (Habitual: 4.33 ± 1.06 v eTRE: 2.82 ± 0.60 mU/L, respectively; p=0.010; Table 3.3), TAG (Habitual: 0.47 ± 0.06 v eTRE: 0.43 ± 0.05 mmol/L, respectively; p=0.013;

Table 3.3), and glycerol (Habitual: 0.08 ± 0.01 v eTRE: 0.06 ± 0.01 mmol/L, respectively; p=<0.001; Table 3.3) as compared to habitual eating. In contrast, eTRE was associated with significantly increased mean FFA during the nocturnal period (Habitual: 0.37 ± 0.05 v eTRE: 0.43 ± 0.04 mmol/L, respectively; p=0.039; Table 3.3) as compared to habitual eating. There were no differences in nocturnal glucose, lactate, FGF21, or VEGF between conditions

Hourly circulating plasma concentrations of glucose, insulin, lactate, FFA, TAG and glycerol

eTRE was associated with alterations in evening profiles of a variety of circulating factors (Figure 3.4a-f). Specifically, eTRE was associated with significantly lower glucose at hour since wake 13 and 15 as well insulin at hour since wake. 13-15 compared to habitual eating (Figure 3.4a-b). In contrast, eTRE was associated with a significant increase in FFA levels in the evening and at night for hour since wake 12, 14-17 (Figure 3.4d). There were no differences in hourly circulating levels of lactate, TAG or glycerol between conditions (Figure 3.4d-f).

Lipidomics

The lipidomic profiles are still being analyzed. By visual inspection, it appears that eTRE may be associated with a decrease in TAG and 1,2-DAGs compared to habitual eating.

Insulin sensitivity

Insulin sensitivity based on the Matsuda Index were not different between week 1 and week 2 (Habitual: 15.25 ± 2.30 v eTRE: 16.14 ± 2.36; p=0.544).

DISCUSSION

The purpose of this study was to investigate the temporal changes in circulating hormones and metabolites during 8h isocaloric eTRE in healthy young adults. We found that compared to 13h habitual eating, eTRE was associated with a decrease in interstitial glucose, insulin, and glycerol, particularly during the biological night. Additionally, we observed reductions in 1,2-DAGs and TAGs in the eTRE condition. This study sheds light on the fundamental biology and temporal changes that occur in response to meal timing, supporting the potential of eTRE as a dietary strategy to improve cardiometabolic health.

Our findings that eTRE was associated with a decrease in 24h interstitial glucose, particularly during the nocturnal period, align with previous research. For example, a study conducted in 19 adults with type 2 diabetes who underwent a 4-week, 9h isocaloric TRE regimen found that TRE decreased both 24h and nocturnal interstitial glucose concentrations compared to habitual eating (30). Additionally, an acute study involving 8 adults with overweight found that eTRE, defined in this study as eating between 0800 and 1400h, decreased 24h and nocturnal interstitial glucose levels. Previous studies examining TRE have predominately focused on populations with higher prevalence of cardiometabolic diseases such as people with type 2 diabetes and overweight. Our study provides novel insights by examining eTRE effects in a homogeneous healthy, young adult population. This population allows for a clearer

understanding of the mechanisms underpinning the impact acute isocaloric eTRE on cardiometabolic health. Lower interstitial glucose levels, particularly at night, may be important for cardiometabolic health.

Our study found that eTRE was associated with a decrease in circulating insulin, glycerol, 1,2-DAGs and TAGs without a change in insulin sensitivity or glucose tolerance compared to habitual eating. Studies examining the impact of eTRE on circulating metabolites are limited. One study conducted in healthy adults during a randomized crossover study in free-living conditions compared delayed eating, defined as eating between 1200 and 2300h, to control eating, defined as eating between 0800-1900h, and found that late eating increased fasting TAGs (16). However, this study was limited to fasting TAGs making it difficult to understand the temporal changes in TAGs from alterations in meal timing. In contrast, our study provides novel insights into the dynamic changes in circulating metabolites during the nocturnal period.

Changes in circulating hormones and metabolites have been examined in other contexts. For example, extensive research has examined the acute impact of alterations in sleep patterns on circulating hormones and metabolites. Data from studies on acute insufficient sleep and altered sleep timing reveal significant impacts on hormonal and metabolic profiles, particularly during sleep. For example, a highly controlled laboratory study conducted in 19 healthy men found that reducing sleep to 4.5h for 4 nights increased circulating non-esterified free fatty acid levels during sleep and reduced insulin sensitivity upon waking compared to sufficient sleep conditions (31). Additionally, data from controlled laboratory studies show that shifting sleep to the biological daytime is associated with increased circulating glucose and TAG levels at night (32), reduced

glucose tolerance (33), and altered 24h lipid profiles (34). Similar to previous studies in the context of sleep research, our study provides insights into how eTRE impacts circulating hormones and metabolites, especially during the nocturnal period. Altogether, data from these studies underscore the importance of understanding how acute changes in behaviors like meal and sleep timing impact cardiometabolic health.

Timing of caloric intake may serve as a key mechanism underlying the improvements in cardiometabolic parameters associated with eTRE. In our study, participants were proscribed meal times based on time since awake, starting at 1h after waking. As a result, participants eliminated food intake in the late evening. Previous evidence has suggested that late-night eating (e.g., within 2h of bedtime) is associated with higher body fat percentage and body mass index (9). Further, eating in the earlier part of the day has been suggested to be more align with circadian rhythms in metabolism (19). Glucose regulation has previously been shown to vary across the 24h period, with improved glucose tolerance observed earlier in the day. For example, a study conducted in healthy adults examined the response of an oral glucose tolerance tests to different times of the day including the morning, afternoon, and night (35). Data from this study found that glucose tolerance was highest in the morning compared to the afternoon or night (35). Thus, the data from our study supports consolidating energy intake to the biological day and eliminating energy intake during the biological nighttime may improve cardiometabolic health.

Limitations

This study has several limitations. First, our analysis included only 13 individuals. Although our sample size is similar to other highly controlled inpatient trials, our results need to be replicated in a larger trial that includes more participants, which

will also allow for examinations of sex differences in response to eTRE. Second, although acute studies in inpatient settings offer the advantage of controlling for many confounding variables, real-world adaptations to shorter eating schedules may differ. Therefore, longer-term studies are needed to assess whether changes in food timing provide lasting metabolic benefits. Third, we were unable to provide participants with controlled food intake. Although we were not able to provide a diet based on resting metabolic rate, we did collect detailed information on energy consumed throughout the study, which was then provided to each participant during week 2 to minimize variability of food between conditions. However, differences in food choice and amount may have been present between conditions, which would not be possible to observe using our current methods. However, weight remained stable over the course of the study, suggesting that participants indeed maintained an isocaloric diet. Finally, our trial did not randomize condition order, and all participants were first assessed after one week of habitual eating, followed by one week of eTRE, thus it is possible that an order effect could be present in our outcome variables.

Future Directions

In the United States, >50% of people are living with one or more cardiometabolic diseases (1–3). Alterations in meal timing is increasingly recognized as one factor contributing to heightened cardiometabolic disease prevalence(4). Strategies to alter meal timing such as eTRE have shown promising improvements to cardiometabolic health (13–17, 22). However, previous studies have not investigated the temporal changes in circulating hormones and metabolites in a homogenous population. This study provides insights into the temporal changes in circulating metabolites like glucose,

insulin, and lipid species, especially during the nocturnal period.

Although this study examined eTRE in the context of healthy adults, future research should investigate eTRE in populations where late-night eating is prevalent. One population that may particularly benefit from eTRE are shift workers. Shift workers, who work outside of the traditional 8am-5pm schedule, are at a heightened risk for cardiometabolic disease compared to day shift workers (5, 36). The heightened risk for cardiometabolic diseases is likely due to inappropriate timing of energy intake and sleep. Early evidence from a controlled laboratory study suggests that restricting to daytime hours during simulated night shift work can mitigate the glucose intolerance associated with night shift work (37). However, the impact of daytime eating in a free-living shift work population is less understood. Therefore, future research should continue to explore eating interventions, like TRE, in at-risk populations such as shift workers.

TABLES

Table 3.1. Baseline characteristics	
n	13
Female	8
Age, y	27 ± 4
Weight, kg	70 ± 11
Height, cm	173 ± 8
BMI, kg/m^2	23 ± 3
Body fat, %	27 ± 6

Table 3.1. Baseline characteristics. Data are presented as mean ± SD. BMI, body mass index.

	Week 1 (habitual eating)	Week 2 (eTRE)	*p* value
Eating duration (h)	12.90 ± 0.04	8.13 ± 0.13	<0.0001*
Weight (kg)	70.3 ± 3.1	70.1 ± 3.2	0.508
BMI (kg/m²)	23.4 ± 0.8	23.3 ± 0.8	0.440
Glucose (mg/dL)	80.16 ± 3.90	78.74 ± 4.23	0.806
Insulin (mU/L)	2.49 ± 2.25	3.25 ± 2.46	0.811
Lactate (mg/dL)	7.81 ± 0.80	7.18 ± 0.86	0.588
FFA (mmol/L)	0.31 ± 0.05	0.31 ± 0.05	0.899
TAG (mmol/L)	0.41 ± 0.07	0.45 ± 0.08	0.727
Glycerol (mmol/L)	0.07 ± 0.02	0.10 ± 0.02	0.240
FGF21 (pg/mL)	88.84 ± 36.57	104.90 ± 37.72	0.752
VEGF (pg/mL)	34.32 ± 30.77	101.21 ± 32.94	0.142
SBP (mmHg)	106 ± 4	101 ± 4	0.377
DBP (mmHg)	63 ± 2	59 ± 3	0.209
HR (bpm)	65 ± 5	62 ± 5	0.106

Table 3.2. Effect of eTRE on eating duration, weight, activity, and fasting glucose, insulin, lactate, FFA, TAG, glycerol, FGF21, and VEGF levels. Data are presented as least-squares means ± SEM. P-values represent linear mixed models between week 1 and week 2. *$p \leq 0.05$.

	Week 1 (habitual eating)	Week 2 (eTRE)	p
Evening: hours since wake 12-15			
Glucose (mg/dL)	94.14 ± 4.93	85.56 ± 3.12	0.014*
Insulin (mU/L)	12.61 ± 3.18	6.18 ± 1.57	0.005*
Lactate (mg/dL)	10.52 ± 0.81	9.91 ± 0.70	0.232
FFA (mmol/L)	0.29 ± 0.05	0.41 ± 0.07	0.004*
TAG (mmol/L)	0.64 ± 0.09	0.63 ± 0.08	0.259
Glycerol (mmol/L)	0.14 ± 0.03	0.11 ± 0.01	0.154
FGF21 (pg/mL)	69.18 ± 16.44	58.81 ± 15.30	0.139
VEGF (pg/mL)	76.09 ± 19.11	111.45 ± 45.21	0.089
Nocturnal: hours since wake 16-24			
Glucose (mg/dL)	82.38 ± 4.54	82.01 ± 2.47	0.455
Insulin (mU/L)	4.33 ± 1.06	2.82 ± 0.60	0.010*
Lactate (mg/dL)	7.23 ± 0.84	6.91 ± 0.74	0.303
FFA (mmol/L)	0.37 ± 0.05	0.43 ± 0.04	0.039*
TAG (mmol/L)	0.47 ± 0.06	0.43 ± 0.05	0.013*
Glycerol (mmol)	0.08 ± 0.01	0.06 ± 0.01	<0.001*
FGF21 (pg/mL)	114.86 ± 42.93	125.48 ± 53.53	0.497
VEGF (pg/mL)	60.49 ± 16.26	68.41 ± 15.35	0.388

Table 3.3. Effect of eTRE on mean evening and nocturnal levels of glucose, insulin, lactate, FFA, TAG, glycerol, FGF21 and VEGF levels. Data are least-squared mean ± SEM. P-values represent linear mixed models between week 1 and week 2. *$p \leq 0.05$; #$p \leq 0.001$.

FIGURES

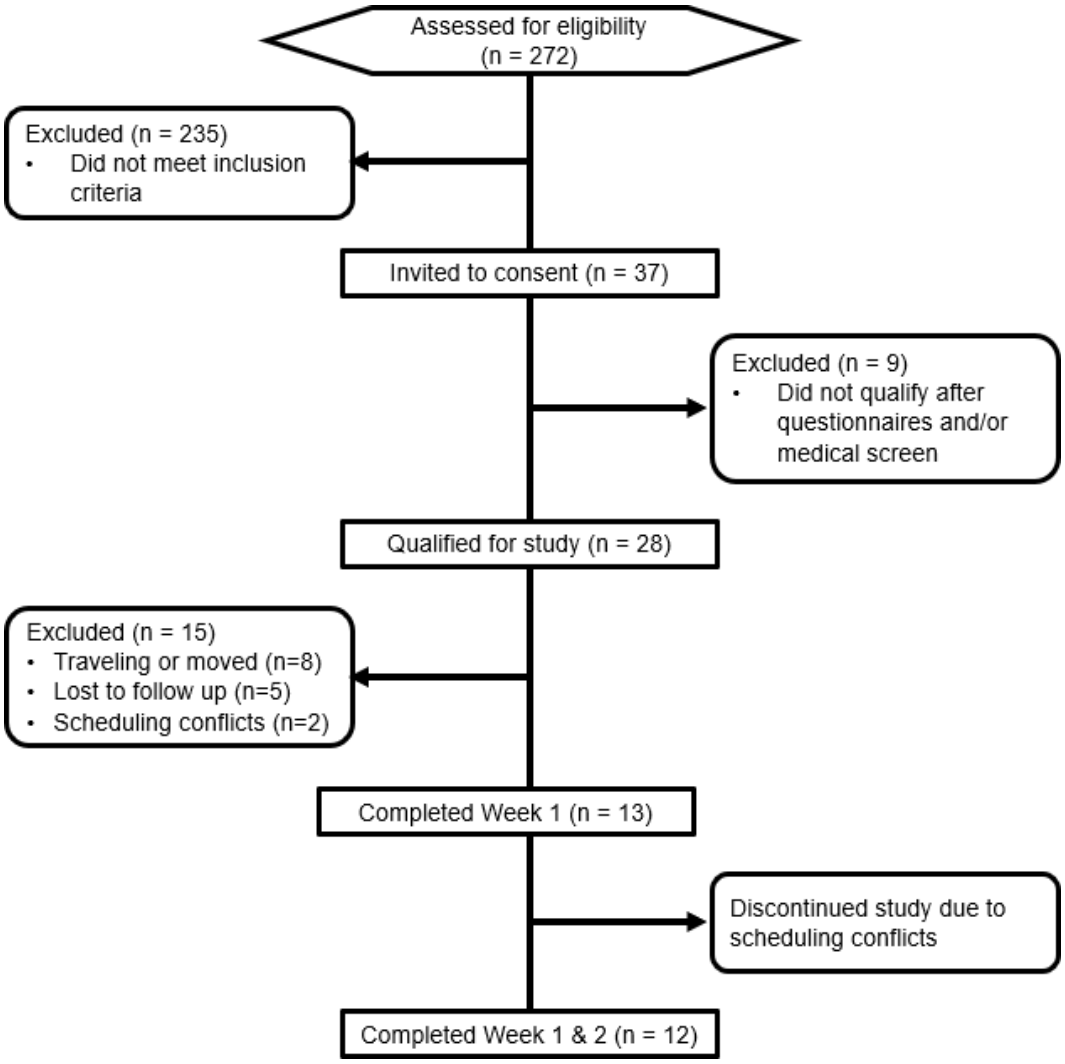
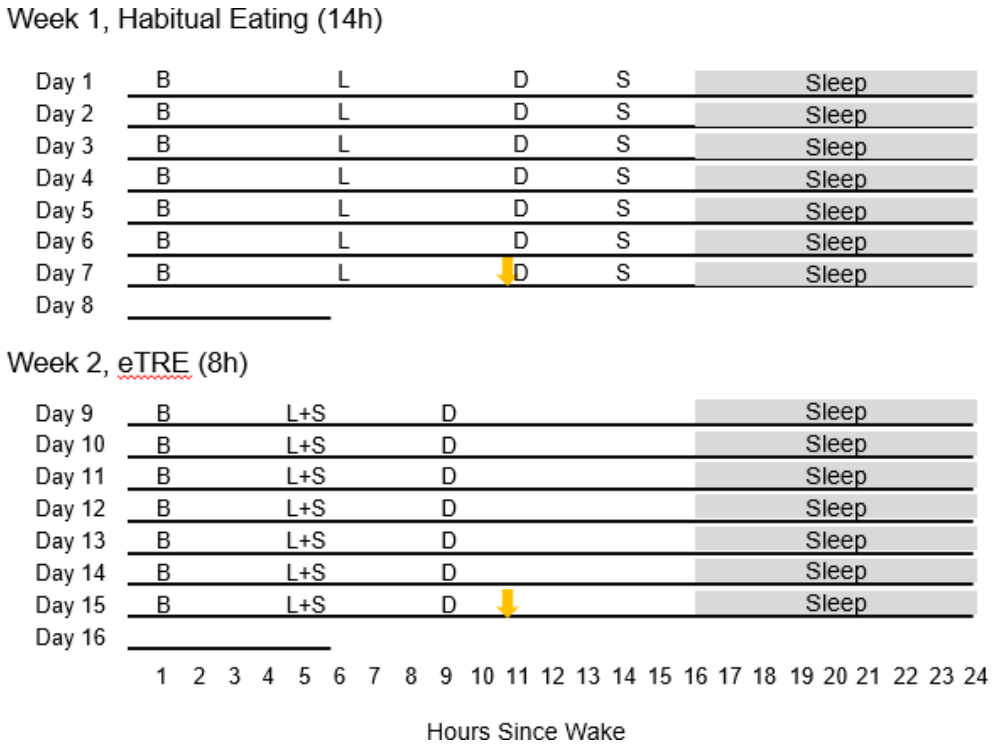


Figure 3.1. Study flow chart. A total of 272 subjects were assessed for eligibility; 28 qualified for the study based on questionnaires and/or medical screen; 15 participants withdrew or were disqualified; 13 participants completed Week 1; 1 participant did not complete Week 2.

A.


Week 1, Habitual Eating (14h)
Day 1 B L D S Sleep
Day 2 B L D S Sleep
Day 3 B L D S Sleep
Day 4 B L D S Sleep
Day 5 B L D S Sleep
Day 6 B L D S Sleep
Day 7 B L D S Sleep
Day 8
Week 2, eTRE (8h)
Day 9 B L+S D Sleep
Day 10 B L+S D Sleep
Day 11 B L+S D Sleep
Day 12 B L+S D Sleep
Day 13 B L+S D Sleep
Day 14 B L+S D Sleep
Day 15 B L+S D Sleep
Day 16
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Hours Since Wake

B.

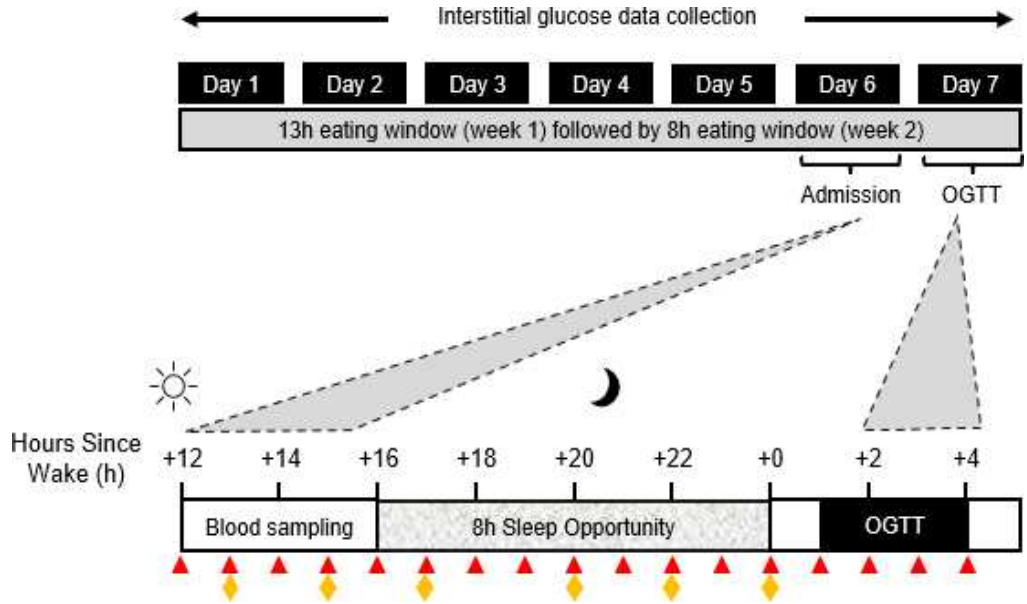

Interstitial glucose data collection
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
13h eating window (week 1) followed by 8h eating window (week 2)
Admission
OGTT
Hours Since Wake (h)
+12 +14 +16 +18 +20 +22 +0 +2 +4
Blood sampling
8h Sleep Opportunity
OGTT

Figure 3.2. Overview of Study Protocol. A. Outpatient Study protocol for typical eating (week 1; top) and eTRE (week 2; bottom). B, L, D, S: breakfast, lunch, dinner, snack; Yellow arrows indicate admission to lab; Gray boxes indicate scheduled sleep opportunities. **B.** Inpatient study protocol. Red triangles indicate hourly blood draws; yellow diamonds indicate lipidomic.

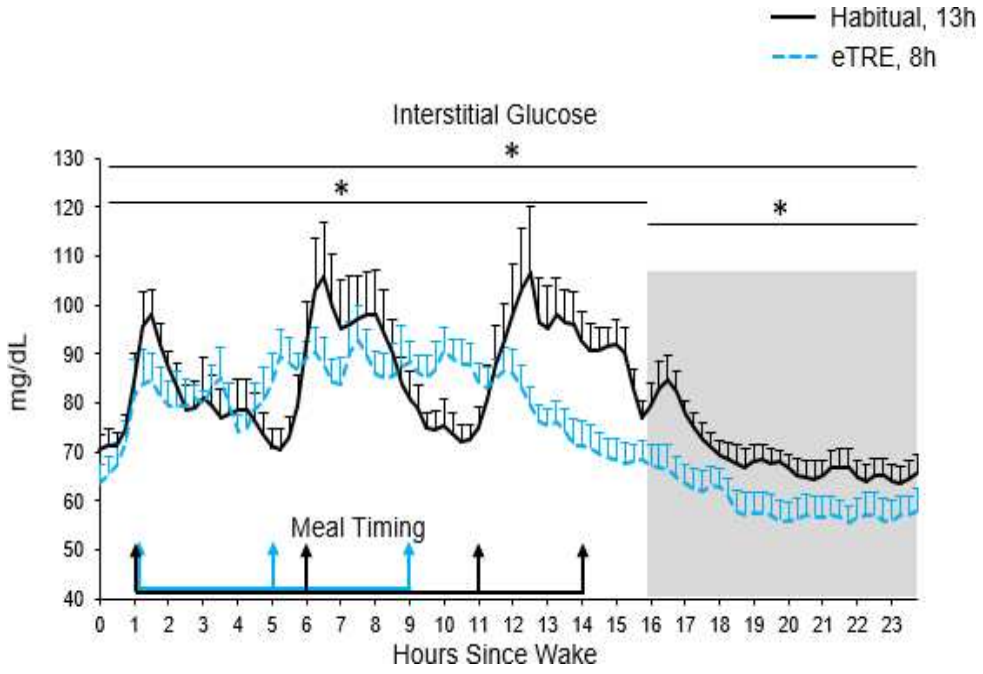


Figure 3.3. Interstitial glucose. Impact of 1 week of eTRE on interstitial glucose concentrations. Black arrows indicate meal timing during 13h eating window; blue arrows indicate meal timing during 8h eating window; gray boxes indicate 8h sleep opportunity. Data are presented as least-squares mean ± SEM. *$p \leq 0.05$; #$p \leq 0.001$.

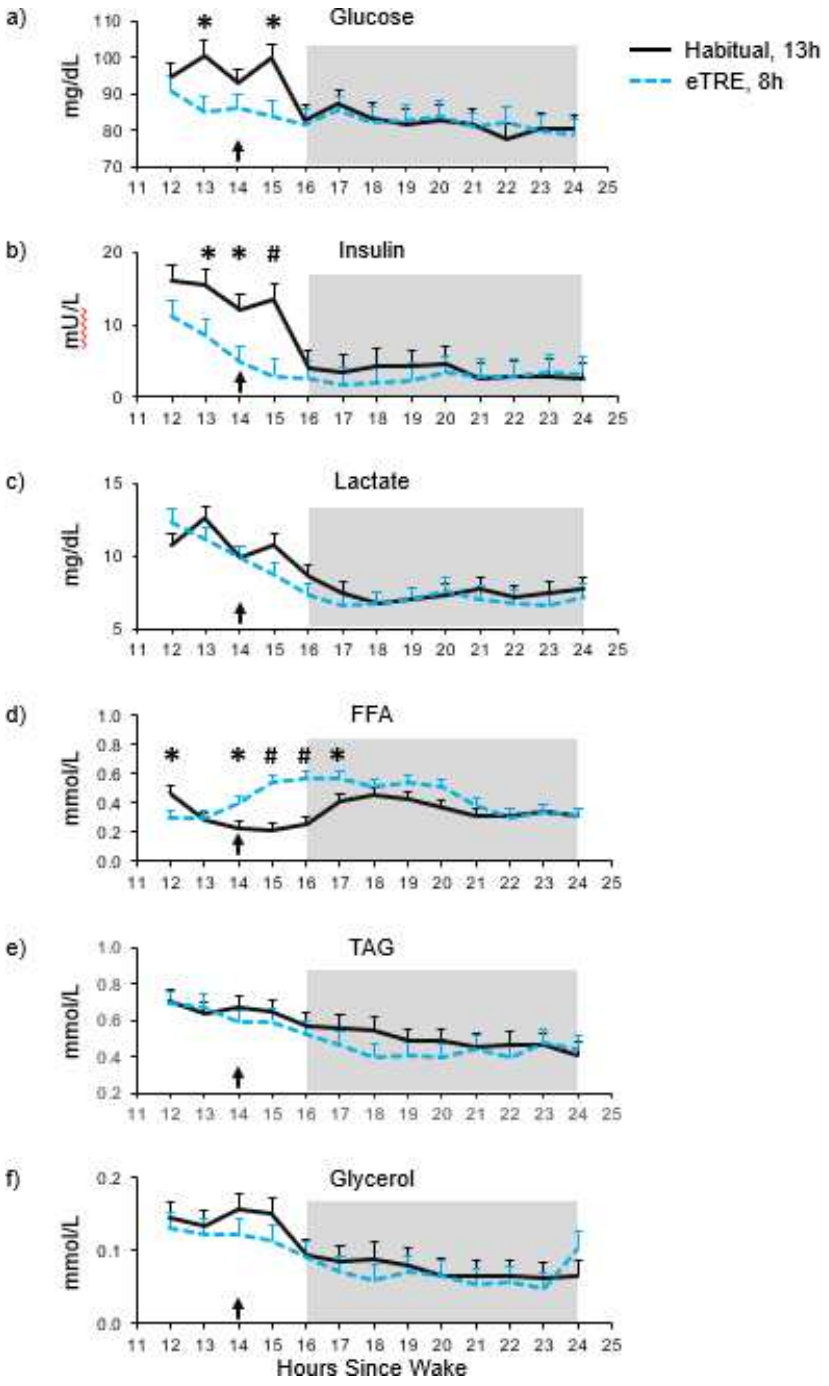


Figure 3.4. Circulating hormones and metabolites. Impact of eTRE on glucose (a), insulin (b), lactate (c), free-fatty acids (d), TAG (e), and glycerol (f). Black solid line: 13h eating window; blue dashed line: 8h eating window; gray boxes indicate 8h sleep opportunity.Data are presented as least-squares mean ± SEM. *$p \leq 0.05$; #$p \leq 0.001$.

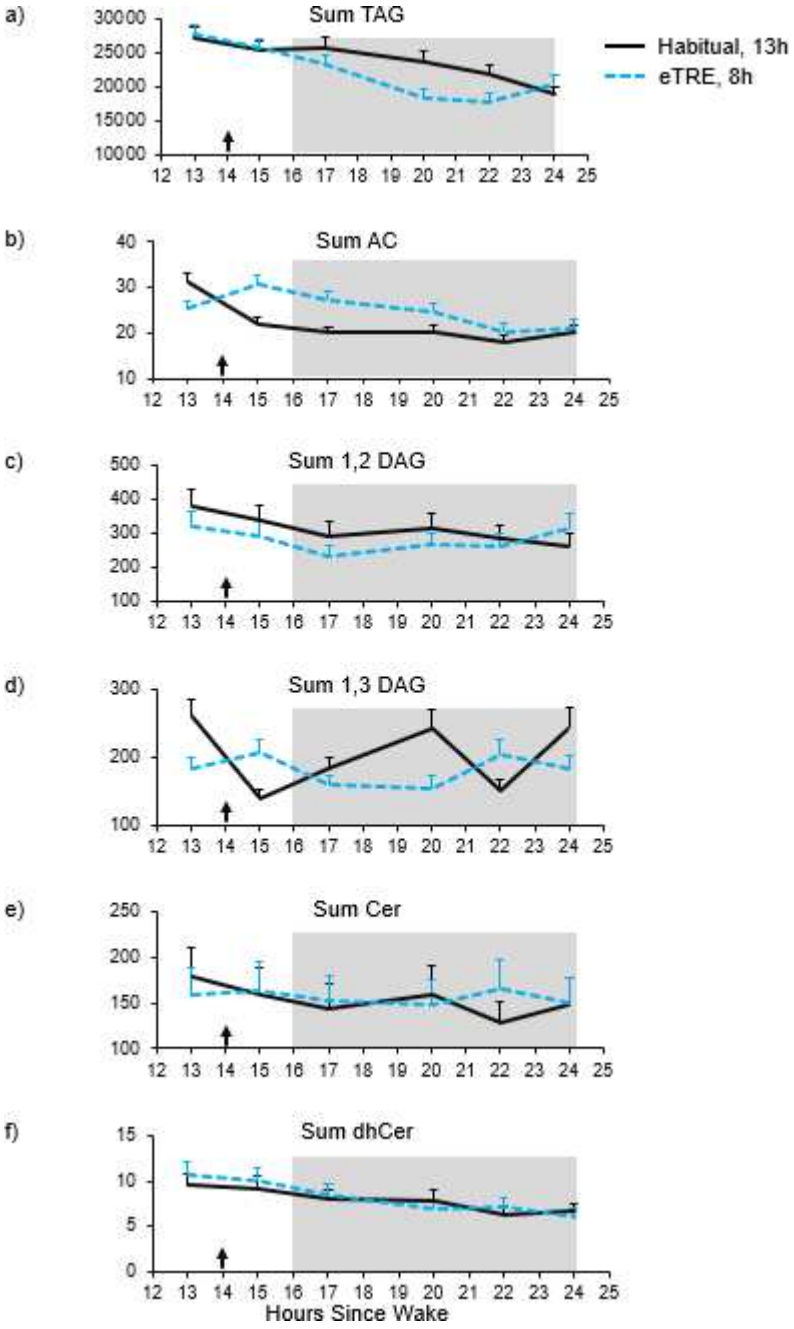


Figure 3.5. Circulating Lipids. Impact of eTRE on sum TAG (a), sum AC (b), sum 1,2 DAG (c), sum 1,3 DAG (d), sum ceramides (e), and sum dCer (f). 1 Black solid line: 13h eating window; blue dashed line: 8h eating window; black arrow indicates last meal during week 1; gray boxes indicate 8h sleep opportunity. Data are presented as least-squares mean ± SEM. *$p \leq 0.05$; #$p \leq 0.001$.

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CHAPTER 4 – CONCLUSION & FUTURE DIRECTIONS

CONCLUSION

"If I have seen further, it is by standing on the shoulders of Giants."

-Isaac Newton

Sleep is a universal biological process regulated by our circadian rhythm to the 24h day and is essential for optimal health and well-being. Chronic disruptions in sleep and circadian rhythms also referred to as sleep and circadian disruption, are becoming increasingly recognized as risk factors for cardiometabolic diseases, including T2D and CVD. In fact, sleep and circadian disruption confer similar or higher risk for T2D as traditional risk factors, such as being overweight, having a family history of diabetes, or being physically inactive(1).

Night shift workers are a particularly vulnerable population because they have an elevated risk for T2D and CVD due to repeated exposure to sleep and circadian disruption. In fact, people who participate in shift work are 44% more likely to develop type 2 diabetes (T2D) (2) and 40% more likely to develop cardiovascular disease (CVD) (3) compared to people who work day shifts. Night shift workers are classified as people who perform at least 7 consecutive hours between 2400 to 0500 (4) and make up a quarter of the United States workforce (5). Due to work schedule demands, people who work night shifts conduct work operations and often eat during the biological nighttime when the internal circadian system promotes restfulness and sleep during the biological daytime when the circadian system promotes wakefulness.

Epidemiological evidence supports that chronic night shift work is associated with a heightened risk for cardiometabolic disease (3, 6). Additionally, when simulated in a

highly controlled laboratory setting, acute sleep and circadian disruption impairs factors related to cardiometabolic health (7, 8). However, there is a gap in research investigating the acute impact of sleep and circadian disruption in a free-living setting on cardiometabolic risk. This dissertation aimed to fill that gap by exploring the acute relationship between sleep and circadian disruption in free-living settings on factors related to cardiometabolic health. To understand this, we examined the acute impact of night shift work on CVD risk factors in a free-living setting. In a quasi-randomized crossover study, we assessed 24h blood pressure during a day shift and a night shift in rotating shift workers. Data generated from this dissertation revealed that as little as one night of shift work in a free-living setting is sufficient to induce multiple CVD risk factors including increased blood pressure and reduced sleep duration in healthy adults. These results emphasize the importance of addressing the negative impact of shift work on CV health through targeted interventions.

Knowledge gained from this and previous work highlight a need for strategies to mitigate the impact of sleep and circadian disruption on cardiometabolic disease risk, especially in populations where sleep and circadian disruption are often unavoidable. To address this, our next study examined early time restricted eating (eTRE), a strategy previously associated with improvements in cardiometabolic health. However, the mechanisms by which eTRE confers these benefits, particularly under isocaloric conditions, remain less understood. Therefore, our study focused on exploring the acute impact of 8h eTRE starting 1h after waking on circulating factors related to cardiometabolic health, independent of sleep and circadian disruption. Data generated from this dissertation revealed that an 8h eTRE compared to a habitual 14h eating

window acutely improved circulating glucose, insulin, and bioactive lipids. These findings provide mechanistic insights into how altering meal timing may benefit cardiometabolic health. Consequently, these data support the investigation of TRE as a potential countermeasure to the cardiometabolic impairments associated with sleep and circadian disruption.

Data from a recent study suggest that TRE, specifically when consolidated to the biological daytime, can improve metabolic health during simulated night shift work in highly controlled inpatient settings (9). However, the impact of TRE during simulated night shift work on 24h CV health metrics are unknown. To address this, our next study investigated the impact of isocaloric TRE during simulated night shift work on CV health measures, including 24h heart rate and blood pressure, in a highly controlled inpatient setting. In this ongoing study, our data suggest that TRE during simulated night shift work decrease 24h heart rate and blood pressure compared to simulated night shift work without TRE. Our data, along with data from the previous study, supports that aligning meal timing to the biological daytime during acute sleep and circadian disruption is associated with improvements in cardiometabolic parameters, including glucose tolerance and blood pressure. It is important to note, however, that both studies are limited because they were conducted in an acute, highly controlled laboratory setting with healthy adults.

FUTURE DIRECTIONS

Data from previous research, including a study explored in this dissertation, have shown promising evidence that consolidating food intake to the biological daytime

reduces 24h heart rate and blood pressure as well as improves glucose tolerance, during controlled inpatient studies simulating circadian misalignment. This simulated circadian misalignment protocol, also called a slam shift protocol, is employed in research to mimic rapidly transitioning work shift schedules as is typical for night shift workers. However, these studies are acute, lasting only 2-4 days and are typically conducted in healthy, lean individuals. Therefore, a significant knowledge gap remains regarding the impact of consolidating food intake on cardiometabolic factors during sleep and circadian disruption in a population of permanent night shift workers.

Permanent night shift workers, unlike participants in previous studies, are often neither young nor lean and frequently possess several cardiometabolic risk factors. Examining this population is crucial for future research to define strategies that counter the detrimental cardiometabolic effects of sleep and circadian disruption. Future research should include a more diverse population of permanent night shift workers that better represents the workforce, including individuals of varying ages, body composition, and existing health conditions. This will allow findings to be more generalizable to the broader shift work population.

Additionally, future research should also aim to conduct long-term studies that extend beyond the acute timeframe of 2-4 days to observe the effects of chronic sleep and circadian disruption on cardiometabolic health. These studies can provide a more comprehensive understanding of the risks and benefits of long-term intervention strategies.

Consolidating meal intake to the biological daytime as shown with TRE may not be suitable for all populations during sleep and circadian disruption for various reasons.

For example, some shift workers may use snacking as a strategy to stay awake during night hours. Snacking may help maintain alertness and cognitive function (10), which is crucial for performing work tasks that require high levels of attention and concentration. Additionally, night shift workers often face irregular meal schedules (11–13) and limited access to healthy food options (14), which may make adherence to TRE challenging. Additionally, people with eating disorder or who are underweight (BMI below 18.5 kg/m^2) may also not benefits from TRE (15). Altogether, this highlights the need for more research to determine the feasibility of TRE in a real-world setting, especially in shift work populations that have irregular meal schedules and limited access to healthy food options.

Additional strategies to mitigate the cardiometabolic impairments associated with sleep and circadian disruption when such behaviors are unavoidable should be explored. One promising direction that is explored throughout this dissertation is altering meal timing to reduce the mismatch between the body's internal circadian system and external activities, potentially lowering the risk of cardiometabolic diseases. Another potential strategy is altering exercise timing. The timing of exercise is interesting because, while the central clock in the hypothalamus responds to light cues, peripheral clocks are influenced by both the central clock and external cues like feeding and activity. Notably, peripheral clocks in skeletal muscle are one type of peripheral clock that is responsive to exercise. Some evidence suggests that exercise can phase-shift the skeletal clock and impact the expression of tissue-specific genes, including those involved in glucose regulation. Thus, exercise timing may serve as a powerful

therapeutic strategy to address cardiometabolic disease risk during sleep and circadian disruption.

More research is needed to determine the optimal timing for exercise as a therapeutically beneficial intervention. The circadian system modulates cardiovascular function and metabolism function across an approximate 24h cycle. The timing of exercise may significantly impact health outcomes due to circadian influences. Ongoing studies by collaborators, including NIH-funded projects (NIH K99HL148500, PI: Jingyi Qian; NIH R56DK136601, PI: Seth Creasy), are currently investigating the effects of exercise at different times of day on factors related to cardiometabolic health. This research aims to enhance our understanding of the best timing for exercise, particularly in populations at risk for cardiometabolic disease like shift workers. Recent findings suggest that engaging in moderate to vigorous aerobic activity in the evening may be associated with the lowest risk of cardiometabolic disease among adults with obesity and type 2 diabetes (16, 17). However, additional studies are needed to investigate the mechanisms underpinning these findings and determine the optimal timing for exercise in populations experiencing sleep and circadian disruption.

Research investigating the optimal timing for exercise in night shift workers to counteract cardiometabolic impairments is limited. However, it is hypothesized that aligning exercise to the biological daytime in night shift workers may improve cardiometabolic health by lessening the mismatch between the endogenous circadian system and external behaviors. Furthermore, some preliminary evidence in a rodent model suggests that exercise timing could be used to phase-shift peripheral clocks, like peripheral clocks in the skeletal muscle (18). Thus, there is potential to use exercise

timing to phase-shift peripheral clocks like the skeletal muscle clock to align closer to the endogenous circadian system. This would be particularly beneficial in night shift workers who are likely repeatedly exposed to mismatches between the endogenous central clock circadian system and peripheral clocks. Altogether, future research investigating how exercise timing could mitigate the adverse effects of sleep and circadian misalignment are warranted.

Regardless of timing, exercise has previously been shown to favorably improve cardiometabolic health including (19–21). Studies investigating the cardiometabolic benefits of exercise in a night shift work population are limited but promising. Previous data from studies have found that aerobic exercise is associated with decreased blood pressure (22, 23) and may even restore sleep BP dipping (24–26). Further, some evidence has associated aerobic exercise to increased self-reported sleep duration in individuals with sleep complaints (27). Improvements in self-reported sleep duration from exercise may be particularly beneficial to night shift workers who frequently experience insufficient sleep duration and poor sleep quality (28, 29). Interventions like exercise have potential to mitigate the cardiometabolic risks amongst night shift workers.

This is an important avenue of future research because an alarming portion of adults in the United States experience sleep and circadian disruption. A staggering 35% of adults in the United States report insufficient sleep on the week days (30, 31) and nearly a quarter of the United States workforce participates in shift work (5). Strategies to mitigate the cardiometabolic risks associated with unavoidable sleep and circadian disruption are of huge importance. Studies presented throughout this dissertation explore a novel strategy to counteract these impairments. Moving forward,

future research should investigate additional strategies and examine their effectiveness within the context of chronic shift work populations. Sleep and circadian disruption are largely unavoidable in modern society; thus, it is critical to identify strategies to prevent the cardiometabolic risk in a large portion of the population.

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APPENDIX A – COUNTERMEASURES TO THE CARDIOVASCULAR IMPAIRMENTS ASSOCIATED WITH SLEEP AND CIRCADIAN DISRUPTION

OVERVIEW

Introduction: Shift workers make up 20% of the US workforce and have a heightened risk for cardiovascular disease (CVD) compared to people who work during the day. Strategies to mitigate these CV impairments in shift workers are limited. Preliminary data demonstrate that restricting food to a shorter window during waking hours (time restricted eating or TRE), decreases blood pressure (BP) data in people with subclinical risk factors for CVD. However, the effects of TRE during shift work on CV health have not been examined. We therefore investigated the impact of isocaloric TRE during simulated shift work on factors related to CV health including systolic and diastolic BP.

Methods: In an ongoing study, eighteen healthy adults (10F, 24±5y, BMI: 22.5±2.3kg/m^2; mean±SD) participated in a randomized crossover study at the Sleep and Metabolism Laboratory. Participants underwent simulated shift work in two conditions. In one condition, participants consumed food throughout the night (habitual eating), and in the other, food was eliminated from the night and concentrated during the day (TRE). BP was measured every 30 minutes with an ambulatory device (OnTrak, Spacelabs^{TM}, Snoqualmie, WA) to assess 24h, wake, and sleep systolic and diastolic BP.

Results: TRE during simulated shift work was associated with significantly lower systolic (104±2 v 107±2 mmHg; $p=0.0003$) and diastolic (65±1 v 66±1 mmHg; $p=0.0448$) BP compared to habitual eating. The TRE-mediated decrease in 24h systolic and diastolic BP was primarily explained by a decrease in systolic and diastolic BP during the wake period ($p<0.05$), but not the sleep period.

Conclusion: TRE in a highly controlled laboratory setting is sufficient to lower 24h systolic and diastolic BP in healthy adults. Future research should investigate the impact of TRE on CV health in a free-living shift work population.

INTRODUCTION

People who work evening, night or rotating shifts (i.e. shift work) make up a quarter of the United States workforce (1) and are disproportionately impacted by cardiovascular disease (CVD) compared to day shift workers. In fact, night shift workers have a 40% higher risk of CVD compared to people who work day shifts (2–5). The CVD risk in shift working populations cannot be fully accounted for by traditional risk factors like family history, smoking status, physical inactivity, or socioeconomic status (6, 7).

One significant factor contributing to the CV impairments associated with shift work is circadian misalignment. Circadian misalignment refers to the mismatch between the endogenous circadian system and external behaviors such as sleep, energy intake, activity and light. Data from studies using highly controlled inpatient protocols to induce circadian misalignment in healthy adults have consistently demonstrated CV impairments similar to those experienced by shift workers (8, 9). These impairments include elevated blood pressure (BP), which is a leading risk factor for CVD (10).

The timing of energy intake during circadian misalignment, regardless of total energy intake, appears to be a major contributing factor to heightened risk of CVD. A recent study of night shift workers recorded calorie and macronutrient intake for a 24h period during both a night shift and a rest day (11). The findings revealed that more calories were consumed at night and over a longer eating window on days when

participants conducted a night shift compared to rest days (approximately 14h v 11h). Interestingly, however, total 24h energy intake was lower during days when participants conducted night shift work compared to rest days. Additionally, another study involving 116 women found that over the course of 1-year, later timing of the energy intake during the day was associated with poorer CV health and higher BP (12). Altogether, data from these studies suggest that the timing of energy intake, particularly later in the day, may have detrimental effects on CV health that are not attributed to higher energy intake.

Given the prevalence of shift work, there is an urgent need to develop effective countermeasures to combat the CV risk associated with circadian misalignment. Preliminary data suggest that consolidating energy intake to a shorter period during the day, known as time-restricted eating (TRE), can improve metabolic health during circadian misalignment. For example, a recent study conducted in a highly controlled inpatient setting demonstrated that consolidating energy intake to the biological daytime during circadian misalignment mitigated impairments in glucose tolerance associated with shift work (13). This study provides early evidence that TRE may be effective in reducing metabolic risk, but its impact on CV parameters remains to be investigated.

Independent of circadian misalignment, TRE is associated with decreases in CV risk factors in people with subclinical risk factors for CVD. For example, in a 12-week study, 19 participants with metabolic syndrome underwent a TRE regimen where energy intake was consolidated to a self-selected 10h window (16). This study found that participants lowered resting SBP and DBP by 5 and 6 mmHg, respectively. Additionally, in another 5-week study, 8 participants with prediabetes underwent a TRE regimen where energy intake was consolidated to a 6h window ending before 15:00 (17). Participants in this study lowered resting systolic BP (SBP) and diastolic BP (DBP)

by 11 and 10 mmHg, respectively, although there was no change in resting heart rate (HR). These findings support the effectiveness of TRE in lowering resting BP; however, its impact during circadian misalignment is less understood. Furthermore, most investigations have been limited to a single resting BP assessment. Future studies need to explore the impact of TRE on 24h blood pressure, as alterations in BP at specific times of the day are independently associated with elevated CVD risk, particularly during sleep (8, 9, 10).

Therefore, this ongoing study aims to determine the impact of TRE during circadian misalignment on CV health measures, including 24h BP and HR. This study is novel because it examines 24h BP and HR, providing a comprehensive assessment of CV health. Our central hypothesis is that TRE during circadian misalignment will decrease 24h BP and HR, especially during sleep. We are using a randomized crossover study with a rigorous inpatient protocol controlling for diet, activity, and light exposure in healthy adults.

METHODS

Participants

Healthy young, lean adults were recruited for this study. Inclusion criteria included individuals who were 18-35 years old with a body mass index (BMI) of 18.5-24.9 kg/m^2 and who were weight stable (<5 lb change in the past 6 months). Individuals habitually slept 7-9h/h and had a normal metabolic profile (fasting plasma glucose concentration <100 mg/dL, HbA1c ≤5.6%, serum triglyceride concentration <150 mg/dL; and serum HDL-cholesterol concentration ≥40 mg/dL for men and ≥50 mg/dL for women). Participants were non-smokers and medications free, had not traveled outside of Mountain Standard Time in the past 3 weeks, had lived at Fort Collins altitude for >3

months, and had no evidence of clinically significant sleep disorders (as assessed by the Sleep Disorder Questionnaire and confirmed with an overnight sleep disorder screen). Participants had no prior history of shift work within the last year.

Participants were excluded if they had any clinically significant unstable medical or surgical conditions within the last year, had clinically significant sleep disorder or psychiatric conditions, used any medications except oral contraceptives (non-hormonal IUDs allowed), and excessively consumed caffeine (>400 mg per day) or alcohol (males >14 drinks/week or > 5 drinks/day; females >7 drinks/week). Participants were excluded if they excessively participated in moderate to vigorous physical activity (>150 min of ≥65% maximal HR aerobic exercise/week) or had irregular menses for female participants. The Institutional Review Board of Colorado State University approved the protocol and all participants provided written informed consent.

Experimental protocol

The week prior to study admission, participants maintained a consistent self-selected 8h/night sleep schedule. Compliance was verified using actigraphy (Actiwatch Spectrum Pro; Philips Respironics), sleep logs and call-ins to a time stamped recorder. For 3 days before each inpatient condition, participants were provided an outpatient energy balanced lead-in diet of meals and snacks consisting of 15% protein, 30% fat and 55% carbohydrate, designed and provided by the Colorado Clinical and Translation Sciences Institute Nutrition Core. The diet was based on resting metabolic rate assessments conducted at the medical screening and designed to maintain stable body weight throughout the protocols.

Upon admission into the laboratory, participants underwent a randomized

crossover inpatient clinical study. The study consisted of 2, 6-day inpatient conditions conducted in a randomized order in the Sleep and Metabolism Laboratory with at least 1-month between conditions. Continuous monitoring by laboratory staff ensured protocol compliance. Participants were kept in dim light (<8 lux) throughout the protocol to allow for accurate assessment of circadian melatonin rhythm. A medical director was on-call during all study visits. Women were all studied during the menstrual phase of their menstrual cycle verified by cycle logs and blood assay for estradiol and progesterone.

In both conditions, participants adapted to the laboratory on study days 1 and 2. During these days, participants maintained their habitual sleep and wake schedules. On study day 3, participants transitioned to a night shift work schedule, with a 2h nap opportunity followed by wakefulness during the nighttime. On study days 4 and 5, participants underwent simulated night shift work. Participants maintained wakefulness throughout the biological nighttime and were provided a 7.5h sleep opportunity in the biological daytime. The timing of all study protocols, including meal and sleep times, were based on their habitual sleep schedule prior to admission. On study day 6, participants were provided a 12h recovery sleep opportunity in the laboratory or at home. Transportation was provided if participants opted to complete recovery sleep opportunity at home. Recovery sleep compliance was ensured using actigraphy, sleep logs and call-ins to a time stamped recorder.

We studied participants during a simulated night shift protocol with and without the TRE intervention (Figure A.1, detailed inpatient protocol schematic). In one condition, during the participants consumed energy intake during the night as typically done during shift work, at +1, +5, +9, +12, and +16 hour since wake. In the other

condition, nighttime energy intake was eliminated, concentrating all food intake during the daytime hours, while maintaining the same sleep opportunity and diet. Energy intake during TRE was consumed at +1, +2, +4, and +16 hours since wake.

Blood pressure and heart rate monitoring

BP and HR assessments were collected over a 26h period during simulated night shift work in each condition. BP and HR were measured every 30 minutes using an ambulatory OnTrak 90227 monitor (Spacelabs™, Snoqualmie, WA). Data collection started immediately upon waking from daytime sleep on study day 4 and ended 2 hours after waking on study day 5. Participants were instructed to remain still during ambulatory BP measurements to reduce monitor errors and ensure accurate readings. If an error message occurred, the monitor repeated the measurement approximately 2 minutes later. Participants were advised to discontinue the use of the ambulatory BP monitor if the cuff induced discomfort to the extent that it hindered their ability to sleep. To minimize discomfort, the monitor automatically inflated to 130 mmHg; however, if SBP was not detected, the cuff automatically increased pressure in increments of 30 mmHg until SBP was detected. Participants were blinded to the ambulatory BP results during the study.

Blood pressure and heart rate parameters

Twenty-four-hour BP was defined as the mean BP during the entire collection period. Wake and sleep BP were defined as the mean BP during wake and sleep opportunities as defined by the protocol. Wake BP and HR included hours since wake 0-16.5 whereas sleep BP included hours since wake 16.5-24. The BP dipping during

sleep was calculated as the percent change between wake BP and sleep BP as previously described (15).

Statistical analyses

Statistical analyses were conducted using SAS (version 9.4; Cary, NC) with a significance threshold of $\alpha = 0.05$. To examine differences between SBP, DBP, and HR during the 24h period, wake periods, and sleep periods, mixed-effect linear models with a random intercept for participant ID were used. The differences between BP dipping were examined between simulated night shift work + control eating and simulated night shift work + TRE using a mixed-effects linear model.

Time since wake inpatient was determined based on average sleep/wake times prior to admission into the laboratory. BP and HR were aggregated into 30-minute intervals based on time since wake. Mixed-effect linear models with a condition by time (time since wake) interaction were used to examine whether BP and HR were different over the 24h period between night shift and day shift conditions. SBP, DBP, and HR were dependent variables. Results are presented as least-squares mean±standard error of the mean (SEM), except for baseline characteristic data, which are displayed as raw mean±standard deviation (SD).

RESULTS

Participants

In this ongoing study, eighteen participants completed at least one condition of the study (24±5y, BMI: 22.5±2.3kg/m^2; mean±SD; Table A.1). Of those 18, 56% were female (n=10), 5% Asian (n=1), 5% American Indian (n=1), 5% Black/African American

(n=1), 72% White (n=13), 5% two or more races (n=1), and 11% unknown race (n=2). Fourteen participants completed both conditions. In this randomized crossover study, 9 participants completed simulated night shift work with control eating first whereas 9 participants completed simulated night shift work with TRE first. The ambulatory device was worn in subset of 11 participants.

Circadian misalignment and systolic and diastolic blood pressure

TRE during simulated night shift work was associated with significantly lower mean 24h SBP (TRE: 104±2 v control eating: 107±2 mmHg; p=0.0003) and DBP (TRE: 65±1 v control eating: 66±1 mmHg; p=0.0448) compared to habitual eating. Mean wake SBP (TRE: 106±2 v control eating: 110±2 mmHg; p<0.0001) and DBP (TRE: 68±2 v control eating: 69±1 mmHg; p=0.0092) were lower during simulated night shift work with TRE as compared to control eating. However, there were no differences in either mean sleep BP or sleep BP dipping between conditions (Table A.2). SBP and HR profiles were not different between conditions (Figure A.2a and A.2b).

Circadian misalignment and heart rate

TRE during simulated shift work was associated with significantly higher mean 24h HR (TRE: 74±3 v control eating: 73±3 mmHg; p=0.0416) compared to habitual eating. Mean wake HR (TRE: 76±3 v control eating: 74±3 mmHg; p=0.0254) was higher during simulated night shift work with TRE as compared to control eating. However, there were no differences in mean sleep HR between conditions (Table A.2). Finally, HR profiles were not different between conditions (Figure A.2c).

DISCUSSION

The purpose of this study is to examine the impact of isocaloric TRE during simulated night shift work on CVD risk factors in healthy, lean participants with no previous shift work history. In this ongoing study, our findings demonstrate that TRE is associated with a decrease in mean 24h SBP and DBP, as well as wake SBP and DBP, as compared to control eating. These findings are novel because they are the first to suggest that TRE may be an effective countermeasure to the CV impairments associated with night shift work in a homogenous and healthy population.

In our study, compared to control eating, TRE during simulated night shift work is associated with a 3 and 1 mmHg decrease in mean 24h SBP and DBP, respectively. To our knowledge, 24h BP assessments during a TRE intervention have not been previously conducted, nor has research explored the impact of TRE during simulated night shift work on CV parameters. Previous studies investigating TRE in non-shift workers have reported similar reductions in BP (16, 17). Reductions in BP are particularly important for shift workers who have a higher rate of elevated BP (18) and subsequent CVD risk (2–5) compared to day shift workers. Elevated BP is one of the most modifiable risk factors for CVD. In fact, data from previous studies have shown that lowering SBP by 10 mmHg is associated with a 20% decrease in the risk of major cardiovascular events (19). Thus, these preliminary findings suggest that TRE may be a clinically meaningful strategy to mitigate the CV impairments associated with shift work.

In our study, BP exhibits a normal dipping pattern (defined as a 10-20% reduction from pre-sleep to sleeping BP) under simulated night shift work conditions. Data from previous studies have demonstrated that night shift work in free-living settings is associated with blunted BP dipping (defined as <10% reduction from pre-sleep to sleeping BP). In a free-living study conducted in emergency medical services

workers, participants demonstrated blunted BP dipping during a night shift as compared to a non-work day (20). However, in a subset of participants in this study who napped for >60 min during the night shift, BP dipping was in the normal range of 10-20% (20), suggesting that insufficient sleep is a likely mechanism underpinning blunted BP dipping in night shift workers. Insufficient sleep (<7h/night) is common amongst night shift workers. In a study that examined medical residents, participants reported sleeping 3h less on night shifts compared to rest days (21). Similarly, a study conducted in rotating shift workers compared sleep duration and quality using polysomnography following a night shift compared to an afternoon shift and found that participants slept less and had significant reductions in Stage 2 and REM sleep compared to sleep following an afternoon shift (22). In contrast, in our study, participants were provided with an adequate sleep opportunity (7.5 hours) in quiet, dark environments. It is possible that in our study, sleep duration and quality were protected. Insufficient duration and poor sleep quality during night shift work may be key mechanisms driving blunted BP dipping; therefore, strategies to provide adequate sleep duration and quality in a shift work population should be explored.

Our study reveals a modest increase in 24h HR compared to control eating. This contrasts with previous findings that show TRE is not associated with changes in resting HR. A 12 week TRE regimen in people with obesity did not change resting HR compared to a control group (23). The modest increase in HR during TRE in our study may be influenced by the relatively small sample size. A larger sample size may be needed to better understand the HR response to TRE under simulated night shift work.

Limitations

This study has several limitations. First, our study analyses include only 11

participants with the ambulatory device. Although our sample size is small, data is ongoing. We expect to have 25 participants complete this randomized crossover study with the ambulatory device. Second, although acute studies conducted in inpatient settings have the advantage of controlling for many potential confounding variables, they may not reflect real-world adaptations to TRE. Thus, future research is needed to determine the impact in a free-living setting. Third, our findings may not be generalizable to night shift workers due to our healthy, homogenous participants. Participants in this study were younger, and likely healthier than most shift work populations (24). Fourth, ambulatory devices may cause discomfort during sleep, potentially leading to sleep disruptions (25).

Future Directions

Approximately 25% of the United States workforce engages in shift work and experiences a higher risk of CVD compared to those who work during the day. Our ongoing study presents promising evidence that TRE may be an effective strategy to reduce the cardiovascular risk associated with night shift work. Nonetheless, further research is necessary to explore the impact of TRE on cardiovascular health within a free-living shift work population. This study involved healthy, lean participants who were not shift workers. In contrast, permanent night shift workers often do not fit this profile and tend to have multiple CV risk factors. It is essential for future studies to examine this demographic to develop strategies that mitigate the negative CV effects associated with night shift work. Future research should include a more diverse group of chronic night shift workers, representing a variety of ages, body compositions, and health conditions, to ensure that findings are applicable to the broader shift work population.

TABLES

Baseline participant characteristics	
n	18
Female	10
Age, y	24 ± 5
Weight, kg	64.1 ± 11.7
Height, cm	167.8 ± 8.0
BMI, kg/m^2	22.5 ± 2.3
Body fat, %	28.0 ± 7.3

Table A.1. Data are presented as mean ± SD. BMI, body mass index.

	Simulated shift work + control eating	**Simulated shift work +TRE**	**P value**
24h SBP, mmHg	107 ± 2	104 ± 2	**0.0003***
24h DBP, mmHg	66 ± 1	65 ± 1	**0.0448**
24h HR, mmHg	73 ± 3	74 ± 3	**0.0416***
Wake SBP, mmHg	110 ± 2	106 ± 2	**<0.0001***
Wake DBP, mmHg	69 ± 1	68 ± 2	**0.0092***
Wake HR, mmHg	74 ± 3	76 ± 3	**0.0254***
Sleep SBP, mmHg	97 ± 2	98 ± 2	0.5295
Sleep DBP, mmHg	57 ± 2	57 ± 2	0.7983
Sleep HR, mmHg	67 ± 3	68 ± 3	0.5291
SBP dip, %	10 ± 2	7 ± 2	0.3114
DBP dip, %	18 ± 2	16 ± 2	0.4728

Table A.2. Data are presented as least square mean ± SEM. Least square means were analyzed by a linear mixed model. * P value ≤ 0.05. Systolic blood pressure (SBP); diastolic blood pressure (DBP); Heart rate (HR); hour (h); beats per minute
(bpm).

FIGURES

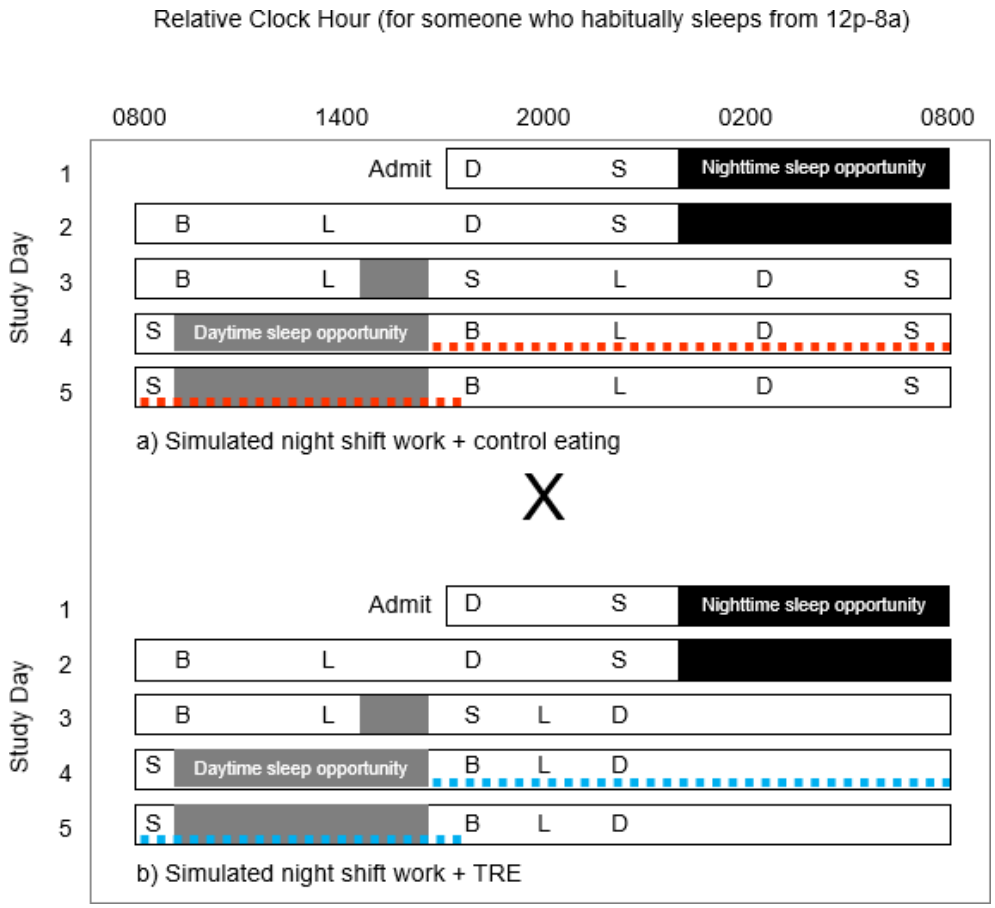

Figure A.1. Detailed inpatient protocol schematic. Simulated night shift work (Control eating; top panel) and simulated night shift work with time-restricted eating (TRE; bottom panel). B, L, D, S: breakfast, lunch, dinner, snack. Orange dotted line indicates 24h BP and HR during simulated night shift work with control eating on Days 5 & 6; Blue dotted line indicates 24h BP and HR during simulated night shift work with TRE; Black boxes indicate sleep opportunities during simulated day shift work; Gray boxes indicate sleep opportunities during simulated night shift work.

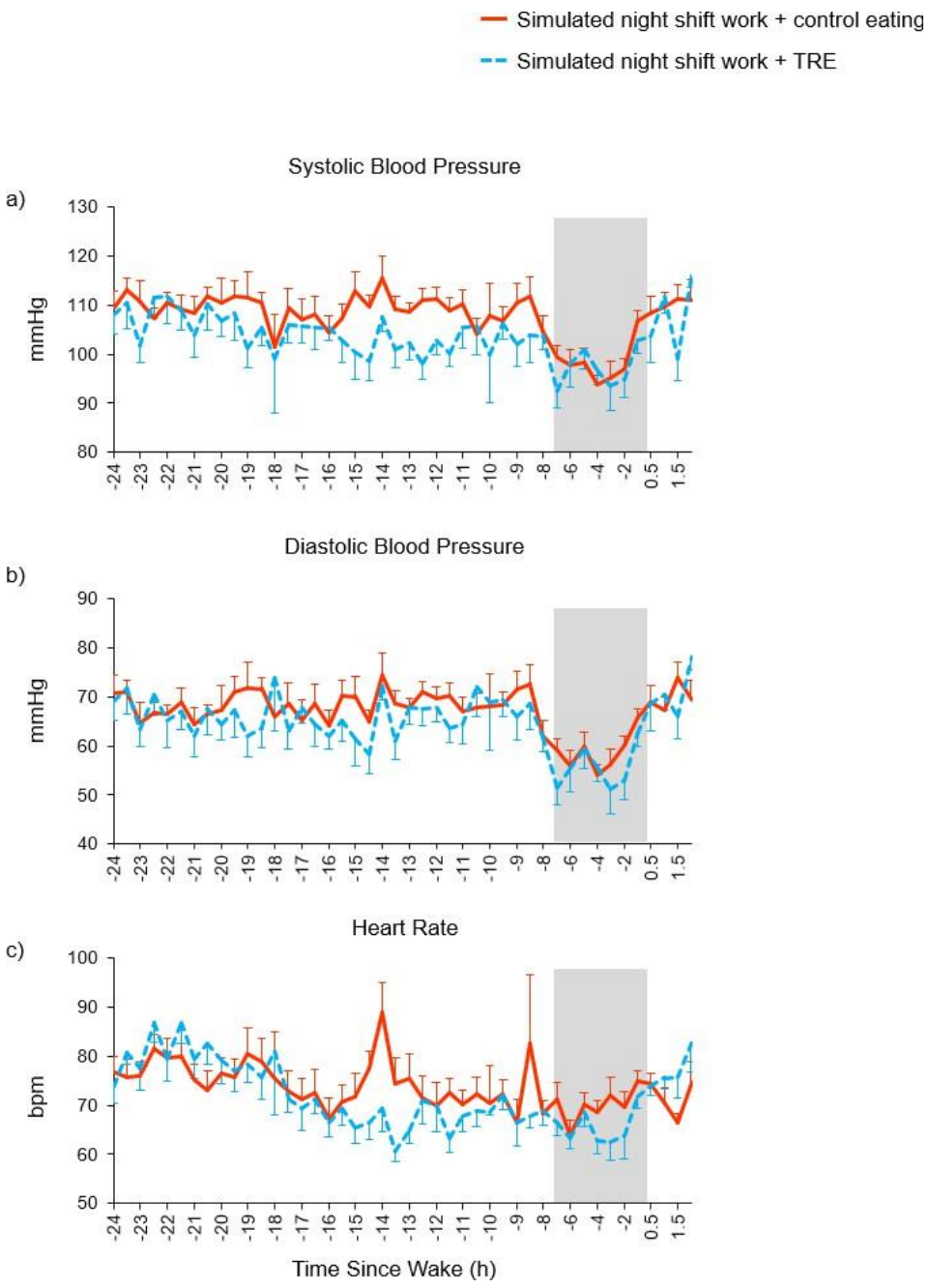

Figure A.2. Systolic blood pressure (top; A), diastolic blood pressure (middle; B) and heart rate (bottom; C) readings assessed every 30 minutes during each condition and analyzed by mixed-effects model. Gray box indicates sleep opportunity during simulated night shift work. Data are presented as least square means ± SEM. **P* value < 0.05.

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