

**AN EXPLORATION OF SELECTED RELATIONSHIPS BETWEEN INSUFFICIENT
AND POOR-QUALITY SLEEP AND DIETARY INTAKE**

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ABSTRACT

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Sleep affects health outcomes, in part, by directly and indirectly influencing undesirable dietary behaviors, which, in this work, is defined as frequent consumption of foods that are inconsistent with health. For example, insufficient sleep leads to more frequent consumption of sweets, a direct effect, and stress negatively influences sleep where insufficient and poor-quality sleep can lead to unhealthy dietary behaviors, and indirect effect. Additionally, sleep influences taste measures, such as sweet taste preference; therefore, sleep may also alter dietary intake through changes in taste preference. Singular relationships between sleep, stress, taste measures, and undesirable dietary behaviors and dietary intake have been investigated; however, more complex relationships between these measures have yet to be explored. Therefore, the overall objective of this research was to explore relationships between sleep and undesirable dietary behaviors in a more holistic manner. This was achieved by investigating the role of sleep in the relationship between stress and dietary behavior as well its influence on taste measures and dietary intake relationships.

Chapter 2 characterizes and compares health behaviors of higher education students from seven different countries during the COVID-19 pandemic and investigates the effects of changes in sleep duration and quality on dietary risk, alcohol misuse, physical activity, and sitting time. The chapter reveals the pressing concern of poor sleep quality that students in higher education were experiencing, and worsened sleep quality was associated with more frequent engagement in

unhealthy dietary behaviors. Therefore, addressing poor sleep quality among higher education student is urgent.

After investigating how changes in sleep predicted dietary risk, alcohol misuse, physical activity, and sitting time, chapter 3 considers mental health measures and explores more complex relationships between stress, sleep, resilience, undesirable dietary behaviors, and alcohol misuse. This study investigates whether sleep duration and quality mediated and resilience moderated the relationships between stress and undesirable dietary behaviors and the relationship between stress and alcohol misuse. Chapter 3 demonstrates that students in higher education are likely to benefit from sleep education and resilience trainings, especially during a stressful period.

Chapter 4 presents two studies. The methodological study examined whether a standardized tool used in evaluating sweet taste preference could be used in determining salt taste preference, and the experimental study used the new tool to investigate the effects of sleep curtailment on salt taste measures and explored the relationship between salt taste measures and dietary intake under the habitual and the curtailed sleep conditions. Results illustrated the adapted forced-choice paired-comparison tracking test can serve as a valid tool for determining salt taste preference. No changes in salt taste function and hedonic measures were observed after a night of curtailed sleep compared to habitual sleep. However, the slope of liking was associated with energy-corrected Na intake only under the habitual sleep condition.

In summary, the work presented in this dissertation explores the relationship between sleep and dietary intake in a complex manner. Overall, the work demonstrates the importance of improving sleep, which could reduce dietary risks, alcohol misuse, and sitting time, and the significance of accounting for sleep in taste studies.

Dedicated to Michael Nolan

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KEY TO ABBREVIATIONS

ANOVA: Analysis of Variance

AUDIT-C: Alcohol Use Disorders Identification Test-Consumption

ASA24: Automated Self-Administered 24-Hour

BF: Body Fat

BMI: Body Mass Index

BRS: Brief Resilience Scale

COVID-19: The Coronavirus Disease

EEG: Electroencephalograph

FDR: False Discovery Rate

FFQ: Food Frequency Questionnaire

IPAQ: International Physical Activity Questionnaire

MET: Metabolic Equivalents

Na: Sodium

NaCl: Sodium Chloride

PSQI: Pittsburgh Sleep Quality Index

PSG: Polysomnography

PSS: Perceived Stress Scale

REM: Rapid Eye Movement

SWS: Slow Wave Sleep

SSB: Sugar-Sweetened Beverage

STC: Starting the Conversation

TIB: Time in Bed

TST: Total Sleep Time

VAS: Visual Analog Scale

CHAPTER 1: INTRODUCTION

Numerous studies demonstrate both insufficient and poor-quality sleep are associated with poor health outcomes (Blair et al., 2015; Cappuccio et al., 2011; Chair et al., 2017; Fatima et al., 2016; Ford & Kamerow, 1989; Hoevenaar-Blom et al., 2011; Itani et al., 2017; Jiao et al., 2013; Knutson et al., 2006; Lemke et al., 2016; Lo & Lee, 2012; Lou et al., 2012; Luoju et al., 2014; O’Leary et al., 2017; Opp & Krueger, 2015; Osorio et al., 2011; Rahe et al., 2015; Sariarslan et al., 2015; Spiegel et al., 2002; Suzuki et al., 2009; Tworoger et al., 2006; Yaggi et al., 2006; Zhao et al., 2013) and sleep problems affect 1 in 3 adults worldwide (Adams et al., 2017; CDC - Data and Statistics - Sleep and Sleep Disorders, 2019; Furihata et al., 2015; C.-L. Lin et al., 2018). Insufficient and poor-quality sleep directly or indirectly relate to all top 10 leading causes of death (Blair et al., 2015; Cappuccio et al., 2011; Hoevenaar-Blom et al., 2011; Jiao et al., 2013; Knutson et al., 2006; Lemke et al., 2016; Luoju et al., 2014; NVSS - Leading Causes of Death, 2020; Opp & Krueger, 2015; Osorio et al., 2011; Tworoger et al., 2006; Yaggi et al., 2006; Zhao et al., 2013). One meta-analysis revealed that short sleep duration was associated with increased risk of diabetes, hypertension, cardiovascular disease, coronary disease, obesity, and overall mortality (Itani et al., 2017). In addition, recent studies also showed insufficient sleep was associated with a higher risk of certain cancers (Jiao et al., 2013; Luoju et al., 2014; Zhao et al., 2013), accidents (Lemke et al., 2016), cognitive impairment (Tworoger et al., 2006), acute and chronic inflammation (Opp & Krueger, 2015; Spiegel et al., 2002), and mental health issues (Ford & Kamerow, 1989; Sariarslan et al., 2015). Further, poor sleep quality increases the risk of type 2 diabetes (Lou et al., 2012), cardiovascular diseases (Chair et al., 2017; Suzuki et al., 2009) obesity (Fatima et al., 2016; Rahe et al., 2015) emotional instability

(O’Leary et al., 2017) and poorer health-related quality of life (Lo & Lee, 2012). In summary, insufficient and poor-quality sleep are associated with increased disease risks.

The negative effects of insufficient and poor-quality sleep on health outcomes can be attributed, in part, to how they influence undesirable dietary behaviors (also referred to as dietary risk), which is defined as frequent consumption of foods that are inconsistent with health in this work. Short sleep and poor sleep quality increase undesirable dietary behaviors, such as increased energy intake, more frequent consumption of sweets (Brondel et al., 2010a; Markwald et al., 2013; Spaeth et al., 2013), and higher consumption of fats and processed foods (Dashti et al., 2015). Additionally, short sleep and poor sleep quality increase the risk of alcohol misuse (Kenney et al., 2014). Therefore, not getting adequate sleep and sleeping poorly can lead to unhealthy dietary behaviors and eventually weight gain as well as other poor health outcomes.

Given that poor sleep quality and insufficient sleep lead to unhealthy dietary behaviors and are associated with poor health outcomes, the fact that multiple studies reported sleep quality and duration of students in higher education declined during the COVID-19 pandemic are concerning (Benham, 2021; J. Deng et al., 2021). Higher education students with poor sleep quality consumed fewer fruit and dairy servings, and poor sleep quality was associated with higher energy intake and poorer diet quality (Lotfi & Al-Hosseini, 2015; Zuraikat Faris M. et al., 2020). Additionally, poor sleep, including both insufficient and poor quality sleep, may also contribute to low physical activity levels (Dzierzewski et al., 2014; Kline, 2014; Sherrill et al., 1998; Strand et al., 2013) and is associated with sedentary behaviors (Must & Parisi, 2009). Therefore, it is imperative to characterize health behaviors of higher education students, including sleep, diet, and physical activity, during the COVID-19 pandemic and investigate how changes in sleep affect these health behaviors.

Among multiple factors affecting sleep, stress plays a major role that negatively influences sleep (Johnson et al., 2006; E.-J. Kim & Dimsdale, 2007; Van Reeth et al., 2000), especially during a stressful period, like the COVID-19 pandemic. Globally, higher education students report high levels of stress (America Psychology Association, n.d.; Hodselmans et al., 2018; Ramachandiran & Dhanapal, 2018), and the COVID-19 pandemic worsened stress among students in multiple countries (Husky et al., 2020; Konstantopoulou & Raikou, 2020; Son et al., 2020). Prior to the pandemic, more than 75% of Malaysian students attending a private university reported experiencing moderate stress while 13% reported high levels of stress (Ramachandiran & Dhanapal, 2018), and over 50% of higher education students in a Dutch university rated their stress as moderate to severe (Hodselmans et al., 2018). Additionally, more than half of the university students in the U.S. reported having difficulties coping with stress based on a national study (Ao, n.d.). Further, more than 60% of students stated that their emotional health worsened during the COVID-19 pandemic compared to before (Active Minds' Student Mental Health Survey, n.d.). Globally, multiple studies observed that the majority of the students in higher education reported higher levels of perceived stress during the pandemic compared to before (Husky et al., 2020; Konstantopoulou & Raikou, 2020; Malik & Javed, 2021; Son et al., 2020; C. Yang et al., 2021; Ye et al., n.d.). Given these reports, the COVID-19 pandemic further worsened the higher stress levels that students in higher education are already experiencing; therefore, increased levels of stress can be a contributor to the declined sleep duration and quality that students reported.

High levels of stress not only negatively influence sleep, but also increase dietary risk, which is defined as more frequent engagement in dietary behaviors that are inconsistent with health (Paxton et al., 2011). Stress leads to higher energy intake (Sims et al., 2008), increased

sugar and fat intake (Barrington et al., 2014; Vriendt et al., 2012), more frequent alcohol abuse (Anthenelli & Grandison, 2012), and poor dietary quality (E. P. Ferranti et al., 2013). During the COVID-19 pandemic, students in higher education reported increased stress; therefore, they may also have experienced increased dietary risk. Given that stress negatively influences sleep and dietary behaviors and insufficient and poor sleep quality lead to higher dietary risk, sleep could serve as a mediator in the relationship between stress and undesirable dietary behaviors. However, the complex relationship between stress, sleep, and undesirable dietary behaviors has not been examined.

Besides sleep, psychological resilience, which is defined by reacting to adversity positivity with quick adaptation (Fletcher & Sarkar, 2013), has been demonstrated to play an important role in reducing the negative effect of stress on dietary behaviors (Houpy et al., 2017; Skrove et al., 2013). Resilience can serve as a positive coping strategy for stress (Ong et al., 2006; Tugade et al., 2004); therefore, individuals with higher resilience may experience fewer negative effects from stress, such as engaging in unhealthy dietary behaviors (Houpy et al., 2017; Skrove et al., 2013). Given the evidence, resilience could moderate the relationship between stress and undesirable dietary behaviors, which has not been investigated.

Sleep may not only affect stress and undesirable dietary behavior relationship but also influence the relationship between taste measures and dietary intake (taste-dietary intake relationship). Despite taste playing a critical role in determining dietary intake (J. E. Hayes et al., 2010; Monteleone et al., 2017; van Langeveld et al., 2017), the ability of taste measures to predict intake is variable (Tan et al., 2021; Tan & Tucker, 2019). Sleep may be a factor that influences the taste-dietary intake relationship. Sleep has been demonstrated to alter measures of taste (Szczygiel, Cho, & Tucker, 2019a, 2019b) and dietary intake (Brondel et al., 2010a; Calvin et

al., 2013; Markwald et al., 2013). For example, after one night of curtailed sleep, increases in the preferred concentrations for sucrose (Szczygiel, Cho, Snyder, et al., 2019; Tajiri et al., 2020) and sucralose were observed (Szczygiel, Cho, & Tucker, 2019a). Additionally, sweeter oat crisps were liked more after curtailed sleep compared to habitual sleep (Szczygiel, Cho, & Tucker, 2019b). In terms of the effects of sleep on dietary intake, one night of short sleep can lead to undesirable dietary behaviors such as increased consumption of sweets and high calorie foods (Calvin et al., 2013; Simon et al., 2015; Spaeth et al., 2013; St-Onge et al., 2011; C.-L. Yang et al., 2019). Taken together, sleep could be a factor that affects the taste-dietary intake relationship, which has not been investigated.

The effects of sleep on sweet taste measures have been studied (Szczygiel, Cho, & Tucker, 2019a, 2019b; Tajiri et al., 2020), but there are limited reports on the effects of sleep on salt taste measures with methodological limitations. One cross-sectional study reported an association between short sleep duration (< 6 hours) and increased odds of self-reported altered taste perception in general (Gao et al., 2021), and a separate study demonstrated self-reported sleepiness was not associated with salt taste sensitivity but with cravings for savory foods (Lv et al., 2018). Two experimental studies which investigated the effects of sleep deprivation, defined by the complete absence of sleep, and sleep curtailment, referred to as shortened sleep duration, on salt taste function, have been conducted. One study reported no change in salt taste detection threshold after 24, 48, and 72 hours of sleep deprivation (Furchtgott & Willingham, 1956) while the other study demonstrated no differences in salt taste sensitivity measured by detection threshold after long (> 7 hour) versus short (< 7 hour) sleep (S. L. Smith et al., 2016). There are several limitations to these studies. First, none of the studies included hedonic measures of salt taste, which may be more important predictors of dietary intake than thresholds (Tan et al., 2021;

Tan & Tucker, 2019). Second, the complete absence of sleep is not commonly experienced in the general adult population (CDC - Data and Statistics - Sleep and Sleep Disorders, 2021; Chattu et al., 2018). Third, the study that compared short versus long sleep duration did not prescribe the curtailed sleep duration based on the habitual sleep duration of participants, which could have created an unstandardized sleep curtailment duration for individuals. Given the small number of studies and these limitations, research investigating the effects of sleep curtailment on both hedonic and functional measures of salt taste is needed.

One standing issue is present when investigating salt taste. Measures of salt taste are variable in the field and no consensus has been reached in measuring salt taste preference (Tan et al., 2021). Therefore, in order to study the effects of sleep on salt taste measures, the present dissertation first tested the utility of a standardized method to measure salt taste preference.

Based on the literature reviewed above, the overall objective of the present dissertation was to investigate the effects of sleep on undesirable dietary behaviors, and the overarching objective would be carried out through three aims that explore the effects of sleep on stress-undesirable dietary behaviors relationship and on taste-dietary intake relationship. The first aim of this body of work was to characterize the health behaviors of higher education students during the COVID-19 pandemic and to examine whether changes in sleep duration and quality would be associated with changes to other health behaviors. The second aim was to determine whether sleep quality or duration mediated and whether resilience moderated the relationship between perceived stress and undesirable dietary behaviors and the relationship between perceived stress and alcohol misuse. The third aim was to examine the effects of sleep curtailment on salt taste hedonic measures (liking and preferred concentration) and function (intensity) and to explore

whether salt taste measures were associated with energy corrected dietary intake. These aims were tested in the following chapters.

CHAPTER 2: HEALTH BEHAVIORS OF HIGHER EDUCATION STUDENTS FROM 7 COUNTRIES: POORER SLEEP QUALITY DURING THE COVID-19 PANDEMIC PREDICTS HIGHER DIETARY RISK

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2.1 Abstract

Health behaviors of higher education students can be negatively influenced by stressful events. The global COVID-19 pandemic presents a unique opportunity to characterize and compare health behaviors across multiple countries and to examine how these behaviors are shaped by the pandemic experience. Undergraduate and graduate students enrolled in universities in China, Ireland, Malaysia, South Korea, Taiwan, the Netherlands, and the United States (U.S.) were recruited into this cross-sectional study. Eligible students filled out an online survey comprised of validated tools for assessing sleep quality and duration, dietary risk, alcohol misuse, and physical activity between late April and the end of May 2020. Health behaviors were fairly consistent across countries, and all countries reported poor sleep quality. However, during the survey period, the COVID-19 pandemic influenced the health behaviors of students in European countries and the U.S. more negatively than Asian countries, which could be attributed

to the differences in pandemic time course and caseloads. Students who experienced a decline in sleep quality during the COVID-19 pandemic had higher dietary risk scores than students who did not experience a change in sleep quality ($p=0.001$). Improved sleep quality was associated with less sitting time ($p=0.010$). Addressing sleep issues among higher education students is a pressing concern, especially during stressful events. These results support the importance of making education and behavior-based sleep programming available for higher education students in order to benefit students' overall health.

2.2 Key words

Sleep, dietary risk, physical activity, alcohol misuse, COVID-19, undergraduate and graduate students

2.3 Introduction

Young adulthood is a time when many health behaviors solidify, making this developmental period a prime opportunity for public health practitioners to shape lifelong healthy habits (Plotnikoff et al., 2015). While higher education student experiences vary across the globe, one important change from adolescence that many students face is increased independence in structuring one's time (Balduf, 2009; G. Deng & Xuan, 2009; W.-C. Wang et al., 2011). In addition to academics, time is also spent on a variety of factors including socializing; working, volunteering, or extracurricular activities; and health behaviors, like sleeping, eating, and physical activity. Struggling with time management demands contributes to many undergraduate and graduate students reporting elevated levels of perceived stress (Geisler & Allwood, 2018; Song et al., 2020). These high levels of perceived stress are a global phenomenon; for example, prior to the pandemic more than three-quarters of Malaysian students

reported experiencing moderate stress (Ramachandiran & Dhanapal, 2018) while nearly 40% of U.S. students reported high levels of perceived stress (VanKim & Nelson, 2013). Stress plays an important role in health behaviors, like insufficient sleep (Charles et al., 2011; Du et al., 2020; Geisler & Allwood, 2018) and increased alcohol consumption (Becker, 2012; Keyes et al., 2012; Perreira & Sloan, 2001), which can lead to negative health outcomes, such as cardiovascular disease (Glaus et al., 2013; Hoevenaar-Blom et al., 2011), diabetes (Knutson et al., 2006; Yaggi et al., 2006), and cancers (Jiao et al., 2013; Luoju et al., 2014; Pelucchi et al., 2011; Zhao et al., 2013). An improved understanding of the health behaviors of young adults, especially under conditions of heightened stress, is needed to assist public health practitioners in designing programming to promote the development of lifelong healthy habits.

While the prevalence of higher education students reporting elevated stress is high, the current COVID-19 pandemic has further increased stress for many students (Grubic et al., 2020; Traumatic Stress in the Age of COVID-19: A Call to Close Critical Gaps and Adapt to New Realities., n.d.; Zolotov et al., 2020). Previous studies have demonstrated that large-scale disasters, including pandemics, led to profound health behavior changes (Huckins et al., 2020; Mattioli, Sciomer, et al., 2020), including unhealthy eating behaviors, poor sleep, and lower physical activity levels (Bonnet et al., 2005; Germain et al., 2004; M. Ji et al., 2020; C. H. Liu et al., 2020; Shen et al., 2020; Stults-Kolehmainen & Sinha, 2014; Y. Yang et al., 2020). Other studies noted increased mental health distress during disasters (Bromet et al., 2017; Neria et al., 2008; Vlahov et al., 2004). Because the student population is at increased risk for elevated stress and mental health concerns ((6) (PDF) Socio-Demographic Determinants and Prevalence of Depression, Anxiety, and Stress among Malaysian University Students, n.d.; Evans et al., 2018; C. Liu et al., 2007; Murphy et al., 2018), and because many students experienced disruption of

their established daily schedules, the current study sought to characterize higher education student health behaviors in multiple countries and to examine how these behaviors were affected by the COVID-19 pandemic.

During the COVID-19 pandemic, popular press and scholarly work reported that sleep duration and quality suffered (Çitak & Pekdemir, 2020; “Sleep Habits Post Lockdown in the U.S. - New Study (2020),” n.d.; “Sleep Problems Becoming Risk Factor as Pandemic Continues,” 2020). The inability to obtain sufficient, good quality sleep has been demonstrated to be an independent risk factor for a variety of diseases and conditions, including obesity (Y. Liu et al., 2013), type 2 diabetes (Kita et al., 2012; Shankar et al., 2010), hypertension (Shankar et al., 2010), alcohol misuse (Chaput et al., 2012), and depression (Williams et al., 2020). Stressful events, like a pandemic, can interfere with sleep (Y. Huang & Zhao, 2020). Thus, the present study also explored whether changes in sleep quality and duration during the COVID-19 pandemic were related to health behaviors. It was hypothesized that a decline in sleep quality and a reduction in sleep duration would be associated with increased dietary risk scores and alcohol misuse, decreased physical activity levels, and increased sitting time while improved sleep quality and increased duration would be associated with the opposite.

2.4 Materials and methods

2.4.1 Study design

Higher education students, including both undergraduate and graduate students, who were at least 18 years old and were enrolled in universities in China, Ireland, Malaysia, Taiwan, South Korea, the Netherlands, and the U.S. were recruited into this cross-sectional study.

Countries included in the present study were based on availability. Survey data was collected in April and May 2020, when most states in the U.S. had adopted shelter in place orders, and most

areas in Ireland, Malaysia, and the Netherlands had also enacted shelter in place orders due to the COVID-19 pandemic. During this time, China, Taiwan, and South Korea had recently revoked their shelter in place orders.

The study was approved by the Michigan State University Human Research Protection Program (East Lansing, MI, USA,) STUDY00004285, 4/7/2020; International Medical University Joint Committee on Research and Ethics (Kuala Lumpur, Malaysia), 481/2020, 5/14/2020; Faculty of Governance and Global Affairs Ethics Committee (The Hague, South Holland, Netherlands), 2020-009-LUC-Cho, 5/25/2020; Indiana University Institutional Review Board for the Protection of Human Subjects (Indiana, PA, USA), IRB Log 20-101, 5/15/2020; Institute Research Ethics Committee, Institute of Technology, Sligo (Sligo, Ireland), ref 2020015, 5/5/2020; University of Taipei Institutional Review Board (Taipei, Taiwan), IRB-2020-045, 6/16/2020; and Bowling Green State University Office of Research Compliance (Bowling Green, OH, USA; 1599753 (US students), 4/29/2020; 1599753 (Chinese students), 5/22/2020; 1599753 (Korean students), 5/11/2020. Consent was obtained from all participants prior to the start of the study.

2.4.2 Demographics

Information regarding age; gender; undergraduate vs. graduate status, including year classification; domestic versus international status; weight; and height was collected.

2.4.3 Assessment of dietary risk and alcohol misuse

Diet was evaluated using the Starting the Conversation (STC) questionnaire. The STC is an eight-item simplified food frequency questionnaire (FFQ), which was used to determine participants' dietary risk score based on common dietary patterns (Paxton et al., 2011). Examples

of questions include: How many times a week did you eat fast food meals or snacks? How many regular sodas or glasses of sweet tea did you drink each day? The STC provides a global score of dietary risk, with higher scores indicating greater risk. Food terms were adjusted to account for cultural differences among countries. For example, the term “snack chips” was changed to “crisps” in the Ireland survey.

Alcohol misuse score was determined using the Alcohol Use Disorders Identification Test Alcohol Consumption questionnaire (AUDIT-C), which is a validated screener for problem drinking over the past year (Bush et al., 1998). A standard drink equivalents reference chart was included in the survey to help participants quantify drink servings (National Institute on Alcohol Abuse and Alcoholism (NIAAA), 2015). A score of 4 and higher for men and 3 and higher for women suggests misuse (AUDIT-C Frequently Asked Questions, n.d.). The alcohol assessment questions were only shown to students who were of legal drinking age in their respective countries.

2.4.4. Assessment of sleep quality and duration

Assessment of sleep quality and duration over the past month was conducted using the Pittsburgh Sleep Quality Index (PSQI), which has been validated (Beaudreau et al., 2012; Buysse et al., 2008; Guo et al., 2016; Marques et al., 2013). In addition to the PSQI, weekday and weekend sleep duration were also quantified in order to distinguish sleeping duration differences between weekdays and weekends.

2.4.5. Assessment of physical activity and sitting time

Physical activity levels and sitting over the past week were measured using the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003). The total physical

activity levels were calculated and reported using metabolic equivalents (METs) minutes per week. The total METs minutes per week were calculated using METs of vigorous, moderate, or walking activity multiplied by the corresponding activity duration; therefore, METs minutes per week reflects both physical activity intensity and duration. Total METs minutes per week was selected to reflect the overall physical activity levels of participants as the measure captures both physical activity intensity and duration. As such, physical activity “level” is used to refer to METs minutes per week. Sitting times were reported as minutes per day.

2.4.6. Assessment of the influence of the COVID-19 pandemic on the factors described above

At the end of each survey section, a question about how the COVID-19 pandemic had affected participants’ diet, alcohol consumption, sleep quality, sleep duration, and physical activity frequency and intensity was asked. Examples of questions included: Have you made changes to your diet during the COVID-19 quarantine compared to before the quarantine? Possible answers included: healthier than before, less healthy than before, or my diet did not change. Has the intensity of your exercising changed during the COVID-19 quarantine compared to before the quarantine? Possible answers included: More intense, less intense, or my exercise intensity did not change.

2.4.7. Statistical analysis

Outlier screening was conducted for each variable, and outliers, defined as greater or less than mean values ± 3 standard deviations, were excluded from data analysis. After outlier exclusion, all examined variables were approximately normally distributed based on normal probability plot, kurtosis, and skewness. Adjusted totals were calculated by averaging variable means of each country to account for differences in country sample size (Wuensch, 2014).

Differences between countries for age, BMI, sleep quality and duration, dietary risk, alcohol misuse risk, physical activity, and sitting time were assessed using one-way ANOVA and Bonferroni post-hoc tests. Statistical significance for these tests was determined by $p < 0.05$. Categorical variables of gender, undergraduate vs. graduate status, domestic versus international status, and health behaviors influenced by the COVID-19 pandemic were compared between countries using a chi-square test of homogeneity followed by z-test comparisons of country proportions. Bonferroni adjusted p-values were used to determine significance. Specific p values for proportion comparisons were not given by SPSS; therefore, the p value cut-offs were calculated based on Bonferroni adjustment rules of alpha divided by number of comparisons (Shan & Gerstenberger, 2017; Wright, 1992). Linear regression with dummy coding of categorical independent variables was conducted to examine whether sleep quality and duration changes were related to dietary risk scores, alcohol misuse scores, physical activity levels (duration and frequency reflected by total METs per week), and sitting time. The differences in PSQI scores and sleep duration were compared between the sleep quality change groups and between the sleep duration change groups using one-way ANOVA. Normal P-P plot and scatter plots of regression standardized residuals were produced, and the plots showed that assumptions of normality and homoscedasticity were met. Linear relationships between independent variables and the outcome variable were observed. Four linear regression models adjusting for age, gender, BMI, and countries were built using the Enter method. All analyses were completed using IBM SPSS version 26 (IBM Corporation, Armonk, New York, USA).

2.5 Results

2.5.1. Demographic and anthropometric information

A total of 2,663 students studying in China, Ireland, Malaysia, South Korea, Taiwan, the Netherlands, and the U.S. initiated the survey, and a total of 2,254 students completed it and were included in the analyses (Table 1). The majority of countries surveyed had more female participants except South Korea and Taiwan, and most of the students were undergraduate students except for South Korea. Students from all countries, besides the Netherlands, were predominantly domestic students, meaning that they attended a higher education institution in their country of citizenship. Students from the U.S. reported a higher body mass index (BMI) compared to all other countries. Overall, demographic information among the countries was fairly consistent, but BMI (based on self-reported height and weight) differed.

Table 1. Demographic and anthropometric information of higher education students from 7 countries

	China	Ireland	Malaysia	South Korea	Taiwan	The Netherlands	U.S.	Total	Adjusted total
n	111	192	91	89	377	114	1280	2254	-----
Female (%)	67.6 ^a	71.9 ^a	79.1 ^a	39.3 ^b	41.1 ^b	80.7 ^a	73.0 ^a	66.6 [†]	74.5
Male (%)	32.4 ^a	27.6 ^a	20.9 ^a	60.7 ^b	53.6 ^b	19.3 ^a	24.1 ^a	30.8 [†]	33.7
Age (year) (Mean±SD)	20.4±2.5 ^c	24.7±7.6 ^a	22.2±4.1 ^{b,c}	25.5±4.6 ^a	20.8±3.1 ^c	20.1±1.3 ^c	22.9±5.9 ^b	22.5±5.5	22.4
BMI (kg/m ²) (Mean±SD)	20.2±2.9 ^d	24.1±6.3 ^b	22.2±4.6 ^{b,c,d}	22.7±3.4 ^{b,c}	22.5±3.2 ^c	22.0±3.6 ^{c,d}	26.0±6.4 ^a	24.4±5.6	22.8
Undergraduate (%)	84.7 ^{a,b,c}	80.2 ^c	96.7 ^{b,d}	46.1 ^e	95.5 ^d	100.0 ^d	74.3 ^{a,c}	79.9	88.6
Domestic student (%)	95.5 ^{a,b}	93.2 ^{a,b}	81.3 ^c	94.4 ^{a,b,c}	95.5 ^b	40.4 ^d	87.0 ^{a,c}	87.0	91.2

Means with different superscripts are significantly different, $p < 0.05$. Chi-square test of homogeneity was used for comparisons of gender, undergraduate vs. graduate status, and domestic vs. international status. One-way ANOVA was used for comparisons of age and BMI. Domestic student was defined as a student attending a higher education institution in their country of citizenship. †2.6% of students self-identified as other, which included transgender, genderqueer, other, and choose not to disclose. Missing values: gender n=0; age n=2; BMI n=16; undergraduate vs. graduate status n=0; domestic vs. international status n=0.

2.5.2. Differences in health behaviors by country

Sleep quality and average, weekday, and weekend sleep duration were largely consistent across countries. However, the highest and lowest absolute values often differed from each other (Table 2). Sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI) where a higher score indicates poorer sleep quality (Buysse et al., 1989). All countries reported PSQI scores greater than 5, which indicates poor sleep quality. The following sleep measures were significantly different between the highest and lowest absolute values reported, but these values did not differ between most other countries: PSQI score (U.S. vs. Taiwan, $p < 0.001$), average sleep duration (the Netherlands vs. Taiwan, $p < 0.001$), and weekday sleep duration (the Netherlands vs. South Korea, $p = 0.032$). Additionally, the following proportions were significantly different between the highest and lowest absolute proportions, but these proportions did not differ between most other countries: the proportion of students classified as poor sleepers (U.S. vs. China, $p < 0.05$) and the proportion of students who did not meet the minimum recommended sleep duration of 7 hours per day (Hirshkowitz et al., 2015) (South Korea vs. the Netherlands, $p < 0.05$). To summarize, sleep quality was poor for students from all countries, and in most countries, more than 25% of students were not meeting sleep duration guidelines.

Table 2. Country comparisons of sleep quality and duration

	China	Ireland	Malaysia	South Korea	Taiwan	The Netherlands	U.S.	Total*	Adjusted total
n	111	190	91	89	375	114	1272	2242	-----
PSQI (Mean±SD)	5.5±2.6 ^{b,c}	7.4±3.6 ^a	5.9±3.1 ^{b,c}	6.4±3.1 ^{a,b,c}	5.5±3.1 ^c	6.8±3.4 ^{a,b}	7.4±3.6 ^a	6.8±3.5	6.4
Average sleep (hrs/d) (Mean±SD)	7.7±0.8 ^{a,b}	7.5±1.2 ^{a,b,c}	7.2±1.2 ^{b,c,d}	7.1±1.1 ^{c,d}	7.0±1.1 ^d	7.7±1.0 ^a	7.6±1.3 ^a	7.5±1.2	7.5
Weekday sleep (hrs/d) (Mean±SD)	7.5±0.9 ^{a,b}	7.3±1.2 ^{a,b}	7.0±1.2 ^{a,b}	6.7±1.3 ^b	7.0±1.3 ^b	8.2±4.6 ^a	7.6±2.5 ^{a,b}	7.5±3.5	7.4
Weekend sleep (hrs/d) (Mean±SD)	8.2±1.1	8.0±1.4	7.8±1.5	8.3±1.5	8.2±3.4	8.2±1.1	8.2±1.6	8.1±1.9	8.1
Poor sleep quality (%)	46.8 ^{a,b,c,d}	64.7 ^{c,d,e}	49.5 ^{a,b,c,d}	60.7 ^{b,d,e}	42.7 ^a	55.3 ^{a,b,c,d,e}	67.2 ^e	60.3	55.3
Short sleep duration (%)	14.4 ^a	26.6 ^{a,b}	34.1 ^{b,c}	46.1 ^c	43.2 ^c	12.3 ^a	24.2 ^{a,b}	27.8	28.7

Means with different superscripts are significantly different, $p < 0.05$. One-way ANOVA was used for comparisons of PSQI, average sleep hours, weekday and weekend sleep hours. Chi-square test of homogeneity was used for comparisons of percentages of students who reported poor sleep quality and short sleep duration. PSQI = Pittsburg Sleep Quality Index. PSQI scores range from 0 = best to 21 = worst sleep quality. PSQI scores > 5 were defined as poor sleep quality. Short sleep duration was defined as sleep duration < 7 hrs/day (average of weekday and weekends). *Included all valid data for which PSQI scores could be computed. Missing values: PSQI n=12; average sleep duration n=21; weekday sleep duration n=2; weekend sleep duration n=2; poor sleep quality (%) n=12; short sleep duration (%) n=21.

Overall dietary risk and alcohol misuse scores were largely consistent by country, but the highest and the lowest absolute values were different from each other (Table 3). Students from the U.S. reported the highest absolute dietary risk scores, which differed from China where students reported the absolute lowest risk scores ($p < 0.001$); however, the two scores were not different from other countries. The following alcohol misuse measures were significantly different between the highest and lowest absolute values reported, but these values did not differ between most other countries: male students' alcohol misuse scores (Ireland vs. Malaysia, $p < 0.001$) and the proportions of male students classified as alcohol misusers (Ireland vs. Malaysia, $p < 0.05$). Female students in China and Malaysia ($p < 0.05$ for both) reported a lower alcohol misuse score compared to all other countries, and the proportion of female students who were classified as misusers was lower in China and Malaysia ($p < 0.05$ for both) compared to all other countries.

Table 3. Country comparisons of dietary risk and alcohol consumption

	China	Ireland	Malaysia	South Korea	Taiwan	The Netherlands	U.S.	Total*	Adjusted total
n	111	192	91	89	-----	114	1280	1877	-----
Dietary risk (Mean±SD)	6.6±2.3 _b	6.9±3.1 ^b	7.1±2.4 ^b	7.4±2.9 ^{a,b}	-----	7.0±2.5 ^b	8.2±2.7 ^a	7.9±2.8	7.3
n (provided alcohol use data)	107	190	91	86	377	114	796	1761	-----
Alcohol misuse score (Mean±SD) (male†)	2.3±2.5 _c	4.4±2.5 ^a	1.2±1.6 ^c	4.2±3.3 ^{a,b}	4.0±3.3 ^{a,b}	4.4±2.3 ^{a,b}	3.3±3.0 _b	3.6±3.1	3.4
Alcohol misuse score (Mean±SD) (female††)	1.2±1.7 _a	3.6±2.1 ^b	0.9±1.4 ^a	3.1±2.8 ^{b,c}	2.7±2.4 ^c	3.7±2.3 ^b	3.1±2.4 _{b,c}	2.9±2.4	2.6
Classified as misuser (%) (male†)	25.0% ^{a, b,c,d}	49.1% ^d	5.3% ^c	48.1% ^{b,d}	42.6% ^{b,d}	45.5% ^{a,b,c,d}	23.7% ^{a,c}	33.3%	34.2%
Classified as misuser (%) (female††)	8.0% ^a	50.7% ^b	6.9% ^a	42.9% ^{b,c}	36.8% ^b	53.3% ^b	23.3% ^c	28.0%	31.7%

Means with different superscripts are significantly different, $p < 0.05$. †For male students, $n=35$ China, $n=53$ Ireland, $n=19$ Malaysia, $n=54$ South Korea, $n=202$ Taiwan, $n=22$ the Netherlands, $n=211$ the U.S. ††For female students, $n=72$ China, $n=136$ Ireland, $n=72$ Malaysia, $n=32$ South Korea, $n=155$ Taiwan, $n=92$ the Netherlands, $n=561$ the U.S. Dietary risk was defined by score on the Starting the Conversation food frequency questionnaire. 0 = best to 16 = worst dietary quality. Alcohol misuse was defined by score on the Alcohol Use Disorders Identification Test Consumption questionnaire. 0 = no alcohol use to 12 = highest alcohol use. Scores ≥ 3 in females and ≥ 4 in males were defined as misuse. * Missing values for dietary risk, $n=377$. Dietary risk data from Taiwan were not collected due to technical difficulties. Missing values for alcohol misuse, $n=493$, represented students who did not meet the legal drinking age of their country.

Physical activity level, measured by self-reported total metabolic equivalents (METs) minutes per week, and sitting time displayed considerable consistency across countries; however, the highest and the lowest values differed from each other (Table 4). While Irish students were the most active, and Malaysian students the least ($p < 0.001$), activity levels reported by these two countries were not different from most other countries. In addition, students from Malaysia reported a higher amount of sitting time compared to most other countries.

2.5.3. Differences in COVID-19 pandemic-influenced health behaviors by country

The influence of the COVID-19 pandemic on health behaviors was similar across countries with few differences observed (Table 5). The absolute proportion of students who indicated they were eating less healthfully during the pandemic compared to before was lowest in Taiwan compared to all other countries ($p < 0.05$); whereas, the highest value, observed among U.S. students, did not differ among the remaining countries. The following behaviors were significantly different between the highest and lowest absolute values reported, but these values did not differ between most other countries: the proportion of students drinking more alcohol (U.S. vs Malaysia, $p < 0.05$), sleeping worse and sleeping less (Ireland vs. Taiwan, $p < 0.05$), and exercising less (China vs. Ireland, $p < 0.05$). In addition, the proportion of students reporting decreased exercise intensity did not differ by country. Generally, in terms of health behaviors, students in European countries and the U.S. were more negatively affected by the COVID-19 pandemic during the study period compared to students in Asian countries.

Table 4. Country comparisons of physical activity level and sitting time

	China	Ireland	Malaysia	South Korea	Taiwan	The Netherlands	U.S.	Total*	Adjusted total
n	105	177	88	87	-----	109	1174	1742	-----
Total METs (mins/wk) (Mean±SD)	1966.5 ±2710.9 ^{b,c}	3748.1 ±3772.3 ^a	1639.2 ±2502.9 ^c	2842.6 ±3317.9 ^{a,b} .c	-----	2849.7 ±2442.6 ^{a,b,c}	2892.9 ±3549.5 ^b	2859.8 ±3441.0	2656.5
Sitting time (mins/d) (Mean±SD)	285.7 ±204.4 ^a	389.7 ±189.7 ^b	492.8 ±255.5 ^c	368.5 ±200.2 ^{a,b}	-----	427.1 ±165.5 ^{b,c}	423.8 ±204.3 ^b	413.0 ±206.7	397.9

Means with different superscripts are significantly different, $p < 0.05$. One-way ANOVA was used for comparisons of total METs and sitting time. Physical activity level was defined by metabolic equivalents (METS) reported on the International Physical Activity Questionnaire. *Physical activity data from Taiwan were not collected due to technical difficulties.

Table 5. Percentage of students reporting undesirable changes in health behaviors during the COVID-19 pandemic compared to before the pandemic

		China (%)	Ireland (%)	Malaysia (%)	South Korea (%)	Taiwan (%)	The Netherlands (%)	U.S. (%)	Total	Adjusted total
n		111	192	91	89	377	114	1280	2254	-----
Diet	Less healthy	22.5 ^a	35.8 ^{a,b}	36.3 ^{a,b}	24.7 ^a	6.1 ^c	33.3 ^{a,b}	45.6 ^b	35.2	30.5
Alcohol consumption*	Drinking more	5.6 ^{a,b,c}	16.3 ^{c,d}	2.2 ^b	5.8 ^{a,b,c}	6.1 ^b	17.5 ^{a,b,d}	26.1 ^d	16.8	8.9
Sleep quality	Sleeping worse	16.2 ^{a,b}	41.6 ^c	29.7 ^{b,c}	19.1 ^b	8.0 ^a	48.2 ^c	38.8 ^c	32.1	21.7
Sleep duration	Sleeping less	12.6 ^{a,b,c,d,e}	27.4 ^{d,e}	22.0 ^{c,e}	10.1 ^{a,b,c}	8.2 ^b	20.2 ^{a,c,d,e}	18.5 ^{a,c,d,e}	17.1	17.0
Exercise frequency*	Exercising less	56.8 ^a	37.0 ^b	50.5 ^{a,b}	44.9 ^{a,b}	-----	50.0 ^{a,b}	51.5 ^a	49.9	48.5
Exercise intensity*	Less intense	49.5 ^a	39.7 ^a	42.9 ^a	38.2 ^a	-----	43.0 ^a	46.4 ^a	45.2 ^a	43.3

2.5.4. Examination of relationships between sleep quality and duration changes and health behaviors

PSQI scores and average sleep duration were compared between students who reported sleeping worse, sleeping better, and no change in sleep quality during the COVID-19 pandemic compared to before. Students who reported sleeping worse during the pandemic experienced poorer sleep quality, as evidenced by higher PSQI scores, compared to students who reported that sleep quality did not change or who reported sleeping better (Table 6). Average sleep duration was different between the three groups, with students who reported sleeping better reporting the longest sleep duration while students who reported sleeping worse reported the shortest.

Sleep quality differed between students who reported sleeping less, sleeping more, and no change in sleep duration during the COVID-19 pandemic compared to before. Students who reported sleeping less during the pandemic reported the highest PSQI scores (worse sleep quality), while students who reported better or no change in sleep duration reported lower PSQI scores that did not differ between each other (Table 6). As expected, average sleep duration differed between the three groups; those who reported sleeping less reported the shortest duration, those who reported sleeping more reported the longest duration, and those reporting no change reported an intermediate duration. Additionally, those who reported sleeping less had the highest PSQI score compared to those who slept more and did not change in sleep duration.

Students who experienced reduced sleep quality during the pandemic had a higher dietary risk score compared to students who reported improved or no change in sleep quality (Table 7). Students who slept less and slept more had a higher dietary risk score compared to students who reported no change in sleep duration. To summarize, reduced sleep quality was associated with

higher dietary risk scores, but change in sleep duration was not a reliable predictor of dietary risk.

Table 6. PSQI and average sleep duration of sleep quality and duration change groups

Groups	n (%)	PSQI (Mean±SD)	Average sleep duration (hrs/day) (Mean±SD)
Sleep quality change			
Worse	719 (32.1)	9.2±3.4 ^a	7.3±1.3 ^a
Better	348 (15.5)	5.5±2.9 ^b	7.8±1.1 ^b
Did not change	1175 (52.4)	5.8±2.9 ^b	7.5±1.1 ^c
Sleep duration change			
Less	383 (17.1)	9.4±3.8 ^a	6.7±1.2 ^a
More	1002 (44.7)	6.6±3.2 ^b	8.0±1.1 ^b
Did not change	857 (38.2)	6.0±3.2 ^c	7.3±1.0 ^c

Means with different superscripts are significantly different, $p < 0.05$. PSQI = Pittsburgh Sleep Quality Index.

Compared to students who reported no change in sleep quality, students who slept better and slept worse did not differ in alcohol misuse scores, and students who slept less and slept more both had higher alcohol misuse scores (Table 7). Therefore, change in sleep quality was not associated with alcohol misuse scores, and change in sleep duration was not a reliable predictor of alcohol misuse scores.

In terms of physical activity levels and sitting time, students who reported improved sleep quality reported less sitting time but no difference in physical activity levels compared to students who reported no change in sleep quality. There was no difference in physical activity levels and sitting time for students who reported reduced sleep quality when compared to students who reported no change (Table 7). Additionally, students who slept more reported lower physical activity levels and more sitting time while there was no difference in physical activity

levels and sitting time for students who slept less when compared to students who reported no change in sleep duration. To summarize, sleeping better during the COVID-19 pandemic was associated with higher physical activity levels and less sitting time while sleeping more was associated with lower physical activity levels and more sitting time.

Table 7. Outcomes of linear regression models examining whether sleep changes were related to health behaviors.

Predictors	Dietary risk B (p-value)	Alcohol misuse scores B (p-value)	Physical activity (METs/wk) B (p-value)	Sitting time (mins/d) B (p-value)
(Constant)	6.756 (<0.001)	2.005 (<0.001)	2517.932 (<0.001)	368.664 (<0.001)
Sleep quality change				
Worse	0.486 (0.001)	0.310 (0.051)	-272.907 (0.123)	19.909 (0.082)
Better	-0.323 (0.081)	10.162 (0.413)	18.737 (0.934)	-36.064 (0.010)
Did not change (reference)	---	---	---	---
Sleep duration change				
Less	0.409 (0.040)	0.473 (0.019)	-128.772 (0.582)	4.436 (0.770)
More	0.408 (0.008)	0.459 (0.004)	-568.192 (0.002)	22.841 (0.050*)
Did not change (reference)	---	---	---	---

n=1877 for dietary risk, n=1761 for alcohol misuse scores, n=1736 for physical activity, and n=1872 for sitting time. One model was built for each dependent variable, and the models controlled for age, BMI, gender, and countries. *The exact p value is 0.049880. Dietary risk was defined by score on the Starting the Conversation, a simplified food frequency questionnaire. 0 = best to 16 = worst dietary quality. Alcohol misuse was defined by score on the Alcohol Use Disorders Identification Test Consumption questionnaire. 0 = no alcohol use to 12 = highest alcohol use. Scores ≥ 3 in females and ≥ 4 in males were defined as misuse. Physical activity level and sitting time were reported on the International Physical Activity Questionnaire. METs = metabolic equivalents.

2.6 Discussion

Health behaviors of students across the seven countries were largely similar; although, a few differences were noted. The negative effects of the COVID-19 pandemic on health behaviors disproportionately affected students in European countries and the U.S. compared to students in Asian countries; this likely resulted from the timeframe of the study (see discussion below). As hypothesized, students who indicated poorer sleep quality during the pandemic had higher dietary risk scores and engaged in less physical activity, while improved sleep quality was associated with higher physical activity levels and less sitting time. Contrary to our hypothesis, sleeping more during the pandemic was associated with lower physical activity levels and greater sitting time. These results suggest that working to improve the sleep quality of higher education students could be an important target for improving overall health by supporting healthier behaviors. This conclusion needs further testing due to the cross-sectional nature of the present study.

2.6.1. Differences in the overall influence of the COVID-19 pandemic by country

Students from European countries and the U.S. were generally more negatively affected by the COVID-19 pandemic compared to students in Asian countries, and students from Taiwan seemed to be the least negatively affected in terms of diet, alcohol consumption, and sleep. The study took place between April and May 2020, during which COVID-19 cases were exponentially growing in the U.S. and in some European countries while the growth of cases started to decline or plateau in China, Malaysia, South Korea, and Taiwan (New Cases of COVID-19 In World Countries, n.d.). Additionally, popular media reported that Taiwan had experienced just 690 novel coronavirus cases and seven deaths as of early December 2020 while the U.S. reported 14 million cases and more than 283,000 deaths (Rauhala, n.d.). As the case

number grew in the U.S. and European countries and more deaths were being reported, students likely experienced increased stress. This supposition is supported by several studies that reported increased mental health concerns among higher education students in China during the Spring of 2020 when the peak number of COVID-19 cases in China emerged (Cao et al., 2020; Sahu, n.d.; Torales et al., 2020; C. Wang, Pan, Wan, Tan, Xu, Ho, et al., 2020; C. Wang, Pan, Wan, Tan, Xu, McIntyre, et al., 2020) and worsened mental health among higher education students and young adults in the U.S. reported both by popular media (Ao, n.d.) and the Centers for Disease Control and Prevention (Czeisler, 2020). Additionally, during the data collection period, the European countries and the U.S. enacted mandatory quarantine, which could also contribute to increased stress (Mattioli, Nasi, et al., 2020) while some Asian countries had lifted the lockdown order. These differences in pandemic-related stress could contribute to some of the differences observed between the countries surveyed.

2.6.2. Health behaviors among students in higher education during the COVID-19 pandemic

Both insufficient sleep and poor sleep quality are health concerns among higher education students (Assaad et al., 2014; Peltzer & Pengpid, 2015); however, the present study observed that poor sleep quality is a more immediate problem for this population. The average PSQI score for all countries was greater than 5, which is consistent with sleep problems (Buysse et al., 2008) and consistent with previous reports regarding high prevalence of poor sleep quality among higher education students (Becker, 2012; Chang et al., 2016; L. Li et al., 2017; van der Heijden et al., 2018). Sleep duration was less of a concern than sleep quality for most countries except South Korea and Taiwan, where more than 40% of students failed to meet the minimal recommended sleep guidelines of 7 hours per night. Unlike other reports (Cao et al., 2020; New Cases of COVID-19 In World Countries, n.d.; Sahu, n.d.), more students in the present study met

the recommended minimum sleep duration guidelines of 7 hours. For example, one meta-analysis of Chinese students' sleeping patterns reported 43.9% of students failing to sleep at least 7 hours per day (L. Li et al., 2017), but the prevalence of insufficient sleep among our Chinese cohort was only 14.4%. Another study, where the majority of students were from the Netherlands, revealed that nearly 25% of students slept less than 7 hours (Kelders, 2019). This contrasts with the present findings where 12.3% of students in the Netherlands did not meet minimum recommendations. Further, a study of higher education students in Malaysia revealed that nearly 60% of students reported sleeping less than 7 hours per day compared to the present study's findings of 34.1% (Peltzer & Pengpid, 2015). The differences in sleeping duration results observed in the present study compared to previously published work could be attributed to the large proportion of students (37.0-55.9% across the different countries) in the present study who reported sleeping more during the COVID-19 pandemic compared to before. While getting an adequate amount of sleep is important, improving sleep quality among students in higher education appears to be a more pressing concern, especially during a stressful event like the COVID-19 pandemic.

Dietary risk scores of students were largely consistent across all countries with few differences observed. These findings are consistent with the current literature (Davoren et al., 2015; T. T.-K. Huang et al., 2004; Jiang et al., 2018; C.-K. Lee et al., 2019; Mansouri et al., 2020; Sa et al., 2020; Van Diepen et al., 2011; Wan Mohamed Radzi et al., 2019). Students from the U.S. reported the highest absolute dietary risk score, which is consistent with the higher prevalence of overweight and obesity among American students and higher BMI compared to students in the other countries studied (Davoren et al., 2015; T. T.-K. Huang et al., 2004; Jiang et al., 2018; C.-K. Lee et al., 2019; Mansouri et al., 2020; Sa et al., 2020; Van Diepen et al., 2011;

Wan Mohamed Radzi et al., 2019). Even though the prevalence of overweight and obesity was higher among U.S. students, previous work reported that the majority of students from the U.S. and other countries did not meet the daily recommended servings of fruit and vegetable intake (Bogerd et al., 2019; Brevard & Ricketts, 1996; Driskell et al., 2005; Kutty et al., 2015; Racette et al., 2005). Additionally, approximately one in three students in the present study reported eating less healthfully during the COVID-19 pandemic compared to before, and almost half of the U.S. students reported eating less healthfully during the COVID-19 pandemic. These findings suggest poor dietary behaviors among students in higher education are global issues, and the COVID-19 pandemic appears to have worsened the problem for a substantial proportion of students.

While a lower proportion of students were classified as alcohol misusers compared to other recent reports (Davoren et al., 2015; Jang et al., 2018; S.-Y. Kim et al., 2015; Mekonen et al., 2017; Verhoog et al., 2020), the findings still warrant concern. For example, a recent report observed two-thirds of Irish students misused alcohol (classified as an AUDIT-C score of greater than 6 among male students and greater than 5 among female students by the study's authors) (Davoren et al., 2015) while the present study reported half of both male and female Irish students were alcohol misusers based on AUDIT-C cut-off scores of greater than 4 for male and 3 for female according to the National Institute on Drug Abuse (Bradley et al., 2003; Bush et al., 1998; Instrument: AUDIT-C Questionnaire | NIDA CTN Common Data Elements, n.d.). Given these lower cut-offs, there is considerable discrepancy between the current study's findings and those of the Davoren group (Davoren et al., 2015). Further, while the current results classified 48.1% of male and 42.9% of female South Korean students as alcohol misusers, others reported 65% of South Korean students were problem drinkers (Jang et al., 2018). It is possible that the

pandemic played a role in these differences as one of the primary reasons for drinking among students involves socializing (Park et al., 2009; Terlecki et al., 2014). The lower prevalence of alcohol misuse could be due, in part, to social distancing rules related to the pandemic that reduced students' ability to socialize. Additionally, it could also be due to changes in living situations, as COVID-19 caused 36% of college students to move, which could contribute to reduced opportunities to socialize (Healthy_Minds_NCHA_COVID_Survey_Report_FINAL.Pdf, n.d.). Despite the reduced prevalence of problem drinking in this sample, alcohol misuse continues to be a grave concern in many countries.

The absolute total METs/week of students in Ireland met the recommended 3000 to 4000 METs per week for disease prevention and health improvement (Kyu et al., 2016) while students in other countries did not. These results were lower than what was previously reported in European countries and the U.S. but similar to what was reported in Asian countries; for example, students in the U.S. reported an average physical activity of 6000 METs/week (Chiang et al., 2013), and students in several European countries reported approximately 3500 to 4600 METs/week (Ćosić Mulahasanović et al., 2018; Mulahasanović et al., 2018), while students in China reported just over 1000 METs/week (H. Liu & Dai, 2017). During the COVID-19 pandemic, approximately half of students in the present study reported lower physical activity levels, which could explain the discrepancy between physical activity levels reported in the current study and previous studies. Overall, the COVID-19 pandemic appeared to negatively influence many students' physical activity levels.

2.6.3. Health behavior analysis

The present study observed that students reporting decreased sleep quality during the pandemic reported higher dietary risk scores, while students reporting improved sleep quality reported less sitting time. The results are consistent with previous studies reporting that higher education students with poor sleep quality consumed less fruit and dairy servings, and poor sleep quality was associated with higher energy intake and poorer diet quality among adult women including young adults (Lotfi & Al-Hosseini, 2015; Zuraikat Faris M. et al., 2020). Poor sleep, including both insufficient and poor quality sleep, may also contribute to low physical activity levels (Dzierzewski et al., 2014; Kline, 2014; Sherrill et al., 1998; Strand et al., 2013) and is associated with sedentary behaviors (Must & Parisi, 2009). Therefore, dietary habits and physical activity levels of students are likely to be improved when students' sleep quality improves.

While both sleep duration and quality are important for health (Dzierzewski et al., 2014; R. Ferranti et al., 2016; Kline, 2014; P.-H. Lin et al., 2018; Sherrill et al., 1998; Strand et al., 2013; Zuraikat Faris M. et al., 2020), the current study observed that poor sleep quality appeared to be a more critical risk factor to health than sleep duration. Most literature suggests that sufficient sleep is associated with a higher quality diet and higher physical activity levels (Dzierzewski et al., 2014; Kline, 2014; P.-H. Lin et al., 2018; Sherrill et al., 1998; Strand et al., 2013), but the current study found that sleep duration change was not consistently related to dietary risk, and sleeping more was associated with lower physical activity levels and more sitting time. These discrepancies might be explained by the high PSQI scores (> 6) reported by all three sleep duration change groups, as higher PSQI scores are associated with unhealthy dietary behaviors (R. Ferranti et al., 2016; Zuraikat Faris M. et al., 2020), less physical activity, and more sedentary behaviors (Dolezal et al., 2017; Kline, 2014). Additionally, the present study

observed that nearly 45% of students reported sleeping more during COVID-19 and 32% of students reported sleeping worse. It is possible that some students compensated for poor sleep quality with increased duration, as previous work reported that longer sleep duration was related to poor subjective sleep quality (Lemola et al., 2013). Therefore, in the present study sample, sleep quality change, rather than sleep duration change, appears to be more closely associated with dietary behaviors and sitting time.

Alcohol misuse is common among higher education students (Davoren et al., 2015; C.-Y. Ji et al., 2012; Mekonen et al., 2017), and previous work indicated college students who reported poor sleep quality consumed alcohol more frequently and excessively and experienced more negative consequences (Babor et al., 2000; Galambos et al., 2009; Lund et al., 2010; Schoenborn & Adams, 2008; M. D. Stein & Friedmann, 2005). Additionally, insufficient sleep impairs cognitive functioning that serves as a protective mechanism against alcohol misuse (Linde & Bergström, 1992; Nilsson et al., 2005; Roehrs et al., 1999; Wimmer et al., 1992). The present study, however, found that changes in sleep quality and duration did not predict higher or lower alcohol misuse scores, which could be explained by the high PSQI scores of all groups compared, as PSQI scores and sleep duration were negatively correlated in this study. Given that all groups compared for changes in sleep duration and quality reported a PSQI score above 5, differences in alcohol misuse might not be detectable.

2.6.4. Public health message

While sleeping adequately is important for health, enhancing sleep quality is also critical; yet, improving higher education students' sleep quality has received little attention (Center for Collegiate Mental Health (CCMH) 2017 Annual Report, n.d.; Prichard & Hartmann, 2019). Higher education students in all countries studied suffered from poor sleep quality, which has

been associated with lower grades, impaired learning ability, worse mood, greater stress, more risk-taking behaviors, and poorer overall health (Afandi et al., 2013; Du et al., 2020; Hartmann & Prichard, 2018; Hershner, 2020; Lund et al., 2010). One study of over 55,000 students reported that stress, binge drinking, and drug use had similar or even smaller associations with academic success compared to sleep disturbance; yet, these risk factors received far more attention by university administrators compared to sleep problems (Hartmann & Prichard, 2018). Improved sleep quality of students is associated with not only better health but also better academic performance, improved graduation rates, and improved future earnings (Prichard & Hartmann, 2019). Further, one study predicted that the economic gain of improving students' sleep exceeds the cost of implementing sleep education programs (Prichard & Hartmann, 2019). Thus, improving sleep among students can likely serve as an effective and efficient way to improve students' future health and economic success.

A variety of strategies can be adopted by universities to improve students' sleep including sleep hygiene education (Hershner & O'Brien, 2018; Levenson et al., 2016), cognitive behavioral therapy for insomnia (CBTi), relaxation, mindfulness, and hypnotherapy (Friedrich & Schlarb, 2018). CBTi is an intervention that involves cognitive, behavioral, and educational interventions (Cognitive Behavioral Therapy for Insomnia (CBT-I), n.d.). CBTi focuses on helping individuals suffering from insomnia to identify and modify thoughts, feelings, and behaviors that contribute to sleep problems. Two systematic reviews have concluded that CBTi-based sleep interventions delivered the largest improvements in a multitude of sleep measures among college students compared to other interventions (Friedrich & Schlarb, 2018) and that online delivery of CBTi is effective (Bowen, 2019). Given the ongoing global pandemic and social distancing requirements in many countries, an online CBTi-based sleep education program

could be a feasible way to improve students' sleep especially when more than 30% of students reported sleeping worse during the pandemic. Ensuring sufficient, good quality sleep not only protects against a variety of chronic diseases (Kita et al., 2012; Y. Liu et al., 2013; Shankar et al., 2010) but also against more immediate health concerns like poor diet quality (Zuraikat Faris M. et al., 2020), mental health concerns (Williams et al., 2020), and alcohol misuse (Chaput et al., 2012). Therefore, university administrators should consider implementing effective strategies, such as CBTi, among university students to improve sleep quality and promote the overall health of students.

2.6.5. Strengths and limitations

There are several strengths to this study. First, the study involved a large sample of students from seven different countries on three continents, which increases the generalizability of the results. Second, the study utilized validated surveys for assessing health behaviors of higher education students. Third, the health behavior differences by country were controlled for in the linear regression models. Fourth, the study included both undergraduate and graduate students, which increased the generalizability of the results to a larger student body.

There are limitations to the study. First, the cross-sectional nature of the study precludes conclusions about the causal relationships between sleep changes and health behaviors changes. Second, the COVID-19 questions included in the survey were not validated; however, validated questionnaires were not available for addressing the specific questions in the study due to the emerging nature of the pandemic. Third, countries included in the study were under different quarantine rules and stages of the pandemic during the data collection period, and these differences could contribute to disparities in and differences in health behaviors. Finally, students

who took the study survey were required to have English proficiency, which might have resulted in not all students who wished to participate being able to do so.

2.7 Conclusions

The present study identified numerous similarities and only a few differences in health behavior risks among higher education students living in seven different countries. Sleeping worse was associated with higher dietary risk scores while sleeping better was associated with less sitting time. Collectively, these data demonstrate that poor sleep quality is a pressing concern among higher education students around the world, especially during the COVID-19 global pandemic. Based on previous work, online CBTi-based sleep education programs appear to be a socially distanced and relatively inexpensive way to improve students' sleep outcomes and overall health.

CHAPTER 3: THE EFFECTS OF SLEEP QUALITY AND RESILIENCE ON PERCEIVED STRESS, DIETARY BEHAVIORS, AND ALCOHOL MISUSE: A MEDIATION-MODERATION ANALYSIS OF HIGHER EDUCATION STUDENTS FROM ASIAN, EUROPE, AND NORTH AMERICA DURING THE COVID-19 PANDEMIC

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3.1 Abstract

Background: The COVID-19 pandemic has increased the already high levels of stress that higher education students experience. Stress influences health behaviors, including those related to dietary behaviors, alcohol, and sleep; yet the effects of stress can be mitigated by resilience. To date, past research studying the connections between dietary behaviors, alcohol misuse, sleep, and resilience commonly investigated singular relationships between two of the constructs. The aim of the current study was to explore the relationships between these constructs in a more holistic manner using mediation and moderation analyses. Methods: Higher education students from China, Ireland, Malaysia, South Korea, Taiwan, the Netherlands, and the United States were enrolled in a cross-sectional study from April to May 2020, which was during the beginning of the COVID-19 pandemic for most participants. An online survey, using validated tools, was distributed to assess perceived stress, dietary behaviors, alcohol misuse,

sleep quality and duration, and resilience. Results: 2254 students completed the study. Results indicated that sleep quality mediated the relationship between perceived stress and dietary behaviors as well as the relationship between perceived stress and alcohol misuse. Further, increased resilience reduced the strength of the relationship between perceived stress and dietary behaviors but not alcohol misuse. Conclusion: Based on these results, higher education students are likely to benefit from sleep education and resilience training, especially during stressful events.

3.2 Keywords

Food intake; nutrition; substance use; college; university students; COVID-19; pandemic; health behaviors; mental health

3.3 Introduction

Globally, higher education students report high levels of stress (America Psychology Association, n.d.; Hodselmans et al., 2018; Ramachandiran & Dhanapal, 2018), which increases the risk of engaging in unhealthy behaviors (Britz & Pappas, 2010; J.-H. Kim & Brown, 2018). Given these high levels of stress, reports from recent studies suggesting the COVID-19 pandemic worsened stress among students in higher education in multiple countries is especially concerning (Husky et al., 2020; Konstantopoulou & Raikou, 2020; Son et al., 2020; Ye et al., n.d.). Unhealthy behaviors related to stress include both poor dietary behaviors (Oliver et al., 2000; Wallis & Hetherington, 2009; Zellner et al., 2006, 2007) and alcohol misuse (Keyes et al., 2012), which can lead to health and social risks (CDC, 2019). Sleep and psychological resilience are two factors that have the potential to alter the relationships between stress and health behaviors. Stress negatively influences sleep (Johnson et al., 2006; E.-J. Kim & Dimsdale, 2007;

Van Reeth et al., 2000) while short sleep and poor sleep quality increase undesirable dietary behaviors, such as increased energy intake and more frequent consumption of sweets (Brondel et al., 2010a; Markwald et al., 2013; Spaeth et al., 2013). Additionally, short sleep and poor sleep quality increase the risk of alcohol misuse (Kenney et al., 2014). Thus, sleep may serve as a mediator in these relationships. Besides sleep, psychological resilience has also been shown to affect the relationships between stress and health behaviors (Houpy et al., 2017; Skrove et al., 2013). Psychological resilience, defined as the processes of adapting and coping quickly when facing a significant source of stress (Fletcher & Sarkar, 2013), reduces the negative effect of stress on health behaviors by improving the ability to cope and manage stress (Carver, 1998; Houpy et al., 2017; Luthar et al., 2000; Skrove et al., 2013). Based on previous work, it is clear that stress, dietary behaviors, alcohol misuse, sleep, and resilience are interconnected.

Current research studying the relationships between these variables commonly investigates a singular relationship between two of the constructs (Brondel et al., 2010a; Houpy et al., 2017; Johnson et al., 2006; Keyes et al., 2012; Oliver & Wardle, 1999; Skrove et al., 2013; Spaeth et al., 2013; Van Reeth et al., 2000); however, the relationships between these constructs are far more complicated. Thus, a more comprehensive understanding of the relationships between stress, dietary behaviors, alcohol misuse, sleep, and resilience is needed to better guide the development of programming to improve the health of higher education students.

To improve our understanding of the interactions between these variables, mediation and moderation analyses were conducted. Mediation analysis examines whether the relationship between an independent variable and a dependent variable is related to an intermediate variable (mediator) (MacKinnon et al., 2007), and moderation analysis determines whether the direction and strength of the relationship between two variables are subject to a moderating variable (A. F.

Hayes, 2017). The objectives of the study were to examine whether (1) sleep quality or duration mediated the relationship between perceived stress and dietary behaviors, (2) sleep quality or duration mediated the relationship between perceived stress and alcohol misuse, (3) resilience moderated the direct and the indirect relationships between perceived stress and dietary behaviors, (4) resilience moderated the direct and the indirect relationships between perceived stress and alcohol misuse. Based on the documented relationships between stress, dietary behaviors, alcohol misuse, sleep, and resilience, hypotheses included:

- (1) Higher levels of perceived stress were likely to be associated with poorer dietary behaviors through decreased sleep quality and sleep duration.
- (2) Higher levels of perceived stress were likely to be associated with higher alcohol misuse through decreased sleep quality and sleep duration.
- (3) Higher levels of resilience were likely to reduce the negative effects of perceived stress on poor dietary behaviors.
- (4) Higher levels of resilience were likely to reduce the negative effects of perceived stress on alcohol misuse.

3.4 Materials and methods

3.4.1 Study design

Undergraduate and graduate students who were at least 18 years old studying at universities in China, Ireland, Malaysia, Taiwan, South Korea, the Netherlands, and the United States (U.S.), were recruited for this study. Students who were less than 18 years old and were not currently enrolled in the surveyed universities were excluded from the study. Students were recruited via university research advertisement systems. The study took place in April and May 2020 during which time period most states in the U.S. and most areas in Ireland, Malaysia, and

the Netherlands had adopted “shelter in place” orders. Most areas of China and Taiwan were under “shelter in place” orders, but some areas had lifted the order. Cross-sectional data were collected online via survey platforms, and all surveys were administered in English.

The study was approved by the Michigan State University Human Research Protection Program (East Lansing, MI, USA), STUDY00004285, 4/7/2020; International Medical University Joint Committee on Research and Ethics (Kuala Lumpur, Malaysia), 481/2020, 5/14/2020; Faculty of Governance and Global Affairs Ethics Committee (The Hague, South Holland, Netherlands), 2020-009-LUC-Cho, 5/25/2020; Indiana University Institutional Review Board for the Protection of Human Subjects (Indiana, PA, USA), IRB Log 20-101, 5/15/2020; Institute Research Ethics Committee, Institute of Technology, Sligo (Sligo, Ireland), ref 2020015, 5/5/2020; University of Taipei Institutional Review Board (Taipei, Taiwan), IRB-2020-045, 6/16/2020; and Bowling Green State University Office of Research Compliance (Bowling Green, OH, USA; 1599753 (US students), 4/29/2020; 1599753 (Chinese students), 5/22/2020; 1599753 (Korean students), 5/11/2020. Consent was obtained from all participants prior to the start of the study.

3.4.2 Demographics and biological information

Age, sex, class status (undergraduate vs. graduate), citizen status (international vs. domestic), and self-reported weight and height were collected. Graduate students included those pursuing masters, doctoral, and professional degrees. International students were attending a higher education institution in a country other than their citizenship; whereas, domestic students were attending a higher education institution in the country of their citizenship. Body mass index (BMI) was calculated from the re-reported weight and height.

3.4.3 Assessment of perceived stress and resilience

Perceived stress was assessed using the Perceived Stress Scale-10 (PSS-10). The PSS-10 is a validated survey (Cohen et al., 1983) that provides a score ranging from 0 to 40. Based on PSS-10 scoring, scores from 0 to 13, 14 to 26, and 27 to 40 indicate low, moderate, and high levels of stress, respectively.

Resilience was measured using the Brief Resilience Scale (BRS), which is a validated tool that measures psychological resilience (B. W. Smith et al., 2008). The global resilience score ranges from 0 to 5, with a higher score indicating higher resilience.

3.4.4 Assessment of dietary behaviors and alcohol misuse

Dietary behaviors were assessed using an eight-item simplified food frequency questionnaire (FFQ)—Starting the Conversation (STC) (Paxton et al., 2011). The STC provides a global score of dietary behaviors ranging from 0 to 16; the higher the score, the more dietary behaviors that are not consistent with health.

Alcohol misuse was assessed using the Alcohol Use Disorders Identification Test Alcohol Consumption questionnaire (AUDIT-C), which provides a global alcohol mis-use score. The AUDIT-C has been validated in many countries among university students (Aertgeerts et al., 2000; Bush et al., 1998; Davoren et al., 2015). A standard drink equivalents reference chart was provided at the beginning of the AUDIT-C survey section to help participants quantify drink servings (National Institute on Alcohol Abuse and Alcoholism (NIAAA), 2015). AUDIT-C scores range from 0 to 12, with a score of 4 and higher for men and 3 and higher for women indicating alcohol misuse (AUDIT-C Frequently Asked Questions, n.d.). Survey logic was set to show these questions only to students who met the minimum drinking age.

3.4.5 Assessment of sleep quality and duration

Subjective sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI), which has been well validated in many populations, including university students (Beaudreau et al., 2012; Buysse et al., 2008; Guo et al., 2016; Marques et al., 2013). PSQI scores range from 0 to 21. Higher scores indicate poorer sleep quality, and a score of ≥ 5 indicates poor sleep quality (Buysse et al., 1989). Weekday and weekend sleep duration were assessed by asking participants how many hours they usually sleep during the weekdays and weekends. Average sleep duration was calculated using the equation, $[(\text{weekday sleep duration} \times 5) + (\text{weekend sleep duration} \times 2)] / 7$. Participants were classified as meeting sleep duration guidelines if they reported sleeping at least 7 h/night (Hirshkowitz et al., 2015).

3.4.6 Assessment of the influence of the COVID-19 pandemic on the factors described above

Questions about how the COVID-19 pandemic affected participants' dietary intake, alcohol consumption, perceived stress, resilience, sleep quality, and sleep duration were asked. Examples of questions included: Are your answers to the perceived stress questions affected by the COVID-19 pandemic? Possible answers included: During the pandemic, I have less perceived stress than usual; during the pandemic, I have more perceived stress than usual; no change of stress during the pandemic. Has the COVID-19 pandemic influenced your alcohol intake? Possible answers included: Yes, I have been drinking more; yes, I have been drinking less; no, it has not influenced my alcohol intake; I don't drink alcohol.

3.4.7 Mediation and moderation models

To test the hypotheses stated in the introduction, four moderated mediation models were proposed (Figure 1).

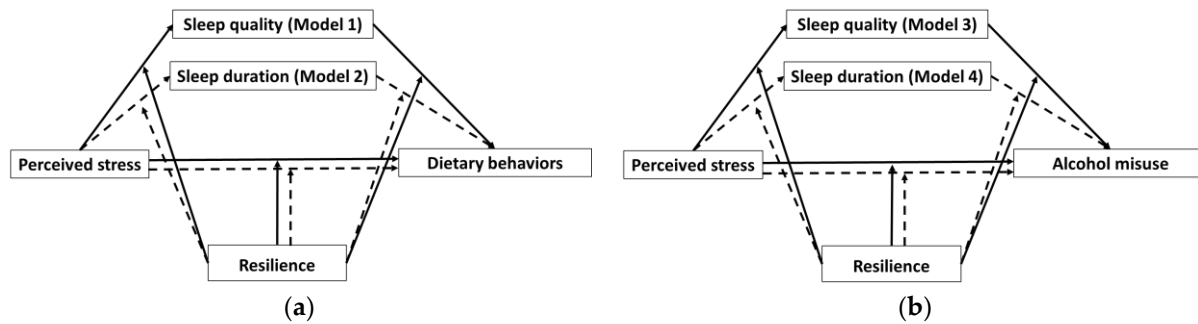


Figure 1. Proposed moderated mediation models.

(a) Perceived stress on dietary behaviors through the mediation of sleep quality and the moderation of resilience (Model 1, solid line) and perceived stress on dietary behaviors through the mediation of sleep duration and the moderation of resilience (Model 2, dashed line). (b) Perceived stress on alcohol misuse through the mediation of sleep quality and the moderation of resilience (Model 3, solid line) and perceived stress on alcohol misuse through the mediation of sleep duration and the moderation of resilience (Model 4, dashed line).

3.4.8 Statistical analysis

Only completed surveys were included in data analysis, and data were analyzed using IBM SPSS Version 26 (IBM Corporation, Armonk, New York, USA). Descriptive statistics were performed, and data were presented in percentage (%) or mean \pm standard deviations (SD). Zero-order correlations were conducted to examine the relationships between all constructs included in mediation and moderation analysis models. Statistical significance was determined using Bonferroni adjusted p values. A total of 28 comparisons were performed in the correlation analysis; therefore, statistical significance was determined at $p < 0.0018$ ($0.05 \div 28$).

Moderated mediation analyses were conducted using the SPSS PROCESS Macro developed by Hayes (A. F. Hayes, 2017). All models were adjusted for age, sex, and BMI. The number of bootstraps performed for bias corrected bootstrap confidence intervals was set at 10,000. All variables entered in the models were approximately normally distributed after excluding outliers, which was defined by above or below mean \pm 3SD. PROCESS was performed for each model by first selecting Model 59 (A. F. Hayes, 2012, 2017) and then

entering one in-dependent variable (perceived stress), one mediator (sleep quality or sleep duration), one moderator (resilience), and one dependent variable (dietary behaviors or alcohol misuse). Statistical significance was determined by $p < 0.05$ for all analyses and the 95% confidence interval (CI) not crossing zero for the indirect effect testing of the mediation analyses.

To interpret the results of the mediation analyses, the following conditions were used. (1) A variable is considered a mediating variable when a significant indirect effect of an independent variable on a dependent variable through the mediator (bootstrap results) is noted. (2) The singular direct relationships between an independent, mediating, and dependent variable do not need to be significant in order for the mediation effect to be significant (A. F. Hayes, 2017).

For moderation analyses, the following principles were applied when conducting the analyses. (1) For significant moderation effects, a statistically significant transition point can be identified using the Johnson-Neyman method (Potthoff, 1964); (2) A moderator can moderate the relationship between an independent and a dependent variable directly or indirectly. Direct effects of a moderator are observed when the relationship between the independent and dependent variables is conditional to the moderator while holding the mediator constant. In contrast, testing for indirect effects of a moderator examines two conditional relationships. First, testing examines whether the relationship between an independent variable and a mediator is conditioned to a moderator. Second, testing examines whether the relationship between a mediator and a dependent variable is conditioned to a moderator. In summary, the differences between direct and indirect effects are that an indirect effect includes the mediator in the analysis while the direct effect does not.

To interpret the results of the moderation analyses, the following condition was used. Significant moderation between any two variables depends on the significant association between the two variables. For example, if a moderator significantly moderates the relationship between an independent variable and a mediator, then the relationship of the independent variable on the mediator must be significant (A. F. Hayes, 2017).

3.5 Results

3.5.1 Demographics

A total of 2663 students initiated the survey, and a total of 2254 students completed the study and were included in the data analysis (Table 8). The completion rate for the study was 84.6%. The majority of the students were female (66.7%), undergraduate (79.9%), domestic (87.0%), and studying in the U.S. (58.7%) (Table 9). Social restrictions varied between the locations during the data collection period, but all locations surveyed adopted an online instructional format for at least some classes.

3.5.2 Correlations between examined variables

The Pearson zero-order correlation analyses showed that dietary behaviors were positively correlated with perceived stress and sleep quality, negatively correlated with resilience, and not correlated with sleep duration (Table 10). These findings indicate that poorer dietary behaviors (higher STC scores) were associated with greater perceived stress (higher PSS-10 scores), poorer sleep quality (higher PSQI scores), and lower resilience (lower BRS scores). Alcohol misuse was positively correlated with greater perceived stress and poorer sleep quality but not correlated with sleep duration and resilience. Additionally, dietary behavior scores and alcohol misuse scores were not correlated. Perceived stress was positively associated with sleep

quality and negatively associated with resilience, which means that greater perceived stress was associated with poorer sleep quality and less resilience. Further, both sleep quality and sleep duration as well as sleep quality and resilience were negatively associated. These results indicate that poorer sleep quality was associated with shorter sleep duration and less resilience. For the covariates, age was negatively associated with perceived stress and sleep duration but positively associated with resilience, which means that younger age was associated with greater perceived stress, longer sleep duration, and less resilience. Additionally, BMI was positively associated with dietary behaviors, alcohol misuse, perceived stress, sleep quality, and age, which indicates that higher BMI was associated with poorer dietary behaviors, more frequent alcohol consumption, greater perceived stress, poorer sleep quality, and older age.

Table 8. Demographic and biological information

Sex n (%)	Undergraduate vs. Graduate n (%)	Domestic vs. International n (%)	Age (y) Mean ± SD	BMI (kg/m²) Mean ± SD
M = 694 (30.8) F = 1502 (66.7) Other * = 44 (2.0) UD = 14 (0.5) Total = 2254	U = 1802 (79.9) G = 452 (20.1)	D = 1962 (87.0) I = 292 (13.0)	22.5 ± 5.5	24.4 ± 5.6

Note: n = number of participants; M = male, F = female, UD = undisclosed; U = undergraduate students, G = graduate students; D = domestic students, I = international students; BMI = body mass index; SD = standard deviation. * Other included transgender, genderqueer, additional gender category or other, and choose not to disclose.

Table 9. Social restriction information and sample size by location

Location	Social Restriction Measures in Place	n (% to Total)
China	Mixture of online and return to in-person classes	111 (4.9)
Ireland	Online classes only	192 (8.5)
Malaysia	Online classes only	91 (4.0)
South Korea	Mixture of online and return to in-person classes	89 (3.9)
Taiwan	Mixture of online and return to in-person classes	377 (15.0)
The Netherlands	Online classes only	114 (5.1)
United States	Online classes only	1278 (58.7)

Note: Universities surveyed included Hangzhou Normal University in China; Athlone Institute of Technology (AIT), Institute of Technology Sligo (IT Sligo), Letterkenny Institute of Technology (LYIT), Trinity College Dublin (TCD), University of Limerick (UL), Waterford Institute of Technology (WIT), Institute of Technology Tralee (ITT), Dublin City University (DCU), University College Dublin (UCD), Hibernia College, National University of Galway (NUIG), Technological University Dublin (TUD/TU Dublin), Cork Institute of Technology (CIT), Galway-Mayo Institute of Technology (GMIT), University College Cork (UCC), and Griffith College Dublin in Ireland; International Medical University (IMU) in Kuala Lumpur, Malaysia; Hanyang University, Chungnam National University, Seokyeong University, and University of Seoul in South Korea; University of Taipei in Taiwan; Leiden University College in the Netherlands; Michigan State University, Bowling Green State University, and Indiana University of Pennsylvania in the United States.

3.5.3 Health behavior classification of higher education students

More than three fourths of students were classified as experiencing moderate or high stress according to the PSS-10 (Cohen et al., 1983) (Table 11). The majority of students were classified as poor sleepers according to the PSQI (Buysse et al., 1989); however, the majority of students also met the minimum recommended sleep duration of 7 h per night. In terms of alcohol misuse, over one-fourth of male students were classified as alcohol misusers while more than one-fifth of female students were classified as alcohol misusers according to the AUDIT-C (Bush et al., 1998).

Table 10. Zero-order correlations between constructs tested and covariates

Measures ^a	Mean ± SD	1	2	3	4	5	6	7	8
(1) Dietary behaviors (STC scores)	7.8 ± 2.8	-	0.08	0.20 *	0.18 *	0.02	-0.20 *	-0.04	0.18 *
(2) Alcohol misuse (AUDIT-C scores)	3.1 ± 2.7		-	0.09 *	0.14 *	-0.02	0.002	-0.04	0.12 *
(3) Perceived stress (PSS-10 scores)	20.6 ± 6.8			-	0.43 *	-0.05	-0.50 *	-0.12 *	0.12 *
(4) Sleep quality (PSQI scores)	6.8 ± 3.5				-	-0.33 *	-0.28 *	0.04	0.19 *
(5) Sleep duration (hours)	7.5 ± 1.2					-	0.05	-0.11 *	-0.06
(6) Resilience (BRS scores)	3.2 ± 0.7						-	0.09 *	-0.03
(7) Age (years)	22.5 ± 5.5							-	0.17 *
(8) BMI (kg/m ²)	24.4 ± 5.6								-

^a Numbers in parentheses correspond to column numbers. * Indicates a significant correlation at the $p < 0.0018$ level based on Bonferroni adjustment for multiple comparisons. STC = Starting the Conversation, higher scores indicate poorer dietary behaviors (range 0–16); AUDIT-C = Alcohol Use Disorders Identification Test Alcohol Consumption, higher scores indicate more frequent alcohol misuse (range 0–12); PSS-10 = Perceived Stress Scale-10, higher scores indicate greater stress (range 0–40); PSQI = Pittsburgh Sleep Quality Index, higher scores indicate worse sleep quality (range 0–21); BRS= Brief Resilience Scale, higher scores indicate greater resilience (range 0–5); BMI = body mass index; SD = standard deviation.

Table 11. Distribution of health behavior classifications

	Alcohol Misuse	Stress Levels	Sleep Quality	Sleep Duration
Percentage of students for each classification [†]	22.8% misuse female 32.1% misuse male 77.2% no misuse female 67.9% no misuse male	15.0% low stress 63.2% moderate stress 21.8% high stress	60.0% poor sleeper 40.0% good sleeper	72.2% met sleep duration 27.8% did not meet sleep duration

[†] Alcohol misuse was classified as an AUDIT-C score of 4 (of 12) and higher for men and 3 (of 12) and higher for women. Stress levels were classified using PSS-10 scores, and scores from 0 to 13, 14 to 26, and 27 to 40 indicate low, moderate, and high levels of stress, respectively. Poor sleepers were classified using the PSQI scores being ≥ 5 (of 21). Students who reported sleeping at least 7 h per day were classified as meeting sleep duration guidelines.

3.5.4 Influence of the COVID-19 pandemic on health behaviors and mental health of higher education students

Participants’ self-assessments indicated that the COVID-19 pandemic had negative impacts on diet and sleep quality of higher education students but not on alcohol consumption and sleep duration. More than one-third of the students reported worsened diet, and close to one-third of the students reported worsened sleep quality during the COVID-19 pandemic compared to before (Table 12). However, less than 20% of students reported increased alcohol consumption, and more than 20% of students reported drinking less during the COVID-19 pandemic compared to before. Additionally, close to half of students reported sleeping more during the COVID-19 pandemic compared to before. The net effect, which was the difference between students who reported worse/better and more/less, showed that more students reported diet and sleep quality to be worse than before, but alcohol use was lower, and sleep duration was longer compared to before the pandemic.

In terms of mental health, close to two-thirds of the students reported more perceived stress and close to one-third of the students reported less resilience during the COVID-19 pandemic compared to before. The net effect indicated that more students reported perceived stress and resilience to be worse than before.

Table 12. Distribution of health behaviors, perceived stress, and resilience changes during the COVID-19 pandemic

	Diet	Sleep Quality		Alcohol Use *	Sleep Duration	Perceived Stress	Resilience
Worse	35.2%	32.0%	More	16.8%	44.6%	60.2%	19.5%
Better	26.6%	15.5%	Less	21.6%	17.1%	13.0%	29.5%
No change	38.2%	52.3%	No change	39.7%	38.2%	26.8%	51.0%
Net effect	8.6% worse	16.5% worse		4.8% better	27.5% better	47.2% worse	10.0% worse

* Alcohol consumption n = 1761; 21.9% (n = 386) students reported they do not consume alcohol.

3.5.5 Mediation and moderation analysis

The mediation analysis of Model 1 showed a significant direct effect of perceived stress on dietary behaviors, and perceived stress and dietary behaviors were positively correlated ($p < 0.001$). Perceived stress was also positively associated with sleep quality scores ($p < 0.001$), which means that as perceived stress increased, sleep quality de-creased since higher PSQI scores indicate worse sleep quality (Table 13). Sleep quality was not associated with dietary behaviors in this model ($p = 0.916$). However, sleep quality significantly mediated the relationship between perceived stress and dietary behaviors, which was evidenced by the bootstrap result showing a significant indirect effect of perceived stress on dietary behaviors through its effect on sleep quality ($B = 0.02$, 95% CI 0.008 to 0.023). Based on the mediation conditions described in the methods section, sleep quality was still a significant mediator, even though sleep quality was not associated with dietary behaviors.

Table 13. Model 1 mediation analysis

Variables	B	SE	t	p Value
Perceived stress → sleep quality	0.35	0.04	8.42	<0.001
Sleep quality → dietary behaviors	0.01	0.08	0.11	0.916
Perceived stress → dietary behaviors	0.12	0.04	3.12	0.002
Bootstrap	Effect	SE	LL 95% CI	UL 95% CI
Sleep quality	0.02	0.004	0.008	0.023

Two moderation pathways were included in the moderation analyses of whether resilience moderated the relationship between perceived stress and dietary behaviors directly or indirectly through sleep quality (Table 14). The number of paths reflects the number of outcome variables (sleep quality and dietary behaviors) (A. F. Hayes, 2017). Path 1 analysis indicated that resilience significantly moderated the relationship between perceived stress and sleep quality,

based on the significant interaction effect of perceived stress and resilience on sleep quality ($p < 0.001$). As resilience increased, the positive association between perceived stress and sleep quality weakened per the Johnson-Neyman test of indirect effect for Path 1. Further, resilience indirectly moderated the relationship between perceived stress and dietary behaviors by moderating the relationship between perceived stress and sleep quality ($p < 0.001$). The results of the Path 2 analysis indicated that resilience directly moderated the relationship between perceived stress and dietary behaviors, as indicated by the significant interaction effect of perceived stress and resilience on dietary behaviors ($p = 0.023$). As resilience increased, the association between perceived stress and dietary behaviors weakened, and the association disappeared at the resilience score of 3.87 per the Johnson-Neyman test of direct effect for Path 2. Of note, 18.3% of students reported a resilience score above 3.87 on the 5-point scale, while 81.7% scored below based on the Johnson-Neyman test. However, resilience did not indirectly moderate this relationship by moderating the relationship between sleep quality and dietary behaviors because the interaction effect of sleep quality and resilience on dietary behaviors was not significant ($p = 0.313$).

To summarize, the mediation and moderation analysis of Model 1 showed that perceived stress and dietary behaviors were positively correlated, and this relationship was associated with sleep quality. This means that greater perceived stress was associated with poorer dietary behaviors, and the negative effect of perceived stress on dietary behaviors was related to poor sleep quality. Increased resilience may serve as a buffer to weaken the relationship between perceived stress and dietary behaviors, and the direct relationship between perceived stress and dietary behaviors diminished when resilience scores were above 3.87.

The mediation analysis of Model 2 showed a significant direct effect of perceived stress on dietary behaviors, and perceived stress and dietary behaviors were positively correlated ($p < 0.001$). Perceived stress was also negatively associated with sleep duration ($p = 0.005$) (Table 15). However, sleep duration was not associated with dietary behaviors in this model ($p = 0.726$). Additionally, sleep duration did not mediate the relationship between perceived stress and dietary behaviors as indicated by the non-significant bootstrap results ($B = -0.002$, 95% CI -0.004 to 0.0002) of testing whether perceived stress indirectly associated with dietary behaviors through its relationship with sleep duration.

Table 14. Model 1 moderation analysis

Variables				
Moderation Path 1	B	SE	t	p Value
Resilience → sleep quality	-0.65	0.29	-2.21	0.03
Perceived stress ×resilience → sleep quality	-0.05	0.01	-4.35	<0.001
Moderation Path 2	B	SE	t	p Value
Perceived stress ×resilience → dietary behaviors	-0.03	0.01	-2.27	0.023
Sleep quality ×resilience → dietary behaviors	0.02	0.02	1.01	0.313
Conditional Indirect Effect of Resilience in Moderation Path 1	Resilience Scores	Effect (SE)	LL 95% CI	UL 95% CI
	2.44	0.22 (0.02)	0.19	0.25
	3.21	0.18 (0.01)	0.16	0.21
	3.97	0.14 (0.01)	0.12	0.17
Conditional Indirect Effect of Resilience Johnson-Neyman Test	Resilience Scores	Indirect Effect (SE)	LL 95% CI	UL 95% CI
	1.00	0.30 (0.03)	0.24	0.36
	1.80	0.26 (0.02)	0.21	0.30
	2.60	0.21 (0.02)	0.19	0.24
	3.40	0.17 (0.01)	0.15	0.20
	4.20	0.13 (0.02)	0.10	0.16
	4.40	0.12 (0.02)	0.09	0.16
	4.60	0.11 (0.02)	0.07	0.15
	4.80	0.10 (0.02)	0.06	0.14
	5.00	0.09 (0.02)	0.05	0.14
Conditional Direct Effect of Resilience in Moderation Path 2	Resilience Scores	Effect (SE)	LL 95% CI	UL 95% CI
	2.44	0.06 (0.01)	0.03	0.09
	3.21	0.04 (0.01)	0.02	0.06
	3.97	0.02 (0.01)	-0.004	0.05
Conditional Direct Effect of Resilience Johnson-Neyman Test	Resilience Scores	Direct Effect (SE)	LL 95% CI	UL 95% CI
	1.00	0.10 (0.03)	0.04	0.15
	1.80	0.08 (0.02)	0.04	0.12
	2.60	0.06 (0.01)	0.03	0.08
	3.40	0.04 (0.01)	0.01	0.06
	3.87 *	0.02 (0.01)	0.00	0.05
	4.40	0.01 (0.02)	-0.02	0.04
	4.60	0.01(0.02)	-0.03	0.04
	4.80	0.001 (0.02)	-0.04	0.04
	5.00	-0.005 (0.02)	-0.05	0.04

*p = 0.05

Table 15. Model 2 mediation analysis

Variables	B	SE	t	p Value
Perceived stress → sleep duration	-0.04	0.02	-2.79	0.005
Sleep duration → dietary behaviors	0.06	0.18	0.35	0.726
Perceived stress → dietary behaviors	0.12	0.03	3.59	<0.001

Bootstrap	Effect	SE	LL 95% CI	UL 95% CI
Sleep duration	-0.002	0.001	-0.004	0.0002

Two moderation pathways were included in the moderation analyses of whether resilience moderated the relationship between perceived stress and dietary behaviors directly or indirectly through sleep duration (Table 9). Path 1 analysis indicated that resilience did not moderate the relationship between perceived stress and sleep duration since the interaction term of perceived stress and resilience was not associated with sleep duration ($p = 0.09$); therefore, the Johnson-Neyman test was not performed for Path 1. Path 2 analysis indicated that resilience directly moderated the relationship between perceived stress and dietary behaviors ($p = 0.016$), which was consistent with Model 1. Based on the Johnson-Neyman test of the direct effect for Path 2, as resilience increased, the association between perceived stress and dietary behaviors weakened, and the association disappeared at the resilience score of 4.16 (Table 16). In this instance, 10.3% of students reported a resilience score above 4.16 on the 5-point scale based on the Johnson-Neyman test. The cut-off resilience score was slightly higher compared to Model 1 due to differences in constructs included in the models (sleep quality vs. sleep duration). However, resilience did not indirectly moderate the relationship between perceived stress and dietary behaviors by moderating the relationship between sleep duration and dietary behaviors because the interaction effect of sleep duration and resilience on dietary behaviors was not significant ($p = 0.851$).

To summarize, the mediation and moderation analysis of Model 2 demonstrated that perceived stress and dietary behaviors were positively correlated, and this was not associated with sleep duration. This means that greater perceived stress was associated with poorer dietary behaviors, and the negative effect of perceived stress on dietary behaviors was not related to short sleep duration. Once again, greater resilience weakened the relationship between perceived stress and dietary behaviors.

Table 16. Model 2 moderation analysis

Variables				
Moderation Path 1	B	SE	t	p Value
Resilience → sleep duration	-0.21	0.11	-1.89	0.06
Perceived stress × resilience → sleep duration	0.01	0.005	1.72	0.09
Moderation Path 2	B	SE	t	p Value
Resilience → dietary behaviors	0.01	0.50	0.03	0.978
Perceived stress × resilience → dietary behaviors	-0.02	0.01	-2.41	0.016
Sleep duration × resilience → dietary behaviors	0.01	0.06	0.19	0.851
Conditional Indirect Effect of Resilience in Moderation Path 1				
	Resilience Scores	Effect (SE)	LL 95% CI	UL 95% CI
	2.44	-0.03 (0.01)	-0.04	-0.01
	3.21	-0.02 (0.01)	-0.03	-0.01
	3.97	-0.01 (0.01)	-0.02	-0.002
Conditional Direct Effect of Resilience in Moderation Path 2				
	Resilience Scores	Effect (SE)	LL 95% CI	UL 95% CI
	2.44	0.07 (0.01)	0.04	0.09
	3.21	0.05 (0.01)	0.03	0.07
	3.97	0.03 (0.01)	0.01	0.05

Table 16 Contd.

Conditional Direct Effect of Resilience Johnson-Neyman test	Resilience Scores	Direct Effect (SE)	LL 95% CI	UL 95% CI
	1.00	0.10 (0.03)	0.05	0.15
	1.80	0.08 (0.02)	0.05	0.12
	2.60	0.06 (0.01)	0.04	0.09
	3.40	0.04 (0.01)	0.02	0.06
	4.16 *	0.03 (0.01)	0.00	0.05
	4.40	0.02 (0.01)	-0.01	0.05
	4.60	0.01(0.02)	-0.02	0.05
	4.80	0.01 (0.02)	-0.03	0.04
	5.00	0.005 (0.02)	-0.03	0.04

*p = 0.05

The mediation analysis of Model 3 showed that perceived stress positively correlated with sleep quality ($p < 0.001$), but perceived stress was not correlated with alcohol misuse ($p = 0.299$), and sleep quality was also not associated with alcohol misuse ($p = 0.109$) (Table 17). However, sleep quality significantly mediated the relationship between perceived stress and alcohol misuse, given the significant mediation bootstrap result ($B = 0.02$, 95% CI 0.009 to 0.025). Based on the mediation conditions described in the methods section, the mediation effect was still significant, even though the association between sleep quality and alcohol misuse and the association between perceived stress and alcohol misuse were not significant.

Table 17. Model 3 mediation analysis

Variables	B	SE	t	p Value
Perceived stress → sleep quality	0.39	0.05	8.44	<0.001
Sleep quality → alcohol misuse	0.14	0.08	1.60	0.109
Perceived stress → alcohol misuse	-0.05	0.04	-1.04	0.299
Bootstrap	Effect	SE	LL 95% CI	UL 95% CI
Sleep quality	0.02	0.004	0.009	0.025

Two moderation pathways were included in the moderation analyses of whether resilience moderated the relationship between perceived stress and alcohol misuse directly or indirectly through sleep quality (Table 18). Path 1 was the same as Path 1 in Model 1 and had the same results, where resilience moderated the relationship between perceived stress and sleep quality ($p < 0.001$). Path 2 analysis indicated that resilience did not moderate the relationship between perceived stress and alcohol misuse directly or indirectly because both the interaction effect of perceived stress and resilience on alcohol misuse ($p = 0.112$) and the interaction effect of sleep quality and resilience on alcohol misuse ($p = 0.573$) were not significant.

To summarize, the mediation and moderation analyses of Model 3 showed that perceived stress was associated with alcohol misuse only through its relationship with sleep quality. This means that greater perceived stress was associated with more frequent alcohol misuse, and the negative effect of perceived stress on alcohol misuse was related to poor sleep quality. Resilience did not serve as a buffer to alter the relationship between perceived stress and alcohol misuse, but it did weaken the relationship between perceived stress and sleep quality (PSQI).

The mediation analysis of model 4 indicated that perceived stress was not correlated with sleep duration ($p = 0.310$) and alcohol misuse ($p = 0.975$), and sleep duration was not correlated with alcohol misuse ($p = 0.789$) (Table 19). Additionally, sleep duration did not mediate the relationship between perceived stress and alcohol misuse, which was evidenced by the non-significant mediation bootstrap result ($B = 0.0002$, 95% CI -0.0006 to 0.0013).

Two moderation pathways were included in the moderation analyses of whether resilience moderated the relationship between perceived stress and alcohol misuse directly or indirectly through sleep duration (Table 20). Path 1 was the same as Path 1 in Model 2. Path 1 analysis indicated that resilience did not moderate the relationship between perceived stress and sleep duration since the interaction term of perceived stress and resilience was not associated with sleep duration ($p = 0.431$); therefore, the Johnson-Neyman test was not performed for Path 1. The Path 2 moderation analyses of Model 4 indicated that resilience did not moderate the relationship between perceived stress and alcohol misuse directly or indirectly because both the interaction effect of perceived stress and resilience on alcohol misuse ($p = 0.344$) and the

interaction effect of sleep duration and resilience on alcohol misuse ($p = 0.917$) were not significant. Therefore, the Johnson-Neyman test was not performed for Path 2.

Table 18. Model 3 moderation analysis

Variables				
Moderation Path 1	B	SE	t	p Value
Resilience → sleep quality	-0.93	0.32	-2.91	0.004
Perceived stress × resilience → sleep quality	-0.06	0.01	-4.55	<0.001
Moderation Path 2	B	SE	t	p Value
Resilience → alcohol misuse	-0.08	0.28	-0.29	0.771
Perceived stress × resilience → alcohol misuse	0.02	0.01	1.59	0.112
Sleep quality × resilience → alcohol misuse	-0.01	0.03	-0.56	0.573
Conditional Indirect Effect of Resilience in Moderation Path 1	Resilience Scores	Effect (SE)	LL 95% CI	UL 95% CI
	2.49	0.23 (0.02)	0.20	0.27
	3.18	0.19 (0.01)	0.16	0.21
	3.88	0.14 (0.02)	0.12	0.18
Conditional Indirect Effect of Resilience Johnson-Neyman Test	Resilience Scores	Indirect Effect (SE)	LL 95% CI	UL 95% CI
	1.00	0.33(0.03)	0.26	0.39
	1.80	0.28 (0.02)	0.23	0.32
	2.60	0.23 (0.02)	0.20	0.26
	3.40	0.18 (0.01)	0.15	0.20
	4.20	0.13 (0.02)	0.09	0.16
	4.40	0.12 (0.02)	0.07	0.15
	4.60	0.10 (0.02)	0.06	0.15
	4.80	0.09 (0.03)	0.04	0.14
	5.00	0.08 (0.03)	0.02	0.13
Conditional Direct Effect of resilience in moderation path 2	Resilience Scores	Effect (SE)	LL 95% CI	UL 95% CI
	2.49	0.01 (0.02)	-0.02	0.04
	3.18	0.02 (0.01)	-0.003	0.04
	3.88	0.03 (0.02)	0.01	0.06

Table 19. Model 4 mediation analysis

Variables	B	SE	t	p Value
Perceived stress → sleep duration	-0.02	0.02	-1.02	0.310
Sleep duration → alcohol misuse	-0.06	0.21	-0.27	0.789
Perceived stress → alcohol misuse	0.001	0.04	0.03	0.975
Bootstrap	Effect	SE	LL 95% CI	UL 95% CI
Sleep duration	0.0002	0.0004	-0.0006	0.0013

To summarize, the mediation and moderation analysis of Model 4 demonstrated that perceived stress was not associated with alcohol misuse through its relationship with sleep duration. Resilience did not have any effect on the relationship between perceived stress and sleep duration and the relationship between perceived stress and alcohol misuse.

Table 20. Model 4 moderation analysis

Variables	B	SE	t	p Value
Moderation Path 1				
Resilience → sleep duration	0.01	0.12	-1.02	0.310
Perceived stress × resilience → sleep duration	0.004	0.01	0.79	0.431
Moderation Path 2				
Resilience → alcohol misuse	-0.07	0.58	-0.13	0.900
Perceived stress × resilience → alcohol misuse	0.01	0.01	0.95	0.344
Sleep duration × resilience → alcohol misuse	0.01	0.07	0.10	0.917
Conditional Direct Effect of Resilience in Moderation Path 2	Resilience Scores	Effect (SE)	LL 95% CI	UL 95% CI
	2.49	0.03 (0.02)	0.001	0.06
	3.18	0.04 (0.01)	0.02	0.06
	3.88	0.04 (0.01)	0.03	0.07

3.6 Discussion

Insufficient sleep is associated with an increased energy intake (Brondel et al., 2010b) and an increased risk of obesity (Gangwisch et al., 2005). This study evaluated the effect of modest sleep curtailment on hunger, food cravings, food reward, and portion size, all of which have been shown to contribute to excess intake and possible weight gain, but previous interventional studies have used more extreme curtailments (Brondel et al., 2010b) or even total deprivation (Benedict et al., 2012a; Hogenkamp et al., 2013; Rihm et al., 2018). The modest curtailment in this study resulted in significantly reduced TIB, TST, SWS, and REM sleep durations as well as reduced subjective sleep quality. The percentage of REM and SWS ranged from ~21–~29% during both nights, which is consistent with healthy sleep (Carskadon & Dement, n.d.). The reduction in sleep duration resulted in participants feeling hungrier, reporting both increased food cravings and increased susceptibility to food reward, and selecting larger portions from meal items during lunch time, even though the same amount of breakfast foods and snacks were consumed at the same time on both days. This study examined the complex relationships between perceived stress, dietary behaviors, alcohol misuse, sleep quality and duration, and resilience. Results indicated that sleep quality, but not sleep duration, mediated the relationship between perceived stress and dietary behaviors as well as the relationship between perceived stress and alcohol misuse. Additionally, increased resilience weakened the relationship between perceived stress and dietary behaviors, but resilience did not serve as a buffer to alter the relationship between perceived stress and alcohol misuse. Therefore, improving sleep quality and resilience among higher education students are likely to reduce poor dietary behaviors especially during a high stress situation, like the COVID-19 pandemic. Additionally, improving sleep quality would also appear likely to reduce alcohol misuse among higher education students. However, these possible interventions will need to be empirically tested.

3.6.1. Mediation effects of sleep quality and sleep duration on the relationship between perceived stress and dietary behaviors

As hypothesized, sleep quality mediated the relationship between perceived stress and dietary behavior. Previous work reported that stress negatively impacts dietary behaviors through reward signal pathways in the brain (Adam & Epel, 2007), where high stress motivates consumption of highly palatable foods. While there are many factors that influence the reward system in the brain, sleep plays a major role in its regulation (Gujar et al., 2011; Krause et al., 2017; Neisewander, 2020). Poor sleep quality heightens reward system responses (Telzer et al., 2013), which results in higher consumption of energy and added sugar (Boozari et al., 2020; Zuraikat Faris M. et al., 2020) and lower intake of fruits and vegetables (Min et al., 2018). Thus, the role of sleep as a mediator in the relationship of perceived stress and dietary behaviors has a plausible physiological mechanism.

Like poor sleep quality, insufficient sleep also results in poor dietary behaviors (Benedict et al., 2012b; Brondel et al., 2010b; Greer et al., 2013; Hanlon et al., 2005; Markwald et al., 2013; Moubarac et al., 2013; Spaeth et al., 2013; St-Onge et al., 2011; C.-L. Yang et al., 2019). This may be explained by the fact that, like poor sleep quality, insufficient sleep also amplifies the brain's reward system (Gujar et al., 2011; Neisewander, 2020). However, the present study re-reported a non-significant mediation effect of sleep duration on the relationship between perceived stress and dietary behaviors. This null finding could be explained by the fact that the average sleep duration of the students (7.5 ± 1.2 h) exceeded the minimum recommended sleep duration of 7 h per day (Hirshkowitz et al., 2015). Previous large cross-sectional studies reported similar results compared to the present study, where students living in different countries, including China (Peltzer & Pengpid, 2016), the Netherlands (Steptoe, 2006) and the U.S. (Steptoe, 2006), generally reported sufficient sleep. Higher education students tend to get enough

sleep, on average, when counting both weekday and weekend sleeping hours since students engage in catch-up sleep during the weekends or when schedules allow (Borisenkov et al., 2019; Haraszti et al., 2014; Jankowski, 2015; Moubarac et al., 2013). Unlike the present study, previous studies noting a significant relationship between sleep duration and poor dietary behaviors frequently reported insufficient sleep in the study populations (Brondel et al., 2010b; Markwald et al., 2013; Spaeth et al., 2013). Thus, adequate sleep duration of students in the present study could be the reason why sleep duration did not mediate the relationship between stress and dietary behaviors.

3.6.2. Mediation effects of sleep quality and sleep duration on the relationship between perceived stress and alcohol misuse

As hypothesized, sleep quality mediated the relationship between perceived stress and alcohol misuse. This finding is consistent with other work which reported that sleep quality mediated the relationship between poor mental health, which included de-pression, anxiety, and stress, as well as frequent alcohol use (Kenney et al., 2013). Alcohol consumption among 18 to 25 year-olds is widespread and is commonly used as a strategy to cope with stress (National Survey on Drug Use and Health (NSDUH-2018) | SAMHDA, n.d.). While stress can trigger more frequent alcohol misuse, stress also leads to poor quality sleep (Johnson et al., 2006; E.-J. Kim & Dimsdale, 2007). Additionally, the influence of sleep on alcohol misuse behaviors has been attributed, in part, to the alteration of neurocognitive function by poor sleep (Thomas et al., 2000). Poor sleep reduces neural activity in the prefrontal cortex, which carries out higher order executive functions, such as decision making, inhibitory function, and self-monitoring (Linde & Bergström, 1992; Nilsson et al., 2005; Thomas et al., 2000; Wimmer et al., 1992). Thus, sub-optimal executive function may lead to dangerous behaviors, such as excessive drinking, when

sleep is compromised (Vail-Smith et al., 2009). Further, a study of university students revealed that poor sleep predicted drinking to cope with stress (Digdon & Landry, 2013), which can potentially set up a vicious cycle since frequent alcohol consumption negatively influences sleep quality by disrupting normal sleep cycles (Thakkar et al., 2015). Therefore, sleep quality serves as an intermediate factor that explains why perceived stress and alcohol misuse are connected.

Contrary to our hypothesis, sleep duration did not mediate the relationship between perceived stress and alcohol misuse. Like poor sleep quality, insufficient sleep has been shown to alter the decision-making process, leading to more frequent risky behaviors (Linde & Bergström, 1992; Nilsson et al., 2005; Thomas et al., 2000; Wimmer et al., 1992). The failure to observe sleep duration as a mediator between stress and alcohol misuse in the present study could, again, be attributed to the fact that the student cohort reported sufficient sleep. Unlike the present study, previous work where associations between sleep duration and alcohol misuse were observed frequently reported insufficient sleep among participants (Chaput et al., 2012; Mike et al., 2016; Strine & Chapman, 2005). Taken together, sufficient sleep observed among the present student cohort could explain the non-significant mediation effect of sleep duration on the relationship between perceived stress and alcohol misuse.

Despite students having sufficient sleep on average in the present study, the average sleep quality was poor, which is consistent with other studies (Balduf, 2009; Brevard & Ricketts, 1996; Driskell et al., 2005; Racette et al., 2005; Verhoog et al., 2020). The present study occurred during the beginning of the COVID-19 pandemic, when many locations surveyed adopted “shelter in place” orders, and the majority of the students were under quarantine, which could change sleep patterns (Bush et al., 1998; Jang et al., 2018; S.-Y. Kim et al., 2015; Mekonen et al., 2017). As shown in the present study, 44.6% of students reported sleeping more

during the COVID-19 pandemic compared to before. Additionally, recent studies noted that university students spent more time in bed during the pandemic compared to before but with poorer sleep quality (Bush et al., 1998; Jang et al., 2018; S.-Y. Kim et al., 2015), which is also consistent with our finding that nearly one-third of students reported worsened sleep quality. Previous work shows that longer sleep duration was related to poorer subjective sleep quality (Bradley et al., 2003); thus, it is possible that the adequate sleep duration observed in the present student cohort reflects poor sleep quality. Taken together, our findings and the previous evidence suggest poor sleep quality may be an emergent issue to be addressed among higher education students in order to reduce the negative effect of stress on dietary behaviors and the negative effect of stress on alcohol misuse.

3.6.3. Moderation effects—resilience

Besides sleep quality, resilience has been noted to play an important role in reducing the negative effect of stress on dietary behaviors (Grubic et al., 2020; Zolotov et al., 2020), and this was corroborated in the present study. Higher resilience weakened the relationship between stress and undesirable dietary behaviors, which could be explained by resilience serving as a positive coping strategy for stress (Instrument: AUDIT-C Questionnaire | NIDA CTN Common Data Elements, n.d.; Park et al., 2009). Previous work showed that individuals who display greater resilience cope better with adversity; therefore, the negative effects of stressful events, like poor dietary behaviors, are reduced with increased resilience (Grubic et al., 2020; Zolotov et al., 2020). Thus, higher education students are likely to benefit from improved resilience to reduce the negative effect of stress on dietary behaviors.

Contrary to our hypothesis, the present study noted that resilience did not play a role in reducing the negative effect of stress on alcohol misuse. While alcohol misuse can be a

consequence of stress (Hoevenaar-Blom et al., 2011), resilience did not moderate the relationship between perceived stress and alcohol misuse, which could be attributed to the lower alcohol misuse among the student cohort in the present study during the COVID-19 pandemic compared to previous studies (Healthy_Minds_NCHA_COVID_Survey_Report, n.d.; Terlecki et al., 2014; Vlahov et al., 2004). In the present study, most students reported either reduced or no change in alcohol consumption during the COVID-19 pandemic compared to before. Among higher education students, socializing is the most important driving factor for alcohol consumption (Ćosić Mulahasanović et al., 2018; Mulahasanović et al., 2018). The COVID-19 pandemic reduced the opportunity for socializing among higher education students, and many students changed living situations, which could further restrict socializing (H. Liu & Dai, 2017). Thus, these factors likely contributed to lower risk of alcohol misuse among higher education students during the COVID-19 pandemic. In summary, the non-significant moderating effect of resilience on the relationship between perceived stress and alcohol misuse is likely attributed to the lower alcohol misuse among higher education students during the COVID-19 pandemic.

3.6.4. Public health messages

Findings from the present study suggest that poor sleep quality is associated with health concerns. Currently, interventions for sleep quality improvement have received little attention by those working with higher education students (Lotfi & Al-Hosseini, 2015; Zuraikat Faris M. et al., 2020). Poor sleep quality is not only associated with poor dietary behaviors and alcohol misuse, but also associated with poor academic performance (Kline, 2014; Strand et al., 2013), obesity (Sherrill et al., 1998), higher risk of chronic diseases (Dzierzewski et al., 2014), and poorer overall health (Must & Parisi, 2009). One large study of higher education students showed that sleep disturbance had a stronger association with academic success compared to

stress, binge drinking, and drug use; however, addressing sleep concerns in the student population is not routinely, or even frequently, done (R. Ferranti et al., 2016). The present study showed that improving sleep quality may be an effective strategy to improve the health of higher education students and warrants further exploration of how improving sleep quality can improve other health outcomes.

Sleep hygiene education, cognitive behavioral therapy (CBT), relaxation, mindfulness, and hypnotherapy are effective strategies to improve sleep among higher education students (Dolezal et al., 2017; Lemola et al., 2013; P.-H. Lin et al., 2018); thus, universities should consider incorporating these strategies to improve sleep quality of these students. One systematic review indicated increased effectiveness of CBT compared to other interventions in improving sleep quality and duration (Lemola et al., 2013), and another systematic review showed that online CBT for insomnia is effective in improving sleep quality and duration among adults with chronic insomnia by identifying and modifying factors that contribute to poor sleep, including stress (C.-Y. Ji et al., 2012). Given that students suffered from a decline in sleep quality during the pandemic (Bush et al., 1998; Jang et al., 2018; S.-Y. Kim et al., 2015), an online CBT sleep education program can serve as a feasible way to address a critical health concern of higher education students. As poor sleep quality mediated the relationship between stress and dietary behaviors and the relationship between stress and alcohol misuse, improving sleep quality during a stressful event may prevent health decline of higher education students during this time. Thus, universities should consider initiating and adopting a feasible form of sleep education to improve the health of higher education students.

Higher education students are also likely to benefit from receiving resilience training to improve health. Resilience training is more commonly used among healthcare and medical

students rather than general higher education students (Galambos et al., 2009; Lund et al., 2010). Limited research conducted among general higher education students indicated that resilience training was just as effective as CBT in improvement of mental health among university students (Schoenborn & Adams, 2008), and one interventional study showed that a mindfulness-based intervention to increase resilience significantly decreased stress compared to traditional mental health support offered by university counseling centers (Babor et al., 2000). Therefore, resilience training should be considered as a student service in university counseling centers given that it shows promise in reducing stress.

3.6.5. Strengths and limitations

There are several strengths to this study. First, the large sample size provided adequate power to conduct the mediation and moderation analyses. Second, using the mediation and moderation analyses allowed for the exploration of more complex relationships known to exist between stress, dietary behaviors, alcohol misuse, sleep, and resilience. Third, the study included students from Asia, Europe, and North America and included both undergraduate and graduate students, which increased the generalizability of the results. Finally, the study used validated questionnaires.

There are limitations to the study. First, the cross-sectional nature of the study does not allow for the attribution of causality between the variables examined. Second, weight and height were self-reported because in-person testing was not allowed during the pandemic, and the possibility of misreporting cannot be ruled out. Third, the social-restriction rules were different among the locations surveyed during the data collection period. Due to the complexity of social restriction rules among and even within locations, it is difficult to include social restrictions as a covariate; however, the information was collected and reported in Table 2. Fourth, the STC

questionnaire, which measures eating frequencies of both healthy and unhealthy foods, does not measure problematic eating behaviors such as uncontrolled or emotional eating. Thus, the dietary behaviors construct presented in this paper only refers to eating frequencies of certain foods. Fifth, questions regarding how the COVID-19 pandemic impacted health behaviors and mental health of higher education students were not validated due to there being no validated questionnaires available when the study was conducted. Sixth, most students in the study self-identified as female undergraduate domestic students, and students from the U.S. and Taiwan were over-represented compared to other participants in this study. The sample population in the study could limit the generalizability of the study results. Seventh, information regarding mental illness and treatment was not collected in the survey due to privacy concerns; therefore, mental illness diagnoses and treatments were not adjusted for in the models examined. Finally, all surveys were administered in English, which restricted the participation of students with low English proficiency.

3.7 Conclusions

The present study identified that sleep quality, but not sleep duration, mediated the relationship between perceived stress and dietary behaviors and the relationship between perceived stress and alcohol misuse. Additionally, increased resilience weakened the relationship between perceived stress and dietary behaviors; the negative effect of high perceived stress on dietary behaviors disappeared at higher resilience scores. These findings suggest that incorporating sleep quality improvement training and resilience training into university health promotion programs would likely be effective strategies to improve the health of higher education students, especially during stressful events. However, these conclusions need to be further tested with interventional approaches.

Chapter 4: THE EFFECTS OF ACUTE SLEEP CURTAILMENT ON SALT TASTE MEASURES AND RELATIONSHIPS WITH ENERGY-CORRECTED SODIUM INTAKE: A RANDOMIZED CROSS-OVER TRIAL WITH METHODOLOGY VALIDATION

4.1 Abstract

Taste plays a major role in determining food choices; however, taste measures are not always associated with dietary intake. Sleep may be a factor that influences the taste-dietary intake relationship. The effect of sleep on salt taste measures has not been adequately studied, and no consensus methodology has been developed for measuring salt taste preference. Therefore, a randomized cross-over trial (RCT) with methodology validation was conducted. The methodology validation component of the present study sought to examine whether an adapted version of a sweet taste forced-choice paired-comparison test could be used to determine salt taste preference. This test determines preferred concentration by taking the geometric mean of two taste trials. Validation analyses were conducted using data collected from the habitual sleep condition. The RCT examined the effects of sleep curtailment on salt taste liking, intensity, and preferred aqueous NaCl concentration and explored whether these measures were associated with energy-corrected sodium (Na) intake. Participants slept a curtailed night (33% sleep duration reduction) and a habitual night wearing a single-channel electroencephalograph. Salt taste tests were conducted the day after each sleep condition using 5 aqueous NaCl solutions. One 24-hour dietary recall was obtained after each taste test. Validation results indicated that the adapted forced-choice paired-comparison tracking test can serve as a reliable tool for determining salt taste preference as each NaCl concentration intensity rating was rated differently ($p < 0.001$), and the preferred salt concentrations from the two trials were not different from each other ($p = 0.078$) nor were they different from the geometric mean of the two trials

($p=0.948$ for trial 1, $p=0.089$ for trial 2). Additionally, the preferred salt concentration was associated with liking slope ($p<0.001$). The RCT demonstrated no changes in salt taste function (slopes of intensity ratings: $p=0.844$) and hedonic measures (slopes of liking ratings: $p=0.074$; preferred NaCl concentrations: $p=0.092$) after a night of curtailed sleep compared to habitual sleep. However, sleep curtailment disrupted the association between liking slope and energy-corrected Na intake observed during habitual sleep ($p<0.001$). Researchers should consider working toward more standardized taste assessments to facilitate comparison between studies and accounting for sleep when exploring taste-diet relationships.

4.2 Keywords

Salt taste preference; salt taste function; sleep curtailment; taste measures; study protocol; method; forced-choice paired-comparison tracking test

4.3 Introduction

Despite taste playing a critical role in determining dietary intake (J. E. Hayes et al., 2010; Monteleone et al., 2017; van Langeveld et al., 2017), the ability of taste measures to predict intake is variable (Tan et al., 2021; Tan & Tucker, 2019). Using salt taste measures as an example, some studies reported hedonic measures, including liking and preference of salty stimuli, were positively correlated with dietary salt and salty food intake (Cornelis et al., 2017; J. E. Hayes et al., 2010; Pilic et al., 2020; Stone & Pangborn†, 1990) while other studies reported salt liking did not predict dietary salt intake (Pangborn & Pecore, 1982; Shepherd et al., 1984). Relationships between diet and salt taste measures reflecting function, like taste thresholds and intensity ratings, were also variable; some studies reported these measures did not predict intake (Inoue et al., 2017; Ishida et al., 1985; H. Lee et al., 2014; Leong et al., 2018; Martinelli et al.,

2020; Veček et al., 2020) while others reported they did (Azinge et al., 2011; Cattaneo et al., 2019; Han et al., 2017; Low et al., 2016). Given the variability of findings, exploring factors that could be obscuring taste-diet relationships is worthwhile.

Sleep is one physiological process that has been shown to alter measures of taste (Szczygiel, Cho, Snyder, et al., 2019; Szczygiel, Cho, & Tucker, 2019a) as well as dietary intake (Brondel et al., 2010a; Calvin et al., 2013; Markwald et al., 2013). Increases in preferred concentrations for sucrose (Szczygiel, Cho, Snyder, et al., 2019; Tajiri et al., 2020) and sucralose were observed after one night of curtailed sleep (Szczygiel, Cho, Snyder, et al., 2019). When testing sweet taste using solid food, sweeter oat crisps were liked more after curtailed sleep compared to habitual sleep (Szczygiel, Cho, & Tucker, 2019b). In summary, one night of short sleep altered hedonic measures of sweet taste, which can lead to undesirable dietary behaviors such as increased consumption of sweets and high calorie foods (Calvin et al., 2013; Simon et al., 2015; Spaeth et al., 2013; St-Onge et al., 2011; C.-L. Yang et al., 2019).

While alterations in sweet taste after sleep curtailment have been studied, there is limited investigation into the effects of sleep on salt taste. One cross-sectional study reported an association between short sleep duration (< 6 hours) and increased odds of self-reported altered taste perception in general (Gao et al., 2021). A separate study demonstrated self-reported sleepiness was not associated with salt taste sensitivity but with cravings for savory foods (Lv et al., 2018). There are two experimental studies which investigated the effects of sleep deprivation, defined by the complete absence of sleep, and sleep curtailment, referred to as shortened sleep duration, on salt taste function. One study reported no change in salt taste detection threshold after 24, 48, and 72 hours of sleep deprivation (Furchtgott & Willingham, 1956) while the other study demonstrated no differences in salt taste sensitivity measured by detection threshold after

long (> 7 hour) versus short (< 7 hour) sleep (S. L. Smith et al., 2016). As far as we know, the studies discussed above are the only investigations done on the topic of the effects of sleep on salt taste measures.

There are several limitations to the previous studies investigating the effects of sleep on salt taste measures. First, none of the studies included hedonic measures of salt taste, which may be more important predictors of dietary intake than thresholds (Tan et al., 2021; Tan & Tucker, 2019). Second, the complete absence of sleep is not commonly experienced in the general adult population (CDC - Data and Statistics - Sleep and Sleep Disorders, 2021; Chattu et al., 2018). Third, the study that compared short versus long sleep duration did not prescribe the curtailed sleep duration based on the habitual sleep duration of participants, which could have created an unstandardized sleep curtailment duration for individuals, i.e., some individuals were curtailed less than others. This methodological approach could reduce the ability to detect differences, should they exist. Given the small number of studies and these limitations, research investigating the effects of sleep curtailment on both hedonic and functional measures of salt taste is needed.

The present report includes two components to address three aims: part one validates a salt taste preference evaluation method, and part two is experimental. First, since a standardized salt preference test does not exist (Tan et al., 2021), the methodology validation component of the study aimed to examine whether an adapted version of a forced-choice paired-comparison test developed for sweet taste (Mennella et al., 2011) could be used in determining salt taste preference. Second, the experimental component aimed to investigate the effects of sleep curtailment on salt taste hedonic measures (liking and preferred concentration) and function (intensity) was undertaken. Third, an exploration of whether any of the taste tests were

associated with energy-corrected sodium intake was conducted. The researchers hypothesized that:

1. The adapted forced-choice paired-comparison tracking test could serve as a valid and reliable tool to determine salt taste preference.
2. Salt taste liking, measured by the slope of liking ratings of five salty solutions across different concentrations would be steeper after a night of curtailed sleep compared to a night of habitual sleep; however, salt taste function, measured by the slope of intensity ratings would not be affected. Additionally, preferred salt concentration, measured by the newly developed test, would increase.
3. Hedonic measures of salt taste (liking slope and preferred salt concentration) and salt taste function (intensity slope) would be positively associated with energy-corrected Na intake after both the habitual and the curtailed night of sleep.

4.4 Methods

4.4.1 Participants

Participants, including both males and females, between the ages of 18 and 45 with no diagnosed sleep conditions who reported habitually sleeping 7 to 9 hours per night and had regular weekday bedtimes were recruited for the study. Potential participants who had conditions that may affect taste function and dietary intake, such as type 2 diabetes and cardiovascular diseases, were excluded from the study. A screening questionnaire was used to check eligibility. Sleep quality measured with the Pittsburg Sleep Quality Index (PSQI) was examined at screening for recruiting approximately equal numbers of good and poor sleepers. The validated Pittsburgh Sleep Quality Index (PSQI) questionnaire was used to examine subjective sleep

quality (Buysse et al., 1989). PSQI scores range from 0 to 21 with higher scores indicating worse sleep quality; scores ≥ 5 indicate poor sleep quality while scores < 5 indicate good sleep quality.

4.4.2 Study protocol and timeline

Eligible participants were invited to attend a consent visit during which they were randomized to start either with a habitual or curtailed night of sleep. After the consent visit, each participant came to the laboratory twice for taste testing. One visit took place on the day after the habitual night of sleep, and the other visit occurred on the day after the curtailed night. These two visits were at least seven days apart to provide a washout period to recover from the one night of shortened sleep. After each lab visit, participants were asked to fill out one 24-hour dietary recall the following morning using the Automated Self-Administered 24 – Hour (ASA24®) Dietary Assessment (Subar et al., 2012) to record everything consumed during the day of the taste test, including meals, snacks, and beverages. ASA24® has been validated and widely used in clinical studies (Baranowski et al., 2011; Frankenfeld et al., 2012; Subar et al., 2010, 2012, 2012).

For the sleep protocol, participants were instructed to wear the Zmachine (General Sleep, Columbus, OH, USA), a single channel electroencephalogram (EEG) that monitors sleep duration and stages, during sleep on both nights. The Zmachine has been validated against polysomnography (PSG) (Y. Wang et al., 2015) and is used in sleep research studies (Pedersen et al., 2015; Szczygiel, Cho, & Tucker, 2019a, 2019b; C.-L. Yang et al., 2019). Participants were instructed to put the Zmachine on 30 minutes before going to bed. For the habitual night, bedtime and waketime were determined based on typical self-reported bed and wake times while curtailed bed and wake times were calculated by reducing self-reported total habitual sleep time by 33% and delaying bedtime (Szczygiel, Cho, & Tucker, 2019a, 2019b). For example, one

participant reported typically going to bed at 10:00 p.m. and waking up at 7:00 a.m. The total bedtime for the participant was 9 hours. For the curtailed night of sleep, a 3-hour reduction of sleep (33% of reduction in sleep duration) was applied, and the bed and wake times were 1:00 a.m. and 7:00 a.m., respectively.

4.4.3 Consent visit

During the consent visit, eligible participants completed the demographic questionnaire and were measured for weight, height, and percent body fat (%BF). Demographic questions included gender, race, ethnicity, and age. The height of participants was measured using a standing stadiometer (HM200P, Charder, Taichung, Taiwan). Weight and %BF were evaluated using a bioelectrical impedance scale (TBF-400, Tanita, Arlington Heights, IL, USA).

4.4.4 Lab visits

Participants returned to the lab after one night of curtailed sleep and one night of habitual sleep. These two lab visits were identical. At each visit, the sleep recording data were reviewed in the Zmachine data viewer for each participant prior to taste testing to confirm that the participant followed the appropriate (habitual vs. curtailed) sleep protocol. Participants who did not follow the sleep protocol, which was defined as more than a 30-minute discrepancy between actual sleep time and protocol determined sleep time, were asked to repeat a night of sleep following the relevant protocol at least a week later. After confirmation that the participant had adhered to the assigned protocol for that night, they were asked how they would rate the previous night's sleep quality and duration on a visual analog scale (VAS) of 0 to 100. Zero indicated far below average while 100 indicated far above average.

4.4.5 Taste testing

The five NaCl solutions presented to participants included concentrations of 0.05, 0.09, 0.15, 0.19, and 0.25 M NaCl, which were selected to reflect the spectrum of salt taste in a real-world food environment (L. J. Stein et al., 2012). For example, the taste of saltiness in milk represents the lowest concentration salt solution (0.05M) while the most concentrated salt solution (0.25M) tastes like the saltiness in pickle juice.

First, five 10 mL salty solutions made with NaCl and distilled water in different concentrations were presented to participants in 30 mL plastic portion cups, in order of increasing concentration (0.05, 0.09, 0.15, 0.19, and 0.25 M). Concentrations were pilot tested in the laboratory prior to the study to reflect commonly experienced dietary salt exposures (L. J. Stein et al., 2012) and to ensure they were distinguishable from each other. Participants were asked to put each solution in their mouth and swish for as long as needed to thoroughly evaluate the solution and then expectorate. Next, participants were asked to rate the liking of each solution on a VAS of 0 to 100, where 0 reflected not at all and 100 represented extremely. Immediately after the liking task, participants were asked how intense they thought the salty solutions were on a VAS of 0 to 100, where 0 indicated not at all and 100 signified extremely. After rating their liking and intensity, participants were asked to rinse their mouth with distilled water after each sample until no saltiness was perceived.

The preferred salt concentration test followed examination of liking and intensity. This test adapted the two-series forced-choice paired-comparison tracking procedure developed by Mennella et al (2011). The forced-choice paired-comparison tracking procedure has been validated in determining preferred sweet concentrations (Mennella et al., 2011). The same 5 concentrations of aqueous NaCl solutions as those used for the liking and intensity tests were used. In the first series of tests, participants were presented a pair of salt solutions, lower

concentration first, and asked to taste both solutions while rinsing in between with distilled water. After tasting both solutions, participants informed researchers regarding which salt solution they preferred. As with the sweet taste forced-choice paired-comparison tracking protocol, the 0.09 M and 0.19 M NaCl solutions, were presented to participants first for each trial. Then, each subsequent pair presented contained the participants' previously preferred concentration paired with an adjacent solution, either higher or lower in concentration. At each presentation, the lower concentration was presented first. The tests were repeated until one preferred concentration was sequentially selected twice while the adjacent concentrations to the preferred concentration had been tasted. This preferred concentration was recorded as the preferred concentration for trial 1. Participants were given two minutes to rest in between trial 1 and the second trial to avoid fatigue. For trial 2, the series of tests were repeated; however, the higher concentration of each pair of solutions were presented to participants first to reduce the possibility of an order effect (Mennella et al., 2011). The preferred concentration for the second series of tests was recorded as the preferred concentration for trial 2. The geometric mean of the preferred concentration for trial 1 and 2 was calculated for each participant to avoid position bias and improve the accuracy in estimating the preferred concentration (Mennella et al., 2011).

4.4.6 Statistical analysis

Descriptive statistics were performed. Variables are presented as mean \pm standard deviations unless specified otherwise. The geometric mean of preferred salt concentrations from trial 1 and 2 under each sleep condition was calculated and used in analyses. Paired t-tests were used to determine the differences of salt taste function (slope of intensity ratings), preference (slope of liking ratings and preferred concentration), sodium intake, energy-corrected sodium intake, macronutrient intake between the curtailed and the habitual night, after confirming the

linearity of these variables were met. Additionally, time in bed, total sleep time, deep sleep, and REM sleep were compared between the curtailed and the habitual night of sleep to verify that the sleep protocol was implemented correctly. To examine whether the adapted forced-choice paired-comparison tracking test is a valid tool in determining salt taste preference, only data from the habitual night of sleep was used. First, the geometric means of preferred salt concentration were compared between visit 1 and visit 2 using one-way ANOVA to ensure no order effect was present. Then, the intensity rating of each concentration of NaCl solution was compared to all others using paired t-tests, and a false discovery rate (FDR) of $q = 0.05$ was employed to reduce the risk of Type 1 error (Benjamini & Hochberg, 1995). The geometric mean of preferred salt concentrations for trial 1, trial 2, and the overall sample were compared between each other. In addition, bivariate correlations were used to examine the associations between the liking slope and the preferred salt concentration. Further, a zero-order Pearson correlation matrix was created to examine the relationships between age, BMI, %BF, PSQI, total sleep time, liking slope, intensity slope, preferred salt concentration, and energy-corrected Na intake. FDR was again used to correct for multiple comparisons. Data analysis was completed using SPSS version 27 (IBM Corporation, Armonk, NY, USA). $P < 0.05$ was used to determine statistical significance in all analyses.

4.5 Results

4.5.1 Anthropometric and demographic information for both studies

A total of 59 participants completed the study and were complied with the sleep protocols (Table 21). More than two-thirds of the participants were female, nearly half were white, more than one-third were Asian, the average BMI was considered to be in the healthy range.

Table 21. Anthropometric and demographic summary

Sex	N	%
Male	18	30.5
Female	41	69.5
Race		
Asian	23	39.0
Black	5	8.5
White	27	45.8
Other	3	5.1
Ethnicity		
Hispanic	2	3.4
Non-Hispanic	51	86.4
Anthropometrics		
Mean ± SD	Range	
Body mass index (kg/m ²)	23.3 ± 4.4	15.5 – 36.6
Body fat (%)	24.7 ± 10.8	3.0 – 49.5
Age (y)	26.2 ± 6.0	19 - 41
Sleep measures		
Mean ± SD	Range	
Pittsburg Sleep Quality Index (score)	4.0 ± 1.7	0 – 8
Sleep duration (h, self-reported)	8.1 ± 0.6	7.1 – 9.1

Race missing n=1, ethnicity missing n=6, BF% missing n=1

4.5.2 Validation of the adapted forced-choice paired-comparison tracking test in determining salt taste preference

Analyses indicated the preferred salt concentration was not different between lab visit 1 and 2, which confirmed that an order effect was not detectable (Table 22). To evaluate the utility and reliability of the adapted procedure, it was necessary to ensure that all salt solution concentrations were perceptibly different in terms of intensity. This was confirmed ($p < 0.001$ for all concentration comparisons, Figure 2). Next, we ensured that preferred concentrations across the two trials were not significantly different from each other; that is, the preferred salt concentration determined in Trial 1 was not different from Trial 2 ($p = 0.078$) or from the geometric mean of the two trials ($p = 0.948$). The concentration of Trial 2 also did not differ from

the geometric mean ($p=0.089$). Out of the 59 participants, 48 (82%) participants either selected the same concentration for Trial 1 and 2 ($n=27$, 46%) or selected neighboring concentrations in Trial 1 and 2 ($n=21$, 36%). Additionally, the preferred salt concentration positively correlated with the liking slope ($r=0.593$, $p<0.001$).

Table 22. Comparison of preferred salt concentration after the habitual night

	Geometric mean of the preferred salt concentration Visit 1 ($n=27$) (mean \pm SD)	Geometric mean of the preferred salt concentration Visit 2 ($n=32$) (mean \pm SD)	Geometric mean of the preferred salt concentration for the overall sample ($n=59$) (mean \pm SD)	P value for comparing visit 1 vs. 2
Habitual night	0.71 \pm 0.38	0.70 \pm 0.36	0.70 \pm 0.37	0.920

Note: Because the sleep conditions were randomized, some individuals experienced the habitual sleep night first and others experienced it second. Visit 1 indicates participants slept a habitual night for their first visit while visit 2 indicated the habitual night was slept at the second visit. To ensure that the order of the sleep conditions did not influence ratings after the habitual night, the visits were compared.

The time in bed as well as total sleep, slow wave sleep (SWS), and REM sleep times were different between the habitual and the curtailed group nights (Table 23). Additionally, the reduction of total sleep time from the habitual to the curtailed night was 36.1%, which confirmed that the desired 33% sleep duration reduction was met, indicating that the sleep protocol for each night was implemented correctly by participants.

4.5.3 No difference in salt taste hedonic measures and function, sodium and macronutrient intake, and food cravings between the curtailed and habitual nights

Slopes of liking ($p=0.074$, Figure 3) and intensity ratings ($p=0.844$, Figure 4) were not different between the habitual and the curtailed nights of sleep. Further, neither preferred

concentration of saltiness (habitual 0.12 ± 0.06 M vs. curtailed 0.13 ± 0.06 M, $p = 0.092$), sodium intake, nor energy-corrected sodium intake differed after the night of habitual sleep compared to the curtailed night (Table 24). In terms of dietary intake, energy consumption (kcal/d), carbohydrate (g/d), protein (g/d), fat (g/d), and Na (mg/d) intake were not different between sleep conditions.

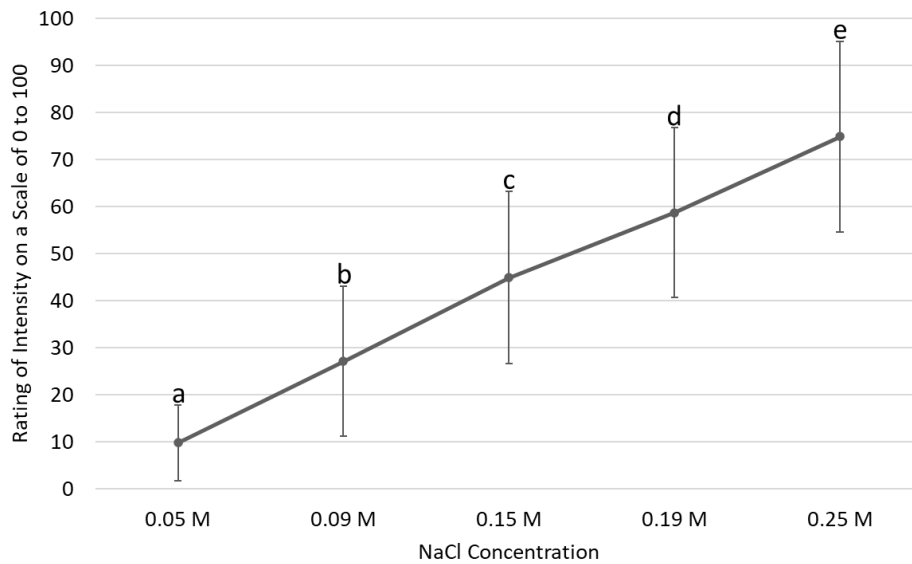


Figure 2. Intensity ratings of salt solutions across all concentrations on the habitual night
 Note: Different letters indicate significant differences between concentrations.

Table 23. Summary of objective and subjective sleep measures

		Habitual	Curtailed	% Reduction	p – value
Objective sleep measures (h)	Time in bed	8.6 ± 0.8	5.5 ± 0.7	36.0%	< 0.001
	Total sleep time	7.2 ± 0.6	4.6 ± 0.7	36.1%	< 0.001
	SWS sleep	1.5 ± 0.4	1.3 ± 0.5	13.3%	0.002
	REM sleep	1.8 ± 0.6	1.1 ± 0.4	38.9%	< 0.001
Subjective sleep measures (0 – 100 scale)	Sleep quality satisfaction	64.1 ± 17.5	41.4 ± 20.4	35.4%	< 0.001
	Sleep duration satisfaction	67.9 ± 17.6	27.3 ± 14.1	59.8%	< 0.001

SWS=slow wave sleep; REM=rapid eye movement sleep.

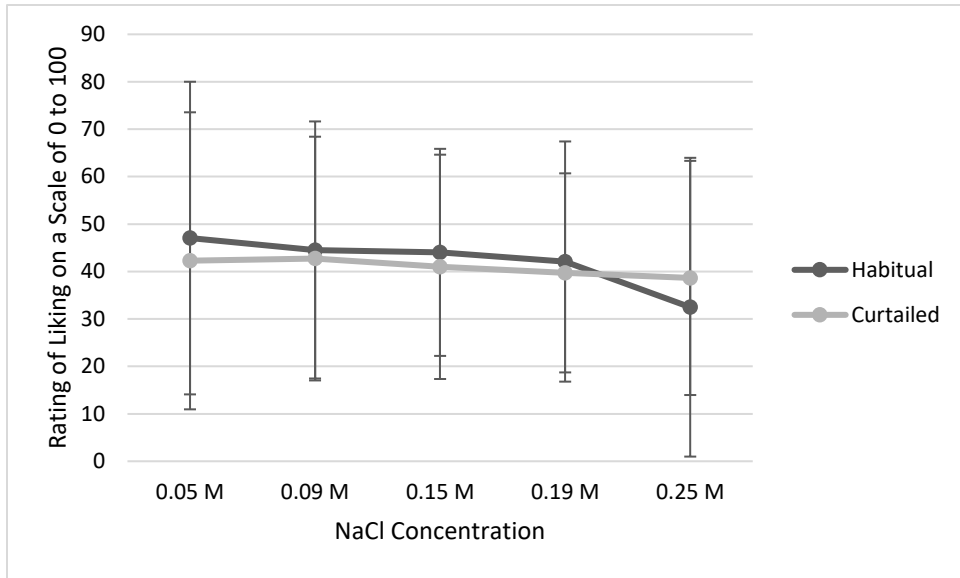


Figure 3. Liking ratings of salt solutions across all concentrations on the habitual vs. curtailed night
 Note: The error bars represent standard deviations.

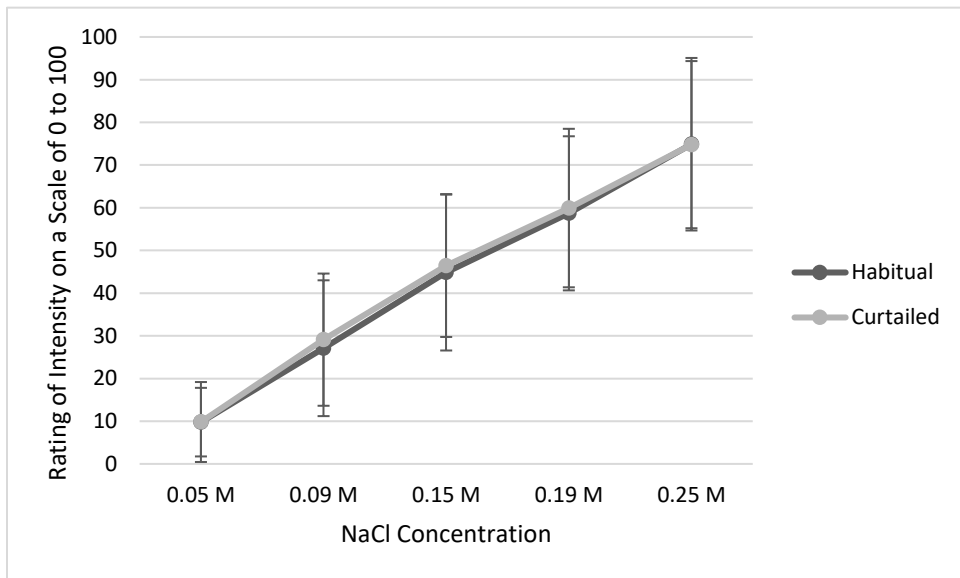


Figure 4. Intensity ratings of salt solutions across all concentrations on the habitual vs. curtailed night
 Note: The error bars represent standard deviations.

Table 24. Energy, macronutrient, and sodium intake

	Habitual	Curtailed	p – value
Na (mg/d)	3251.9 ± 1866.6	3156.9 ± 1400.0	0.621
Energy-corrected Na (mg/Kcal)	1.7 ± 0.5	1.7 ± 0.6	0.986
Energy (Kcal/d)	1984.3 ± 1101.6	1948.6 ± 818.0	0.757
Carbohydrate (g/d)	249.0 ± 156.7	235.6 ± 120.3	0.454
Protein (g/d)	73.5 ± 54.0	70.8 ± 37.7	0.657
Fat (g/d)	76.7 ± 47.5	77.6 ± 36.9	0.864

4.5.4 Correlations between hedonic measures and function of salt taste and energy-corrected sodium intake

A steeper liking slope, which indicated a more rapid increase in salt liking as the NaCl concentration increased, was associated with higher preferred salt concentration and higher sodium intake after one night of habitual sleep (Table 25).

Age, BMI, %BF, sleep quality and duration were not associated with hedonic measures of salt, intensity, or energy-correlated Na intake. Additionally, salt taste intensity and preferred salt concentration were not associated with energy-corrected Na intake.

Under the curtailed sleep condition, liking slope positively correlated with preferred salt concentration; however, sleep curtailment disrupted the relationship between liking and energy-corrected Na intake (Table 26). All other relationships did not change when compared to the habitual sleep condition.

Table 25. Zero-order correlations under the habitual sleep condition

Measures	1	2	3	4	5	6	7	8	9
(1) Age (years)	-	0.213	0.295 [†]	-0.087	-0.138	-0.129	0.161	-0.078	-0.239
(2) BMI (Kg/m ²)		-	0.798**	0.012	-0.029	-0.004	0.098	-0.001	0.021
(3) BF%			-	-0.046	0.046	-0.110	0.121	-0.030	-0.119
(4) PSQI				-	0.020	0.072	-0.174	0.177	-0.154
(5) Total sleep time (h)					-	-0.104	-0.248	0.075	0.024
(6) Liking slope						-	0.009	0.593**	0.338**
(7) Intensity slope							-	-0.199	0.005
(8) Preferred salt concentration (M)								-	0.165
(9) Energy-corrected Na intake (mg/Kcal)									-

*p<0.05, **p<0.01, †no longer significant after the FDR adjustment.

Table 26. Zero-order correlations under the curtailed sleep condition

Measures	1	2	3	4	5	6	7	8	9
(1) Age (years)	-	0.213	0.295 [†]	-0.087	-0.084	-0.315 [†]	0.158	-0.021	0.037
(2) BMI (Kg/m ²)		-	0.798**	0.012	-0.214	0.074	0.294 [†]	-0.014	0.217
(3) BF%			-	-0.046	-0.195	-0.119	0.259 [†]	-0.170	0.077
(4) PSQI				-	-0.061	0.136	0.162	0.060	0.203
(5) Total sleep time (h)					-	-0.171	-0.259 [†]	-0.032	-0.255
(6) Liking slope						-	-0.029	0.671**	0.132
(7) Intensity slope							-	-0.099	0.299 [†]
(8) Preferred salt concentration (M)								-	-0.016
(9) Energy-corrected Na intake (mg/Kcal)									-

*p<0.05, **p<0.01, †no longer significant after the FDR adjustment.

4.6 Discussion

The purpose of the present study was to examine whether the adapted version of the forced-choice paired-comparison tracking procedure could be used as a reliable and valid tool for determining salt taste preference and to evaluate the effects of sleep on salt taste hedonic measures and function. The results from the methodology validation component of the study demonstrated that the adapted forced-choice paired-comparison tracking procedure could serve as a reliable and valid test to determine salt taste preference. The RCT illustrated there were no changes in salt taste function, as measured by the slope of intensity, or hedonic measures, as measured by the slope of liking or preferred salt concentration were seen after one night of curtailed sleep compared to one night of habitual sleep. However, the slope of liking was associated with energy-corrected Na intake, but only under the habitual sleep condition. These results suggest that salt taste function and hedonics, as measured by intensity, slope of liking, and preferred concentration, are not affected by one night of curtailed sleep, and the lack of associations between salt taste measures and dietary intake reported by the current literature (Tan et al., 2021; Tan & Tucker, 2019) could be attributed, in part, to not accounting for sleep duration.

4.6.1 Validity of the adapted forced-choice, paired-comparison tracking procedure in determining salt taste preference

The forced-choice, paired-comparison tracking procedure has been validated to determine sweet taste preferences for adults (Mennella et al., 2011) while the present study is the first to adapt and validate the forced-choice paired-comparison tracking test procedure in assessing salt taste preference. Three validation steps were undertaken. First, participants were able to distinguish the intensity of the salt solutions. Second, two trials to determine preferred

concentrations were performed, and the concentrations selected for each trial were not different from each other or from the geometric mean of the two trials. Additionally, more than 80% of participants picked either the same or neighboring concentrations for the first and the second trial, indicating the results were reproducible between the two trials. Third, the preferred salt concentration was positively associated with the liking slope, which suggests that the adapted forced-choice paired-comparison tracking test could be used in place of assessing liking slope, should the researcher wish to conduct only one hedonic measure. Given these outcomes, the present study supports the use of the adapted forced-choice paired-comparison tracking test in determining salt taste preference.

There are strengths and weaknesses of using liking slopes and preferred concentrations to assess salt taste hedonics. Liking slopes use multiple data points to describe a function, which provides a more holistic understanding of taste responses over a range of concentrations. This more holistic evaluation could explain why energy-corrected Na intake was associated with liking slope but not preferred concentration. However, liking is commonly evaluated with Likert scales or visual analog scales (VAS) (Lawless & Heymann, 2010b). For Likert scales, the options are usually limited to 5 to 7 choices, which may not truly reflect the attitude or feeling of participants (Bishop & Herron, 2015). Additionally, it is arbitrary to translate the results of Likert scales into continuous numbers, which is the standard protocol for scoring Likert scales (Bishop & Herron, 2015). In terms of VAS, even though such scales can provide continuous data, the common disadvantage of a visual analog scale is that participants may use the scale differently, for example, two participants may wish to indicate that the stimulus is liked “slightly”, but that same perception could be marked on very different locations on the scale (Lawless & Heymann, 2010b). Preferred concentration testing, as conducted in this study, is

limited in that it identifies only one concentration, which can be heavily influenced by the concentrations selected for testing as well as the differences between each concentration. In contrast, a strength of using the preferred salt taste concentration as a hedonic measure of salt taste is that it overcomes much of the subjectiveness of liking ratings. Therefore, if only relying on one test, researchers should carefully consider which one best meets their needs in the context of the proposed research question. Results from the present study suggest that if a researcher is attempting to predict sodium intake from taste hedonics, the slope of liking responses is the better choice.

There is no consensus on salt taste test procedures, which makes comparing results from one study to another difficult (Tan et al., 2021; Tan & Tucker, 2019). For example, some studies measured salt liking on a 9-point hedonic scale, using 1 representing dislike extremely while 9 representing like extremely (Lucas et al., 2011) while others measured liking using a general Labeled Magnitude Scale (Hayes et al., 2010). In terms of salt taste preference, some studies used the method of ad libitum mixing of salt into beef broth until the preferred concentration was achieved (e.g., Veček et al., 2020) while others assessed self-reported salt preference in foods (e.g., Lee et al., 2014) and frequency of consuming common salty foods (e.g., Li et al., 2017). The use of inconsistent methods in determining hedonic measures of salt taste make it challenging to compare studies. The advantage of using the concentrations delineated in the present study is that they have been shown to be distinguishable from each other and span a range of concentrations that encompass commonly encountered sensations when eating or drinking (L. J. Stein et al., 2012).

4.6.2 The effects of sleep on hedonic measures of salt taste

Our hypothesis that sleep curtailment would affect hedonic measures of salt taste was based on prior studies on sweet taste. Previous work reported acute sleep curtailment increased the slope of sweet liking ratings (Szczygiel, Cho, & Tucker, 2019a, 2019b) and the preferred sweet taste concentration (Szczygiel, Cho, & Tucker, 2019a, 2019b; Tajiri et al., 2020). Neural and behavioral reactivity to pleasurable experiences increase after sleep deprivation and curtailment (Gujar et al., 2011). For example, under conditions of shortened sleep, consuming sweets becomes more pleasurable after one night of short sleep; therefore, liking and consumption of sweets increase. No other studies have investigated the effects of sleep curtailment on hedonic measures of salt taste; therefore, we based our hypothesis on previous studies investigating sweet taste.

Contrary to our hypothesis, sleep curtailment for one night did not affect hedonic measures of salt taste, including liking and preferred salt concentration, which could be attributed to differences in the activation of the reward system of the brain by these two tastes (Spetter et al., 2010). Previous studies noted the anterior insula of the brain, the putative primary taste cortex (Pritchard et al., 1986), was activated more with NaCl than with sucrose solutions (Spetter et al., 2010). The anterior insula plays a role in negative valence-specific responses in taste (Small et al., 2003), responding more to taste that is unpleasant. Salt solutions are often rated as less pleasant compared to sucrose solutions (Spetter et al., 2010). Additionally, under sleep deprivation or curtailment, the ability for the anterior insula to discriminate reward versus punishment decreased (Krause et al., 2017; Mullin et al., 2013; Venkatraman et al., 2007). Given the anterior insula is activated more for salty taste and is less sensitive under short sleep, sleep curtailment may not increase the liking of salt. In summary, differences in the effects of sleep on

salty vs sweet hedonics could be because of differences in the valence and neural activity produced by these tastes.

4.6.3 The effects of sleep on salt taste function

Sleep curtailment did not affect salt taste intensity assessments, which aligns with our hypothesis and agrees with what was reported regarding the effects of sleep curtailment on sweet taste function (Holt et al., 2000; Jayasinghe et al., 2017; Leong et al., 2018; Low et al., 2016; Mattes, 1985; Sartor et al., 2011; S. L. Smith et al., 2016; Stevenson et al., 2016; Szczygiel, Cho, & Tucker, 2019a, 2019b). Two previous studies examined the effects of sleep duration alteration on salt taste function; one implemented sleep deprivation (Furchtgott & Willingham, 1956) while the other investigated the effects of short sleep (< 7 hours) versus long sleep (> 7 hours) on salt taste function (S. L. Smith et al., 2016). Both studies reported sleep duration had no effects on salt taste sensitivity; however, it is difficult to compare these results with ours because of the differences in taste measures assessed. The present study examined salt taste sensitivity at a suprathreshold level using an intensity rating method while the other two studies focused on examining detection thresholds (Furchtgott & Willingham, 1956; S. L. Smith et al., 2016). Detection thresholds are assessed using salt concentrations that span a range from undetectable to detectable (Buddies, n.d.; Lawless & Heymann, 2010a) with the goal to determine the lowest salt concentration that can be detected. Therefore, detection threshold testing is a completely different test compared to intensity ratings. In terms of the effects of sleep on sweet taste function, previous studies demonstrated sweet taste function, measured by intensity rating (Szczygiel, Cho, & Tucker, 2019a, 2019b) or detection threshold (S. L. Smith et al., 2016), did not differ after one night of curtailed sleep. These findings suggest that taste function, including

both salt and sweet taste, is relatively robust after one night of short sleep. Future work should focus on investigating the effects of chronic sleep curtailment on sweet and salt taste functions.

4.6.4 Associations between salt taste measures and dietary intake

The association between salt taste liking slope and energy-corrected Na intake was only present under the habitual sleep condition, which provides one possible explanation for the lack of associations between taste measures and dietary intake reported in the literature (Tan et al., 2021; Tan & Tucker, 2019). Almost all studies investigating the relationships between taste measures and dietary intake fail to account for sleep curtailment or deprivation (Tan et al., 2021; Tan & Tucker, 2019). In the present study, we observed that the association between salt taste liking slope and energy-corrected Na intake disappeared after one night of curtailed sleep, which suggests that sleep curtailment disrupted the previously observed association between liking and intake. Given 35% of the U.S. adults do not achieve adequate sleep (CDC - Data and Statistics - Sleep and Sleep Disorders, 2021), failing to account for short sleep duration could obscure taste test-dietary intake relationships. Therefore, the findings from the present study suggest that sleep duration should be considered when investigating relationships between taste and diet.

4.6.5 Strengths and limitations

There are several strengths of this work. First, the study included objective sleep data to validate sleep duration. Second, both salt taste hedonic and function measures were examined, which provide a more comprehensive evaluation of salt taste. Third, sleep curtailment was individualized based on habitual sleep duration of participants, which ensured the consistency of sleep curtailment between participants. Fourth, the study incorporated a one-week wash-out period between the two taste test sessions for study 2 to avoid carry-over effects. Finally, the

study employed the randomized crossover design, which minimized confounding bias that could come from the differences in demographic and anthropometric characteristics between separate groups.

Several limitations are noted. Results from the study may have limited generalizability, as mostly young adults were tested. Future studies should consider testing the forced-choice paired-comparison tracking procedure in other populations, such as teenagers and older adults. Additionally, the study included only testing short-term sleep curtailment, and only one 24-hour dietary recall was obtained after the habitual and curtailed nights of sleep; hence, this may not represent habitual intake. Some taste measures, such as sensitivity, appear to be robust to acute sleep curtailment; however, future studies should consider investigating the effects of chronic sleep curtailment on salt taste measures. Future research should consider using multiple dietary assessment methods and/or consider using indirect methods of measuring intake, such as urinary sodium.

4.7 Conclusion

The present study demonstrated that the forced-choice paired-comparison tracking procedure could be used as a reliable and valid tool for determining salt taste preference. Additionally, acute sleep curtailment did not affect salt taste function or hedonic measures; however, the slope of salt taste liking was associated with energy-corrected Na intake only under the habitual sleep condition. These findings suggest that sleep duration should be considered in taste studies examining relationships between taste and dietary intake. Salt taste measures appear to be robust after acute sleep curtailment; therefore, future studies should consider examining the effects of chronic sleep curtailment on salt taste. Further, researchers should consider adopting the NaCl concentrations used in the present study for salt taste testing and use the forced-choice

paired-comparison tracking tool as a standard procedure to measure salt taste preference to facilitate direct comparisons between future taste studies.

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Conclusions

The results demonstrate the relationships between sleep and undesirable dietary behaviors by identifying the mediating role that sleep plays in the stress-undesirable dietary behavior relationships and how sleep may play a factor in the salt taste measures and sodium intake relationship. The following topics were examined in each chapter:

- A. The effects of changes in sleep duration and quality during the COVID-19 pandemic on other health behaviors, including dietary risk, alcohol misuse, physical activity, and sitting time, among higher education students. (Chapter 2).
- B. The mediating effects of sleep duration and quality and the moderating effects of resilience on the relationship between perceived stress and undesirable dietary behaviors and on the relationship between perceived stress and alcohol misuse (Chapter 3).
- C. The effects of sleep curtailment on salt taste function and hedonic measures and on the relationship between salt taste measures and dietary intake (Chapter 4).

Based on the research conducted, the following are the most important findings:

- A. Sleep quality was poor for students from all countries, and in most countries, more than 25% of students were not meeting sleep duration guidelines.
- B. Students who indicated poorer sleep quality during the pandemic had higher dietary risk scores, while improved sleep quality was associated with less sitting time.

- C. Sleeping more during the pandemic was associated with lower physical activity levels and greater sitting time.
- D. Sleep quality mediated the relationship between perceived stress and undesirable dietary behaviors measured by the STC questionnaire.
- E. Sleep quality also mediated the relationship between perceived stress and alcohol misuse.
- F. Increased resilience reduced the strength of the relationship between perceived stress and undesirable dietary behaviors measured by the STC questionnaire but not alcohol misuse.
- G. The adapted forced-choice paired-comparison tracking procedure could serve as a reliable test to determine salt taste preference.
- H. No changes in salt taste function, as measured by the slope of intensity, or hedonic measures, as measured by the slope of liking or preferred salt concentration were seen after one night of curtailed sleep compared to one night of habitual sleep.
- I. The slope of liking was associated with energy-corrected Na intake only under the habitual sleep condition.

The study reported in chapter 2 characterized and compared the health behaviors, including sleep, diet, alcohol misuse, and physical activity of higher education students in seven countries. Further, the study examined the effects of changes in sleep duration and quality on dietary risk, alcohol misuse, physical activity, and sitting time. The results demonstrated sleep quality was poor for students from all countries, and in most countries, more than 25% of students were not meeting sleep duration guidelines. Additionally, findings indicated that

students who reported poorer sleep quality during the COVID-19 pandemic had higher dietary risk scores, indicating more frequent engagement in unhealthy eating behaviors. On the other hand, students who reported improved sleep quality during the pandemic compared to before reported less sitting time. In terms of changes in sleep duration, sleeping more was associated with less physical activity and greater sitting time. The results presented in chapter 2 suggest that poor sleep quality reported by higher education students during the COVID-19 pandemic is concerning because poorer sleep quality is associated with higher dietary risk scores. Programs improving sleep quality of higher education students should be considered.

Based on the findings in chapter 2 that demonstrated that poorer sleep quality was associated with higher dietary risk scores, chapter 3 further examined more complex relationships between sleep, dietary behaviors, stress, and resilience. Mediation and moderation analyses were employed for examining the complex relationship in chapter 3. The results illustrated that sleep quality, but not sleep duration, mediated the relationship between perceived stress and undesirable dietary behaviors and the relationship between perceived stress and alcohol misuse. Additionally, increased resilience reduced the strength of the relationship between perceived stress and undesirable dietary behaviors but not alcohol misuse, and the negative effect of high perceived stress on undesirable dietary behaviors disappeared at higher resilience scores. These results suggest incorporating programs that focus on sleep quality and resilience improvement would likely be effective strategies to improve the health of higher education students, especially during stressful events.

Chapter 4 examined 1) whether an adapted version of a forced-choice paired-comparison test could be used in determining salt taste preference; 2) investigated the effects of sleep curtailment on salt taste function and hedonic measures; and 3) explored the relationship

between salt taste measures and energy-corrected Na intake under habitual and curtailed sleep conditions. The study used a modest sleep curtailment protocol, which aimed to reduce habitual sleep duration by 33%. The protocol successfully and significantly reduced the total sleep time of participants on the curtailed night compared to the habitual night. When looking into the habitual night only, the study illustrated that the adapted forced-choice paired-comparison tracking procedure could serve as a reliable test to determine salt taste preference. When comparing the two sleep conditions, no changes in salt taste function, as measured by the slope of intensity, or hedonic measures, as measured by the slope of liking or preferred salt concentration were observed after one night of curtailed sleep compared to one night of habitual sleep. However, the slope of liking was associated with energy-corrected Na intake only under the habitual sleep condition. These results suggest sleep duration should be assessed and accounted for in taste studies, especially when examining taste and diet relationships. Further, the methodology validation results suggest that further studies should consider adopting the NaCl concentrations used in our study for salt taste testing and use the forced-choice paired-comparison tracking tool as a standard procedure to measure salt taste preference to facilitate direct comparisons between future taste studies.

Several noticeable findings in this dissertation warrant further research; therefore, recommendations for future research are listed below.

5.2 Future directions

5.2.1 Determine the effectiveness of online CBTi-based sleep education programs in improving sleep among higher education students globally

The study in chapter 2 identified the pressing issue of poor sleep quality reported by higher education students from seven different countries and the association between sleeping

worse and higher dietary risk scores. Therefore, it is important to initiate or adopt interventions that improve sleep quality of higher education students. Based on previous work, online CBTi-based sleep education programs can be a socially distanced and relatively inexpensive way to improve students' sleep outcomes (Attridge et al., 2020; Bowen, 2019; Friedrich & Schlarb, 2018). However, the effectiveness of the online CBTi-based sleep education program has not been tested among higher education students in countries other than the U.S. Therefore, future studies should consider determining the effectiveness of online CBTi-based sleep education programs among higher education students in other countries and possibly make cultural adjustments, such as adapting culturally appropriate verbiage and language of the questions asked when helping individuals identifying barriers to sleep (Gjersing et al., 2010), to these programs.

5.2.2 Examine the effects of sleep quality improvement training on dietary behaviors

The study presented in chapter 3 identified that sleep quality, but not sleep duration, mediated the relationship between perceived stress and undesirable dietary behaviors and the relationship between perceived stress and alcohol misuse; however, the effects of sleep improvement programs on dietary behaviors have not been examined empirically. Incorporating sleep quality improvement training into university health promotion programs would likely be an effective strategy to improve the health of higher education students, especially during stressful events. Future studies should consider delivering a sleep improvement program to higher education students first, then possibly expanding to other populations, and measuring dietary behavior changes, dietary intake, and dietary risk scores before and after the program delivery.

5.2.3 Examine the effects of resilience training on dietary behaviors among high stress university students

Chapter 3 demonstrated that increased resilience weakened the relationship between perceived stress and undesirable dietary behaviors; however, these relationships have not been empirically tested. Research indicated that resilience training was just as effective as CBT in improvement of mental health among university students (Zamirinejad et al., 2014), and one interventional study showed that a mindfulness-based intervention to increase resilience significantly decreased stress compared to traditional mental health support offered by university counseling centers (Galante et al., 2018). However, whether resilience training can reduce dietary risk among university students with high stress levels has not been examined. Therefore, future studies should consider delivery of a resilience training program among students who reported high levels of stress and assess dietary risk scores and intake changes before and after program delivery.

5.2.4 Examine the effects of chronic sleep curtailment on salt taste measures

Chapter 4 observed acute sleep curtailment (one night) did not affect salt taste function or hedonic measures; however, the effects of chronic sleep curtailment on these measures are unknown. The study in chapter 4 illustrated hedonic measures of salt taste were robust after acute sleep curtailment, which was different from hedonic measures of sweet taste (Szczygiel, Cho, & Tucker, 2019a, 2019b; Tajiri et al., 2020). The differences between the effects of acute sleep curtailment on salt and sweet taste hedonic measures could be attributed to activation of different brain regions by the two types of tastes (Krause et al., 2017; Mullin et al., 2013; Spetter et al., 2010; Venkatraman et al., 2007). However, no study has been conducted to investigate the

effects of long-term sleep curtailment on hedonic measures of salt taste. Therefore, future studies should focus on examining the effects of chronic sleep curtailment on salt taste measures.

5.2.5 Standardize NaCl concentrations for salt taste tests and adopt the forced-choice paired-comparison tracking tool as a standard procedure to measure salt taste preference

Chapter 4 demonstrated the adapted version of the forced-choice paired-comparison tracking procedure could be used as a reliable tool for determining salt taste preference, and the validation study used five different NaCl concentrations that are reflective of the spectrum of salt taste in the real-world food environment. There is no consensus on salt taste test procedures, which makes comparing results from one study to another difficult (Tan et al., 2021; Tan & Tucker, 2019). For instance, ad libitum mixing of salt into broth was used in determining preferred salt concentration in one study (Veček et al., 2020) while self-reported salt preference in different foods was used as a method to determine preferred salt concentration in another study (H. Lee et al., 2014). The advantage of using the concentrations delineated in the present work is that they have been shown to be distinguishable from each other and span a range of concentrations that encompass commonly encountered sensations when eating or drinking (L. J. Stein et al., 2012). Given this evidence, future studies should consider adopting the NaCl concentrations described in chapter 4 for salt taste tests and adopt the forced-choice paired-comparison tracking tool as a standard procedure to measure salt taste preference.

5.2.6 Assess and account for sleep duration when investigating taste and diet relationships

In chapter 4, the study illustrated the salt taste liking slope was only associated with the energy-corrected Na intake after one night of habitual sleep, which suggests the lack of association between taste measures and dietary intake reported by the body of literature (Tan et al., 2021; Tan & Tucker, 2019) could be attributed to failing to account for short sleep duration.

Given 35% of U.S. adults do not achieve adequate sleep (CDC - Data and Statistics - Sleep and Sleep Disorders, 2021), failing to account for short sleep duration could obscure taste test-dietary intake relationships. Almost all studies investigating the relationships between taste measures and dietary intake fail to assess and account for sleep curtailment or deprivation (Tan et al., 2021; Tan & Tucker, 2019). Therefore, future studies investigating relationships between taste and diet should consider assessing and accounting for sleep duration.

The findings from the studies included in this dissertation provide a better understanding of the relationship between sleep and dietary intake and proposed future study directions for investigating the effects of sleep on intake empirically. Primarily, the work identified the issue of poor-quality sleep among higher education students during the COVID-19 pandemic and illustrated how poorer sleep quality predicted higher dietary risk scores while better sleep quality was associated with shorter sitting time. Additionally, this investigation identified that sleep quality mediated and resilience moderated the relationship between perceived stress and undesirable dietary behaviors (also referred as dietary risk in this work). Finally, this research noted curtailed sleep disrupted the expected relationship between measures of salt liking and energy-corrected sodium intake. Overall, these studies contribute to a better understanding of how sleep plays a role in predicting dietary intake and the taste-diet relationship, and practical applications contributed by this work include delivering sleep improvement programs among higher education students and testing their effects on dietary intake and accounting for sleep duration for taste-diet relationship studies.

APPENDICES

Appendix A: The IRB approval letters

A. 1 The IRB approval letter “Health Behaviors of Higher Education Students from 7 Countries: Poor sleep quality during the COVID-19 Pandemic Predicts Higher Dietary Risk” and “The Effects of Sleep Quality and Resilience on Perceived Stress, Dietary Behaviors, and Alcohol Misuses: A Mediation-Moderation Analysis of Higher Education Students from Asia, Europe, and North American during the COVID-19 Pandemic”

**MICHIGAN STATE
UNIVERSITY**

**EXEMPT DETERMINATION
Revised Common Rule**

April 7, 2020

To: Robin Marie Tucker

Re: **MSU Study ID:** STUDY00004285
Principal Investigator: Robin Marie Tucker
Category: Exempt 2(ii)
Exempt Determination Date: 4/7/2020
Limited IRB Review: Not Required.

Title: University Students' Resilience

This study has been determined to be exempt under 45 CFR 46.104(d) 2(ii).

The State of Michigan and Michigan State University (MSU) have placed temporary restrictions on human subject research conducted by MSU employees or agents. All MSU human research activities conducted by MSU employees or agents that take place in Michigan and cannot be done at home or place of residence with no inter-personal interaction with participants and others like research staff must stop unless the project is a clinical trial activity, that if discontinued, would negatively impact the patient's care, or projects related to COVID-19, particularly if they have a timeline for deployment that could address the crisis. Ongoing clinical trial activity, which if discontinued, would negatively impact the patient's care may continue with already enrolled participants. New enrollment in clinical trials conducted in Michigan is not permitted without additional institutional approval.

For MSU human research activities that take place outside of Michigan, the university has stated that unless there is the potential for direct therapeutic benefit to the participant (drug or device), any in-person participant interaction must immediately pause. This applies to both exempt and non-exempt research studies.

For all human research activities, research procedures involving no direct in-person interactions with participants may continue (e.g. data analysis, online surveys, telephone interviews), so long as any research procedure conducted in Michigan are done at home or place of residence and follow the restrictions set forth in Executive Order that temporarily suspends activities that are not necessary to sustain or protect life.

Please note that the situation is rapidly evolving and may further change. Visit <http://hrpp.msu.edu/COVID-19/index.html> for the latest information and updates, including the restrictions and their duration as the situation evolves.

Principal Investigator (PI) Responsibilities: The PI assumes the responsibilities for the protection of human subjects in this study as outlined in Human Research Protection Program (HRPP) Manual Section 8-1, Exemptions.



**Office of
Regulatory
Affairs
Human Research
Protection Program**

4000 Collins Road
Suite 136
Lansing, MI 48910

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Email: irb@msu.edu
www.hrpp.msu.edu

MSU is an affirmative-action,
equal-opportunity employer.

Continuing Review: Exempt studies do not need to be renewed.

Modifications: In general, investigators are not required to submit changes to the Michigan State University (MSU) Institutional Review Board (IRB) once a research study is designated as exempt as long as those changes do not affect the exempt category or criteria for exempt determination (changing from exempt status to expedited or full review, changing exempt category) or that may substantially change the focus of the research study such as a change in hypothesis or study design. See HRPP Manual Section 8-1, Exemptions, for examples. If the study is modified to add additional sites for the research, please note that you may not begin the research at those sites until you receive the appropriate approvals/permissions from the sites.

Please contact the HRPP office if you have any questions about whether a change must be submitted for IRB review and approval.

New Funding: If new external funding is obtained for an active study that had been determined exempt, a new initial IRB submission will be required, with limited exceptions. If you are unsure if a new initial IRB submission is required, contact the HRPP office. IRB review of the new submission must be completed before new funds can be spent on human research activities, as the new funding source may have additional or different requirements.

Reportable Events: If issues should arise during the conduct of the research, such as unanticipated problems that may involve risks to subjects or others, or any problem that may increase the risk to the human subjects and change the category of review, notify the IRB office promptly. Any complaints from participants that may change the level of review from exempt to expedited or full review must be reported to the IRB. Please report new information through the study's workspace and contact the IRB office with any urgent events. Please visit the Human Research Protection Program (HRPP) website to obtain more information, including reporting timelines.

Personnel Changes: After determination of the exempt status, the PI is responsible for maintaining records of personnel changes and appropriate training. The PI is not required to notify the IRB of personnel changes on exempt research. However, he or she may wish to submit personnel changes to the IRB for recordkeeping purposes (e.g. communication with the Graduate School) and may submit such requests by submitting a Modification request. If there is a change in PI, the new PI must confirm acceptance of the PI Assurance form and the previous PI must submit the Supplemental Form to Change the Principal Investigator with the Modification request (available at hrpp.msu.edu).

Closure: Investigators are not required to notify the IRB when the research study can be closed. However, the PI can choose to notify the IRB when the study can be closed and is especially recommended when the PI leaves the university. Closure indicates that research activities with human subjects are no longer ongoing, have stopped, and are complete. Human research activities are complete when

investigators are no longer obtaining information or biospecimens about a living person through interaction or intervention with the individual, obtaining identifiable private information or identifiable biospecimens about a living person, and/or using, studying, analyzing, or generating identifiable private information or identifiable biospecimens about a living person.

For More Information: See HRPP Manual, including Section 8-1, Exemptions (available at hrpp.msu.edu).

Contact Information: If we can be of further assistance or if you have questions, please contact us at 517-355-2180 or via email at IRB@msu.edu. Please visit hrpp.msu.edu to access the HRPP Manual, templates, etc.

Exemption Category. The full regulatory text from 45 CFR 46.104(d) for the exempt research categories is included below.¹²³⁴

Exempt 1. Research, conducted in established or commonly accepted educational settings, that specifically involves normal educational practices that are not likely to adversely impact students' opportunity to learn required educational content or the assessment of educators who provide instruction. This includes most research on regular and special education instructional strategies, and research on the effectiveness of or the comparison among instructional techniques, curricula, or classroom management methods.

Exempt 2. Research that only includes interactions involving educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures, or observation of public behavior (including visual or auditory recording) if at least one of the following criteria is met:

(i) The information obtained is recorded by the investigator in such a manner that the identity of the human subjects cannot readily be ascertained, directly or through identifiers linked to the subjects;

(ii) Any disclosure of the human subjects' responses outside the research would not reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, educational advancement, or reputation; or

(iii) The information obtained is recorded by the investigator in such a manner that the identity of the human subjects can readily be ascertained, directly or through identifiers linked to the subjects, and an IRB conducts a limited IRB review to make the determination required by 45 CFR 46.111(a)(7).

Exempt 3. (i) Research involving benign behavioral interventions in conjunction with the collection of information from an adult subject through verbal or written responses (including data entry) or audiovisual recording if the subject prospectively agrees to the intervention and information collection and at least one of the following criteria is met:

(A) The information obtained is recorded by the investigator in such a manner that the identity of the human subjects cannot readily be ascertained, directly or through identifiers linked to the subjects;

(B) Any disclosure of the human subjects' responses outside the research would not reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, educational advancement, or reputation; or

(C) The information obtained is recorded by the investigator in such a manner that the identity of the human subjects can readily be ascertained, directly or through identifiers linked to the subjects, and an IRB conducts a limited IRB review to make the determination required by 45 CFR 46.111(a)(7).

(ii) For the purpose of this provision, benign behavioral interventions are brief in duration, harmless, painless, not physically invasive, not likely to have a significant adverse lasting impact on the subjects, and the investigator has no reason to think the subjects will find the interventions offensive or embarrassing. Provided all such criteria are met, examples of such benign behavioral interventions would include having the subjects play an online game, having them solve puzzles under various noise conditions, or having them decide how to allocate a nominal amount of received cash between themselves and someone else.

(iii) If the research involves deceiving the subjects regarding the nature or purposes of the research, this exemption is not applicable unless the subject authorizes the deception through a prospective agreement to participate in research in circumstances in which the subject is informed that he or she will be unaware of or misled regarding the nature or purposes of the research.

Exempt 4. Secondary research for which consent is not required: Secondary research uses of identifiable private information or identifiable biospecimens, if at least one of the following criteria is met:

(i) The identifiable private information or identifiable biospecimens are publicly available;

(ii) Information, which may include information about biospecimens, is recorded by the investigator in such a manner that the identity of the human subjects cannot readily be ascertained directly or through identifiers linked to the subjects, the investigator does not contact the subjects, and the investigator will not re-identify subjects;

(iii) The research involves only information collection and analysis involving the investigator's use of identifiable health information when that use is regulated under 45 CFR parts 160 and 164, subparts A and E, for the purposes of "health care operations" or "research" as those terms are

defined at 45 CFR 164.501 or for "public health activities and purposes" as described under 45 CFR 164.512(b); or

(iv) The research is conducted by, or on behalf of, a Federal department or agency using government-generated or government-collected information obtained for nonresearch activities, if the research generates identifiable private information that is or will be maintained on information technology that is subject to and in compliance with section 208(b) of the E-Government Act of 2002, 44 U.S.C. 3501 note, if all of the identifiable private information collected, used, or generated as part of the activity will be maintained in systems of records subject to the Privacy Act of 1974, 5 U.S.C. 552a, and, if applicable, the information used in the research was collected subject to the Paperwork Reduction Act of 1995, 44 U.S.C. 3501 et seq.

Exempt 5. Research and demonstration projects that are conducted or supported by a Federal department or agency, or otherwise subject to the approval of department or agency heads (or the approval of the heads of bureaus or other subordinate agencies that have been delegated authority to conduct the research and demonstration projects), and that are designed to study, evaluate, improve, or otherwise examine public benefit or service programs, including procedures for obtaining benefits or services under those programs, possible changes in or alternatives to those programs or procedures, or possible changes in methods or levels of payment for benefits or services under those programs. Such projects include, but are not limited to, internal studies by Federal employees, and studies under contracts or consulting arrangements, cooperative agreements, or grants. Exempt projects also include waivers of otherwise mandatory requirements using authorities such as sections 1115 and 1115A of the Social Security Act, as amended. (i) Each Federal department or agency conducting or supporting the research and demonstration projects must establish, on a publicly accessible Federal Web site or in such other manner as the department or agency head may determine, a list of the research and demonstration projects that the Federal department or agency conducts or supports under this provision. The research or demonstration project must be published on this list prior to commencing the research involving human subjects.

Exempt 6. Taste and food quality evaluation and consumer acceptance studies: (i) If wholesome foods without additives are consumed, or (ii) If a food is consumed that contains a food ingredient at or below the level and for a use found to be safe, or agricultural chemical or environmental contaminant at or below the level found to be safe, by the Food and Drug Administration or approved by the Environmental Protection Agency or the Food Safety and Inspection Service of the U.S. Department of Agriculture.

Exempt 7. Storage or maintenance for secondary research for which broad consent is required: Storage or maintenance of identifiable private information or identifiable biospecimens for potential secondary research use if an IRB conducts a limited IRB review and makes the determinations required by 45 CFR 46.111(a)(8).

Exempt 8. Secondary research for which broad consent is required: Research involving the use of identifiable private information or identifiable biospecimens for secondary research use, if the following criteria are met:

(i) Broad consent for the storage, maintenance, and secondary research use of the identifiable private information or identifiable biospecimens was obtained in accordance with 45 CFR 46.116(a)(1) through (4), (a)(6), and (d);

(ii) Documentation of informed consent or waiver of documentation of consent was obtained in accordance with 45 CFR 46.117;

(iii) An IRB conducts a limited IRB review and makes the determination required by 45 CFR 46.111(a)(7) and makes the determination that the research to be conducted is within the scope of the broad consent referenced in paragraph (d)(8)(i) of this section; and

(iv) The investigator does not include returning individual research results to subjects as part of the study plan. This provision does not prevent an investigator from abiding by any legal requirements to return individual research results.

¹Exempt categories (1), (2), (3), (4), (5), (7), and (8) cannot be applied to activities that are FDA-regulated.

²Each of the exemptions at this section may be applied to research subject to subpart B (Additional Protections for Pregnant Women, Human Fetuses and Neonates Involved in Research) if the conditions of the exemption are met.

³The exemptions at this section do not apply to research subject to subpart C (Additional Protections for Research Involving Prisoners), except for research aimed at involving a broader subject population that only incidentally includes prisoners.

⁴Exemptions (1), (4), (5), (6), (7), and (8) of this section may be applied to research subject to subpart D (Additional Protections for Children Involved as Subjects in Research) if the conditions of the exemption are met. Exempt (2)(i) and (ii) only may apply to research subject to subpart D involving educational tests or the observation of public behavior when the investigator(s) do not participate in the activities being observed. Exempt (2)(iii) may not be applied to research subject to subpart D.

A. 2 The IRB approval letter “The effects of sleep on salt taste measures and intake relationships”

MICHIGAN STATE
UNIVERSITY

Initial Study APPROVAL
Revised Common Rule

October 20, 2021

To: Robin Marie Tucker

Re: **MSU Study ID:** STUDY00004101
IRB: Biomedical and Health Institutional Review Board
Principal Investigator: Robin Marie Tucker
Category: Expedited 4 & 7
Submission: Initial Study STUDY00004101
Submission Approval Date: 10/20/2021
Effective Date: 10/20/2021
Study Expiration Date: **None; however modification and closure submissions are required (see below).**

Title: Saltiness perception after sleep curtailment

This submission has been approved by the Michigan State University (MSU) Biomedical and Health Institutional Review Board. The submission was reviewed by the Institutional Review Board (IRB) through the Non-Committee Review procedure. The IRB has found that this study protects the rights and welfare of human subjects and meets the requirements of MSU's Federal Wide Assurance (FWA00004556) and the federal regulations for the protection of human subjects in research (e.g., 2018 45 CFR 46, 21 CFR 50, 56, other applicable regulations).



**Office of
Regulatory
Affairs**
**Human Research
Protection Program**

4000 Collins Road
Suite 136
Lansing, MI 48910

517-355-2180
Fax: 517-432-4503
Email: irb@msu.edu
www.hrpp.msu.edu

How to Access Final Documents

To access the study's final materials, including those approved by the IRB such as consent forms, recruitment materials, and the approved protocol, if applicable, please log into the Click™ Research Compliance System, open the study's workspace, and view the "Documents" tab. To obtain consent form(s) stamped with the IRB watermark, select the "Final" PDF version of your consent form(s) as applicable in the "Documents" tab. Please note that the consent form(s) stamped with the IRB watermark must typically be used.

Expiration of IRB Approval: The IRB approval for this study does not have an expiration date. Therefore, continuing review submissions to extend an approval period for this study are not required. **Modification and closure submissions are still required (see below).**

Modifications: Any proposed change or modification with certain limited exceptions discussed below must be reviewed and approved by the IRB prior to implementation of the change. Please submit a Modification request to have the changes reviewed.

New Funding: If new external funding is obtained to support this study, a Modification request must be submitted for IRB review and approval before new funds can be spent on human research activities, as the new funding source may have additional or different requirements.

Immediate Change to Eliminate a Hazard: When an immediate change in a research protocol is necessary to eliminate a hazard to subjects, the proposed change need not be reviewed by the IRB prior to its implementation. In such situations, however, investigators must report the change in protocol to the IRB immediately thereafter.

Reportable Events: Certain events require reporting to the IRB. These include:

- Potential unanticipated problems that may involve risks to subjects or others
- Potential noncompliance
- Subject complaints
- Protocol deviations or violations
- Unapproved change in protocol to eliminate a hazard to subjects
- Premature suspension or termination of research
- Audit or inspection by a federal or state agency
- New potential conflict of interest of a study team member
- Written reports of study monitors
- Emergency use of investigational drugs or devices
- Any activities or circumstances that affect the rights and welfare of research subjects
- Any information that could increase the risk to subjects

Please report new information through the study's workspace and contact the IRB office with any urgent events. Please visit the Human Research Protection Program (HRPP) website to obtain more information, including reporting timelines.

Personnel Changes: Key study personnel must be listed on the MSU IRB application for expedited and full board studies and any changes to key study personnel must be submitted as modifications. Although only key study personnel need to be listed on a non-exempt application, all other individuals engaged in human subject research activities must receive and maintain current human subject training, must disclose conflict of interest, and are subject to MSU HRPP requirements. It is the responsibility of the Principal Investigator (PI) to maintain oversight over all study personnel and to assure and to maintain appropriate tracking that these requirements are met (e.g. documentation of training completion, conflict of interest). When non-MSU personnel are engaged in human research, there are additional requirements. See HRPP Manual Section 4-10, Designation as Key Project Personnel on Non-Exempt IRB Projects for more information.

Prisoner Research: If a human subject involved in ongoing research becomes a prisoner during the course of the study and the relevant research proposal was not reviewed and approved by the IRB in accordance with the requirements for

research involving prisoners under subpart C of 45 CFR part 46, the investigator must promptly notify the IRB.

Site Visits: The MSU HRPP Compliance office conducts post approval site visits for certain IRB approved studies. If the study is selected for a site visit, you will be contacted by the HRPP Compliance office to schedule the site visit.

For Studies that Involve Consent, Parental Permission, or Assent Form(s):

Use of IRB Approved Form: Investigators must use the form(s) approved by the IRB and must typically use the form with the IRB watermark.

Copy Provided to Subjects: A copy of the form(s) must be provided to the individual signing the form. In some instances, that individual must be provided with a copy of the signed form (e.g. studies following ICH-GCP E6 requirements). Assent forms should be provided as required by the IRB.

Record Retention: All records relating to the research must be appropriately managed and retained. This includes records under the investigator's control, such as the informed consent document. Investigators must retain copies of signed forms or oral consent records (e.g., logs). Investigators must retain all pages of the form, not just the signature page. Investigators may not attempt to de-identify the form; it must be retained with all original information. The PI must maintain these records for a minimum of three years after the IRB has closed the research and a longer retention period may be required by law, contract, funding agency, university requirement or other requirements for certain studies, such as those that are sponsored or FDA regulated research. See HRPP Manual Section 4-7-A, Recordkeeping for Investigators, for more information.

Closure: If the research activities no longer involve human subjects, please submit a Continuing Review request, through which study closure may be requested. Closure indicates that research activities with human subjects are no longer ongoing, have stopped, and are complete. Human research activities are complete when investigators are no longer obtaining information or biospecimens about a living person through interaction or intervention with the individual, obtaining identifiable private information or identifiable biospecimens about a living person, and/or using, studying, analyzing, or generating identifiable private information or identifiable biospecimens about a living person.

For More Information: See the HRPP Manual (available at hrpp.msu.edu).

Contact Information: If we can be of further assistance or if you have questions, please contact us at 517-355-2180 or via email at IRB@msu.edu. Please visit hrpp.msu.edu to access the HRPP Manual, templates, etc.

Expedited Category. Please see the appropriate research category below for the full regulatory text.

Expedited 1. Clinical studies of drugs and medical devices only when condition (a) or (b) is met.

(a) Research on drugs for which an investigational new drug application (21 CFR Part 312) is not required. (Note: Research on marketed drugs that significantly increases the risks or decreases the acceptability of the risks associated with the use of the product is not eligible for expedited review.)

(b) Research on medical devices for which (i) an investigational device exemption application (21 CFR Part 812) is not required; or (ii) the medical device is cleared/approved for marketing and the medical device is being used in accordance with its cleared/approved labeling.

Expedited 2. Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture as follows:

(a) from healthy, nonpregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn may not exceed 550 ml in an 8 week period and collection may not occur more frequently than 2 times per week; or

(b) from other adults and children, considering the age, weight, and health of the subjects, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.

Expedited 3. Prospective collection of biological specimens for research purposes by noninvasive means.

Examples: (a) hair and nail clippings in a nondisfiguring manner; (b) deciduous teeth at time of exfoliation or if routine patient care indicates a need for extraction; (c) permanent teeth if routine patient care indicates a need for extraction; (d) excreta and external secretions (including sweat); (e) uncannulated saliva collected either in an unstimulated fashion or stimulated by chewing gumbase or wax or by applying a dilute citric solution to the tongue; (f) placenta removed at delivery; (g) amniotic fluid obtained at the time of rupture of the membrane prior to or during labor; (h) supra- and subgingival dental plaque and calculus, provided the collection procedure is not more invasive than routine prophylactic scaling of the teeth and the process is accomplished in accordance with accepted prophylactic techniques; (i) mucosal and skin cells collected by buccal scraping or swab, skin swab, or mouth washings; (j) sputum collected after saline mist nebulization.

Expedited 4. Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)

Examples: (a) physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the subject or an invasion of the subject's privacy; (b) weighing or testing sensory acuity; (c) magnetic resonance imaging; (d) electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, doppler

blood flow, and echocardiography; (e) moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual.

Expedited 5. Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(4). This listing refers only to research that is not exempt.)

Expedited 6. Collection of data from voice, video, digital, or image recordings made for research purposes.

Expedited 7. Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(2) and (b)(3). This listing refers only to research that is not exempt.)

Expedited 8. Continuing review of research previously approved by the convened IRB as follows:

(a) where (i) the research is permanently closed to the enrollment of new subjects; (ii) all subjects have completed all research-related interventions; and (iii) the research remains active only for long-term follow-up of subjects; or

(b) where no subjects have been enrolled and no additional risks have been identified; or

(c) where the remaining research activities are limited to data analysis.

Expedited 9. Continuing review of research, not conducted under an investigational new drug application or investigational device exemption where categories two (2) through eight (8) do not apply but the IRB has determined and documented at a convened meeting that the research involves no greater than minimal risk and no additional risks have been identified.

Appendix B: Consent forms

B. 1 Consent forms for “Health Behaviors of Higher Education Students from 7 Countries: Poor sleep quality during the COVID-19 Pandemic Predicts Higher Dietary Risk” and “The Effects of Sleep Quality and Resilience on Perceived Stress, Dietary Behaviors, and Alcohol Misuses: A Mediation-Moderation Analysis of Higher Education Students from Asia, Europe, and North American during the COVID-19 Pandemic”

Informed Consent to Participate in Research

You are being asked to participate in a research study. Researchers are required to provide a consent form to inform you about the research study, to convey that participation is voluntary, to explain risks and benefits of participation, and to empower you to make an informed decision. You should feel free to ask the researchers any questions you may have. If you have any questions please email your questions to Dr. Robin Tucker, PhD, RD, FAND at tucker98@msu.edu

Study Title: University Students' Resilience

Researchers and Title: Robin Tucker, PhD, RD, FAND

Address and Contact Information: 2110 S. Anthony Hall, 517-353 3408, tucker98@msu.edu

PURPOSE OF RESEARCH

You are being asked to participate in a research study that is investigating the relationship between sleep, diet, and physical activity and resilience to stress. You have been selected as a possible participant in this study because you are either an undergraduate student or a graduate student at Michigan State University, and you are 18 years old and older. From this study, the researchers hope to better understand how diet, sleep, and physical activity impact resilience to stress for University students. The study will include a total of 200 university students.

WHAT YOU WILL DO

If you agree to participate, you will be asked to complete a series of surveys. The surveys include questions about:

Who you are

Your weight and height

Exposure to and how you handle stress

How COVID-19 has impacted your stress levels

Your diet and alcohol consumption (21-years-old or older only)

Your sleep quality and physical activity

It will take approximately 45 minutes to complete the series of surveys.

Please take the survey by yourself in a private location, where you feel comfortable. Please take the survey on a personal computer or a password protected public computer.

COMPENSATION

Within 7 days of successful completion of the survey, you will receive a \$10 Amazon.com gift card.

You must provide an MSU.edu email address. And, the Amazon.com gift card will be delivered to your msu.edu email address.

POTENTIAL RISKS

There are minimal risks to this study. Some participants might feel uncomfortable being exposed

to questions asking about mental health. Some participants might feel uncomfortable reporting current weight and height. If you feel uncomfortable answering any of the questions in the survey, feel free to skip those questions. If you need to seek help from University counseling services, please contact Michigan State University Counseling & Psychiatric Services at 517-355-8270, Olin Health Center, 463 E Circle Dr, East Lansing, MI 48824. There is a risk of breach of confidentiality (see below).

PRIVACY AND CONFIDENTIALITY

The data for this project will be kept confidential. Although we will make every effort to keep your data confidential there are certain times, such as a court order, where we may have to disclose your data. The principal investigator and research student assistants will have access to the data. The Human Research Protection Program (HRPP) may review records to ensure researcher compliance with federal law and university policy. Your data will be stored in a password-protected computer file. Electronic files will be stored on a computer in password-protected documents and will not be destroyed. Once the study is complete, any personally identifiable information will be removed from the data files. The results of this study may be published or presented at professional meetings, but the identities of all research participants will remain anonymous.

YOUR RIGHTS TO PARTICIPATE, SAY NO, OR WITHDRAW

Participation is completely voluntary. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You may discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled. You have the right to say no. You may change your mind at any time and withdraw. You may choose not to answer specific questions or to stop participating at any time.

If you have concerns or questions about this study, such as scientific issues, how to do any part of it, or to report an injury, please contact the researcher: Dr. Robin Tucker, Michigan State University, 2110 S. Anthony Hall, 474 S. Shaw Ln., East Lansing, MI 48824, tucker98@msu.edu, 517-353-3408

If you have questions or concerns about your role and rights as a research participant, would like to obtain information or offer input, or would like to register a complaint about this study, you may contact, anonymously if you wish, the Michigan State University's Human Research Protection Program at 517-355-2180, Fax 517-432-4503, or e-mail irb@msu.edu or regular mail at 4000 Collins Rd, Suite 136, Lansing, MI 48910.

By clicking the "next" button and continuing to the survey you are consenting to participate in this survey.

B. 2 Consent form “The effects of sleep on salt taste measures and intake relationships”

Research Participant Information and Consent Form

You are being asked to participate in a research study. Researchers are required to provide a consent form to inform you about the research study, to convey that participation is voluntary, to explain risks and benefits of participation, and to empower you to make an informed decision. You should feel free to ask the researchers any questions you may have.

Study Title: Saltiness perception after sleep curtailment
Researcher and Title: Robin Tucker, PhD, RD, Assistant Professor
Department and Institution: Food Science & Human Nutrition, Michigan State University
Address and Contact Information: 2110 S. Anthony Hall, 517-353-3408, tucker98@msu.edu
Sponsor: None

1. PURPOSE OF RESEARCH

- You are being asked to participate in a research study that is investigating how sleep influences salt taste liking and preference of different salt concentrations.
- You have been selected as a possible participant in this study because you are between the ages of 18 and 45, do not have any sleep problems, do not have any diagnosed sleep conditions, have a regular weekday bedtime, sleep 7-9 hours on a regular night, can modify your normal bedtime and wake-up time, can participate across two weeks of testing, and do not have a pacemaker, cardiac defibrillator, use oxygen or any electronic medical devices.
- From this study, the researchers hope to learn if differences in sleep duration and quality are associated with differences in salt taste perception. Information gained from this study may help scientists identify whether sleep influences how saltiness is perceived.
 - Your participation in this study will take about 18.5 hours – about 16 hours of which will occur while you are sleeping at home. Only 2.5 hours will take place in the laboratory over the course of two weeks. In the entire study, 160 people are being asked to participate.

2. WHAT YOU WILL DO

- Come to Trout Hall, room 111 to meet with the research staff. Dr. Tucker or her associates will ask you to read, ask questions, and sign this consent form, if you agree to participate. You should only sign this form once all of your questions have been answered.
- Once you have signed the form:
 - You will complete online questionnaires that ask about your sleep duration and quality, food cravings, and stress levels for the past several weeks.
 - You will have your height, weight, and percent body fat measured.
 - You will receive instructions on how to use the Zmachine.
 - The Zmachine measures how long and how well you sleep. The Zmachine measures the electrical activity of your brain to assess sleep patterns. Use of the Zmachine is painless.
 - You will use the Zmachine for 2 nights – after each night, you will visit the lab for taste testing. We will work with you to schedule 1 night where you will get normal, habitual sleep and another night where you will go to bed later and wake up earlier, totaling about a 33% reduction in sleep compared to your normal night.
 - Use of the Zmachine involves the placement of three electrodes on your skin – 2 behind your ear and 1 on the back of your neck – at least 30 minutes before bed.
 - You will first clean the areas where the electrodes are to be placed with an alcohol swab. Allow the areas to dry.
 - Peel the clear backing off of the sensor and adhere it to the bony part of the back of your ear with the snap facing down. Secure by rubbing the outer edge of the sensor. Do not touch the middle of the pad, as this will prevent the sensor from working. Repeat this procedure on the other side, and once more for the back of your neck. Avoid placing the sensor over hair. If this is impossible on the neck, then the back of one shoulder can be used instead.

- Do not try to re-use or reapply a sensor. Always use a new one.
- When you are ready for bed, connect the sensors to the wires of the Zmachine by snapping the wires to the snaps on the sensors. The two white wires can be connected to either ear sensor. The black wire must be connected to the neck sensor. Next, connect the sensor cable to the Zmachine. Once this has been done, the Zmachine will turn on and check the sensors. Once all sensors are shown to be working, you can set the Zmachine on a nightstand, place it in the bed next to you, or put it in a pocket and go to bed. Laying on the wires or cable is perfectly acceptable.
- In the morning, disconnect the sensor cable from the Zmachine. Unsnap the wires from the sensor pads. Slowly peel the sensor pads from the skin. Dispose of the pads and gently clean your skin with soap and water or an alcohol pad.
- Charge the Zmachine with the charging cable provided. This must be done after the first night. The Zmachine is not designed to work more than one night without being recharged.
- If the Zmachine becomes disconnected during the night, you will need to repeat the testing on another night.
- Please note: The Zmachine cannot diagnose sleep disorders/abnormalities, and if you need sleep evaluation you should pursue this with your physician.
- Please note: You should keep the Zmachine in a secure location out of reach from young children and pets. The Zmachine should only be used by you, the consenting participant.

The day before lab testing, you will need to pick up the Zmachine from the lab.

The night before both lab testing visits:

- Connect the Zmachine as before.
- Go to bed at your usual time and wake at your usual time OR go to bed and wake up at the times specified by the researchers (you will be randomly assigned to start either with a normal night of sleep or a shorter one).

At each lab appointment

- Your lab appointment will last about an hour and will take place at the GM Trout building room 111, 469 Wilson Rd, East Lansing, MI. Your lab visit will take place in the morning after you record your sleep with the Zmachine.
- Bring your Zmachine with you to your lab appointment.
- Upon arriving, we will ask you to:
 - Give us your Zmachine to confirm you wore it for the past night and that all data were collected. If there is a problem with the machine, you will need to repeat the testing.
 - Tell us how tired you are and answer other questions regarding last night's sleep, how sleepy you are at that moment.
- During testing, we will have you perform several taste tests:
 - Taste testing
 - You will be asked to wear a face shield during taste testing to protect against COVID-19 transmission. You and the researcher will remain at least 6 feet apart during taste testing.
 - You will be given a series of five small cups containing about a mouthful of salt water. These solutions will contain varying concentrations of salt that range in concentration from weakly salty to approximately ocean water. While wearing nose clips, we will ask you to swish each solution in your mouth and then spit the solution out. In between each solution, you will swish and spit water. After each solution has been tasted, we will ask you to report how much you 1) like the solution and (first test) 2) how intense you perceive the saltiness to be (second test).
 - The third taste test involves tasting different concentrations salt solutions and telling us which one you prefer. You will swish and spit as before. There are five possible solutions, but depending on your responses, you might not taste all of them. Most people taste about 8 pairs of solutions. The concentrations are the same as those used in the liking and intensity tests.

- Dietary Recall Questionnaire (ASA24)
 - Each day following the taste tests, you will be asked to fill out a short diet questionnaire (ASA24) online. The researchers will provide the website link, username, and passcode to you for accessing the diet questionnaire.

3. POTENTIAL BENEFITS

You will not directly benefit from your participation in this study. However, your participation in this study may contribute to the understanding of how sleep and sleep curtailment influences taste and salt liking.

4. POTENTIAL RISKS

- The potential risks of participating in this study are:
 - There is a risk of a breach of confidentiality.
 - Some of the tastes you experience could be unpleasant.
 - Sleep curtailment may be unpleasant for some; you could feel tired as a result.
 - Reduction in sleep duration for one day may negatively influence routine activities, such as driving, and academic or work performance.
 - Some people might experience irritation or allergic reactions from the sensors. If this occurs, immediately discontinue the use of the Zmachine and inform the researchers.
 - Some people may feel uncomfortable getting their weight, height, and body fat measured.

5. PRIVACY AND CONFIDENTIALITY

- The data for this project will be kept confidential.
- Although we will make every effort to keep your data confidential there are certain times, such as a court order, where we may have to disclose your data. The principal investigator and research student assistants will have access to the data. The Human Research Protection Program (HRPP) may review records to [insure](#) researcher compliance with federal law and university policy.
- Your data will be stored in a locked filing cabinet and in a password-protected computer file. Hard copies of the data will be retained for 3 years after the project ends, after which they will be destroyed by shredding. Electronic files will be stored on a computer in password-protected documents and will not be destroyed. Your name will never be on any of the electronic files. The results of this study may be published or presented at professional meetings, but the identities of all research participants will remain anonymous. Your data will be kept confidential to the 'maximum extent allowable by law.

6. YOUR RIGHTS TO PARTICIPATE, SAY NO, OR WITHDRAW

- Participation is voluntary. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You may discontinue participation at any time without penalty or loss of benefits, as stated in the benefits section of this document, to which you are otherwise entitled.
- You have the right to say no.
- You may change your mind at any time and withdraw.
- You may choose not to answer specific questions or to stop participating at any time.

7. COSTS AND COMPENSATION FOR BEING IN THE STUDY

- There are no costs to participate in this study.
 - Upon completion of the 2 nights of sleep recording and 4 laboratory visits and the return of the Zmachine and its accessories, you will receive a \$50 Amazon gift card sent to your email address. If you only complete the first lab visit, you will receive a \$20 Amazon gift card, also sent to your email address.

8. THE RIGHT TO GET HELP IF INJURED

If you are injured as a result of your participation in this research project, Michigan State University will assist you in obtaining emergency care, if necessary, for your research related injuries. If you have insurance for medical care, your insurance carrier will be billed in the ordinary manner. As with any medical insurance, any costs that are not covered or in excess of what are paid by your insurance, including deductibles, will be your responsibility. The University's policy is not to provide financial compensation for lost wages, disability, pain or discomfort, unless required by law to do so. This does not mean that you are giving up any legal rights you may have. You may contact Dr. Robin Tucker (tucker98@msu.edu, 517-353-3408) with any questions or to report an injury.

9. FUTURE RESEARCH

Information that identifies you might be removed from the Z-machine and the identifiable private information record. After such removal, the information including sleep data, weight, height, BMI, and survey data could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you. If you allow your de-identified data to be used in future studies, please initial here _____.

Or, if you would like to participate in the current study but not allow your de-identified data to be used for future research, please initial here _____.

10. CONTACT INFORMATION

If you have concerns or questions about this study, such as scientific issues, how to do any part of it, or to report an injury, please contact the researcher: Dr. Robin Tucker, Michigan State University, 2110 S. Anthony Hall, 274 S. Shaw Ln., East Lansing, MI 48824, tucker98@msu.edu, 517-353-3408).

If you have questions or concerns about your role and rights as a research participant, would like to obtain information or offer input, or would like to register a complaint about this study, you may contact, anonymously if you wish, the Michigan State University's Human Research Protection Program at 517-355-2180, Fax 517-432-4503, or e-mail irb@msu.edu or regular mail at 4000 Collins Rd, Suite 136, Lansing, MI 48910.

11. DOCUMENTATION OF INFORMED CONSENT

Your signature below means that you voluntarily agree to participate in this research study.

Signature

Date

You will be given a copy of this form to keep.

BIBLIOGRAPHY

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