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The Impact of Sleep Quality Combined with Physical Activity on Autonomic Function (24-hour HRV) in College Students

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Abstract

Long-term poor sleep quality is associated with health outcomes that can lead to autonomic nervous system dysfunction. Both cardiorespiratory fitness and physical activity (PA) are associated with improvements in autonomic function and heart rate variability (HRV). Markers of fitness and sleep were explored to determine the degree of contribution to autonomic dysfunction. A total of 15 subjects, (age = 20.6±0.5; BMI = 23.9±1.0) completed 7 days of physical activity and sleep assessment via accelerometry. Participants then completed a graded exercise test to determine cardiorespiratory fitness (VO_{2max}), followed by a 24-H HRV measurement. HRV through LF:HF ratio was negatively associated with moderate physical activity (MPA) (4.6% ± 0.7), percent in MPA, total moderate to vigorous physical activity (MVPA), and percentage spent in MVPA (2.6 ± 0.4 vs. 4.1 ± 1.7, p = 0.04). Students that obtained less than 7 hours of sleep tended to participate in lighter intensities of physical activity (333.6 ± 71.1 vs. 261.5 ± 68.3). Individuals that obtained more than 7 hours of sleep spent more time in vigorous exercise (3.2 ± 3.1 vs. 6.7 ± 4.5). Having a lower HRV status is associated with less time participating in MVPA. This demonstrates the importance of MVPA written for the general population and should be applied to college students, especially those that may lack proper sleep.

Chapter 1

Introduction

Summary of the Literature

Sleep is an inherent necessity of human life and has a significant impact on physiological function. Sleep is believed to be a restorative period that influences immune, endocrine, and central nervous systems as well as aspects of metabolism (Luyster et al., 2012). Despite recommendations that adults should acquire between 7–9 hours of sleep per day, much of the college population falls short of these guidelines (Hirshkowitz et al., 2015). According to Lund et al, over 60% of college students are poor-quality sleepers as assessed by the Pittsburgh Sleep Quality Index (PSQI) (Lund et al., 2010). This may be associated with deficits in attention and academic performance, drowsy driving, risk-taking behavior, and depression, impaired social relationships, and poorer health, all commonly found within this population (Gaultney et al., 2010). Hargens et al. studied the relationship between sleep quality, physical activity, and sedentary behavior in college students to assess the relationship between poor sleep and physical activity (Hargens et al., 2021). It was concluded that poor sleep was associated with increased sedentary behavior the following day. When compared to weekdays, there was a "sleep compensation" effect of sleeping more and moving less on the weekend (Hargens et al., 2021). It is also important to recognize that those with a total sleep time (TST) less than 6 hours were found to have greater sedentary bouts and minutes per day, postulating that poor sleep hinders physical activity.

There is growing evidence that poor sleep quality is both a common precursor for and consequence of poor health. Not only can long-term poor sleep quality be associated with insulin

resistance and weight gain, which are both precursors to type 2 diabetes (Luyster et al., 2012), it can also be caused by risk factors associated with metabolic disease. For example, Beydoun et al. identified smoking, physical inactivity, diet, and obesity to be predictors of short sleep duration (Beydoun et al., 2017). Conversely, VanHelder et al. found that sleep deprivation of 30 to 72 hours did not have an effect on cardiovascular and respiratory responses to exercise, or on aerobic and anaerobic capacity (Vanhelder., 1989). However, it is important to note that this was investigated in military trained individuals taught to withstand high amounts of stress and tactical training. When considering the general population, these effects may have had a larger effect size. Second, ratings of perceived exertion (RPE) increased during exercise in those that were sleep-deprived, although this study did not conclude that RPE was a strong enough indicator.

Sleep can be broken up into two distinct cycles of sleep, NREM (non-rapid eye movement) and REM (rapid eye movement). NREM consists of three stages, N1, N2, and N3, with N3 characterized as restorative deep sleep. The restorative nature of N3 is due to marked reductions of sympathetic activity which causes a reduction in heart rate and blood pressure, creating stable breathing. Thus, persons with low amounts of N3 are found to have an increased risk for cardiovascular disease (CVD) (VanHelder et al., 1989). As previously established, hypertension is a key contributor to CVD and a risk factor for autonomic dysfunction. Sharafkhaneh et al found that 60% of veterans with obstructive sleep apnea had diagnosed hypertension, concluding that poor sleep quality can elevate blood pressure (Sharafkhaneh et al., 2004). Furthermore, obstructive sleep apnea was present in at least 30% of adults with hypertension.

Subsequently, poor sleep quality has also been associated with a decrease in autonomic function. The autonomic nervous system is a key player in the peripheral nervous system that

regulates involuntary physiological processes such as heart rate and blood pressure (Waxenbaum et al., 2022). Meisinger et al. found a higher incidence of heart attack among women with short sleep duration and difficulty falling asleep (Meisinger et al., 2007). In men, Mallon et al. found an increase of coronary heart disease in men who had difficulty sleeping (Mallon et al., 2002). Both findings can be correlated with short sleep duration, low sleep efficiency, and insomnia causing lower levels of cardiac parasympathetic tone and/or higher levels of sympathetic tone (Castro-Diehl et al., 2016).

A common method to assess autonomic function is through heart rate variability (HRV), defined as the amount of time between heartbeats (variability of R-R intervals). RR interval variability is useful for assessing risk of cardiovascular failure and is characterized as the distance from one R wave of a single cardiac cycle to the R wave of the next cardiac cycle (Kleiger et al., 2005). HRV is a property of interdependent regulatory systems, which means they operate on different time scales in order to adapt to environmental and psychological challenges. HRV reflects the regulation of autonomic balance, blood pressure (BP), gas exchange, and gut, heart, and vascular tone (Shaffer et al., 2017). A high measurement of HRV indicates that an individual is efficient at adapting to micro changes in the environment. However, if an individual presents a low HRV, this may be a marker of poor health status (Shaffer et al., 2017). HRV is an important variable to consider when examining cardiorespiratory function during exercise. While it is known that autonomic dysfunction is a marker of poor health, it is unclear how a person's cardiorespiratory fitness, physical activity habits, and sleep quality impact autonomic function (Goldberger et al., 2019).

When measuring HRV, it is important to point out technique and duration. Currently, the gold standard technique is 24-h HRV due to the utilization of time-domain, frequency-domain,

and non-linear measurements. Time-domain measurements quantify the amount of variability in measurements of the interbeat interval; frequency-domain measurements estimate the distribution of absolute or relative power into four frequency bands; and non-linear measurements quantify the unpredictability of a time series (Shaffer et al., 2017). A primary measurement, the root mean square of successive RR interval difference (RMSSD), reflects the beat-to-beat variance in HR (Shaffer et al., 2017). Due to its primary role in time-domain measurement to estimate vagally mediated changes in HRV, Twenty-four-hour RMSSD measurements are strongly correlated with high statistical power (Shaffer et al., 2017). Additionally, the standard deviation of NN intervals (SDNN) is a predictor of both SNS and PNS activity.

Researchers have also proposed other HRV strategies such as an ultra-short HRV measurement. Ultra-short HRV refers to analysis lasting five minutes or less. However, HRV measurement can span from 5 minutes to 24 hours. 24h HRV is classified as long term, 5 min is considered short-term, and less than 5 min is classified as ultra-short-term (Shaffer et al., 2017). Contrary to 24-H HRV measurement, the reliability and validity of ultra-short HRV analysis has yet to be investigated (Pecchia et al., 2018). Further, it is known that some HRV features lose statistical significance if analyzed during an ultra-short period (Heart Rate Variability, 1996). This is due to the fact that it has been established that spectral analyses need to last at least 10 minutes more than the slower significant signal oscillation period. However, ultra-short term HRV analysis has slower significant oscillations periods of 25s, thus presenting a concern for the validity of the result. Finally, SDNN is more accurate when calculated over 24 h and has been considered the “gold standard” for calculating cardiac risk when completed for 24h (Shaffer et al., 2017). Longer recording provides data about cardiac reactions to a greater range of

environmental stimulation. In this investigation, 24-H HRV features will be used to ensure a full capture of autonomic function that will minimize erroneous results for the reasons stated.

To our knowledge, most of the current literature gathers exercise/fitness data via questionnaire or brief interview when assessing the effects of exercise on sleep. We seek to explore the relationship between markers of fitness (cardiorespiratory fitness and physical activity), and sedentary behavior (sedentary time) to determine the degree to which these contribute to autonomic dysfunction. To control for confounding variables, our study will assess VO_{2max}, sedentary data and sleep data with 7-day accelerometer data, and 24-hour HRV data with two validated devices.

Purpose

The purpose of this proposed study is to examine how autonomic function through heart rate variability is manifested in college students. We would like to explore the relationship between these factors and a 24-H measurement, which have not been previously explored in this population, and how they impact autonomic function. We aim to identify the extent of sleep quality and physical activity habits on a potential mechanism linking autonomic function and MVPA requirements. We predict that participating in cardiorespiratory fitness and physical activity will increase heart rate variability in college students.

Assumptions

One way to identify early continuous autonomic fitness is through the use of heart rate variability (HRV), which is a non-invasive way to assess autonomic nervous system function through the analysis of beat-to-beat variation in heart rate. Previous research has already identified alterations in HRV with Type 2 diabetes, suggesting CAN. We know that those who

are physically active and have higher cardiorespiratory fitness tend to have better HRV profiles. Additionally, we know that poor sleep quality negatively impacts HRV (Serhiyenko et al., 2018).

Limitations

This study will be utilizing the Wellue Health electrocardiography device. The Wellue Health electrocardiography device is an FDA approved and cleared device for use in 24-hour monitoring of heart rate and rhythm that has recently been introduced in the lab. The precision of measurement may be limited by the reliability and validity of the Wellue device due to the absence of pre-established accuracy trials.

Delimitations

The findings of this study may be limited to the college population and may not be applicable to other populations with various comorbidities.

Definition of Terms

Heart rate variability (HRV) is the variation of the time between two heartbeats. Morning chronotype (MC) is characterized as being most productive from 7:00 and 9:00 am. Evening chronotype (EC) is characterized as being productive from (EC) 6:00 - 8:00 pm. Intermediate chronotype (IC) will consist of individuals that are productive between 12:00 and 2:00 pm.

Chapter 2

Methodology

Selection of Sample

Following IRB approval, we will recruit 10 - 20 subjects (male and female) from the James Madison University (JMU) campus and surrounding communities. To be eligible for study participation, the subjects must meet the following criteria: 1. No identified sleep disorder, as determined by the Pittsburgh Sleep Quality Index Questionnaire (PSQI) (Buysse et al., 1989); 2. 18 - 65 years old; 3. Subjects are willing to give informed consent, and to understand, participate and comply with the study requirements 4. Do not currently smoke tobacco or e-cigarette products; 5. Cleared to participate in moderate-to-vigorous exercise using criteria from the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription, and completion of the Physical Activity Readiness Plus Questionnaire (PAR Q+) (American College of Sports Medicine, 2021); 6. Are not limited to perform maximal exercise by orthopedic limitations; 7. Have received both doses of COVID-19 vaccination. If subjects do not wish to disclose their vaccination status, this will serve as exclusionary criteria; 8. Do not have a diagnosed cardiovascular or pulmonary disease. We seek to recruit students currently enrolled at James Madison University. Before testing is initiated, subjects will be given consent forms to read and sign that provide a comprehensive description of the study, the risks and benefits associated with the study, and the ways in which confidentiality will be maintained.

Preliminary Testing

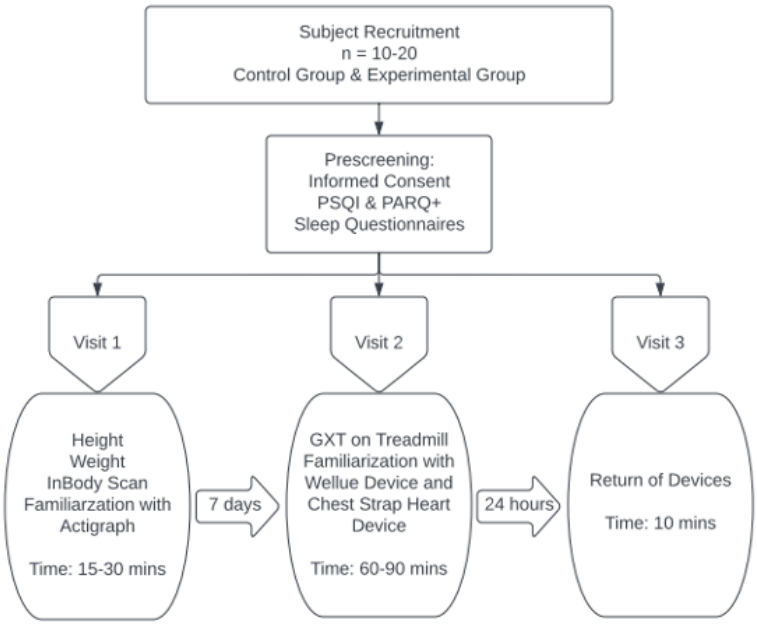
Following informed consent and approval for physical activity, subjects will complete the Sleep Chronotype Questionnaire (Horne & Ostberg, 1976), a self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms) and the Epworth Sleepiness

Scale questionnaire (Johns, 1991). The Epworth Sleepiness scale assesses level of daytime sleepiness.

Sleep chronotype describes whether a person identifies more as a "morning person", an "evening person", or an "intermediate”. Sleep chronotype is an individualized trait that varies between individuals. This is a genetically influenced factor, independent of environmental influences, and can correspond to an individual's circadian rhythm (Di Somma et al., 2021). To maximize validity of VO2max results, sleep chronotype will be utilized to present an opportunity for individuals to exercise during their peak hours of the day.

Once sleep chronotype is determined from the questionnaires, participants will be scheduled for their first lab visit. A graded exercise test (GXT) will be conducted at a time of day that coincides with their sleep chronotype. For morning subjects, tests will take place between 7:00 and 9:00 am, for evening subjects 6:00 - 8:00 pm, and for intermediate, tests will take place between 12:00 and 2:00 pm.

Study Design



Experimental Trials

Participants will be asked to report to the Integrative Nutrition and Physiology Lab (INAP) and/or the Human Performance Lab (HPL) a total of 3 times. All measures will be taken or overseen by these research staff members and will take place in the INAP and/or HPL.
At the arrival of the first visit, measurement of body weight and height, and body composition will be taken. Body weight will be measured using a physician's scale and recorded to the nearest 0.1 kg. Height will be measured using a wall stabilized stadiometer and recorded to the nearest 0.5 cm. Body composition (% body fat, lean body mass, and fat mass) will be assessed via multi-frequency bioelectrical impedance (Model 770, InBodyUSA, Seoul, Korea). Following completion of anthropometric measures, subjects will be instructed on the proper wear and use of the accelerometer (Model wGT3X-BT, ActigraphCorp, Pensacola, FL, US), which they will wear for 7 consecutive days. They will be instructed to wear the device on their right hip during all waking hours (excluding any water-based activities), and on their non-dominant wrist at night. They will be asked to document their time in bed and time out of bed each day, which will be accomplished through an online survey that they can input at the precise times.

Following 7 days of physical activity and sleep assessment, subjects will report to INAP, for their second visit to complete a GXT on a Cosmed Quark Cardiopulmonary Exercise Test Treadmill (QuarkCPET, Cosmed, Rome, Italy). The GXTs will utilize a Bruce Ramp Protocol (Kaminsky & Whaley, 1998). Briefly, this protocol begins at a slow walking pace, with small incremental increases in speed and/or grade every 60 seconds. Workloads will be increased until subjects voluntarily request to stop due to volitional fatigue or are unable to continue at the speed/grade. Oxygen uptake (VO_2), heart rate, and blood pressure will be assessed throughout the GXT.

Heart Rate Variability

Following completion of the GXT, subjects were instructed on the proper wear and use of a device that measured heart rate, electrocardiography, and HRV. The device was worn via chest strap (Wellue Health AI-ECG Analysis, ER1, Wellue, Kentucky, US) and automatically turned one once a heart rate was detected. Each subject was asked to wear the device for 24 hours continuously, going about their normal daily routine. Four primary heart rate variability measurements will be utilized to assess autonomic function: root mean square of successive differences between normal heartbeats (RMSSD), the proportion of time successive heartbeat intervals exceed 50 (pNN50), the logarithm of high-frequency HRV (LogHF), and low frequency to high frequency ratio (LF:HF ratio). Both RMSSD and pNN50 are time-domain measurements, meaning that they quantify the amount of HRV observed during a particular period, in this case, 24 hours. Specifically, RMSSD reflects the beat-to-beat variance in HR and when measured over a 24-H period, it is strongly correlated with pNN50 (Shaffer et al., 2017). pNN50 is a calculation based on differences between successive N-N intervals represents high frequency heart rate oscillations. Conversely, LogHF and LF:HF ratio are frequency-domain measures. LF:HF ratio estimates the balance between the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). LogHF expresses high-frequency band power but can be expressed as the natural logarithm of original units to achieve a more normal distribution. LogHF estimates vagal modulation of heart rate, with the majority of the contribution coming from the PNS (Shaffer et al., 2017).

Statistical Analysis

Relationships through Spearman Rho correlations were utilized to compare physical activity habits, total sleep time, sleep efficient, and 24-H HRV to one another. To compare sleep

with 24-H HRV and sleep with physical activity data, median split T-Tests were completed in SPSS. A p-value of .05 was used to establish statistical significance.

Chapter 3

Manuscript

The Impact of Sleep Quality Combined with Physical Activity on Autonomic Function (24-hour HRV) in College Students

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Abstract

Introduction: Long-term poor sleep quality is associated with health outcomes that can lead to autonomic nervous system dysfunction. Both cardiorespiratory fitness and physical activity (PA) are associated with improvements in autonomic function and heart rate variability (HRV). However, current literature assesses PA via questionnaire and measures HRV for shorter durations, which limits the validity and reliability of these measure. The purpose of this study was to explore the relationship between markers of fitness and sleep to determine the degree of contribution to autonomic dysfunction.

Methods: A total of 15 subjects, (age = 20.6±0.5; BMI = 23.9±1.0) completed 7 days of physical activity and sleep assessment via accelerometry. Participants then completed a graded exercise test to determine cardiorespiratory fitness (VO_{2max}), followed by a 24-H HRV measurement.

Results: HRV through LF:HF ratio was negatively associated with moderate physical activity (MPA) (4.6% ± 0.7), percent in MPA, total moderate to vigorous physical activity (MVPA), and percentage spent in MVPA (2.6 ± 0.4 vs. 4.1 ± 1.7, p = 0.04). Students that obtained less than 7 hours of sleep tended to participate in lighter intensities of physical activity (333.6 ± 71.1 vs. 261.5 ± 68.3). Individuals that obtained more than 7 hours of sleep spent more time in vigorous exercise (3.2 ± 3.1 vs. 6.7 ± 4.5).

Conclusions: Having a lower HRV status is associated with less time participating in MVPA. This demonstrates the importance of MVPA written for the general population and should be applied to college students, especially those that may lack proper sleep.

Introduction

Sleep is a necessity of human life and has a significant impact on physiological function due to its influences on the immune, endocrine, and central nervous systems (Luyster et al., 2012). Despite recommendations that adults acquire between 7–9 hours of sleep per day, much of the college population falls short of these guidelines (Hirshkowitz et al., 2015). According to Lund et al, over 60% of college students are poor-quality sleepers as assessed by the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 2010). Not only is long-term poor sleep quality associated with insulin resistance and weight gain, it can also be caused by risk factors associated with metabolic disease (Luyster et al., 2012) and vice versa. For example, Beydoun et al. identified smoking, physical inactivity, diet, and obesity to be predictors of short sleep duration (Beydoun et al., 2017). In 2021, Hargens et al. studied the relationship between sleep quality, physical activity, and sedentary behavior in college students (Hargens et al., 2021). Poor sleep quality was associated with increased sedentary time and subjects with a total sleep time (TST) less than six hours had a greater number of sedentary bouts and sedentary minutes the following day (Hargens et al., 2021).

Subsequently, poor sleep quality has also been associated with autonomic imbalance (Bonnet & Arand, 1998). The autonomic nervous system is a key player in the peripheral nervous system that regulates involuntary physiological processes such as heart rate and blood pressure (Waxenbaum et al., 2022). Meisinger et al. found a higher incidence of heart attacks among women with short sleep duration and difficulty falling asleep (Meisinger et al., 2007). In men, Mallon et al. found an increase of coronary heart disease in men who had difficulty sleeping (Mallon et al., 2002). In support of both findings, short sleep duration, low sleep

efficiency, and insomnia was associated with lower levels of cardiac parasympathetic tone and/or higher levels of sympathetic tone (Castro-Diehl et al., 2016). A common method to assess autonomic function is through heart rate variability (HRV), defined as the amount of time between heartbeats (variability of R-R intervals). HRV reflects the regulation of autonomic balance, blood pressure (BP), gas exchange, and gut, heart, and vascular tone (Shaffer et al., 2017). A high measurement of HRV indicates that an individual is efficient at adapting to micro-changes in the environment. However, if an individual presents a low HRV, this may be a marker of poor health status (Shaffer et al., 2017). While it is known that autonomic dysfunction is a marker of poor health, it is unclear how a person's cardiorespiratory fitness, physical activity habits, and sleep quality impact autonomic function (Goldberger et al., 2019).

In this population, most autonomic function data is gathered via ultra-short HRV (<5 minutes) or short-term HRV (~5 minutes). However, the gold standard technique is 24 hour (24-H) HRV due to the utilization of time-domain, frequency-domain, and non-linear measurements. Contrary to 24-H HRV measurement, the reliability and validity of ultra-short HRV analysis has yet to be fully determined (Pecchia et al., 2018), however the available evidence demonstrates that some HRV features lose statistical significance if analyzed during an ultra-short period (Pecchia et al., 2018). This is because spectral analyses need to last at least 10 minutes more than the slower significant signal oscillation period. However, ultra-short term HRV analysis has slower significant oscillations periods of 25s, thus presenting a concern for the validity of the result. Longer recording provides data about cardiac reactions to a greater range of environmental stimulation.

To our knowledge, a majority of current literature in this population gathers exercise/fitness data via questionnaire or brief interview when assessing the effects of exercise

on sleep. Similarly, autonomic function is typically assessed by short or ultra-short-term heart rate variability. Thus, the purpose of this proposed study is to examine how sleep quality impacts autonomic function in college students and the effect that physical activity may have on autonomic function when utilizing gold standard methods to assess each. It is hypothesized that poor sleep quality will worsen HRV, with physical activity and cardiorespiratory fitness attenuating that effect.

Methodology

Subjects

Participants were recruited from the James Madison University (JMU) campus and surrounding communities through bulk email requests to all JMU faculty and students, word of mouth, and flyers. To be eligible for study participation, subjects met the following criteria: 1. No indicative sleep orders, as assessed by the PSQI (Buysse et al., 1989); 2. 18 – 26 years old; 3. Willingness to give informed consent, and to understand, participate and comply with the study requirements 4. Did not currently smoke tobacco or e-cigarette products; 5. Cleared to participate in moderate-to-vigorous exercise using criteria from the American College of Sports Medicine's Guidelines for Exercise Testing and Prescription, and completion of the Physical Activity Readiness Plus Questionnaire (PAR Q+) (American College of Sports Medicine, 2021); 6. Were not restricted to perform maximal exercise by orthopedic limitations; 7. Had received both doses of COVID-19 vaccination. 8. Did not have a diagnosed cardiovascular or pulmonary disease. Before testing was initiated, subjects were given consent forms to read and sign that provide a comprehensive description of the study, the risks and benefits associated with the study, and the ways in which confidentiality will be maintained.

Preliminary Testing

Following informed consent and approval for physical activity and screening for sleep disorders, subjects completed the completed the Epworth Sleepiness Scale questionnaire (Johns, 1991) to assess the level of daytime sleepiness. Once questionnaires were completed, participants were scheduled for their first lab visit.

Experimental Trials

Twenty subjects volunteered for the study (9 male, 11 female). Five subjects were excluded from the data due to improper wear of technology. (2 male, 3 female). Participants were asked to report to the Integrative Nutrition and Physiology Lab (INAP) and/or the Human Performance Lab (HPL) a total of three times.. Body composition (% body fat, lean body mass, and fat mass) was assessed via multi-frequency bioelectrical impedance (Model 770, InBodyUSA, Seoul, Korea). Following completion of anthropometric measures, subjects were instructed on the proper wear and use of the accelerometer (Model wGT3X-BT, ActigraphCorp, Pensacola, FL, US), which they wore for 7 consecutive days. They were instructed to wear the device on their right hip during all waking hours (excluding any water-based activities), and on their non-dominant wrist at night. They were also asked to document their time in bed and time out of bed both day and night right before going to bed and right after waking up, which was accomplished through an online survey.

Following 7 days of physical activity and sleep assessment, subjects reported to INAP, for their second visit to complete a GXT to assess VO2max on a Cosmed Quark Cardiopulmonary Exercise Test Treadmill (QuarkCPET, Cosmed, Rome, Italy). The GXTs utilized a Bruce Ramp Protocol (Kaminsky & Whaley, 1998). Briefly, this protocol began at a slow walking pace, with small incremental increases in speed and/or grade every 60 seconds. Workloads were increased until volitional fatigue. Heart rate was measured with a Polar heart

strap and watch (Polar H10 Heart Rate Sensor, Polar, Kempele, Finland) and manual blood pressure was taken throughout the GXT.

Heart Rate Variability

Following completion of the GXT, subjects were instructed on the proper wear and use of a device that measured heart rate, electrocardiography, and HRV. The device was worn via chest strap (Wellue Health AI-ECG Analysis, ER1, Wellue, Kentucky, US) and automatically turned on once a heart rate was detected. Each subject was asked to wear the device for 24 hours continuously, going about their normal daily routine. The Wellue device measures a variety of HRV variables, including the standard deviation of NN intervals (SDNN), the standard deviation of the average NN intervals for each five minutes (SDANN), root mean square of successive differences between normal heartbeats (RMSSD), low-frequency power (LF Power), high-frequency power (HF Power), low frequency to high frequency ratio (LF:HF ratio), the proportion of time successive heartbeat intervals exceed 50 (pNN50), baseline width of the RR interval (TINN), the standard deviation of RR intervals (SDRR), and Ultra-low-frequency band power. However, four primary heart rate variability measurements were utilized to assess autonomic function: RMSSD, pNN50, the logarithm of HF, LF:HF ratio. Both RMSSD and pNN50 are time-domain measurements, meaning that they quantify the amount of HRV observed during a particular period, in this case, 24 hours. Specifically, RMSSD reflects the beat-to-beat variance in HR and when measured over a 24-H period, it is strongly correlated with pNN50 (Shaffer et al., 2017). pNN50 is a calculation based on differences between successive N-N intervals represents high frequency heart rate oscillations. Conversely, LogHF and LF:HF ratio are frequency-domain measures. LF:HF ratio estimates the balance between the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). LogHF

expresses high-frequency band power but can be expressed as the natural logarithm of original units to achieve a more normal distribution. LogHF estimates vagal modulation of heart rate (Shaffer et al., 2017).

Statistical Analysis

All statistical analyses were conducted using IBM SPSS (version 28.0). Spearman Rho correlations were utilized to explore evaluate relationships between physical activity, sleep quality, and HRV. Independent sample t-tests were conducted to examine mean differences in HRV variables when comparing normal sleepers (total sleep time >7 hours and sleep efficiency ≥85%) to poor sleepers. Additionally, mean differences in physical activity and sleep quality were conducted with independent sample t-test utilizing median values of HRV data, to evaluate whether those with lower HRV differed in their sleep quality or physical activity habits compared to those with higher HRV. A p-value of < 0.05 was used to establish statistical significance.

Results

Statistical analyses were performed on fifteen subjects (7 male, 8 female), with demographics outlined in Table 1. Significant differences between male and female for height (177.2 ± 7.4 vs. 166.3 ± 6.5; $p=0.009$), body fat % (14.2 ± 6.8 vs. 23.1 ± 5.4; $p=0.015$), and vigorous physical activity (2.1 ± 2.2 vs. 7.2 ± 3.9; $p=0.009$) are reported.

Table 2 reflects associations between 24-H Heart Rate Variability (HRV) with physical activity and sleep. Significant correlations were found between number of awakenings ($r = 0.821$), sleep efficiency ($r = -0.676$), total minutes in bed ($r = 0.533$), wake after sleep onset ($r = 0.759$) with RMSSD. Additionally, significant correlations were observed with number of awakenings ($r = 0.681$), efficiency ($r = -0.666$), and wake after sleep onset ($r = 0.673$) with

pNN50. Significant associations were observed between LogHF and number of awakenings (r = 0.763), efficiency (r = -0.713), and wake after sleep onset (r = 0.773). No other significant associations were found between HRV and sleep variables. LF:HF ratio was found to be associated with moderate intensity physical activity minutes per day (r = -0.600), percent time in moderate physical activity (r = -0.636), and total moderate-to-vigorous intensity physical activity (MVPA) (r = -0.634). There were no other significant associations between other markers of sleep quality and HRV.

When correlating sleep and physical activity, sleep efficiency was negatively associated with light (r = -0.596) and moderate (r = -0.571) physical activity (Table 3). Second total sleep time was negatively associated with light physical activity (r = -0.675). Third, wake after sleep onset was positively associated with moderate physical activity (r = 0.561) (Table 3).

Comparisons between heart rate variability and moderate to vigorous physical activity through a median split t-test (Figure 1). Significant findings between MVPA and LF:HF Ratio were observed (3.5 ± 1.4 vs. 1.6 ± 1.0) (p=0.02). No other significant comparisons were observed between variables.

Those with a higher LF:HF ratio (an indication of lower vagal activation and greater autonomic imbalance) spent a significantly smaller percentage of time in MVPA (4.6% ± 0.7) compared to those with greater autonomic balance (7.5% ± 3.0, p = 0.032). Additionally, they accumulated fewer average MVPA minutes per hour (2.6 ± 0.4 vs. 4.1 ± 1.7, p = 0.04).

Unexpectedly, when sleep quality was examined via independent t-test, those with greater HRV reported worse sleep quality in several instances. In those with greater RMSSD, they had longer sleep latency, lower sleep efficiency, lower total minutes in bed (yet no

difference in total sleep time), greater WASO, greater number of awakenings and average length of awakenings, a greater sleep fragmentation index, and a greater movement index (Table 4).

Discussion

Results from this study suggest that, in a sample of highly active college students, with higher-than-average cardiorespiratory fitness, participation in MVPA was associated with a greater autonomic nervous system balance, reflected in improved LF:HF ratio through measurement of HRV. These students are meeting ACSM's physical activity requirements and are below the threshold for risk factors associated with cardiovascular disease (BMI, body fat%, non-sedentary, and age) (American College of Sports Medicine, 2020). This aligns with previous stating that physical activity can help attenuate coronary heart disease (CHD), cardiovascular disease (CVD), and myocardial infarction (MI). Specifically, Routledge et al. concluded that exercise training may improve cardiac autonomic regulation when conducting a meta-analysis, including populations at risk for CVD (Routlegde et al., 2010). LF:HF ratio is a measurement of HRV that represents a balance between the sympathetic nervous system (SNS) and the parasympathetic nervous systems (PNS). A lower LF:HF ratio is indicative of a more dominant parasympathetic nervous system, leading to a healthier HRV score. Gutin et al. found that Higher MVPA was associated with a lower LF:HF ratio (Gutin et al., 2005). This may be attributed to physical activity's influence on autonomic balance. As you begin exercise, the SNS increases HR. whereas the PNS withdraws (Gafni et al., 2022). Upon cessation of exercise, the SNS subsides and the PNS re-engages. This process of alternating between the SNS and PNS results in greater ANS balance because the ANS can become more adaptive to regulating the switch between increasing and decreasing HR. Kiviniemi et al., found this same ANS regulation from physical activity on HRV within a fit and healthy group (Kiviniemi et al., 2007). As it relates to the

current study, our participants were young and physically fit, which may have had a larger effect with MVPA and HRV. With more opportunities for physical activity, this can cause the ANS to adapt to parasympathetic contribution, therefore decreasing LF:HF ratio (Daniela et al., 2022). This finding relays the importance of MVPA on a daily basis to maintain cardiovascular fitness, outlined in ACSM's *Guidelines for Exercise Testing and Prescription* (American College of Sports Medicine, 2020).

By contrast, this study found that less efficient sleep, more awakenings, and more WASO were correlated with a higher heart rate variability (RMSSD, pNN50, and LogHF) (Table 2 & Table 4). This result does not align with previous literature that links poor autonomic function with short sleep duration, low sleep efficiency, and insomnia (Castro-Diehl et al., 2016). HR has been shown to decrease during sleep (more reliance on parasympathetic system), but factors such as stage of sleep, movement, and awakenings can all influence stability of HR (Sajjadieh et al., 2020). One potential mechanism for a high HRV but low sleep efficiency could be due to the stage of sleep. The parasympathetic system is more active during NREM sleep, whereas the sympathetic nervous system (lower HRV) is more active during REM sleep (Sajjadieh et al., 2020). If students are showing a higher HRV and are presenting greater wake after sleep onset and are spending more minutes awake at night after initially falling asleep, this may mean that they are not sleeping long enough to reach REM sleep. With a greater duration spent in NREM, contribution from the parasympathetic nervous system may be a key player to increasing HRV. Similarly, REM sleep has been shown to induce sleep paralysis (Farooq & Anjum, 2022). Sleep paralysis is characterized as the "resumption of consciousness occurring while muscle atonia during REM sleep is maintained" (Farooq & Anjum, 2022). Of the college students sampled, this study showed more awakenings and wake after sleep onset, meaning subjects may have been

"tossing and turning." To identify these occurrences, the Actigraph device detects movements as awakenings. However, since REM sleep is associated with muscle paralysis, this may mean that subjects were not getting into REM sleep. These further postulates that subjects are spending more time in NREM, which may increase HRV despite poor sleep efficiency and quality. The current study was not able to measure sleep stages in these subjects, as that is beyond the capability of the Actigraphs. Sitnick et al., studied the accuracy of sleep awakening detection from the Actigraph vs awakenings recorded via videotape. The study concluded that the Actigraph had poor agreement with video recording for detecting awakenings (Sitnick et al., 2008). This postulates that the Actigraph may be predicting awakenings when they are not occurring. A similar study by Smichenko et al., concluded that TST was reported lower with an Actigraph versus observational measurements (Smichenko et al., 2022).

It is important to revisit the profile of these subjects. As previously mentioned, this was a highly fit group (young, satisfactory body fat% and weight, high VO2max, and high step counts). As related to age, Garavaglia et al., reported that autonomic function (HRV) decreases with age due to parasympathetic regulation decreasing after the age of 50 (well above our subject profile) (Garavaglia et al., 2021). Another association can be made about HRV and VO2max. Hedelin et al., studied the relationship between HRV and VO2max and found that there was a positive association between the two (Hedelin et al., 2001). This was specifically shown with a negative association between LF Power, which is characterized by sympathetic nervous system activity, and VO2 ($r= -0.53$). As it relates to our study, having a higher VO2max may have contributed to higher HRV values, despite the poor sleep. These associations may present the importance of physical fitness on autonomic function. Although this sample may be lacking proper sleep

quality, the profile of these subjects may suggest potential for physical activity to outweigh the detriments sleep may have on HRV.

Another factor to consider is that the device used to measure HRV was the Wellue Health AI-ECG Analysis Device, which is an FDA approved and cleared device for use in 24-hour monitoring of heart rate and rhythm. Few studies, however, have utilized the device. So, there is some question as to the validity of this device. Another factor to point out is that sleep was measured for seven days, whereas HRV was a one-time 24-H measurement. A seven-day Actigraph analysis and seven-day sleep log were recorded to determine sleep quality factors and length to establish a pattern between weekday and weekend sleep habits. However, the 7 day PA measurement through the Actigraph and the 24-H HRV measurement were not simultaneously measured, meaning that the one day of 24-H HRV may not directly represent PA activity. Also, participants were asked to log sleep via a sleep log that asked time and date for both time in bed and wake time. Participants were asked to fill this out each night and day, night being classified as attempting sleep (lights turned off, devices off) and morning being exact time when awakening. There were many occasions where subjects would wait to log bed time hours until the following morning which can increase recall bias. If subjects were not exact with times, this could have caused us to mislabel sleep periods when manually entering it into the software. This could contribute to inaccurate sleep duration. Finally, the accelerometers utilized in this study are not waterproof. Any activities that involve being submerged (swimming, showering, etc.) may have not been recorded, leading to a decrease in recorded physical activity.

In conclusion, most of the findings of our study confirm that: 1) this sample of college students is meeting ACSM's recommendations for physical activity, 2) persons who do not get enough sleep tend to participate in lighter physical activity, 3) MVPA levels were associated

with higher HRV scores, and 4) lower sleep quality was associated with higher HRV. These findings emphasize the importance of MVPA recommendation for college students and may attenuate some of the sleep quality deficiencies observed in the population. However, our finding that poor sleep quality was positively associated with higher HRV scores does not endorse our hypothesis that higher sleep quality tends to be associated with higher HRV. Based on these findings, future research should involve comparison between the Wellue device and the Polar device, involve more 24-H HRV measurement in this population, and should involve an exercise intervention to establish physical activity and HRV norms.

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Table 1. Subject Characteristics by Gender

Descriptive	All Subjects (n=15)	Male (n=7)	Female (n=8)
Age	20.6 ± 2.0	20.7 ± 2.1	20.5 ± 2.0
Height (cm)	171.4 ± 8.7	177.2 ± 7.4	166.3 ± 6.5*
Weight (kg)	70.5 ± 15.0	76.9 ± 17.8	64.9 ± 9.9
BMI	23.9 ± 3.8	24.4 ± 4.9	23.4 ± 2.8
Body fat (%)	18.9 ± 7.4	14.2 ± 6.8	23.1 ± 5.4*
VO_2max (ml/kg/min)	48.4 ± 7.8	53.1 ± 6.6	44.3 ± 6.7
Sedentary (mins)	874.4 ± 108.5	882.5 ± 119.3	867.4 ± 106.0
Light PA (mins)	299.9 ± 76.9	300.8 ± 92.4	299.4 ± 67.2
Moderate PA (mins)	72.5 ± 33.9	72.4 ± 45.0	72.5 ± 23.8
Vigorous PA (mins)	4.8 ± 4.1	2.1 ± 2.2	7.2 ± 3.9*
Total MVPA (mins)	77.5 ± 34.1	74.6 ± 44.8	80.0 ± 24.2
Step Count	11489.3 ± 5120.0	12311.5 ± 7156.7	10769.8 ± 2694.4
SDNN (ms)	166.4 ± 59.6	158.9 ± 20.6	174.0 ± 84.5
RMSSD (ms)	61.2 ± 22.5	65.2 ± 22.8	57.3 ± 23.1
pNN50 (%)	23.9 ± 10.9	23.7 ± 8.5	24.2 ± 13.7
LF:HF ratio	2.6 ± 1.5	3.2 ± 1.8	2.0 ± 0.9
Log LF	7.8 ± 0.7	8.1 ± 0.7	7.5 ± 0.7
Log HF	7.0 ± 0.7	7.2 ± 0.7	6.9 ± 0.8
Sleep Latency	6.9 ± 5.7	5.5 ± 1.5	8.1 ± 7.7
Sleep Efficiency	84.2 ± 5.9	83.8 ± 4.7	84.5 ± 7.1
Total minutes in bed	484.4 ± 41.2	480.7 ± 56.8	488.2 ± 26.0
TST	407.5 ± 37.3	402.8 ± 44.1	411.6 ± 32.8
WASO	70.3 ± 28.4	72.4 ± 26.5	68.5 ± 31.7
Number of awakenings	21.7 ± 6.0	22.1 ± 6.5	21.4 ± 6.0

Average awakening length	3.0 ± 0.8	3.1 ± 0.7	3.0 ± 0.8
Sleep fragmentation index	23.3 ± 7.4	21.5 ± 6.0	24.8 ± 8.5

*p <0.05, compared to males, WASO = wake after sleep onset, TST = total sleep time, PA = physical activity, MVPA = moderate to vigorous physical activity

Table 2. Correlations between Sleep & PA with 24-H HRV

Descriptive	RMSSD		pNN50		LogHF		LF: HF Ratio	
	r	*P*	r	*P*	r	*P*	r	*P*
Number of Awakenings	.811	.001*	.681	.007**	.763	.001**	-.307	.286
Sleep Midpoint	-.362	.203	-.237	.414	-.258	.373	.161	.583
Latency	.433	.122	.395	.163	.502	.067	-.230	.429
Efficiency	-.676	.008*	-.666	.009**	-.713	.004**	.287	.320
TST	.014	.963	-.251	.387	-.101	.731	.116	.693
Total Minutes in Bed	.533	.050*	.240	.408	.445	.111	-.114	.699
WASO	.759	.002*	.673	.008**	.773	.001**	-.311	.279
Light PA (mins)	.007	.980	.108	.713	.016	.955	-.104	.723
Moderate PA (mins)	.263	.364	-.030	.919	-.279	.334	-.600	.023*
Vigorous PA (mins)	-.068	.818	-.055	.853	-.206	.480	-.226	.438
% in Light	-.211	.469	-.090	.760	-.028	.925	.054	.856
% in Moderate	.214	.462	-.065	.826	-.311	.279	-.636	.015*
% in Vigorous	-.097	.742	-.091	.758	-.221	.447	-.229	.432
Total MVPA	.254	.380	-.037	.900	-.310	.281	-.634	.015*

WASO = wake after sleep onset, TST = total sleep time, PA = physical activity, RMSSD = root mean square of successive difference between normal heartbeats, pNN50 = proportion of NN50 divided by total number of NN intervals, LogHF = Logarithm of high-frequency power, LF:HF Ratio = low-frequency to high-frequency ratio

Table 3. Correlations between Sleep & PA

Descriptive	Sedentary		Light		Moderate		Vigorous		MVPA		Step Counts		VO2max	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
Number of Awakenings	.150	.594	.464	.081	.439	.101	-.181	.520	.381	.162	.414	.125	.125	.657
Sleep Midpoint	-.143	.611	-.059	.835	.084	.766	-.241	.388	-.092	.744	-.250	.368	-.038	.894
Latency	-.304	.271	.406	.134	.479	.071	-.070	.805	.354	.195	.114	.685	.189	.499
Efficiency	.214	.443	-.596	.019*	-.571	.026*	.266	.337	-.459	.085	-.354	.196	.114	.685
TST	.114	.685	-.675	.006*	-.225	.420	.341	.213	-.095	.737	-.057	.840	.182	.516
Total Minutes in Bed	.086	.761	.050	.860	.246	.376	.070	.805	.213	.447	.346	.206	.000	1.00
WASO	-.154	.585	.450	.092	.561	.030*	-.270	.331	.452	.091	.379	.164	.025	.930

*p <0.05, median of RMSSD at 29.77ms, WASO = wake after sleep onset, TST = total sleep time

Table 4. *t*-test Results Comparing RMSSD and Sleep

	Below	Above	*t*	*P*
Sleep Midpoint	3:59 ± 1:04	3:53 ± 1:52	0.11	.915
Latency	4.2 ± 1.7	7.0 ± 2.7	-2.37*	.035
Efficiency	89.2 ± 3.1	80.5 ± 3.2	5.20*	<.001
Total Minutes in Bed	460.5 ± 35.1	505.4 ± 39.2	-2.26*	.043
TST	412.4 ± 42.4	406.6 ± 35.9	0.28	.788
WASO	43.9 ± 9.9	91.8 ± 15.4	-6.92*	<.001
Number of Awakenings	16.4 ± 1.8	16.4 ± 1.8	-5.79*	<.001
Average Awakening Length	2.5 ± 0.7	3.4 ± 0.4	-3.06*	.010
Sleep Fragmentation Index	18.9 ± 5.8	25.7 ± 5.5	-2.25*	.044
Movement Index	11.2 ± 3.6	15.1 ± 2.6	-2.28*	.042
Fragmentation Index	11.2 ± 6.2	14.6 ± 8.1	-0.88	.399

*p <0.05, median of RMSSD at 29.77ms, WASO = wake after sleep onset, TST = total sleep time

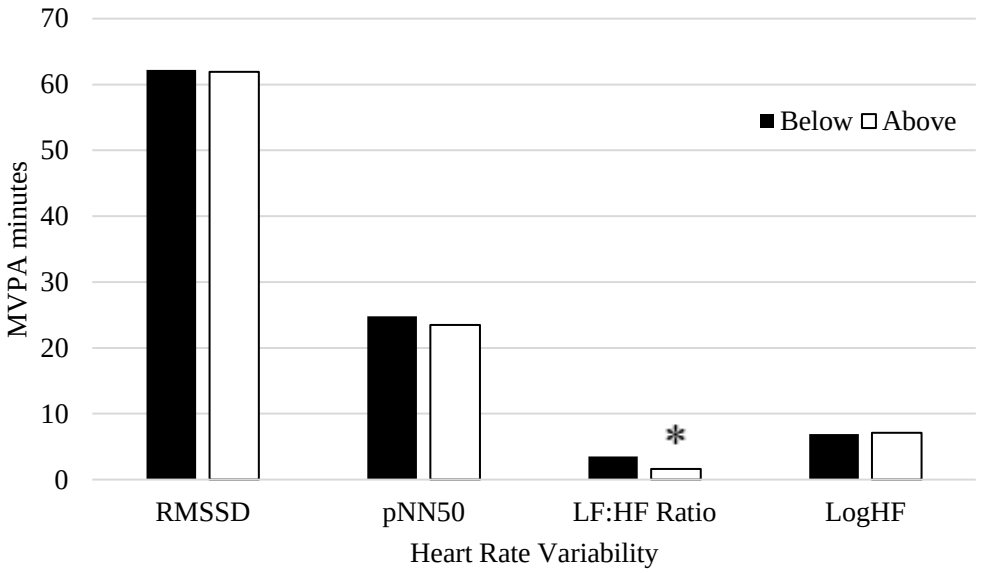

Figure 3. Median split t-test comparing heart rate variability measurements with moderate to vigorous physical activity in college students. Median set at 66.2 minutes

Appendix A. Informed Consent

Purpose: You are being asked to volunteer for a research study conducted by Dr. Trent Hargens from James Madison University entitled, *The Impact of Sleep Quality Combined with Physical Activity on Autonomic Function (24-hour HRV) in College Students*

The goal of this study is to examine the degree to which your cardiorespiratory (or aerobic) fitness, physical activity levels, and sleep quality impacts the function of your autonomic nervous system, which we measure via heart rate monitoring.

You will be asked to visit the Human Performance Laboratory (HPL) in Godwin Hall or the Integrative Nutrition and Physical Activity Laboratory (INAP) in Burruss Hall 3 times over the course of about 2 to 3 weeks. Your total time commitment for participation in this study will be about 3 hours.

Preliminary Testing: Upon completion of this informed consent, you will be asked to complete 4 short questionnaires, 1 that asks about your ability to participate in vigorous exercise, 1 that asks about whether you are a morning person, and evening person or in between, 1 that asks about your current sleep quality, and 1 that asks about your level of daytime sleepiness. Each of these questionnaires should take about 5 minutes to complete.

You will then have your height and weight measured. Upon completion of this, you will have your body composition analyzed via multi-frequency bioelectrical impedance (BIA). BIA will allow us to measure your percent body fat and lean tissue mass. This procedure will take about two minutes to complete. You will stand on the device with minimal clothing and no shoes. You will feel no discomfort associated with this test.

After this, you will complete a maximal treadmill exercise test to determine your maximum oxygen consumption (VO2max). You will be asked to begin walking on a treadmill at a very slow pace. The speed and/or grade will then be increased every 20 to 30 seconds until fatigue is reached, determined by either: 1) your request to stop due to fatigue, or 2) inability to keep up with the speed and grade of the treadmill. Depending on your fitness, you may need to begin jogging/running before the end of the test. You will be verbally encouraged to continue to obtain an accurate measurement of VO2max. Prior to your exercise test, we will check your blood glucose level to make sure you are in an optimal range for vigorous exercise. If you blood glucose is too high or too low, we will postpone this part of the visit until your next visit. To measure oxygen consumption, you will need to breathe through a mouthpiece/breathing apparatus which collects expired air throughout the test (8 – 12 minutes). Prior to your arrival to the HPL that day, you will be asked to refrain from eating for 4 hours prior to your arrival, and to avoid caffeine and alcohol for the same time frame. This session should last about 60 to 90 minutes.

Follow-up Visits: Following this first visit, you will be given instructions on proper wear and use of an accelerometer. An accelerometer is a small device that is to be worn on your waist during all waking hours, except for any water-based activities, and on your non-dominant wrist during sleep. This device will measure the amount of physical activity you do during the day and the quality of your sleep at night. You will be asked to wear this device for 7 straight days and

nights and return the device on the 8th or 9th day. When you return the device, you will then be instructed on how to wear two devices, which you will wear for the next 24 hours straight. The first device will be attached to your upper left chest with 2 small electrodes. The second is a strap that will be worn around the middle of your chest. You will be provided another accelerometer, which will be collecting the data transmitted by the chest strap. You will then go about your normal daily routine and then return these devices 2 days later.

Risks: There are no risks associated with wearing an accelerometer. Also, there are no risks associated with the BIA. The measurement with associated risks are the treadmill exercise tests. There is a risk of abnormal changes during the maximal exercise tests. These changes may include abnormal blood pressure, fainting, heart rhythm disorders, stroke, heart attack, and death. The chance of serious heart problems during maximal exercise among adults is very small (less than 1/10,000 maximal exercise tests). Every effort will be made to minimize risks of an abnormal response by reviewing your health history and providing adequate supervision of the exercise test. All staff are certified by the American Heart Association in BLS (Basic Life Support), and all tests will be supervised by individuals certified by the American College of Sports Medicine.

Benefits: Participation may include knowledge about your health status. Upon request, you will receive information on your cardiovascular fitness and body composition.

Confidentiality: All data and results will be kept confidential. You will be assigned an identification number and a pseudonym in place of your real name. At no time will your name be identified with your individual data. The researcher retains the right to use and publish non-identifiable data. All paper data will be kept secured in a locked cabinet in a locked office. All electronic data will be kept on a password-protected computer in encrypted file folders. Final aggregate results will be made available to participants upon request.

Inquiries: If you have any questions or concerns or you would like to receive a copy of the final aggregate results of this study, please contact Dr. Trent Hargens at hargenta@jmu.edu or (540) 568-5844.

Questions about Your Rights as a Research Subject
Dr. Lindsey Harvell-Bowman
Chair, Institutional Review Board
James Madison University
(540) 568-2611
harve2la@jmu.edu

Freedom of Consent : Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form I requested. I certify that I am

at least 18 years of age. By clicking "Yes" to the question below and submitting this confidential online survey, I am consenting to participate in this research.

Appendix B. PAR-Q+

2017 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

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

GENERAL HEALTH QUESTIONS

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

☑ **If you answered NO to all of the questions above, you are cleared for physical activity.**
Go to Page 4 to sign the PARTICIPANT DECLARATION. You do not need to complete Pages 2 and 3.

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

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

Delay becoming more active if:

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

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FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

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

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

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

2. Do you currently have Cancer of any kind?

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

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

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

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

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

4. Do you have High Blood Pressure?

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

_____

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

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If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active - sign the PARTICIPANT DECLARATION below:

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

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

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

Delay becoming more active if:

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

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

PARTICIPANT DECLARATION

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

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that the Trustee maintains the privacy of the information and does not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact
www.eparmedx.com
Email: eparmedx@gmail.com

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01-01-2017

Appendix C. PSQI

Name:_____ Date: _____

Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. **Please answer all questions.**

1. During the past month, what time have you usually gone to bed at night? _____
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night? _____
3. During the past month, what time have you usually gotten up in the morning? _____
4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.) _____

5. During the past month, how often have you had trouble sleeping because you…	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe:				
6. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
8. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?				
	Very good	Fairly good	Fairly bad	Very bad
9. During the past month, how would you rate your sleep quality overall?				

	No bed partner or room mate	Partner/room mate in other room	Partner in same room but not same bed	Partner in same bed
10. Do you have a bed partner or room mate?				
	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
If you have a room mate or bed partner, ask him/her how often in the past month you have had:				
a. Loud snoring				
b. Long pauses between breaths while asleep				
c. Legs twitching or jerking while you sleep				
d. Episodes of disorientation or confusion during sleep				
e. Other restlessness while you sleep, please describe:				

Appendix D. Morningness-Eveningness Questionnaire

MORNINGNESS-EVENINGNESS QUESTIONNAIRE (MEQ)

Instructions:

- Please read each question very carefully before answering.
- Please answer each question as honestly as possible.
- Answer ALL questions.
- Each question should be answered independently of others. Do NOT go back and check your answers.

1. What time would you get up if you were entirely free to plan your day?

5:00 – 6:30 AM	5
6:30 – 7:45 AM	4
7:45 – 9:45 AM	3
9:45 – 11:00 AM	2
11:00 AM – 12 NOON	1
12 NOON – 5:00 AM	0

2. What time would you go to bed if you were entirely free to plan your evening?

8:00 – 9:00 PM	5
9:00 – 10:15 PM	4
10:15 PM – 12:30 AM	3
12:30 – 1:45 AM	2
1:45 – 3:00 AM	1
3:00 AM – 8:00 PM	0

3. If there is a specific time at which you have to get up in the morning, to what extent do you depend on being woken up by an alarm clock?

Not at all dependent	4
Slightly dependent	3
Fairly dependent	2
Very dependent	1

4. How easy do you find it to get up in the morning (when you are not woken up unexpectedly)?

Not at all easy	1
Not very easy	2
Fairly easy	3
Very easy	4

5. How alert do you feel during the first half hour after you wake up in the morning?

Not at all alert	1
Slightly alert	2
Fairly alert	3
Very alert	4

6. How hungry do you feel during the first half-hour after you wake up in the morning?

Not at all hungry	1
Slightly hungry	2
Fairly hungry	3
Very hungry	4

7. During the first half-hour after you wake up in the morning, how tired do you feel?

Very tired	1
Fairly tired	2
Fairly refreshed	3
Very refreshed	4

8. If you have no commitments the next day, what time would you go to bed compared to your usual bedtime?

Seldom or never later	4
Less than one hour later	3
1-2 hours later	2
More than two hours later	1

9. You have decided to engage in some physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 7:00 – 8:00 am. Bearing in mind nothing but your own internal "clock", how do you think you would perform?

Would be in good form	4
Would be in reasonable form	3
Would find it difficult	2
Would find it very difficult	1

10. At what time of day do you feel you become tired as a result of need for sleep?

8:00 – 9:00 PM	5
9:00 – 10:15 PM	4
10:15 PM – 12:45 AM	3
12:45 – 2:00 AM	2
2:00 – 3:00 AM	1

11. You want to be at your peak performance for a test that you know is going to be mentally exhausting and will last for two hours. You are entirely free to plan your day. Considering only your own internal "clock", which ONE of the four testing times would you choose?

8:00 AM – 10:00 AM	4
11:00 AM – 1:00 PM	3
3:00 PM – 5:00 PM	2
7:00 PM – 9:00 PM	1

12. If you got into bed at 11:00 PM, how tired would you be?

Not at all tired	1
A little tired	2
Fairly tired	3
Very tired	4

13. For some reason you have gone to bed several hours later than usual, but there is no need to get up at any particular time the next morning. Which ONE of the following are you most likely to do?

Will wake up at usual time, but will NOT fall back asleep	4
Will wake up at usual time and will doze thereafter	3
Will wake up at usual time but will fall asleep again	2
Will NOT wake up until later than usual	1

14. One night you have to remain awake between 4:00 – 6:00 AM in order to carry out a night watch. You have no commitments the next day. Which ONE of the alternatives will suite you best?

Would NOT go to bed until watch was over	1
Would take a nap before and sleep after	2
Would take a good sleep before and nap after	3
Would sleep only before watch	4

15. You have to do two hours of hard physical work. You are entirely free to plan your day and considering only your own internal "clock" which ONE of the following time would you choose?

8:00 AM – 10:00 AM	4
11:00 AM – 1:00 PM	3
3:00 PM – 5:00 PM	2
7:00 PM – 9:00 PM	1

16. You have decided to engage in hard physical exercise. A friend suggests that you do this for one hour twice a week and the best time for him is between 10:00 – 11:00 PM. Bearing in mind nothing else but your own internal "clock" how well do you think you would perform?

Would be in good form	1
Would be in reasonable form	2
Would find it difficult	3
Would find it very difficult	4

17. Suppose that you can choose your own work hours. Assume that you worked a FIVE hour day (including breaks) and that your job was interesting and paid by results). Which FIVE CONSECUTIVE HOURS would you select?

5 hours starting between 4:00 AM and 8:00 AM	5
5 hours starting between 8:00 AM and 9:00 AM	4
5 hours starting between 9:00 AM and 2:00 PM	3
5 hours starting between 2:00 PM and 5:00 PM	2
5 hours starting between 5:00 PM and 4:00 AM	1

18. At what time of the day do you think that you reach your "feeling best" peak?

5:00 – 8:00 AM	5
8:00 – 10:00 AM	4
10:00 AM – 5:00 PM	3
5:00 – 10:00 PM	2
10:00 PM – 5:00 AM	1

19. One hears about "morning" and "evening" types of people. Which ONE of these types do you consider yourself to be?

Definitely a "morning" type	6
Rather more a "morning" than an "evening" type	4
Rather more an "evening" than a "morning" type	2
Definitely an "evening" type	0

Appendix E. Epworth Sleepiness Scale

Epworth Sleepiness Scale[11]

How likely are you to nod off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently, try to work out how they would have affected you. It is important that you answer each question as best you can.

Use the following scale to choose the most appropriate number for each situation.

	Would never nod off 0	Slight chance of nodding off 1	Moderate chance of nodding off 2	High chance of nodding off 3
Sitting and reading				
Watching TV				
Sitting, inactive, in a public place (e.g., in a meeting, theater, or dinner event)				
As a passenger in a car for an hour or more without stopping for a break				
Lying down to rest when circumstances permit				
Sitting and talking to someone				
Sitting quietly after a meal without alcohol				
In a car, while stopped for a few minutes in traffic or at a light				

Add up your points to get your total score. A score of 10 or greater raises concern: you may need to get more sleep, improve your sleep practices, or seek medical attention to determine why you are sleepy.

Appendix F. Sleep Log

Sleep Log

Here is where you will enter your times for when you get into bed for the purpose of going to sleep, as well as the time you wake up. Please be as ***precise*** as you can when entering your times. For example, put 11:47 pm as the precise time you turned out the lights for the purpose of going to sleep, rather than input 12:00 am.

Input your Subject ID number provided to you by the research team
_____

Which time are you submitting?
___ Time in Bed
___ Waking Time

Input date and time
_____

Appendix G. Karolinska Sleepiness Scale

Karolinska Sleepiness Scale (KSS) 47

Purpose This scale [1] measures the subjective level of sleepiness at a particular time during the day. On this scale subjects indicate which level best reflects the psycho-physical sate experienced in the last 10 min. The KSS is a measure of situational sleepiness. It is sensitive to fluctuations.

Population for Testing It has been used in studies of shift work, jetlag, for driving abilities [2], attention and performance, and in clinical settings. It is used for both males and females.

It is helpful in assessing the changes in response to environmental factors, circadian rhythm, and effects of drugs. Because the KSS is not a measure of 'Trait' sleepiness, it has not been widely used for clinical purposes.

Administration This is self-report measure. It takes 5 min to complete.

Reliability and Validity In a study conducted by Kaida et al. [3], the authors investigated the validity of the KSS and found that it was highly correlated to EEG and behavioral variables. The results show that KSS has a high validity. However, because the scores of the KSS vary according to earlier sleep, time of day, and other parameters, it is difficult to deduce its test–retest reliability.

Scoring This is a 9-point scale (1 = extremely alert, 3 = alert, 5 = neither alert nor sleepy, 7 = sleepy – but no difficulty remaining awake, and 9 = extremely sleepy – fighting sleep). There is a modified KSS that contains one other item: 10 = extremely sleepy, falls asleep all the time. Scores on the KSS increase with longer periods of wakefulness and it strongly correlate with the time of the day.

Obtaining a Copy A copy can be obtained from the authors.

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Karolinska Sleepiness Scale (KSS)

Extremely alert	1
Very alert	2
Alert	3
Rather alert	4
Neither alert nor sleepy	5
Some signs of sleepiness	6
Sleepy, but no effort to keep awake	7
Sleepy, but some effort to keep awake	8
Very sleepy, great effort to keep awake, fighting sleep	9
Extremely sleepy, can't keep awake	10

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