Differential Effects of Split and Continuous Sleep on Neurobehavioral Function and Glucose Tolerance in Sleep-Restricted Adolescents

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ABSTRACT

Study Objectives: Many adolescents are exposed to sleep restriction on school nights. We assessed how different apportionment of restricted sleep (continuous versus split sleep) influences neurobehavioral function and glucose levels.

Methods: Adolescents, aged 15-19 years, were evaluated in a dormitory setting using a parallel-group design. Following 2 baseline nights of 9-h time-in-bed (TIB), participants underwent either 5 nights of continuous 6.5-h TIB (n=29) or 5-h nocturnal TIB with a 1.5-h afternoon nap (n=29). After 2 recovery nights of 9-h TIB, participants were sleep restricted for another 3 nights. Sleep was assessed using polysomnography (PSG). Cognitive performance and mood were evaluated 3 times per day. Oral Glucose Tolerance Tests (OGTT) were conducted on mornings after baseline sleep, recovery sleep, and the third day of each sleep restriction cycle.

Results: The split sleep group had fewer vigilance lapses, better working memory and executive function, faster processing speed, lower level of subjective sleepiness, and more positive mood, even though PSG-verified total sleep time was less than the continuous sleep group. However, vigilance in both sleep-restricted groups was inferior to adolescents in a prior sample given 9-h nocturnal TIB. During both cycles of sleep restriction, blood glucose during the OGTT increased by a greater amount in the split sleep schedule compared with persons receiving 6.5-h continuous sleep.

Conclusions: In adolescents, modest multi-night sleep restriction had divergent negative effects on cognitive performance and glucose levels depending on how the restricted sleep was apportioned. They are best advised to obtain the recommended amount of nocturnal sleep.

Keywords: adolescents, cognition, continuous sleep, glucose tolerance, partial sleep deprivation, sleep restriction, split sleep, vigilance

Clinical trial: https://clinicaltrials.gov/ct2/show/NCT03333512

Statement of significance:

Many adolescents do not get adequate sleep, but the outcomes of how that restricted sleep is apportioned have not been studied. During a simulated school week with time-in-bed restricted to 6.5 h per day, adolescents with a split sleep schedule (night sleep plus afternoon nap) exhibited better vigilance, working memory / executive function, processing speed, subjective alertness, and mood compared with adolescents with continuous night sleep. However, they exhibited a greater increase in blood glucose during a glucose tolerance test. Under conditions of suboptimal sleep, effects on neurobehavioral outcomes and morning glucose levels diverge depending on how sleep is apportioned.

INTRODUCTION

Many adolescent students sleep less than the recommended duration of 8-10 h[1,2] a night.[3,4] Even those who take compensatory naps may fall short of their daily sleep need for optimal cognitive performance, mood and physical health. Existing sleep restriction studies have mainly examined adults and have evaluated cognition and metabolism separately with protocols that mostly manipulate only nocturnal sleep.[5-8] While naps can benefit neurobehavioral function[9,10] and are commonplace in some societies,[11] they have typically been studied as an 'add-on' or 'booster' as opposed to a scenario where naps are integrated into schedules, such that a person's total sleep opportunity over 24 h is unaltered upon inclusion of the nap. The handful of studies that examined split sleep schedules in working-age adults found that such schedules yield comparable cognitive performance when compared to an equivalent amount of continuous sleep.[12-15] However, no insight has been provided about their metabolic impact, a significant gap given that short sleep is associated with increased risk of diabetes mellitus.[16,17] Expert opinion suggests that optimal sleep duration for maintenance of neurobehavioral function and metabolic health may differ,[18] but there is a dearth of supportive empirical data.

The aim of the present study was to determine whether neurobehavioral function and glucose levels differ in sleep-restricted *adolescents* when sleep is either split (primary night sleep opportunity with a daytime nap) or taken in a single nocturnal sleep episode. This objective was achieved by tracking performance and glucose tolerance during 2 cycles of sleep restriction and recovery, in which adolescents were scheduled to have either continuous sleep (nocturnal time-in-bed [TIB] = 6.5 h) or split sleep (nocturnal TIB = 5 h plus a 1.5-h afternoon nap) during sleep restriction. The two cycles of sleep restriction provided an opportunity to evaluate the degree of recovery following the intervening recovery sleep (9-h TIB) as well as the added alterations in neurobehavioral function [9], and the consistency of glucose tolerance measurements. Neurobehavioral function in these two groups was also

compared to a reference historical control group that received an age-appropriate amount of sleep (9-h TIB) every night over 2 weeks.[19]

METHODS

Participants

The same sample size and inclusion criteria were used in three previous studies from our group:[9,19,20] 15-19 years of age, no known health conditions, no sleep disorders, body mass index of ≤30 kg/m^2, not a habitual short sleeper (actigraphically measured TIB <6 h averaged across weekdays and weekends, with weekend sleep extension ≤1 h), consumption of ≤5 cups of caffeinated beverages a day, and no travel across >2 time zones 1 month prior to the experiment.

A total of 126 adolescents were assessed for eligibility for this 15-day parallel-group study. Of these, 60 (30 males) were randomly assigned to the split sleep group (n = 30) and the continuous sleep group (n = 30). Two participants dropped out, and analyses were based on 58 participants (Figure S1). While the primary goal of the current work was to compare two sleep restriction schedules, the data generated was also appraised in light of the recommended sleep duration for adolescents (8-10h/ night). To this end, we compared the present findings to previously published data on students sleeping 9h TIB at night,[19] recruited using the recruitment criteria employed in the present study.

The three groups were similar in multiple measures assessed during screening, including age, sex, and BMI percentile (based on the Singaporean BMI-for-age growth charts), as well as daily caffeine consumption, morningness-eveningness preference,[21] excessive daytime sleepiness,[22] and symptoms of chronic sleep reduction[23] (P > .10; Table 1). Although the split and the continuous sleep groups did not differ in sleep behavior based on both self-report[24] and actigraphy (Table 1), some slight differences were found with the

control group from our previous protocol 3 years ago. Specifically, the control group seemed to sleep less on weekdays, but extended their sleep more on weekends. Thus, critically, actigraphically assessed TST averaged across the week was comparable across all three groups ($P > 0.66$). Overall, based on actigraphy data, the three groups spent about 6.1 to 7.0 h per night in bed on school nights, with more than an hour of sleep extension on weekends. This was far less than the recommended sleep duration of 8-10 h for adolescents.[1,2] Self-reported nap duration, which was not assessed in the control group, averaged 1 h in both split sleep and the continuous sleep groups (Table 1, $P = 0.75$).

During the week prior to the experiment, napping was not allowed and a 9-h nocturnal sleep schedule (23:00-08:00) was enforced for minimizing the effects of prior sleep loss and for facilitating stable circadian entrainment. The split and the continuous sleep groups did not differ in actigraphically assessed TIB (mean ± SEM for continuous sleep: 8.99 ± 0.06 h vs. split sleep: 9.07 ± 0.08 h, $P = 0.43$) or total sleep time (TST) (7.37 ± 0.08 h vs. 7.44 ± 0.10 h, $P = 0.62$).

Study Protocol

The Need for Sleep (NFS) study 4 was conducted during the vacation period in 2017 in a student dormitory (refer to Supplementary Materials for details of the living environment). The 15-day protocol (Figure 1) started with 2 baseline nights (B_1-B_2) of 9-h nocturnal TIB (23:00-08:00) for adaptation and baseline characterization, followed by 2 cycles of sleep restriction and recovery sleep. The first cycle began with 5 nights of sleep restriction ($SR1_1$-$SR1_5$) and ended with 2 nights of 9-h recovery sleep opportunity ($R1_1$-$R1_2$). During the sleep restriction nights, the continuous sleep group had 6.5 h of nocturnal TIB (00:15-06:45), while the split sleep group had 5 h of TIB at night (01:00-06:00) with a 1.5-h nap opportunity in the mid-afternoon (14:00-15:30) the following day. The second cycle

consisted of 3 nights of sleep restriction with the same TIB manipulation ($SR2_1$-$SR2_3$) and 2 recovery nights ($R2_1$-$R2_2$).

Polysomnographic (PSG) data were collected during selected sleep and nap episodes (Figure 1). Neurobehavioral function was assessed with a cognitive test battery at 10:00, 16:15, and 20:00 every day, except the first and the last days of the protocol. Participants also underwent a 75-g Oral Glucose Tolerance Test (OGTT) on 4 different mornings: following baseline sleep (B_2), sleep restriction ($SR1_3$), recovery sleep ($R1_2$), and re-exposure to sleep restriction ($SR2_3$).

The study was approved by the Institutional Review Board of the National University of Singapore, and conducted according to the principles in the Declaration of Helsinki. All participants and their legal guardians gave informed consent prior to participating in the study.

Actigraphy

A wrist actiwatch (Actiwatch 2, Philips Respironics Inc., Pittsburgh, PA) was worn on the non-dominant hand for 1 week during the preceding school term for screening purposes. Data were collected in 2-min epochs and were scored using Actiware software (version 6.0.7) with a medium wake-sensitivity threshold (activity count ≥ 40). Bedtimes and wake times were determined using event markers on the actogram and corroborated with self-reported sleep and wake times on a sleep diary. Actigraphy was also performed in the 1-week pre-study period for verification of compliance with the prescribed 9-h nocturnal sleep schedule, and during the 15-day protocol.

Polysomnography

Electroencephalography (EEG) was performed using a SOMNOtouch recorder (SOMNOmedics GmbH, Randersacker, Germany) on two channels (C3 and C4 in the international 10-20 system). Contralateral mastoids were used as references. Electrodes placed at Cz and Fpz were used as common reference and ground electrodes respectively. Electrooculography (EOG) and submental electromyography (EMG) were also utilized. Impedance was kept below 5 kΩ for EEG and 10 kΩ for EOG and EMG electrodes. Signal was sampled at 256 Hz and filtered between 0.2 and 35 Hz for EEG, and between 0.2 and 10 Hz for EOG.

Sleep stages and artefactual epochs were automatically scored using the z3score algorithm (https://z3score.com)[25] in conjunction with the FASST toolbox (http://www.montefiore.ulg.ac.be/~phillips/FASST.html), and visually checked by trained technicians, following criteria set by the American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events.[26] Pulse oximetry was used in the first night (B_1) to evaluate oxygen saturation and rule out undiagnosed sleep apnea.

The following sleep parameters (in minutes) were computed for each polysomnographic record: total sleep time (TST), N2 latency (time from lights off to N2 sleep onset), and duration of individual sleep stages (N1, N2, N3, and rapid eye movement [REM] sleep). As an indicator of homeostatic sleep pressure, slow wave activity (SWA) in the first hour of nocturnal sleep from N2 sleep onset was computed from 5-s artifact-free epochs from C3/A2 using custom routines written in Matlab R2012a (The MathWorks, Inc. Natick, MA). For each epoch, power spectral density estimates were obtained using a fast Fourier transform routine (Hamming window; 0.2Hz bin resolution) and integrated from 0.6 to 4Hz using the trapezoidal rule for integral approximation to obtain SWA measures per epoch. SWA was then averaged across all NREM epochs in the first hour and expressed as a percentage of mean SWA in the first hour of the baseline night (B_2). Recordings containing more than 10% artifacts (from epochs scored as NREM sleep) were excluded from further analyses (1% of all records).

Cognitive Performance Test Battery

The test battery lasted approximately 25 min and comprised seven tasks in the following order: the Karolinska Sleepiness Scale,[27] the Symbol Digit Modalities Test,[28] the verbal 1- and 3-back tasks,[7] the Mental Arithmetic Test,[29] the Positive and Negative Affect Scale,[30] and a 10-min Psychomotor Vigilance Task (PVT).[31] All the cognitive tests were programmed in E-Prime 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA).

The PVT was used to measure vigilance, the cognitive domain most affected by sleep loss.[7,19,32] It typically began 15 min into the test battery. A count-up timer that displayed elapsed time in milliseconds was presented in the center of the laptop display at random intervals between 2 and 10 seconds. Participants had to respond to the appearance of the count-up timer as quickly as possible by pressing the spacebar. Beeping tones were presented via a headphone if no response was detected 10 seconds after stimulus onset. Vigilance was indicated by the number of lapses (response time >500 ms). Details about the other cognitive tasks have been previously published.[9,19]

Oral Glucose Tolerance Test

Capillary blood was collected using the finger-prick method (Accu-Chek® Performa blood glucose meter system, Roche, Mannheim, Germany), with glucose measurements taken immediately before and 2 h after administering oral glucose (Trutol 75, Thermo Fisher Scientific Inc., Middletown, VA). The meter meets the ISO 15197 requirements for in vitro diagnostic test systems for blood-glucose monitoring systems for self-testing in managing diabetes (≥95% of the system measurement results must fall within ±15 mg/dl of the results of the manufacturer's measurement procedure at glucose concentrations <100 mg/dl and within ±15% at glucose concentrations ≥100 mg/dL).[33] Each meter was verified for accuracy

of measurement with test strips prior to the conduct of the study. Each OGTT was performed after overnight fasting (>8 h), with the first glucose measurement taken between 08:30-09:00. Outcomes included fasting glucose, 2-h glucose, and glucose excursion, which was defined as the change in blood glucose from the fasting state to 2 h after the oral glucose load.

Statistical Analysis

Subject characteristics. ANOVA and Chi-squared tests were used to detect differences in screening variables among the control, continuous and split sleep groups.

Cognitive performance and PSG-assessed sleep. Cognitive data were analyzed by combining datasets for the split sleep and continuous sleep groups with data from a control group in a previous study in which participants were given 9-h TIB for sleep each night.[19] A general linear mixed model with PROC MIXED in SAS 9.4 (SAS Institute, Cary, NC) was used to determine the effects of group (3 groups), day (from B_2 to $R2_1$), and their interaction on the number of lapses averaged across the three PVTs each day. Performance in the third test battery on day B_1 (the fifth test battery participants had done) was used as a covariate to control for group differences in baseline performance. The same statistical model was applied separately to PVTs taken during the morning, afternoon, and evening. We used the same statistical models for the other neurobehavioral functions.

Similar models were used to determine the effects of group (continuous sleep and split sleep), day (from night B_2 to $R2_1$) and group × day interaction on (1) PSG-assessed TST and duration of sleep stages at night, as well as per 24-h interval, and (2) SWA 1 hour after N2 sleep onset at night. PSG data from the adaptation night (i.e. B_1) were not included in the analyses. Sleep macro-architecture of the nap episodes during the two sleep restriction periods were also investigated. Least square means and standard errors estimated with PROC MIXED were plotted.

Blood glucose. A mixed ANOVA was used to test for interaction and main effects of group (continuous sleep and split sleep) and day of OGTT (B_2, $SR1_3$, $R1_2$, and $SR2_3$) on each glucose measure, with multiple comparison testing performed using the Holm-Sidak method (Sigmaplot 12; Systat Software Inc, San Jose, CA). Glucose values are reported as least square means and standard errors based on results of the ANOVA.

RESULTS

Splitting sleep reduced sleep pressure at night in spite of a lower total sleep time

During the baseline night (B_2) with 9-h TIB, both groups had similar PSG-assessed sleep characteristics, including TST and time spent in N2, N3, and REM sleep (Figure 2; $P > .08$). In both sleep restriction periods, the continuous sleep group had 84 to 101 min more of nocturnal sleep than the split sleep group, because they had more TIB at night (Figure S2A). As expected, the continuous sleep group had more N2, N3, and REM sleep at night compared with the split sleep group ($P < .02$; Figure S2C-E). During their 90-min daytime nap opportunity, the split sleep group slept about 71-79 min on average, comprising predominantly N2 and N3 sleep, with lesser amounts of REM sleep (Figure S2C-E). Overall, splitting sleep shortened total daily TST by 15-21 min compared with continuous sleep ($SR1_1$-$SR1_5$ and $SR2_3$: $P < .007$; Figure 2A). Total N3 sleep duration was preserved and similar in both groups ($P > 0.09$ on all SR days excepting $SR2_1$ when $P = .04$; Figure 2D). Notably, the reduction of homeostatic sleep pressure afforded by afternoon naps was associated with longer nocturnal N2 onset latency ($SR1_3$ to $R1_1$ and $R2_1$: $P < .03$; Figure 3A); and reduced slow wave activity (SWA; $SR1_3$ to $R1_1$ and $SR2_3$-$R2_1$: $P < .05$; Figure 3B).

During the recovery nights ($R1_1$ and $R2_1$), TST (Figure 2A), N3 sleep duration (Figure 2D), N2 onset latency (Figure 3A) and SWA (Figure 3B) were lower in the split sleep group

relative to the continuous sleep group ($P < .005$), likely a result of greater dissipation of homeostatic sleep pressure during the nap opportunity in the preceding afternoon. This was accompanied by an increase in N2 duration ($P < .05$; Figure 2C).

Split sleep was associated with relatively better neurobehavioral function

While vigilance performance as indicated by the number of PVT lapses was similar in the two groups at baseline, the split sleep group exhibited fewer lapses than the continuous sleep group during both cycles of sleep restriction and in the intervening recovery sleep (group × day interaction: $F = 3.47$, $P < .001$; Figure 4A). During the first sleep restriction period, the split sleep group maintained PVT performance ($SR1_1$ vs. $SR1_5$: $P = .10$), while the continuous sleep group showed an increase in lapses ($P < .001$). Both groups showed further deterioration in performance during the second sleep restriction period (e.g. $SR1_3$ vs. $SR2_3$: $P = .02$ and .03).

While exhibiting poorer morning vigilance relative to the control group that received 9 h of TIB in our previous study[19] (Figure 4B; $SR1_3$-$SR1_5$: $P < .05$; $R1_2$-$SR2_3$: $P < .04$), the split sleep group critically had fewer lapses than the continuous sleep group during the first period of sleep restriction ($SR1_2$-$SR1_5$: $P < .02$). Morning performance was similarly impaired in both the split sleep and continuous sleep groups during the second cycle of sleep restriction ($SR2_1$-$SR2_3$: $P > .22$). For tests taken 1 h and 4.75 h after the afternoon nap (Figure 4C-D), the split sleep group had a comparable number of lapses to the control group during both sleep restriction periods ($P > .12$). Participants in the split sleep group outperformed the continuous sleep group in the afternoon on all SR days ($P < .01$) and on all SR evenings ($P < .05$), except for the evening after very first night of sleep restriction ($P = .22$).

Relative to continuous sleep, split sleep was also associated with better working memory and executive function (Figure S3A), better speed of processing (Figure S3B), lower levels of subjective sleepiness (Figure S3C), and more positive mood (Figure S3D).

Continuous sleep was associated with a better morning glucose response

During sleep restriction, the split sleep group showed a greater increase in blood glucose (glucose excursion) during the OGTT than the continuous sleep group (group × day interaction: $F = 3.14$, $P = .03$; Figure 5). Multiple comparison testing showed that the glucose excursion in the split sleep group was significantly greater compared with the continuous sleep group during the first and second cycles of sleep restriction ($SR1_3$: $P = .03$; $SR2_3$: $P = .03$), whereas there was no group difference after baseline sleep or recovery sleep when both groups had 9 h of TIB (difference in means: B_2: $P = .84$; $R1_2$: $P = .66$).

In addition, the split sleep group showed a significantly greater glucose excursion during *both* cycles of sleep restriction compared with their baseline glucose excursion response (B_2 vs. $SR1_3$: $P = .001$; $SR2_3$: $P = .01$), whereas the continuous sleep group did not show any differences in glucose excursion during sleep restriction compared with their baseline response (B_2 vs. $SR1_3$: $P = .96$; $SR2_3$: $P = .94$). For fasting and 2-h glucose levels, the group × day interaction did not reach statistical significance (Figure S4).

DISCUSSION

Although the adolescent participants in this study reported having an average TIB of about 6.1 to 7.0 h a night on weekdays (Table 1), similar to the 6.5 h found in our survey on a local sample of over 2,300 teenage students,[34] when they were given a limited time to sleep over two simulated school weeks, regardless of whether it was a split or a continuous sleep schedule, negative outcomes were observed relative to sleeping the recommended

duration every night. Splitting sleep to a shorter 5-h nocturnal sleep opportunity and a 90-min mid-afternoon nap resulted in less decrement in vigilance in the post-nap afternoon and evening than if they slept their entire daily sleep allocation at night. Vigilance was also less impaired in the morning in the split sleep group during the first cycle of sleep restriction, whereas morning performance was impaired to a similar degree as the continuous sleep group by the second cycle of sleep restriction. Benefits for other neurobehavioral functions (working memory / executive functions, speed of processing, subjective sleepiness, and mood) were also observed. In contrast, the split sleep schedule resulted in poorer morning glucose tolerance compared to continuous nocturnal sleep. Overall, both restricted sleep schedules were inferior to 9h continuous nocturnal sleep.

Split sleep and continuous nocturnal sleep differ in neurobehavioral outcomes

The current findings contrast with those derived from adults, which suggest that when daily TIB is held constant, how sleep is distributed across 24 h has little influence on vigilance performance averaged across the day.[12,13,15,35] A methodological reason for this could be that the present study had more participants per sleep group (29 per group vs. 5-18 per group in previous studies[12-15]) and greater statistical power to find differences between two specific sleep patterns relative to prior work. Another notable difference from previous work, which all used working-age adults, is that our participants were adolescents whose brains are still undergoing development and might respond differently to different sleep schedules.

Consistent with prior work in sleep-restricted adolescents and adults,[9,36] we found that napping led to improved post-nap vigilance that extended into the evening. In addition, in the first week of sleep restriction, the benefit of the nap schedule relative to the continuous sleep schedule appeared to carry forward to the following morning.

It is possible that distributing slow wave sleep across nocturnal sleep and a daytime nap in sleep-restricted adolescents may represent a more efficient way of dissipating homeostatic sleep pressure compared to shortened continuous nocturnal sleep. Our finding of longer sleep latency and lower slow wave activity early in the nocturnal sleep of the split sleep participants is an indicator of some recovery from homeostatic sleep pressure consequent on napping.

While the bulk of the benefit of an afternoon nap may appear to occur after school hours, many of the students who obtain insufficient sleep at night do so for academic reasons. For such persons, learning after official school hours represents a substantial proportion of their regular learning time. Hence, the boost in vigilance has practical significance as it affords better encoding[20] or revision of learning material. Additionally, we recently showed that napping after learning is at least as beneficial to immediate and 1-week post learning recall of educationally realistic memoranda as if cramming were performed in the nap period.[37]

Non-linear effects of cumulative sleep restriction and naps

Influential and informative as it has been, the two-process model of sleep regulation[38] does not explain the time course of lapses following exposure to moderate-to-severe partial sleep deprivation over multiple successive nights. For example, a previous study in adults found no difference in the cumulative effects of 6 h versus 4 h of TIB for sleep each night on lapses until after 5 nights.[8] Similarly, in the present study, the continuous sleep group (6.5-h TIB) exhibited comparable vigilance decline during the first period of sleep restriction to a group that was given 5 h of TIB in a previous study[9] conducted using the same protocol (Figure S5). Intriguingly, the benefit of the additional 1.5 h of nocturnal sleep over 5 h a night, was only revealed during the second week of sleep restriction. Together, these

findings indicate that the cumulative dose-dependent effects of chronic sleep restriction on vigilance are likely non-linear and do not mirror effects on sleep architecture.[8,39,40]

Recently developed models that extend and modify the two-process model appear to perform better at estimating vigilance lapses during exposure to sleep restriction and subsequent recovery sleep.[41] Consistent with our empirical data, the Unified Model of Performance predicted that sleep-restricted adults exposed to 4 h of TIB per day would on average perform better if sleep was split into 2 bouts (2-h nap every 12 h), compared to continuous sleep scheduled during either the night or daytime.[41] Critically, while newer biomathematical models of vigilance can successfully predict that a split sleep schedule can outperform a continuous sleep schedule, they do not explain the divergence in vigilance and glucose tolerance results.

Appropriate sleep may differ according to health category

Sleep loss can affect general, cardiovascular, metabolic, mental, and immunologic health, as well as human performance.[16,17,32,42-44] Existing research has probed each of these categories in isolation but not in combination. Yet, there is a consensus among multi-disciplinary experts that sleep duration appropriate for one category may not necessarily be appropriate for another.[18] The present study in adolescents is *the first* to demonstrate that a moderate level of nocturnal sleep restriction (6.5 h of TIB for sleep) can strongly affect vigilance and yet have little impact on blood glucose concentration. By comparison, shortening the already restricted continuous nocturnal sleep opportunity by splitting it was associated with larger glucose excursion during the OGTT compared with continuous sleep. The 44-49% larger glucose excursion in the split sleep group suggests that shorter nocturnal sleep may have a clinically meaningful impact on glucose responses in otherwise healthy adolescents, with *potential* implications for diabetes risk adolescents are chronically exposed to insufficient nocturnal sleep.

Interestingly, glucose responses in the split sleep group recovered over the simulated weekend in contrast to failure of vigilance performance to return to baseline level hinting at the possibility for different time constants for the recovery of cognition and glucose metabolism.

Limitations

We specifically intended to ascertain how two different sleeping schedules would affect cognitive and metabolic outcomes when adolescents receive successive nights of *inadequate sleep*. It is unclear if the current findings would apply if participants' total sleep duration over 24 h was adequate. Extension of the present results to different temporal distributions of sleep with varied total duration is unclear and should stimulate further research.

Given that adolescents' brains are still developing, these findings should be replicated in adults should they be intended for generalization. Additionally, future work should evaluate how the timing of circadian rhythms is affected by continuous vs. split sleep, and whether this modulates the time course of performance and morning glucose tolerance.

Our findings for glucose tolerance testing were based on a finger-prick test in which insulin was not measured. Therefore, we did not evaluate whether exposure to sleep restriction resulted in decreased insulin sensitivity, which has been reported previously in adolescent boys who underwent partial sleep deprivation.[45] It is possible that blood glucose levels were unchanged in the continuous sleep group due to a compensatory increase in insulin secretion, whereas the response was insufficient to fully offset the effects of sleep restriction in the split sleep group. We did not compare glucose responses between the continuous and split sleep groups in the afternoon or evening, at a time when there could be a post-nap difference between groups. Intravenous blood sampling would be ideal for more accurate results, but impractical as the study was run in a dormitory of adolescents.

However, elevation in glucose excursion in the split sleep group was replicated in the second exposure to sleep restriction making it unlikely that the initial finding occurred by chance. Furthermore, in contrast to results for cognitive performance, our analyses of blood glucose did not include a separate control group with 9 h TIB across the entire 2-week protocol. Rather participants' glucose responses during sleep restriction were compared to their baseline OGTT conducted after 9 h of TIB.

During sleep restriction, the split sleep group had a longer waking interval prior to the OGTT compared with the continuous sleep group. This is an inherent limitation of any comparison between different nocturnal sleep durations. While it might be argued that it is better to have participants wake up at a fixed time prior to the OGTT (i.e. sleep restriction by only delaying bedtime), this approach would lead to delays in circadian phase and hence, differences in circadian timing of OGTTs between conditions**.** Additionally, a fixed wake-up time across the study would affect the ecological validity of our work which sought to simulate a typical school week, in which adolescents wake up much earlier on school days compared with weekends.

Conclusions

Under conditions of limited sleep availability, divergent negative outcomes with respect to neurobehavioral and glucose responses arise depending on whether the same amount of sleep is split or consolidated across the night. Neither sleep restriction schedule is without compromise when compared with a TIB of 9 h. Despite 6.5 h being the average self-reported TIB in the age group studied,[34] adolescents do not appear to be able to sustain this without adverse consequences and are thus advised to obtain the recommended 8-10hrs of nocturnal sleep.

ACKNOWLEDGMENTS

The authors are grateful for James Cousins, Alyssa Ng, Jesisca Tandi, Nicholas Chee, Andrew Dicom, Azrin Bin Jamaluddin, Shirley Koh, Karen Sasmita, Teck Boon Teo, Ksenia Vinogradova, Ruth Leong, James Teng, Kian Wong, Sing Chen Yeo, Litali Mohapatra, David Jeremiah Chee, Shaun Yee, Misya Erwin, Yin Shan Chee, Keren Lee, Su Sandar Tun, Weiyu Lim, Swee Yan Lim, and Joel Mathias for their assistance.

DISCLOSURE STATEMENT

This research was supported by the STaR Investigator Award from the National Medical Research Council, Singapore (STaR/0015/2013) awarded to Dr. Chee, grant NRF2016-SOL002-001 from the National Research Foundation, Singapore, awarded to Drs. Chee, Gooley, and Lo, as well as the Far East Organization. Manpower was also supported

by research contract PA/9016104043 from the Defense Science & Technology Agency, Singapore, awarded to Dr. Gooley, and grant MOE2015-T2-077 from the Ministry of Education, Singapore, awarded to Dr. Gooley. Drs Chee and Ong have a patent for the Z3-score framework. No other conflicts of interest were reported.

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Figure legends

Figure 1. Protocol. In this 15-day protocol, both (A) the continuous sleep group and (B) the split sleep group had 2 adaptation and baseline nights (B_1 and B_2; time-in-bed [TIB] indicated by black bars = 9 h from 23:00 to 08:00). The first cycle of sleep restriction lasted 5 nights ($SR1_1$ to $SR1_5$) followed by 2 nights of recovery sleep ($R1_1$ and $R1_2$; TIB = 9 h). The second cycle consisted of 3 nights of sleep restriction ($SR2_1$ to $SR2_3$) and 2 nights of recovery sleep ($R2_1$ and $R2_2$). During the two SR periods, the continuous sleep group had a nocturnal TIB of 6.5 hours (00:15-06:45), while the split sleep group had a nocturnal TIB of 5 hours (01:00-06:00) and a 1.5-h nap opportunity between 14:00 and 15:30. Asterisks mark nocturnal sleep and daytime nap episodes that were monitored with polysomnography. A cognitive test battery (purple "T") was administered at 10:00, 16:15, and 20:00, except during the first and last days of the protocol. An oral glucose tolerance test (gray bars) was done between 08:30 and 11:00 after the last baseline night (B_2), on the third day of the SR periods ($SR1_3$ and $SR2_3$), and after the first two nights of recovery ($R1_2$).

Figure 2. Sleep duration and macrostructure per 24-h period. The least square means and standard errors estimated with general linear mixed models are plotted for polysomnographically assessed (A) total sleep time (TST) and duration of (B) N1, (C) N2, (D), N3, and (E) rapid-eye-movement (REM) sleep across each 24-h period separately for the split sleep group (blue) and the continuous sleep group (red). Gray shaded areas mark the sleep restriction (SR) periods. *** $P < .001$, ** $P < .01$, and * $P < .05$ for significant group contrasts.

Figure 3. Markers of homeostatic sleep pressure. The least square means and standard errors of (A) N2 sleep latency and (B) slow wave activity (SWA) in the first hour of nocturnal sleep from N2 sleep onset are plotted for the split sleep group (blue) and the continuous

sleep group (red) from the second baseline night (B_2) to the first and second cycles of sleep restriction (SR; gray shaded areas) and recovery (R). *** $P < .001$, ** $P < .01$, and * $P < .05$ for significant group contrasts.

Figure 4. Vigilance performance during repeated sleep restriction with a split or continuous sleep schedule. The numbers of lapses in the Psychomotor Vigilance Task (PVT) are shown (A) averaged across the three tests each day, and separately for tests taken in the (B) morning, (C) afternoon, and (D) evening. PVT results are plotted after the last baseline night (day B_2), during the first cycle of sleep restriction (days $SR1_1$ to $SR1_5$; gray shading) and after recovery nights ($R1_1$ and $R1_2$), to the second cycle of sleep restriction (days $SR2_1$ to $SR2_3$ in gray shading) and recovery sleep ($R2_1$). Observations for the split sleep group are shown in blue and those for the continuous sleep group in red. For comparison, performance in a control group with 9 h of time-in-bed for sleep is shown in gray for data collected in a previous study.[19] The least square means and standard errors estimated with general linear mixed models are plotted. *** $P < .001$, ** $P < .01$, and * $P < .05$ for significant contrasts between the split and the consolidated sleep groups.

Figure 5. Blood glucose response in sleep-restricted adolescents. An Oral Glucose Tolerance Test (OGTT) was performed on mornings following baseline sleep (B_2), sleep restriction ($SR1_3$), recovery sleep ($R1_2$), and re-exposure to sleep restriction ($SR2_3$). The glucose excursion, defined as the change in blood glucose from the fasting state to 2 h after the 75-g oral glucose load, is shown for the split sleep group (n = 25; blue bars) and the continuous sleep group (n = 26; red bars). The mean ± standard error is shown. Asterisks (*) indicate significant between-group differences in the glucose response, and hash marks (#) indicate significant within-subject differences between OGTTs.

Table 1. Characteristics of all manipulation and control groups.

	Split sleep group		Continuous sleep group		Control group		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***P***
N	29	-	29	-	26	-	-
Age (years)	16.55	0.74	16.58	1.12	16.81	1.17	0.60
Gender (% male)	51.70	-	51.70	-	42.30	-	0.73
Body Mass Index (percentile)	45.69	23.25	51.03	26.84	44.04	20.83	0.52
Daily caffeine intake (cups)	0.55	0.69	0.58	0.80	0.54	0.79	0.97
Morningness-Eveningness Questionnaire	50.72	7.07	48.97	7.54	49.96	7.15	0.65
Epworth Sleepiness Scale	7.86	3.78	8.21	3.43	6.19	3.57	0.10
Chronic Sleep Reduction Questionnaire	36.10	4.66	35.24	5.96	33.81	5.13	0.28
Pittsburgh Sleep Quality Index							
Weekday TIB (h)	6.78[1]	0.89	6.85[2]	1.35	5.94[1,2]	1.14	<0.01
Weekend TIB (h)	8.76	1.23	8.93	1.18	9.20	1.30	0.42
Weekday TST (h)	6.47[1]	0.86	6.46	1.19	5.78[1]	1.15	<0.05
Weekend TST (h)	8.41	1.18	8.56	1.20	9.04	1.30	0.15
Nap duration (min)	62.93	65.66	68.52	64.76	-	-	0.75
Global score	4.17	1.77	4.48	1.50	4.58	2.58	0.73
Actigraphy							
Weekday TIB (h)	6.84[1]	1.13	7.00[2]	0.77	6.09[1,2]	0.85	<0.01
Weekend TIB (h)	8.15	1.05	8.45	1.13	8.45	1.25	0.52
Weekday TST (h)	5.50	0.89	5.51	0.75	5.37	0.73	0.77
Weekend TST (h)	6.64[1]	1.00	6.76[2]	1.14	7.53[1,2]	1.14	<0.01
Average TST (h)	5.83	0.73	5.86	0.68	5.99	0.62	0.66

Sleep efficiency (%)	81.04[1]	6.64	79.02[2]	5.57	88.45[1,2]	4.66	<0.001

TIB: Time in Bed, TST: Total Sleep Time

P values from the ANOVA and Chi-squared tests contrasting the three groups are listed. Since nap duration was not assessed for the control group, the associated P value referred to the contrast between the split and the continuous sleep groups.

[1]Significant difference between the split sleep group and the control group (independent-samples t-test, $P < .05$)

[2]Significant difference between the continuous sleep group and the control group (independent-samples t-test, $P < .05$)

Figure 1

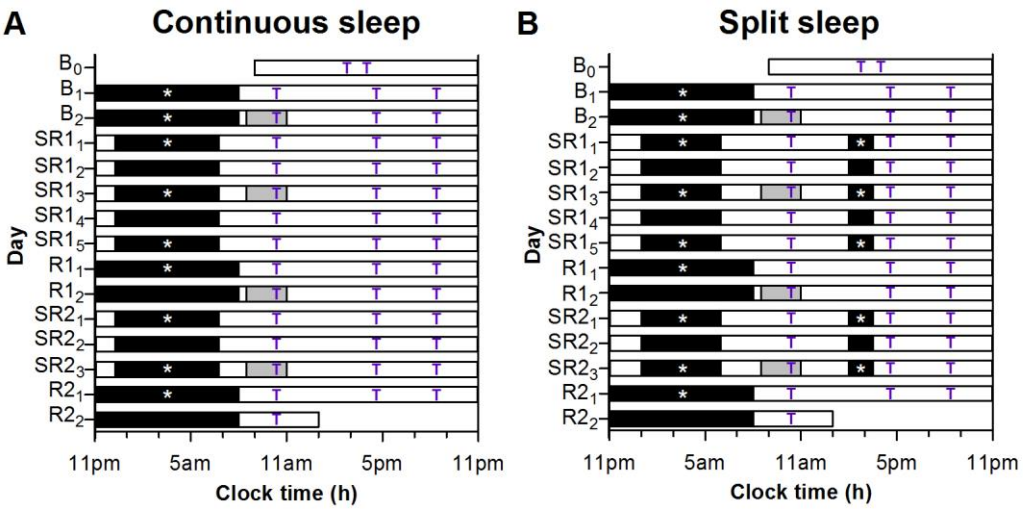

Figure 2

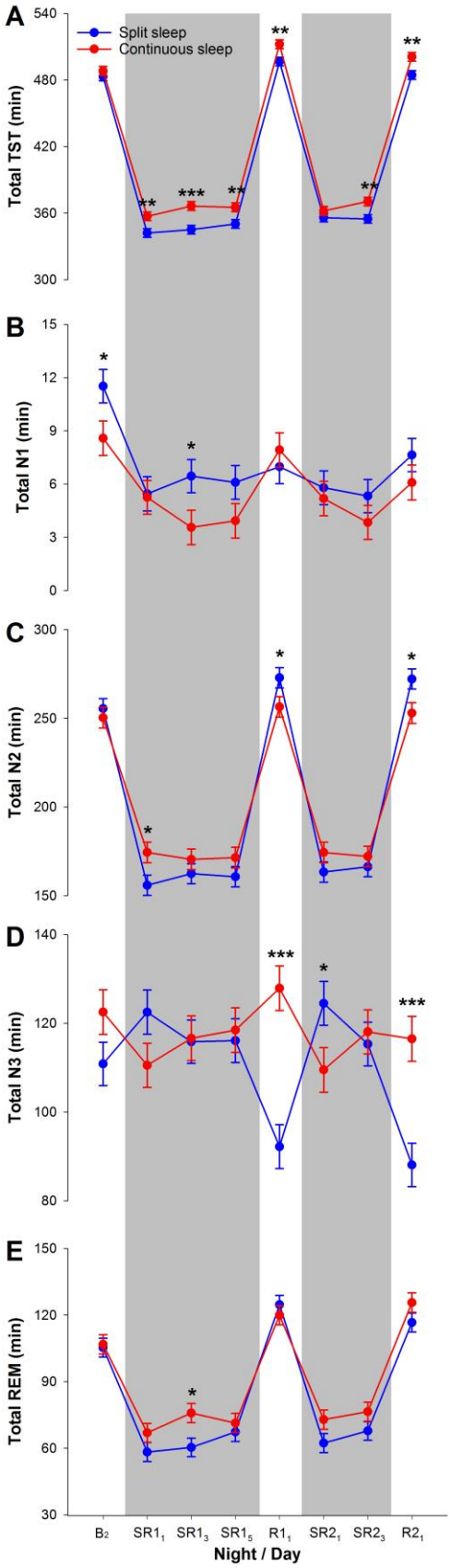

Figure 3

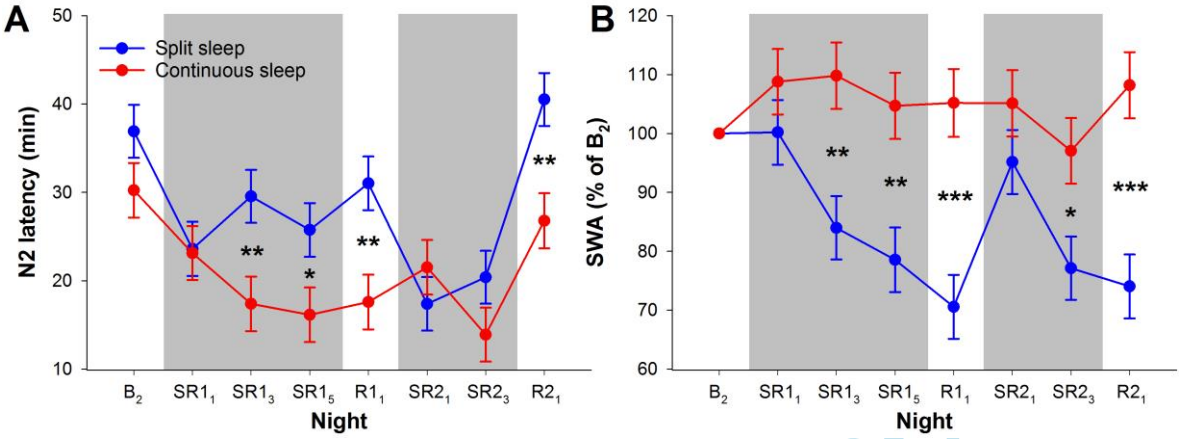

Figure 4

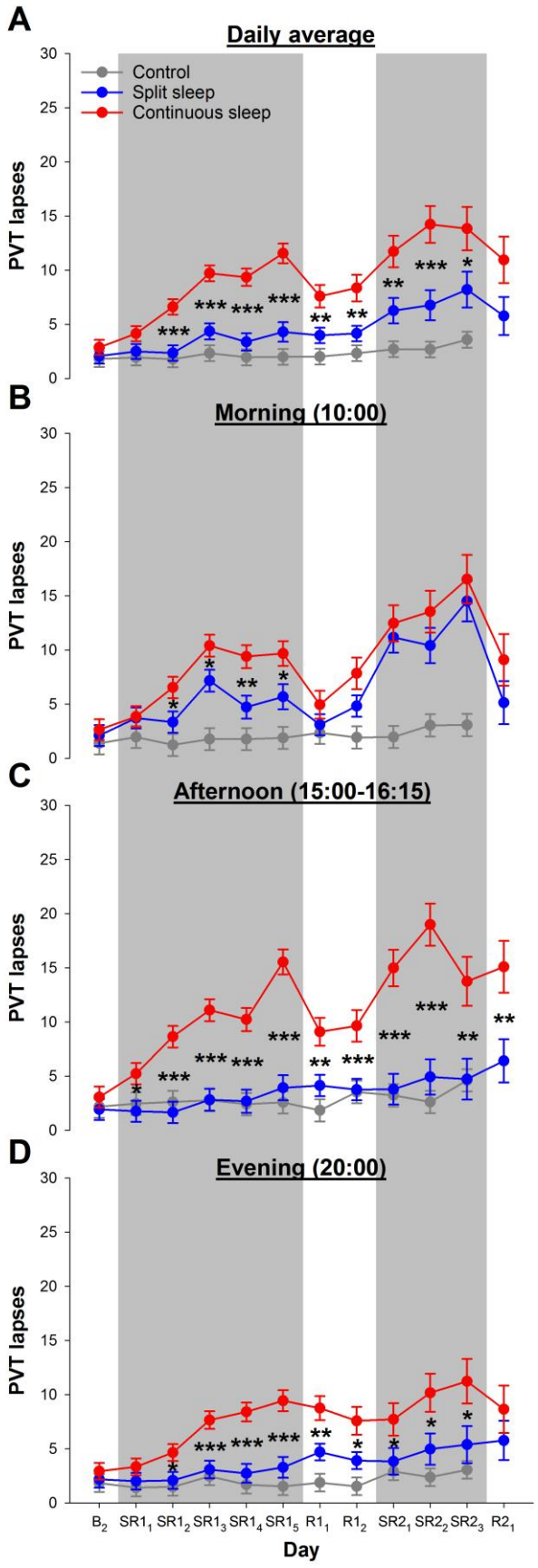

Figure 5