Title: Evening high-intensity interval exercise does not disrupt sleep or alter free-living energy intake despite changes in acylated ghrelin in middle-aged men

Running Title: Evening exercise, sleep and appetite

Authors: Penelope Larsen[1], Frank Marino[1], Kerri Melehan[2,3], Kym J Guelfi[4], Rob Duffield[5], Melissa Skein[1]

Affiliation: [1] School of Exercise Science, Sport and Health, Charles Sturt University, Bathurst, Australia;

[2] Royal Prince Alfred Hospital, Sydney;

[3]Discipline of Sleep Medicine, University of Sydney, Sydney, Australia;

[4]School of Human Sciences (Exercise and Sports Science), University of Western Australia, Perth, Australia;

[5]Sport and Exercise Discipline Group, University of Technology Sydney, Australia.

Keywords: Sleep, appetite, exercise

Word Count: 4790 (excluding reference list)

References: 50

Correspondence: Penelope Larsen

School of Exercise Science, Sport & Health

Charles Sturt University

Panorama Avenue, Bathurst, Australia, 2795

Phone: +61 2 6338 6101

Email: plarsen@csu.edu.au

Subject Area: Sleep and health

Abstract

Many adults remain inactive, despite exercise benefits for sleep and appetite, due to increased time-restraints. Methods to improve exercise compliance include preferential time-of-day or engaging in short-duration, high-intensity interval exercise (HIIE). Hence, this study aimed to compare effects of HIIE time-of-day on sleep and appetite. Eleven inactive men undertook sleep monitoring to determine baseline (BASE) sleep stages and exclude sleep disorders. On separate days, participants completed 30min HIIE (60s work at 100% $\dot{V}O_{2peak}$: 240s rest at 50% $\dot{V}O_{2peak}$) in the 1) morning (MORN; 0600-0700h), 2) afternoon (AFT; 1400-1600h) and 3) evening (EVEN: 1900-2000h). Measures included appetite-related hormones (acylated ghrelin, leptin, peptide tyrosine tyrosine), and glucose pre-exercise, 30min post-exercise, and next morning; overnight polysomnography (PSG; sleep stages); and actigraphy, self-reported sleep and food diaries for 48h post-exercise. There were no between-trial differences for total sleep time (p=0.46). Greater stage N3 sleep was recorded for MORN (23 ± 7%) compared to BASE (18 ± 7%; p=0.02); however, no between-trial differences existed (p>0.05). Rapid eye movement (REM) sleep was lower and non-REM sleep was higher for EVEN compared to BASE (p≤0.05). At 30min post-exercise, ghrelin was higher for AFT compared to MORN and EVEN (p=0.01); while glucose was higher for MORN compared to AFT and EVEN (p≤0.02). No between-trial differences were found for perceived appetite (p≥0.21) or energy intake (p=0.57). Evening HIIE can be performed without subsequent sleep disruptions and reduces acylated ghrelin. However, perceived appetite and energy intake appear to be unaffected by HIIE time-of-day.

Keywords: Sleep, vigorous exercise, appetite regulation

Introduction

Regular exercise is believed to be an important behaviour to assist in the improvement of sleep (Buman *et al.*, 2014). However, there has been coincidental reductions in exercise participation and sleep duration in recent decades which is reportedly due to a commonly cited barrier of 'lacking time' (Rajaratnam & Arendt, 2001; Gibala *et al.*, 2012; Buman *et al.*, 2014). For example, in Australia peak inactivity occurs at 35-54 years of age (ABS, 2015), while 60-64% of this age group also have at least one persistent sleep problem such as not obtaining adequate sleep, feeling unrefreshed upon waking, or waking frequently during the night (Adams *et al.*, 2017). In addition, reduced sleep duration has also played a significant role in the upregulation of the orexigenic hormone acylated ghrelin and downregulation of anorexigenic hormones such as leptin and peptide tyrosine tyrosine (PYY) which is highlighted in acute sleep deprivation studies (Magee *et al.*, 2009; Omisade *et al.*, 2010; St-Onge *et al.*, 2012). To combat this, short duration, high-intensity interval exercise has been encouraged to increase exercise participation (Gibala *et al.*, 2012). Also, the physiological basis for this type of exercise in relation to sleep and appetite is supported by evidence of increased sleep efficiency and reduced sleep onset latency (Dworak *et al.*, 2008; Hayashi *et al.*, 2014); increased anorexigenic signalling and subsequent reduction of energy intake (Sim *et al.*, 2014; Broom *et al.*, 2017); and higher, longer lasting reductions on post-prandial glucose compared to moderate-intensity exercise (Little *et al.*, 2014).

The American Academy of Sleep Medicine (2001) supports the recommendation of regular exercise to aid sleep; although, it is advised to avoid high-intensity or vigorous exercise close to bed time since this may increase arousal and disrupt subsequent sleep. However, the evidence for this is limited and appears to be a common warning which has come from early exercise and sleep research rather than recommendations that have evolved from more recent research (Irish *et al.*, 2015). Instead, experimental findings indicate that sleep is not disturbed by evening high-intensity exercise but may improve some variables including sleep efficiency, stage N3 sleep and sleep onset latency (O'Connor

47 *et al.*, 1998; Youngstedt *et al.*, 1999; Flausino *et al.*, 2012; Myllymäki *et al.*, 2012; Robey *et al.*, 2013;
48 Hayashi *et al.*, 2014). It has been postulated that the acute body-heating, anxiolytic and antidepressant
49 effects of exercise may, in part, explain these observed sleep changes following evening high-intensity
50 exercise (Youngstedt, 2005). Nevertheless, most studies have recruited young, active adults already
51 obtaining recommended sleep quantity and thus do not represent the age-associated changes in sleep
52 patterns experienced by many middle-aged, inactive adults (Copinschi *et al.*, 2014). Given that sleep
53 quantity decreases with age, it is possible that older populations may be more responsive to acute
54 exercise stimuli due to greater room for change (i.e. not hindered by a ceiling effect) (Youngstedt,
55 2005). It may also be important to examine sleep patterns following evening high-intensity exercise in
56 middle-aged populations compared to high-intensity exercise performed in the morning and
57 afternoon (i.e. 4 to 8 hours prior to bed time) (Irish *et al.*, 2015) as discouraging evening high-intensity
58 exercise, particularly of short duration, may remove a preferential time-of-day for exercise or
59 eliminate exercise altogether for time-poor individuals (Buman *et al.*, 2014).

60

61 Further consideration is needed for metabolic functioning following evening high-intensity exercise
62 and potential changes in subsequent sleep. For instance, should evening high-intensity exercise induce
63 poor sleep outcomes such as shortened total sleep time, and increased sleep onset latency and wake
64 after sleep onset it is likely to be associated with elevations in acylated ghrelin concentration and
65 reduced anorexigenic peptide levels including leptin and PYY (Magee *et al.*, 2009; Omisade *et al.*, 2010;
66 St-Onge *et al.*, 2012). In isolation, high-intensity interval exercise, has been shown to have a positive
67 effect on acylated ghrelin, leptin and PYY, and further associated with favourable reductions in energy
68 intake for up to 24 h post-exercise (Thivel *et al.*, 2012; Sim *et al.*, 2014; Panissa *et al.*, 2016). However,
69 in these studies, exercise was performed in the morning and due to circadian variations responses
70 may not reflect hormonal changes following exercise performed in the afternoon or evening. Leptin
71 has been previously examined following a 30 s Wingate anaerobic test performed in the morning and

evening whereby authors observed no difference between trials (Bilski *et al.*, 2016). However, ghrelin and PYY have yet to be investigated in relation to exercise time-of-day.

Given the potential interaction between exercise, sleep and appetite, it may be important to investigate the role of exercise on sleep and appetite simultaneously due to the complex pathways which regulate these physiological processes (Copinschi *et al.*, 2014). As such, the aim of this study was to compare the effect of high-intensity interval exercise performed in the morning, afternoon and evening on sleep, appetite-related hormones and free-living energy intake in inactive, middle-aged men. It was hypothesized that high-intensity afternoon and evening exercise would increase the proportion of stage N3 sleep compared to baseline and morning exercise; while all exercise trials would induce favourable appetite changes (anorexigenic changes in the circulating hormones and reduced energy intake) due to the implementation of a standardised high-intensity exercise protocol.

Methods

Ethical Approval

Each participant was required to provide informed written consent to the protocols, which were approved by the Charles Sturt University Human Ethics Committee (H16136). This study conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database.

Participants

Eleven overweight, inactive men (mean ± SD; age: 49 ± 5 y; apnoea hypopnea index (AHI): 12 ± 4; BMI: 28 ± 3 $kg \cdot m^{-2}$; $\dot{V}O_{2peak}$: 34 ± 8 $ml \cdot kg^{-1} \cdot min^{-1}$) completed this study. Inclusion/exclusion criteria included non-smokers, participating in < 150 min of moderate-intensity exercise per week, had no previous or

current diagnosis of sleep or metabolic disorders, and no medical conditions or medications that affect sleep quality or quantity. Volunteers were also excluded if the baseline PSG studies indicated an AHI of ≥ 15. Initially, 13 men volunteered to participate in the study; however, one participant was excluded due to signs of severe sleep apnoea and one participant withdrew due to an illness unrelated to the study. Sleep was initially assessed by the STOP-BANG questionnaire (Chung *et al.*, 2008), the Epworth Sleepiness Scale (Johns, 1991) and the Pittsburgh Sleep Quality Index (PSQI) (Buysse *et al.*, 1989). Risk of sleep apnoea was further assessed during two consecutive nights of polysomnography (PSG) measurement. Medical clearance was obtained from a General Practitioner and a Pre-Exercise Medical Health Questionnaire was completed by each participant prior to enrolling in the study to ensure no underlying conditions would be exacerbated by vigorous exercise.

Experimental Overview

Participants attended the laboratory for an initial familiarisation session and baseline assessments of anthropometry and peak oxygen consumption ($\dot{V}O_{2peak}$) and habitual sleep and eating patterns were documented for 7 days prior to testing. During this time, two consecutive nights of PSG sleep testing were conducted to exclude sleep apnoea and record normal sleep stages and arousals. Following baseline (BASE), participants completed three experimental trials (4 days duration for each) in a randomised fashion. The experimental trials included 30 min of high-intensity interval exercise (60 s at 100% $\dot{V}O_{2peak}$: 240 s at 50% $\dot{V}O_{2peak}$) (Sim *et al.*, 2014) performed 1) in the morning (MORN: 0600 - 0700 h), 2) afternoon (AFT: 1400 - 1600 h), and 3) evening (EVEN: 1900 - 2000 h). Experimental trials were separated by a minimum of 5 days recovery. Primary outcome measures included post-exercise sleep quality and quantity, changes in plasma concentrations of appetite-related hormones, ratings of perceived appetite, and post-exercise free-living energy intake.

Familiarisation and Baseline Testing

The familiarisation session involved assessments of height, body mass, and waist and hip girths were completed to calculate body mass index (BMI) and waist-to-hip ratio (WHR), respectively. Further, $\dot{V}O_{2peak}$ was assessed using a ramp protocol (Barstow *et al.*, 2000) on a cycle ergometer (Lode B.V., Excalibur Sport, Groningen, The Netherlands) to calculate workloads for the experimental trials. The $\dot{V}O_{2peak}$ test commenced at 50 W for the first 2 min and increased 25 W every minute thereafter with cadence maintained at 70 rpm until volitional exhaustion. During the test, heart rate (HR; F1, Polar, Electro-Oy, Kempele, Finland) was monitored every minute and breath-by-breath pulmonary gas exchange was obtained via a mouthpiece connected to a calibrated metabolic gas oxygen analysis system and custom-developed software (LabVIEW; National Instruments, Austin, TX, USA).

At-home baseline data was obtained for a total of 7 days which included 7 nights actigraphy recorded via a wrist-worn actigraph (Actiware 2, Philips Respironics, Andover, MA), alongside a diary to verify sleep bed and wake times, and food intake. During this time, participants were instructed to maintain usual bed time, wake time, and diet. These data were obtained to provide a representation of habitual sleep quantity, behaviour and diet as a comparative control (Champagne *et al.*, 2013; Bei *et al.*, 2016). The two nights of PSG sleep measurement were conducted during the 7 night baseline period, depending on participant and equipment availability. A level II, take home PSG device was used to exclude sleep disorders and record baseline sleep stages and arousals.

Experimental Trials

During each experimental trial, participants did not engage in physical activity and documented all food and drink consumption 24 h prior to exercise. On the day of exercise, participants abstained from alcohol and caffeine; and fasted overnight for the MORN trial to ensure participant's sleep on the night

prior to exercise was not shortened due to fasting requirements and for 3 h prior to the AFT and EVEN trials. Upon arrival, participants indicated perceived hunger and fullness on validated Visual Analogue Scales (VAS) (Flint *et al.*, 2000) and a capillary blood sample was obtained from the fingertip for the assessment of appetite-related hormones and glucose. Participants then performed the high-intensity interval protocol which consisted of 6 × 60 s maximal sprints (100% $\dot{V}O_{2peak}$) interspersed by 240 s of active recovery (50% $\dot{V}O_{2peak}$) which equated to a total exercise duration of 30 min (Sim *et al.*, 2014). Exercise was performed on a stationary cycle ergometer (Wattbike Trainer, Wattbike Ltd, Nottingham, UK) and intensity was monitored via power output (PO) every minute. Heart rate (F1, Polar, Electro-Oy, Kempele, Finland) responses were also recorded every minute for calculation and reporting of mean HR across the entire exercise protocol. Participants also reported rating of perceived exertion (RPE; 1-10 scale) (Borg, 1982) every 5 min. Immediately post-exercise, participants were instructed to passively rest for 30 min, after which time a second blood sample was obtained, and perceived appetite was recorded to assess the acute effects of exercise on appetite variables. That night, sleep was recorded using a level II, take home PSG device and scored for sleep stages and arousals. Participants returned to the laboratory the following morning (60 min after waking), for a fasted capillary blood sample and reported perceived appetite to examine appetite variables in relation to the preceding night's sleep. Actigraphy, and sleep and food records were maintained for 3 days during each trial, including the day of exercise, one day after exercise, and two days after exercise (refer to Figure 1). Data were examined for sleep quantity and energy intake up to 48 hours post-exercise. Following exercise, participants were free to choose bed times, wake-up times, and food intake to observe sleep and eating responses to the respective trials.

Polysomnography

Polysomnography was performed using recommended electrode and sensor placements (Berry *et al.*, 2016), connected to the Alice PDx system (Philips Respironics, Pittsburg, USA) and analysed using

Sleepware G3 software version 3.7.4 (Philips Respironics, Pittsburg, USA). Electrode and sensor placements included: three electroencephalogram (EEG; F3-A2, C4-A1, and O1-A2) electrodes, unilateral electrooculogram (EOG), chin electromyography (EMG), electrocardiography (ECG; lead I), oxygen saturation via pulse oximetry, thoracic and abdominal respiratory effort via belts, and nasal airflow via pressure transducer. The BASE sleep studies were scored to exclude sleep disorders and data were used for the BASE sleep staging and arousal parameters; whereas, only the sleep staging and arousal data were analysed for experimental sleep studies. All sleep studies were scored based on standard guidelines (Berry *et al.*, 2016) by an experienced sleep technologist who was blinded to the experimental trials. Sleep parameters assessed included time in bed, total sleep time (TST), sleep efficiency (SE) [(sleep duration - wake time) / sleep duration) × 100], sleep onset latency (SOL; time from lights out to the first epoch of sleep), rapid eye movement (REM) onset latency, wake after sleep onset (WASO; total time awake after sleep onset), percent of time spent in each sleep stage (N1: stage 1; N2: stage 2; N3: stage 3; total NREM: non-rapid eye movement sleep; REM), and arousal index.

Actigraphy

Actigraphy was recorded in 1 min epochs (Esliger & Tremblay, 2006) and analysed using Actiware v5.70 software (Philips Respironics, Pittsburgh, USA). Variables obtained included bed time, wake time, time in bed (period between bed time and wake time), TST (time asleep during time in bed), SOL (period between bed time and sleep onset), SE (percent of time in bed spent sleeping), WASO (total time awake after sleep onset), and number of awakenings (Knutson *et al.*, 2007).

Appetite Perception and Hormones

Perceived hunger and fullness were assessed using a VAS comprised of straight lines (100 mm) accompanied by a question anchored with words representing opposing extreme states of hunger and

fullness at either end (Flint *et al.*, 2000). A 600 µl sample of blood was collected from a fingertip using a sterile lancet. To assist vasodilation, the hand was submerged in a bowl of warm water for 5 min prior to blood draw. Blood glucose concentration was measured directly from the fingertip using an Accu-Chek Performa (Roche, Manheim, Germany). The remaining blood was immediately aliquoted into pre-chilled EDTA tubes (Becton Dickinson, Sydney, Australia) treated with serine protease inhibitor (25 µl per 600 µl of blood; Pefabloc® SC, Sigma-Aldrich, St. Louis, USA) then immediately centrifuged at 3000 rpm for 10 min. Plasma obtained was stored at -80˚C and later analysed according to manufacturer's instructions for acylated ghrelin, leptin and PYY_{total} using a commercially available assay kit (Cat. No# HMHEMAG-34K; Milliplex, Millipore Corporation, MA, USA). These hormones were chosen based on previous literature demonstrating their responsiveness to exercise (Broom *et al.*, 2009; Balaguera-Cortes *et al.*, 2011) and association with sleep and appetite (Spiegel *et al.*, 2011). For acylated ghrelin, leptin and PYY_{total} the intra- and inter-assay coefficient of variations were < 10% and < 15%, respectively.

Sleep and Energy Intake Records

Sleep diary entries were used to confirm bed times and wake-up times for actigraphy data. For food records, instructions on the use (including a 1 day example), and the necessity for accurate (i.e. food and drink brands and quantities) and detailed recordings of energy intake immediately after consumption were emphasised. Total energy and macronutrient intake were calculated using commercially available software (Foodworks; Xyris Software, Kenmore Hills, QLD, Australia). Also, absolute (g) and relative data (%) were calculated for carbohydrate, fat and protein intake.

Statistical Analysis

A *priori* sample size calculations for a repeated measures ANOVA was performed using G*Power (version 3.1.9.2) which confirmed that the final sample size of 11 participants was adequate for the input parameters which included the PSG sleep variables as these were the primary study measures. A repeated-measures (trial × time interaction) ANOVA with a Bonferroni correction and Tukey's *post hoc* were used to determine significant differences for performance, physiological and perceptual measures, perceived appetite, glucose and appetite-related hormones, total and macronutrient energy intake, PSG and actigraphy variables. PSG data were further separated to analyse the initial 180 min after sleep onset as the first 1-2 sleep cycles have been shown to be altered by acute stimuli including evening high-intensity exercise (Myllymäki *et al.*, 2012). Analysis was performed using Statistical Package for Social Sciences (SPSS v 20.0, Chicago, USA). Data are reported as mean ± standard deviation (SD) and statistical significance was accepted at $p \leq 0.05$.

Results

Exercise Responses

There was no significant difference for mean power output between MORN (355 ± 106 W), AFT (396 ± 126 W) or EVEN (391 ± 139 W) ($p = 0.11$; Figure 2A). As for trial × time interactions, power output was higher at sprint 1 and sprint 2 for AFT compared to MORN ($p \leq 0.05$). While for EVEN, power output was greater at sprint 2 compared to MORN ($p = 0.01$; Figure 2A). Mean heart rate was 126 ± 13 bpm for MORN, 132 ± 10 bpm for AFT, and 130 ± 9 bpm for EVEN. Mean heart rate for AFT was higher compared to MORN ($p = 0.05$). There was no trial × time interaction for RPE; although, a main effect of time for all trials indicated increased RPE from sprint 1 to sprint 6 ($p \leq 0.01$; Figure 2B).

Sleep Questionnaires, Polysomnography and Actigraphy

The results for the STOP-BANG questionnaire, Epworth Sleepiness Scale and PSQI at baseline were 2 ± 1, 7 ± 4 and 5 ± 2, respectively. Whole night and initial 180 min polysomnography data are presented in Table 1. There were no significant differences for time in bed, total sleep time, sleep efficiency, sleep onset latency, wake after sleep onset, stage N1 and N2 sleep, or arousal index between BASE, MORN, AFT and EVEN ($p > 0.05$). However, there was a greater proportion of stage N3 sleep following MORN compared to BASE ($p = 0.02$). There was a greater proportion of NREM sleep after EVEN compared to BASE for whole night sleep ($p = 0.05$) and initial 180 min of sleep ($p = 0.006$). Also, for the initial 180 min of sleep, proportion of REM sleep was lower for EVEN compared to BASE ($p = 0.006$). Analysis of actigraphy data (Table 2) showed there were no trial × time interactions for all variables ($p > 0.05$). However, there was a main effect of time for all trials which indicated a lower number of awakenings on the night post-exercise compared to one ($p = 0.05$) and two days post-exercise ($p = 0.04$).

Perceived Appetite and Appetite-Related Hormones

There was no trial × time interaction for perceived hunger ($p = 0.51$) or perceived fullness ($p = 0.21$; Figure 3). The hormone and glucose responses for MORN, AFT and EVEN are shown in Figure 4. There was a trial × time interaction for acylated ghrelin, with post hoc analyses revealing significantly higher values pre-exercise for AFT compared to MORN ($p = 0.001$) and EVEN ($p = 0.03$), and for EVEN compared to MORN ($p = 0.004$; Figure 4A). Acylated ghrelin remained higher 30 min post-exercise for AFT compared to MORN and EVEN ($p = 0.01$), while concentrations were higher for EVEN compared to AFT the morning post-exercise ($p = 0.01$; Figure 4A). The percentage change of acylated ghrelin was -34 ± 50% for MORN, for AFT -68 ± 30% and -74 ± 37% for EVEN ($p = 0.06$). Glucose values at 30 min post-exercise were higher for MORN compared to AFT and EVEN ($p \leq 0.02$; Figure 4D). Also, the percentage change in glucose was 26 ± 25% for MORN, AFT for 16 ± 21% and 14 ± 28% for EVEN from pre to 30 min post-exercise ($p = 0.37$). There was no trial × time interaction for leptin or PYY_{total} ($p >$

0.05). Although, there was a main effect of time for leptin in which values were higher at pre-exercise and the morning after exercise compared to 30 min post-exercise for all trials ($p \leq 0.01$; Figure 4B). The percentage change of leptin was -35 ± 20% for MORN, for AFT -34 ± 27% and -29 ± 16% for EVEN ($p = 0.64$). While, the percentage change of PYY_{total} was 20 ± 61% for MORN, for AFT 88 ± 157% and 22 ± 81% for EVEN ($p = 0.17$).

Free Living Energy Intake

Total energy intake and macronutrient intake is presented in Table 3. There were no significant differences between trials for total energy intake ($p = 0.57$), and carbohydrate, fat, protein, sodium, sugar or caffeine intake ($p \geq 0.09$).

Discussion

We investigated the effects of exercise time-of-day on sleep patterns, appetite responses and subsequent free-living energy intake in overweight, inactive men. Our novel findings show that many sleep variables do not differ to high-intensity interval exercise performed at different times of day. Although, the proportion of stage N3 sleep was higher after MORN compared to BASE; and after EVEN there was an increase in NREM sleep and decrease in REM sleep compared to BASE in the initial 180 min of sleep. There was also a favourable decline in acylated ghrelin from pre-exercise to 30 min post-exercise for AFT and EVEN compared to MORN; however, there were only small changes for all trials in leptin and PYY_{total}. Similarly, there were no differences between trials for perceived appetite or energy intake. Sprint power output during the high-intensity interval protocol was significantly higher for AFT and EVEN compared to MORN despite no between-trial differences for mean power output. Collectively, these findings indicate that acute evening high-intensity exercise does not impair subsequent sleep patterns and is unlikely to alter energy intake compared to exercise performed at

other times of day or to no exercise. Although, the greater efforts during maximal sprints in the afternoon and evening may stimulate larger reductions of orexigenic signals compared to morning high-intensity exercise which would be of more benefit to appetite control long-term.

Findings from the present study are consistent with experimental evidence suggesting that vigorous exercise performed close to bed time does not disrupt sleep (O'Connor *et al.*, 1998; Youngstedt *et al.*, 1999; Myllymäki *et al.*, 2011; Flausino *et al.*, 2012; Robey *et al.*, 2013; Hayashi *et al.*, 2014). Following the evening trial, PSG data indicated an increase in NREM sleep and decrease in REM sleep predominantly within the initial 180 min of sleep which have also been previously reported by Netzer *et al.* (2001) and Robey *et al.* (2013). Netzer *et al.* (2001) further presented a correlation between an extension of REM onset latency and reduction of REM sleep percentage in the first half of sleep with an increase in norepinephrine following intense exercise. Although the mechanisms are not fully understood, it is known that noradrenergic cells are tonically active during all sleep stages except for REM sleep (Poe *et al.*, 2010). Given that high-intensity interval exercise, compared to moderate-intensity exercise, is associated with a post-exercise 14.5 fold increase in norepinephrine release (Boutcher, 2010), it is plausible that the presence of such high levels close to bed time are linked to delayed REM sleep. Norepinephine may further enhance and prolong long-term potentiation (i.e. persistent strengthening of synapses based on recent patterns of activity) which occurs during NREM sleep stages and facilitates the events to convert early long-term potentiation to lasting long-term potentiation (Poe *et al.*, 2010). Nonetheless, opposing findings for evening vigorous exercise have been presented by Souissi *et al.* (2012) whereby total sleep time and sleep efficiency were lower, and sleep onset latency and awakenings increased compared to afternoon vigorous exercise. As such, further research is needed to examine the potential influence of covariates including age, gender and training status, that may affect sleep responses to evening high-intensity interval exercise.

Limited differences were observed in appetite responses; although, for the hormone changes, it did appear that afternoon and evening high-intensity exercise induced greater changes in acylated ghrelin, while morning high-intensity exercise altered glucose only. Interestingly, there was large variation in pre-exercise acylated ghrelin concentrations which are likely attributed to the natural circadian rhythm of this hormone which is typically lowest in the morning before progressively increasing until mid-afternoon (Birketvedt *et al.*, 2012; Copinschi *et al.*, 2014). As such, relative changes following exercise compared to pre-exercise values for the respective trials may provide a clearer understanding of time-of-day effects on circulating ghrelin. In this study, the magnitude of change for acylated ghrelin was larger following afternoon and evening trials compared to changes after morning high-intensity interval exercise, but these differences were not significant. Nonetheless, it is possible that the sprint power output differences between the afternoon and evening trials compared to the morning trial induced the observed ghrelin changes from pre-exercise to 30 min post-exercise. In support, Sim *et al.* (2014) observed a significantly greater reduction in ghrelin concentration for a very high-intensity exercise protocol compared to high-intensity interval exercise, moderate-intensity continuous exercise and a non-exercise control trial. Further, lower ghrelin levels continued for the very high-intensity protocol for up to 90 min post-exercise (Sim *et al.*, 2014). Despite implementing the same high-intensity interval protocol for all trials in the current study, sprint power output, particularly for the first 2 sprints, was higher for AFT and EVEN compared to the MORN trial. These data are largely supported by previous findings which report that maximal short-duration performance output nadirs in the morning and peaks in the afternoon in general and athletic populations (Atkinson *et al.*, 1993; Souissi *et al.*, 2002; Souissi *et al.*, 2004; Souissi *et al.*, 2007; Chtourou & Souissi, 2012). Therefore, it may be more beneficial to engage in high-intensity interval exercise in the afternoon and evening as performance output is likely to be greater leading to larger reductions in orexigenic signals.

Despite the reduction in acylated ghrelin levels post-HIIE in the AFT and EVEN trials, there were no associated reduction in perceived appetite or energy intake in this study. Much of the research investigating energy intake following exercise has been conducted during morning hours. Bilski *et al.* (2016) previously measured leptin following morning and evening high-intensity exercise, finding no difference between trials. However, unlike the current study, authors did observe a reduction in perceived hunger and post-exercise energy intake following both morning and evening high-intensity exercise (Bilski *et al.*, 2016). Differences between studies may be due to the provision of an *ad-libitum* meal to examine post-exercise energy intake compared to the self-reported diaries used in the present study which may not be sensitive enough to detect significant changes in energy intake. Furthermore, Bilski *et al.* (2016) only examined energy intake immediately post-exercise while the present study investigated potential long-lasting exercise effects on energy intake (i.e. up to 48 hours post-exercise).

The novel aspect of the current study is the examination of sleep and appetite concurrently following this distinct times-of-day. Even so, there are several limitations which need to be addressed and may assist the direction of future research. The difference in time of fasting for the MORN trial (i.e. 10 h overnight) compared to the AFT and EVEN trials (i.e. 3 h) were likely to have an effect on the diurnal variations of the appetite-related hormones and glucose levels. However, the overnight fast was chosen for the MORN trial to avoid forced sleep restriction which may also alter diurnal changes (Spiegel *et al.*, 2004). Also, there were limited time points for the analysis of acylated ghrelin, leptin, PYY_{total} and glucose; however, the three designated time points are in alignment with capturing acute and prolonged responses across all hormones. Eating and sleep behaviour may have influenced energy intake rather than changes in feeding mechanisms following high-intensity interval exercise performed at different times of the day. As such, future research may benefit from assessing prolonged energy intake in a controlled laboratory setting and collecting more frequent blood samples

to identify diurnal changes of appetite-related hormones and glucose after HIIE performed in the morning, afternoon and evening.

In summary, this study does not support the recommendation of avoidance of evening high-intensity exercise due to its effect on sleep. Rather this study shows high-intensity exercise can be safely performed in the evening without subsequent detriment to sleep duration or arousal index. Also, high-intensity exercise performed in the afternoon and evening are likely to be associated with greater performance output; therefore, greater reductions in orexigenic signals. As such, collectively these observations support the evening as a viable time-of-day for individuals to engage in high-intensity exercise should this be a preferential time-of-day.

References

Adams R.J., Appleton S.L., Taylor A.W., Gill T.K., Lang C., McEvoy R.D. & Antic N.A. (2017). Sleep health of Australian adults in 2016: Results of the 2016 Sleep Health Foundation national survey. *Sleep Health: Journal of the National Sleep Foundation 3*(1), 35-42. https://doi.org/10.1016/j.sleh.2016.11.005

American Academy of Sleep Medicine (2001). ICSD—International classification of sleep disorders, revised: diagnostic and coding manual. *American Academy of Sleep Medicine*.

Atkinson G., Coldwells A., Reilly T. & Waterhouse J. (1993). A comparison of circadian rhythms in work performance between physically active and inactive subjects. *Ergonomics 36*(1-3), 273-281. http://doi.org/10.1080/00140139308967882

Australian Bureau of Statistics (2015). 4364.0.55.001 - National Health Survey: First Results, 2014-15, vol. 2018. http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/by%20Subject/4364.0.55.001~2014-15~Main%20Features~Exercise~29

Balaguera-Cortes L., Wallman K.E., Fairchild T.J. & Guelfi K.J. (2011). Energy intake and appetite-related hormones following acute aerobic and resistance exercise. *Applied Physiology, Nutrition, & Metabolism 36*(6), 958-966. http://doi:10.1139/h11-121

Barstow T.J., Jones A.M., Nguyen P.H. & Casaburi R. (2000). Influence of muscle fibre type and fitness on the oxygen uptake/power output slope during incremental exercise in humans. *Experimental Physiology 85*(1), 109-116.

Bei B., Wiley J.F., Trinder J. & Manber R. (2016). Beyond the mean: A systematic review on the correlates of daily intraindividual variability of sleep/wake patterns. *Sleep Medicine Reviews 28*, 108-124. http://doi:10.1016/j.smrv.2015.06.003

Berry R.B., Brooks R. & Gamaldo C.E. (2016). The AASM manual for the scoring of sleep and asociated events: Rules, terminology and technical specifications, version 2.3. *American Academy of Sleep Medicine, Darien (IL)*.

Bilski J., Jaworek J., Pokorski J., Nitecki J., Nitecka E., Pokorska J., Mazur-Bialy A. & Szklarczyk J. (2016). Effects of time of day and the Wingate test on appetite perceptions, food intake and plasma levels of adipokines. *Journal of Physiology and Pharmacology 67*(5), 667-676.

Birketvedt G.S., Geliebter A., Kristiansen I., Firgenschau Y., Goll R. & Florholmen J.R. (2012). Diurnal secretion of ghrelin, growth hormone, insulin binding proteins, and prolactin in normal weight and overweight subjects with and without the night eating syndrome. *Appetite 59*(3), 688-692. http://doi.org/10.1016/j.appet.2012.07.015

Borg G.A. (1982). Psychophysical bases of perceived exertion. *Medicine & Science in Sports & Exercise 14*(5), 377-381.

Boutcher S.H. (2010). High-intensity intermittent exercise and fat loss. *Journal of Obesity 2011*. http://doi:10.1155/2011/868305

Broom D.R., Batterham R.L., King J.A. & Stensel D.J. (2009). Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *American Journal of Physiology-Regulatory, Integrative & Comparative Physiology 296*(1), R29-R35. http://doi:10.1152/ajpregu.90706.2008

Broom D.R., Miyashita M., Wasse L.K., Pulsford R., King J.A., Thackray A.E. & Stensel D.J. (2017). Acute effect of exercise intensity and duration on acylated ghrelin and hunger in men. *Journal of Endocrinology 232*(3), 411-422. http://doi:10.1530/joe-16-0561

Buman M.P., Phillips B.A., Youngstedt S.D., Kline C.E. & Hirshkowitz M. (2014). Does nighttime exercise really disturb sleep? Results from the 2013 National Sleep Foundation Sleep in America Poll. *Sleep Medicine 15*(7), 755-761. http://doi:10.1016/j.sleep.2014.01.008

Buysse D.J., Reynolds C.F., Monk T.H., Berman S.R. & Kupfer D.J. (1989). The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Research 28*(2), 193-213.

Champagne C.M., Han H., Bajpeyi S., Rood J., Johnson W.D., Lammi-Keefe C.J., … Bray G.A. (2013). Day-to-day variation in food intake and energy expenditure in healthy women: The Dietitian II Study. *Journal of the Academy of Nutrition & Dietetics 113*(11), 1532-1538. http://doi.org/10.1016/j.jand.2013.07.001

Chtourou H. & Souissi N. (2012). The effect of training at a specific time of day: A review. *The Journal of Strength & Conditioning Research 26*(7), 1984-2005. http://doi:10.1519/JSC.0b013e31825770a7

Chung F., Yegneswaran B., Liao P., Chung S.A., Vairavanathan S., Islam S., … Shapiro C.M. (2008). STOP Questionnaire: A tool to screen patients for obstructive sleep apnea. *The Journal of the American Society of Anesthesiologists 108*(5), 812-821. http://doi:10.1097/ALN.0b013e31816d83e4

Copinschi G., Leproult R. & Spiegel K. (2014). The important role of sleep in metabolism. In *How Gut & Brain Control Metabolism*, vol. 42, pp. 59-72. Karger Publishers.

Dworak M., Wiater A., Alfer D., Stephan E., Hollmann W. & Strüder H.K. (2008). Increased slow wave sleep and reduced stage 2 sleep in children depending on exercise intensity. *Sleep Medicine 9*(3), 266-272. http://doi.org/10.1016/j.sleep.2007.04.017

Esliger D.W. & Tremblay M.S. (2006). Technical reliability assessment of three accelerometer models in a mechanical setup. *Medicine & Science in Sports & Exercise 38*(12), 2173-2181. http://doi:10.1249/01.mss.0000239394.55461.08

Flausino N.H., Da Silva Prado J.M., Queiroz S.S., Tufik S. & Mello M.T. (2012). Physical exercise performed before bedtime improves the sleep pattern of healthy young good sleepers. *Psychophysiology 49*(2), 186-192. https://doi.org/10.1111/j.1469-8986.2011.01300.x

Flint A., Raben A., Blundell J.E. & Astrup A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity 24*(1), 38-48. http://doi.org/10.1038/sj.ijo.0801083

Gibala M.J., Little J.P., MacDonald M.J. & Hawley J.A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *The Journal of Physiology 590*(5), 1077-1084. https://doi.org/10.1113/jphysiol.2011.224725

Hayashi Y., Nishihira Y., Higashiura T. & Sotoyuki U. (2014). The effects of different intensities of exercise on night sleep. *Advances in Exercise & Sports Physiology 20*(1), 19-24.

Irish L.A., Kline C.E., Gunn H.E., Buysse D.J. & Hall M.H. (2015). The role of sleep hygiene in promoting public health: A review of empirical evidence. *Sleep Medicine Reviews 22*, 23-36. http://doi.org/10.1016/j.smrv.2014.10.001

Johns M.W. (1991). A new method for measuring daytime sleepiness: The Epworth sleepiness scale. *Sleep 14*(6), 540-545. http://10.1093/sleep/14.6.540

Knutson K.L., Rathouz P.J., Yan L.L., Liu K. & Lauderdale D.S. (2007). Intra-individual daily and yearly variability in actigraphically recorded sleep measures: The CARDIA study. *Sleep 30*(6), 793-796. http://doi.org/10.1093/sleep/30.6.793

Little J.P., Jung M.E., Wright A.E., Wright W. & Manders R.J.F. (2014). Effects of high-intensity interval exercise versus continuous moderate-intensity exercise on postprandial glycemic control assessed by continuous glucose monitoring in obese adults. *Applied Physiology, Nutrition, and Metabolism 39*(7), 835-841. http://doi:10.1139/apnm-2013-0512

Magee C.A., Huang X., Iverson D.C. & Caputi P. (2009). Acute sleep restriction alters neuroendocrine hormones and appetite in healthy male adults. *Sleep & Biological Rhythms 7*(2), 125-127. http://doi.org/10.1111/j.1479-8425.2009.00396.x

Myllymäki T., Kyröläinen H., Savolainen K., Hokka L., Jakonen R., Juuti T., … Rusko H. (2011). Effects of vigorous late-night exercise on sleep quality and cardiac autonomic activity. *Journal of Sleep Research 20*(1pt2), 146-153. http://doi:10.1111/j.1365-2869.2010.00874.x

Myllymäki T., Rusko H., Syväoja H., Juuti T., Kinnunen M. & Kyröläinen H. (2012). Effects of exercise intensity and duration on nocturnal heart rate variability and sleep quality. *European Journal of Applied Physiology 112*(3), 801-809. http://doi:10.1007/s00421-011-2034-9

Netzer N.C., Kristo D., Steinle H., Lehmann M. & Strohl K.P. (2001). REM sleep and catecholamine excretion: A study in elite athletes. *European Journal of Applied Physiology 84*(6), 521-526. http://doi:10.1007/s004210100383

O'Connor P.J., Breus M.J. & Youngstedt S.D. (1998). Exercise-induced increase in core temperature does not disrupt a behavioral measure of sleep. *Physiology & Behavior 64*(3), 213-217. http://doi.org/10.1016/S0031-9384(98)00049-3

Omisade A., Buxton O.M. & Rusak B. (2010). Impact of acute sleep restriction on cortisol and leptin levels in young women. *Physiology & Behavior 99*(5), 651-656. https://doi.org/10.1016/j.physbeh.2010.01.028

Panissa V.L.G., Julio U.F., Hardt F., Kurashima C., Lira F.S., Takito M.Y. & Franchini E. (2016). Effect of exercise intensity and mode on acute appetite control in men and women. *Applied Physiology, Nutrition & Metabolism 41*(10), 1083-1091. http://doi:10.1139/apnm-2016-0172

Poe G.R., Walsh C.M. & Bjorness T.E. (2010). Cognitive neuroscience of sleep. *Progress in Brain Research 185*, 1-19. http://doi:10.1016/B978-0-444-53702-7.00001-4

Rajaratnam S.M.W. & Arendt J. (2001). Health in a 24-h society. *The Lancet 358*(9286), 999-1005. http://doi:10.1016/S0140-6736(01)06108-6

Robey E., Dawson B., Halson S., Gregson W., King S., Goodman C. & Eastwood P. (2013). Effect of evening postexercise cold water immersion on subsequent sleep. *Medicine & Science in Sports & Exercise 45*(7), 1394-1402. http://doi:10.1249/MSS.0b013e318287f321

Sim A.Y., Wallman K.E., Fairchild T.J. & Guelfi K.J. (2014). High-intensity intermittent exercise attenuates ad-libitum energy intake. *International Journal of Obesity*, 1-6. http://doi:10.1038/ijo.2013.102

Souissi M., Chtourou H., Zrane A., Cheikh R.B., Dogui M., Tabka Z. & Souissi N. (2012). Effect of time-of-day of aerobic maximal exercise on the sleep quality of trained subjects. *Biological Rhythm Research 43*(3), 323-330. http://doi:10.1080/09291016.2011.589159

Souissi N., Bessot N., Chamari K., Gauthier A., Sesboüé B. & Davenne D. (2007). Effect of time of day on aerobic contribution to the 30-s wingate test performance. *Chronobiology International 24*(4), 739-748. http://doi:10.1080/07420520701535811

Souissi N., Gauthier A., Sesboüé B., Larue J. & Davenne D. (2002). Effects of regular training at the same time of day on diurnal fluctuations in muscular performance. *Journal of Sports Sciences 20*(11), 929-937. http://doi:10.1080/026404102320761813

Souissi N., Gauthier A., Sesboüé B., Larue J. & Davenne D. (2004). Circadian rhythms in two types of anaerobic cycle leg exercise: Force-velocity and 30-s wingate tests. *International Journal of Sports Medicine 25*(1), 14-19. http://doi:10.1055/s-2003-45226

Spiegel K., Tasali E., Leproult R., Scherberg N. & Van Cauter E. (2011). Twenty-four-hour profiles of acylated and total ghrelin: Relationship with glucose levels and impact of time of day and sleep. *The Journal of Clinical Endocrinology & Metabolism 96*(2), 486-493. http://doi:10.1210/jc.2010-1978

Spiegel, K., Tasali, E., Penev, P., & Van Cauter, E. (2004). Brief Communication: Sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. *Annals of Internal Medicine 141*(11), 846-850. http://doi:10.7326/0003-4819-141-11-200412070-00008

St-Onge M., O'Keeffe M., Roberts A.L., RoyChoudhury A. & Laferrere B. (2012). Short sleep duration, glucose dysregulation and hormonal regulation of appetite in men and women. *Sleep 35*(11), 1503-1510. http://doi:10.5665/sleep.2198

Thivel D., Isacco L., Montaurier C., Boirie Y., Duché P. & Morio B. (2012). The 24-h energy intake of obese adolescents is spontaneously reduced after intensive exercise: A randomized controlled trial in calorimetric chambers. *Public Library of Science One 7*(1), e29840. http://doi:10.1371/journal.pone.0029840

Youngstedt S.D. (2005). Effects of exercise on sleep. *Clinics in Sports Medicine 24*(2), 355-365. http://doi:10.1016/j.csm.2004.12.003

Youngstedt S.D., Kripke D.F. & Elliott J.A. (1999). Is sleep disturbed by vigorous late-night exercise? *Medicine & Science in Sports & Exercise 31*(6), 864-869. http://doi:10.1097/00005768-199906000-00015

Competing Interests

None declared.

Author Contributions

PL and MS developed the study concepts. PL collected data, performed the data analysis and prepared the manuscript. KM scored all the sleep studies. All authors provided important insight on data interpretation and contributed to the manuscript.

Funding

This is not an industry supported study. PL has received research support from the Australian Postgraduate Award. All other authors did not receive any funding in relation to this project.

Acknowledgements

We would like to thank the participants for volunteering for this study.

Table 1. Mean ± SD whole night and initial 180 min polysomnography for baseline (BASE), morning exercise (MORN; *n* = 10), afternoon exercise (AFT; *n* = 11), and evening exercise (EVEN; *n* = 11) trials.

	BASE		MORN		AFT		EVEN	
	Whole Night	**Initial 180 min**	**Whole Night**	**Initial 180 min**	**Whole Night**	**Initial 180 min**	**Whole Night**	**Initial 180 min**
Time in bed (min)	484.6 ± 39.8		450.4 ± 43.5		461.7 ± 34.9		454.5 ± 36.8	
Total sleep time (min)	405.7 ± 54.4	163.7 ± 14.3	387.7 ± 55.9	169.1 ± 6.7	407.1 ± 40.7	167.5 ± 11.3	392.6 ± 33.9	171.1 ± 5.0
Sleep efficiency (%)	83.7 ± 6.9	90.8 ± 7.9	86.0 ± 6.3	93.7 ± 3.7	88.2 ± 5.6	92.8 ± 6.3	86.5 ± 5.4	94.9 ± 2.8
Sleep onset latency (min)	23.1 ± 16.2		19.5 ± 11.7		18.4 ± 15.2		20.4 ± 14.3	
Rapid eye movement latency (min)	84.2 ± 21.0	82.9 ± 21.9	107.9 ± 60.1	107.0 ± 60.4	109.5 ± 34.6	109.5 ± 34.5	81.0 ± 24.0	81.0 ± 24.0
Wake after sleep onset (min)	55.7 ± 32.6	16.7 ± 14.2	43.1 ± 27.6	11.1 ± 6.8	36.2 ± 21.6	13.0 ± 11.3	41.5 ± 24.9	9.2 ± 5.1
Stage N1 sleep (%)	8.4 ± 4.0	6.9 ± 3.4	6.8 ± 3.2	5.4 ± 2.4	6.3 ± 2.3	5.6 ± 3.2	7.0 ± 2.7	5.5 ± 3.1
Stage N2 sleep (%)	53.9 ± 5.9	52.8 ± 7.9	54.2 ± 8.0	50.4 ± 8.9	55.5 ± 7.7	55.3 ± 7.9	56.3 ± 8.9	53.9 ± 14.1
Stage N3 sleep (%)	18.0 ± 7.2	27.7 ± 10.6	22.9 ± 7.3[a]	35.3 ± 10.9	21.0 ± 7.3	31.9 ± 8.2	20.6 ± 7.9	33.0 ± 9.8
Non-rapid eye movement (%)	80.3 ± 3.9	87.3 ± 5.4	83.5 ± 6.7	91.1 ± 5.9	82.8 ± 5.2	92.4 ± 4.2	83.9 ± 4.8[a]	92.4 ± 5.1[a]
Rapid eye movement (%)	19.7 ± 3.9	12.7 ± 5.4	16.4 ± 6.9	8.9 ± 5.9	17.2 ± 5.2	7.6 ± 4.3	16.1 ± 4.8	7.7 ± 5.1[a]
Arousal index (#/h)	12.4 ± 4.2	5.8 ± 5.8	12.8 ± 3.6	5.4 ± 4.1	12.3 ± 4.3	3.6 ± 3.9	10.8 ± 4.1	3.4 ± 2.8

[a] Indicates differences compared to BASE ($p \leq 0.05$).

Table 2. Mean ± SD actigraphy sleep data recorded at home for baseline (BASE), day of morning exercise (MORN-0), one day after MORN (MORN+1), two days after MORN (MORN+2), day of afternoon exercise (AFT-0), one day after AFT (AFT+1), two days after AFT (AFT+2), day of evening exercise (EVEN-0), one day after EVEN (EVEN+1), and two days after EVEN (EVEN+2) (*n* = 11).

	BASE	MORN-0	MORN+1	MORN+2	AFT-0	AFT+1	AFT+2	EVEN-0	EVEN+1	EVEN+2
Bed time (hh:mm)	22:15 ± 0:33	22:15 ± 0:34	22:35 ± 0:38	22:24 ± 0.36	22:29 ± 0:39	22:10 ± 0:39	22:21 ± 0:46	22:24 ± 0:51	22:20 ± 0:39	22:34 ± 0:57
Wake time (hh:mm)	6:24 ± 0:45	5:49 ± 0:32	6:21 ± 0:43	6:22 ± 0:51	6:02 ± 0:35	6:22 ± 1:05	6:27 ± 1:13	6:02 ± 0:38	5:59 ± 0:32	5:56 ± 0:34
Time in bed (hh:mm)	8:02 ± 0:36	7:33 ± 0:34	7:46 ± 0:27	7:59 ± 1:01	7:32 ± 0:37	7:50 ± 0:55	8:01 ± 0:46	7:38 ± 0:43	7:38 ± 0:46	7:22 ± 1:02
Total sleep time (hh:mm)	6:34 ± 0:32	6:26 ± 0:56	6:20 ± 0:37	6:27 ± 0:48	6:23 ± 0:42	6:39 ± 0:48	6:50 ± 0:42	6:25 ± 0:50	6:36 ± 0:48	6:17 ± 0:57
Sleep onset latency (min)	14.3 ± 18.9	30.2 ± 30.4	23.5 ± 19.4	31.7 ± 42.7	24.3 ± 21.4	25.1 ± 9.4	24.1 ± 12.7	26.5 ± 23.4	15.5 ± 13.4	12.5 ± 13.6
Sleep efficiency (%)	82.1 ± 3.6	84.4 ± 9.1	82.3 ± 6.6	82.0 ± 8.9	84.6 ± 8.5	83.1 ± 4.8	84.5 ± 5.8	84.0 ± 7.9	86.3 ± 4.3	85.3 ± 4.4
Wake after sleep onset (min)	41.1 ± 13.9	29.6 ± 9.1	45.4 ± 18.3	46.3 ± 27.6	32.3 ± 17.6	41.7 ± 18.9	36.1 ± 13.9	35.8 ± 17.6	36.0 ± 8.7	38.2 ± 10.1
Number of Awakenings (#)	20.4 ± 3.6	17.9 ± 5.3	23.4 ± 6.6*	22.6 ± 8.6*	17.6 ± 4.9	22.3 ± 6.0*	21.8 ± 6.1*	16.9 ± 4.5	21.0 ± 7.4*	22.0 ± 5.9*

* Indicates a main effect of time for all trials ($p \leq 0.05$).

Table 3. Mean ± SD total energy and macronutrient breakdown for baseline (BASE), day of MORN (MORN-0), one day after MORN (MORN-1), two days after MORN (MORN-2), day of AFT (AFT-0), one day after AFT (AFT+1), two days after AFT (AFT+2), day of EVEN (EVEN-0), one day after EVEN (EVEN+1), two days after EVEN (EVEN+2) (*n* = 11).

	BASE	**MORN-0**	**MORN+1**	**MORN+2**	**AFT-0**	**AFT+1**	**AFT+2**	**EVEN-0**	**EVEN+1**	**EVEN+2**
Total Energy Intake (kJ)	8501 ± 3248	8162 ± 4274	8167 ± 4166	7583 ± 2928	7839 ± 1283	7215 ± 3266	7813 ± 3544	6954 ± 1337	6856 ± 3294	6238 ± 1641
Carbohydrates (g)	204 ± 84	160 ± 14	161 ± 38	175 ± 87	220 ± 88	179 ± 72	169 ± 44	210 ± 113	179 ± 77	138 ± 36
(%)	41 ± 7	41 ± 10	37 ± 5	39 ± 10	43 ± 10	40 ± 1	42 ± 9	44 ± 14	46 ± 10	36 ± 6
Fats (g)	78 ± 37	73 ± 42	60 ± 23	68 ± 29	83 ± 28	66 ± 43	61 ± 34	70 ± 22	51 ± 21	67 ± 32
(%)	34 ± 6	33 ± 8	33 ± 7	34 ± 7	37 ± 6	32 ± 10	30 ± 8	36 ± 11	35 ± 8	35 ± 6
Protein (g)	97 ± 37	92 ± 51	96 ± 41	86 ± 27	93 ± 37	79 ± 38	80 ± 45	93 ± 30	69 ± 30	84 ± 41
(%)	19 ± 3	19 ± 4	20 ± 4	20 ± 5	18 ± 5	18 ± 4	16 ± 4	19 ± 3	17 ± 4	19 ± 4
Sodium (mg)	2078 ± 349	2511 ± 1400	2384 ± 1338	2302 ± 1136	2658 ± 713	1985 ± 1372	1655 ± 665	2890 ± 1210	1956 ± 1089	2112 ± 1053
Sugar (g)	81 ± 41	59 ± 17	72 ± 49	62 ± 18	85 ± 46	66 ± 46	73 ± 49	93 ± 64	81 ± 49	54 ± 17
Caffeine (mg)	146 ± 76	83 ± 69	113 ± 70	129 ± 104	100 ± 86	100 ± 78	125 ± 78	137 ± 107	155 ± 90	164 ± 89