Phase-shifting Effects of Light and Activity on the Human Circadian Clock

Eve Van Cauter, Ph.D.
Orfeu M. Buxton, Ph.D.

Department of Medicine, MC 1027
University of Chicago, 5841 South Maryland Avenue
Chicago, IL 60637

AFOSR/NL
801 North Randolph St, Room 732
Arlington, VA 22203-1977

Basic human research experiments conducted at the University of Chicago examined jet lag and sleep loss, conditions that are highly relevant to Air Force operations. First, a series of studies examined the photic and nonphotic means by which the timing of the human circadian system can be changed. These protocols were typically of 3-4 days duration involving collection of 24-hour profiles of neuroendocrine and other physiological parameters under "constant routine" conditions to determine the phase-shifting effects of exercise and dark/sleep on hormonal markers of circadian phase. The results indicate that periods of either exercise and dark/sleep can change circadian phase, which has potential practical benefits for conditions of jet lag and night work.

Second, the effects of partial sleep loss on metabolic, endocrine, cognitive, cardiovascular, and immune function, neurobehavioral performance, and subjective sleepiness and mood have been examined in separate studies not funded by this grant but nevertheless highly relevant to Air Force operations. These protocols involved 16-day studies with one-week periods of sleep restriction to 4 hours/night and sleep extension to 12 hours/night. The results indicate that sleep loss has a profound deleterious impact on human health and performance that can be improved by sleep extension.
A. OBJECTIVES AND OVERVIEW OF PROGRESS

The overall objective of this project was to carry out studies of jet lag and sleep loss, conditions that are highly relevant to Air Force operations. Two general directions were pursued. First, a series of studies have been completed in this and the previous grant periods to determine the photic and nonphotic means by which the timing of the human circadian system can be changed. Second, the effects of total or partial sleep loss, conditions highly relevant to Air Force operations, on metabolic, endocrine, cognitive, cardiovascular, and immune function, as well as subjective sleepiness and mood and cognitive function have been examined in separate studies.

The specific aims of this project include:

1. to examine the phase-shifting effects of a single daytime period of darkness and sleep presented at various circadian times. Results of this study are described in "Studies completed - Phase-shifting effects of daytime exposure to darkness and sleep".

2. to test the hypothesis that exposure to exercise in the evening (i.e. at the only time not tested in our previous studies) will result in rapid delays of circadian rhythms. Results of this study are described in "Studies completed - Phase-shifting effects of daytime exposure to exercise".

3. to test the hypothesis that partial sleep loss for several consecutive days is associated with significant alterations of EEG patterns, mood, cognitive, metabolic, endocrine, cardiovascular, and immune function during both sleep and wakefulness, and that these alterations can be reversed by extending the sleep period and "paying the sleep debt". Results of this study are described in "Studies completed - Semi-chronic partial sleep loss and extension alters endocrine function."

A total of 21 publications (11 original articles, and 10 reviews and book chapters), 11 abstracts, and 12 transitions has resulted from this effort.

B. STUDIES COMPLETED

B1. Phase-shifting effects of daytime exposure to darkness and sleep

Although extensive rodent data indicate that a variety of nonphotic stimuli that involve changes in the rest-activity state may induce phase shifts of circadian rhythms, the non-photic component of the human circadian system has been studied very little. During the prior grant periods, we showed that exposure of humans in constant conditions to single exercise pulses can result in phase shifts of human circadian rhythms (Van Reeth O, et al. Am J Physiol
266: E964-E974, 1994; Buxton OM et al. Am Journal of Physiology 273 36: E536-E542, 1997). However, a recent study of humans in temporal isolation found that a 12-hr inversion of a dim light dark cycle could not reentrain the subjects (Duffy JF, Kronauer RE, Czeisler CA. J Physiol 495: 289-197, 1996), suggesting that a period of darkness during the subjective day does not shift circadian rhythms. The purpose of this study was therefore to examine the effects of daytime sleep in darkness on the human circadian system.

25 normal men, 20-30 years of age were studied in "constant routine" conditions, including constant recumbent posture, constant glucose infusion in lieu of meals, and constant dim light (= 35 lux). Subjects had a one week prestudy period of normal bedtimes verified by actigraphy and one night of habituation to experimental conditions. Blood sampling at 20 minute intervals began at 16:00 on the day 2 of the study and continued until 08:00 on day 5. Bedtimes were limited to 02:00-08:00 on the second study night to increase sleep propensity. Different groups of subjects (n=6 each) were exposed on day 3 to a single 6 hour period of darkness (09:00-15:00, 14:00-20:00, 19:00-01:00) during which they were encouraged to sleep. Sleep was polygraphically monitored. A control group of subjects (n=7) remained in constant conditions in the absence of a dark/sleep pulse. Plasma profiles of melatonin assayed by RIA were used to assess circadian phase before and after exposure to the dark/sleep pulse.

The 6 morning nap subjects all showed phase delays from day 2 to day3 (mean±SE = -77 ± 10 minutes) about one hour, and significantly (p<.05) greater than the phase shift of the control group over the same interval. No further significant shifts were observed from day 3-4 (mean±SE = -3 ± 10 minutes) in continued constant routine, i.e., in the absence of further phase-shifting stimuli. In the absence of a nap in darkness, the control subjects showed a small mean phase delay from day 2 to day 3 (n=7, mean±SEM= -29 ± 20 minutes), the interval used to determine phase shifts of the morning dark/sleep group. Control subjects showed a small mean phase delay from 2 to day 4 (n=6, mean±SEM= -20 ±9 minutes), the interval used to determine phase shifts of the afternoon and evening dark/sleep groups, which were not significantly different from control.

We found no significant correlations of the total minutes NREM sleep stages I, II, or III (no subjects had stage IV sleep) or total minutes of REM sleep in the 6 morning nap subjects who showed phase delays greater than the control group. The lack of a correlation of phase shift magnitude with NREM or REM sleep amount suggests that sleep per se is not responsible for the phase-shifting effects of a nap in darkness.

In this dark/sleep study, a nap starting in the morning phase delayed the human rhythm of melatonin when subjects were kept in constant dim light during waking intervals. However, we have recently shown that an 8-hr advance of the sleep wake cycle (i.e., a "nap" beginning at 3pm) was associated with a nearly 2hr phase advance of melatonin secretion (publication #1), suggesting that the dark pulse PRC in humans (figure 1) may have both an advance and a delay region, depending on the intensity of the background light
during waking periods. The light intensity of the 8-hr advance study was ±200 lux, suggesting that the presence of an advance region in the human PRC to dark pulses is dependent on the contrast with the dim light of the constant routine.

From the current study, we conclude that morning sleep in darkness can phase delay the human circadian clock. The absence of light conveys a timekeeping cue to the circadian clock which depends on the contrast with the intensity of the light during the rest of the day, even within the range of dim light intensities not thought to convey dramatically different temporal cues to the circadian clock.

The present findings highlight the importance of periods of darkness in phase-shifting methodologies that focus on the use of light, but which often entail periods of darkness that were previously not thought to affect the phase of circadian rhythms alone. These findings have implications for shiftworkers, nightworkers, and continuous operations personnel who may wish to align imposed work and sleep periods with times at which their circadian system will promote alertness and best operational performance or restorative sleep, respectively (publication # 2, 3; abstracts #1, 2).

![Graph](image)

**Figure 1:** Partial Phase Response Curves (PRCs) observed in response to daytime sleep in darkness. By convention, phase advances and phase delays are defined as positive or negative numbers, respectively. The magnitude of the phase shift of the circadian melatonin onset (top panel) and thyrotropin (TSH) onset (bottom panel) for each individual is plotted versus the timing of the midpoint of the 6-hour sleep/dark period relative to the onset of the hormonal phase marker used. The local clock time at zero-hour in the top panels also represents the mean timing of the melatonin or TSH onsets.

A related study of a pharmacological non-photic intervention, i.e., triazolam administration, further supports the notion that nonphotic stimuli can impact human circadian timing. The objective of this study was to determine whether appropriately timed administration of a short-acting benzodiazepine hypnotic which has proven effective in an animal model of jet lag also facilitates adaptation of circadian rhythmicity and sleep-wake homeostasis in a human model of jet lag.

Six normal, healthy men 24-31 years of age participated in two double-blind, placebo-controlled studies of adaptation to an 8-hr delay shift of sleep-wake and dark-light cycles simulating westward travel. Each 9-day laboratory study began with a 3-day habituation period followed by a 24-hr study to
obtain basal hormonal and sleep profiles (23:00-07:00). Subjects were then kept awake until 07:00 the next day and slept in darkness 07:00-15:00 for the next five 24-hr spans post-shift. Oral triazolam (0.5 mg) or placebo given at 04:00 before the first shifted sleep/dark period (3 hours before bedtime) and at 07:00 (at bedtime) on days 2-5 post-shift.

Sleep recordings and 24-hr cortisol and growth hormone profiles were obtained at baseline and on the first, third and fifth days post-shift. Global measures of treatment efficacy were calculated for multiple endpoints representing circadian rhythmicity and sleep-wake homeostasis. With placebo, the shift induced disturbances of sleep and hormonal secretion, and a gradual re-entrainment of circadian rhythmicity. Triazolam significantly facilitated adaptation by accelerating re-entrainment of circadian rhythms (chronobiotic effect) and normalizing markers of sleep/wake homeostasis (hypnotic effect). Appropriately timed administration of a benzodiazepine hypnotic appears to facilitate the adaptation of both circadian rhythmicity and sleep-wake homeostasis to a shifted dark/sleep cycle. Compounds with combined chronobiotic/hypnotic properties may be useful in conditions of jet lag or night work (publication #4).

B2. Phase-shifting effects of daytime exposure to exercise

We have studied a total of 41 normal young subjects who were exposed to sessions of physical exercise at various times of the circadian cycle. In a first study, 17 subjects were studied twice under so-called "constant routine" conditions (i.e. a regimen of constant recumbency, constant dim light (<200 lux) exposure, continuous wakefulness and constant caloric intake under the form of an intravenous glucose infusion), once in the absence of stimulus and once with a 3-h nocturnal exercise session interrupting the constant routine conditions. Phase delays on the order of 1-2 hours were observed when the exercise session occurred 2-5 hours before the timing of the minimum of core body temperature, while smaller phase delays were observed when the exercise session was scheduled around or shortly after the timing of the temperature minimum delays (Van Reeth O, et al. Am J Physiol 266: E964-E974, 1994). This study represented the first clear demonstration of an effect of non-photic stimuli on human circadian rhythms.

A second study was designed to determine the role of intensity and duration of exercise and included 8 healthy male subjects who were each studied three times in constant routine conditions with no exercise, a 3-h period of low intensity exercise identical to that used in the first study, and a 1-h bout of high intensity exercise. Exercise stimulus was centered at a clock time of 1 am. The results confirmed that a 3-h period of low intensity exercise in this range of circadian times result in phase delays of 1-2 hours and demonstrated that high intensity exercise of 1-h duration, a protocol more compatible with the demands of a real life setting, caused similar phase delays (publication #5).
A third study enrolled 22 healthy men and examined the effects of 1-h high intensity exercise in the morning, afternoon, and evening. As in all three exercise studies, exercise capacity was determined for each individual prior to the study and the intensity of the exercise was tailored to the individual VO2max. The exercise stimulus was identical to the stimulus used in the second exercise study as described above. The onsets of the circadian elevations of plasma TSH and melatonin levels were used to determine circadian phase before and after stimulus presentation. The timing of the stimulus relative to endogenous circadian phase was determined a posteriori from the timings of the TSH and melatonin onsets prior to exercise. Figure 2 illustrates the individual data as well as the overall human phase-response curve to exercise delineated by these three studies using the timing of the melatonin onset as marker of circadian phase.

The findings summarized in Figure 2 indicate that 1) the phase-shifting effects of nocturnal exercise are generally in the delaying direction; 2) exercise during the morning and afternoon has no impact on circadian phase distinguishable from control subjects not given exercise.; and 3) evening exercise results in phase advances of melatonin secretion (publication#3).

Figure 2: Phase Response Curves (PRCs) observed in response to exercise at various times of day. The magnitude of the phase shift of the circadian melatonin onset for each individual is plotted versus the timing of the midpoint of 1-hour bouts of high-intensity exercise (filled diamonds) relative to the onset of melatonin secretion. On each panel, the approximate clock time of exercise is shown on top. By convention, phase advances and phase delays are defined as positive or negative numbers, respectively. Phase shifts from day 1-2 are shown in the left panel and phase shifts from day 1-3 are shown in the right panel. Phase shifts in control subjects (open squares) studied in the absence of exercise are shown at the right of each panel.
B3. Sleep restriction and sleep extension alter metabolic and endocrine function, sleep, fatigue, mood, and cognitive function

Sleep loss is a common complaint in adult population. Many studies have focused on the effect of acute sleep deprivation on different physiological and psychological parameters but little is known about the effect of a reduction of the sleep period for a consistent period of time, which is interestingly the kind of sleep deprivation experienced by a substantial segment of the adult population. The aim of the "sleep debt and sleep recovery" study was to test the hypothesis that partial sleep loss for several consecutive days is associated with significant alterations of metabolic function, endocrine profiles, EEG patterns, mood, cognitive function, and immunological functions during both sleep and wakefulness, and that these alterations can be reversed by extending the sleep period and "paying the sleep debt".

The protocol involved 16 consecutive nights in the Clinical Research Center (CRC) including 3 baseline nights with bedtime from 23:00 to 07:00, 6 nights with bedtime from 01:00 to 05:00 (sleep debt) and 7 nights with bedtime from 21:00 to 09:00 (sleep recovery). For the last 2 days in each condition, the subjects remained continuously at bed rest in the CRC for intensive physiological monitoring. On other days, the subjects were allowed to leave the CRC to attend to their usual activities but returned to sleep in the CRC. While in the CRC, identical carbohydrate-rich (62%) meals were consumed at 5-hour intervals (i.e. 09:00, 14:00, 19:00). Beat to beat heart rate interval, blood pressure, and heart rate were recorded continuously. A neurobehavioral assessment battery and mood questionnaires were administered at frequent intervals. During the 2 inpatient days in the CRC at the end of sleep debt and sleep recovery conditions, subjects underwent an Intravenous Glucose Tolerance Test (IVGTT) at 09:00. On the following days, blood samples were collected at 10-30 min intervals for 24 hours for the measurement of glucose and hormone levels, and saliva samples for the measurement of free cortisol levels were collected at 30-min intervals during waking periods. During each of these 60-hour studies spent in the CRC, blood pressure was measured at 15-min intervals during waking periods using an ambulatory blood pressure monitor, vigilance was monitored using the Nightcap and during the last day only by EEG recording. On the morning of the fourth day of sleep curtailment, the subjects were immunized against influenza to determine whether the sleep loss impaired the response to the vaccine. A group of healthy control subjects, not participating in any other aspects of the protocol, but closely matched with the study volunteers for age, sex, height, weight and lifestyle, also received the vaccine. A blood sample was taken before vaccine administration, 10 days and 21-30 days after vaccine administration.

Analysis of the glucose and insulin responses to an IVGTT (Fig.3A) indicated that parameters of glucose metabolism were in the normal range for young healthy men when the subjects were fully rested, whereas parameters measured in the state of sleep debt were consistent with a clinically significant impairment of glucose tolerance. The rate of disappearance of glucose post-
injection ($K_a$) was nearly 40% slower in the sleep debt condition than after recovery (1.45±0.31 versus 2.40±0.41%/min; p<0.02). $K_a$ values around 1.6%/min are typical of older adults with impaired glucose tolerance while values of 2.2-2.9%/min are typical of fit young subjects. Glucose effectiveness ($S_a$), a parameter that quantifies the ability of glucose to mediate its own disposal independently of insulin, (3) was 30% lower after sleep restriction than after sleep recovery (1.7±0.2 versus 2.6±0.2%/min, p<0.0005). This difference in glucose effectiveness is nearly identical to that reported between groups of patients with non-insulin-dependent diabetes and normal Caucasian men (1.4 versus 2.6%/min). The acute insulin response to glucose (AIRG) was reduced by 30% in the sleep debt condition as compared to post-recovery (304±95 versus 432±110pM/min; p<0.04). A decrease in acute insulin response to glucose is an early marker of diabetes. Differences in AIRG of magnitude similar to that observed between the sleep debt and fully rested conditions have been described in aging.

Carbohydrate metabolism was further examined following ingestion of carbohydrate-rich meals on the 6th day of both conditions. The glucose response to breakfast tended to be higher when sleep was curtailed than when sleep was extended (p=0.05), despite similar insulin secretory responses (Fig.3B). The difference in peak glucose levels in response to breakfast between the sleep debt and fully rested conditions (±15mg/dl) is similar to that observed in a previous study comparing young and old adults (20-36 versus 60-72 years), and translates into an approximate 20mg/dl difference in glucose levels 120min after the beginning of a standard glucose tolerance test. This comparison suggests that, under the sleep debt condition, these lean subjects would have responded to a morning standard oral glucose tolerance test in a manner consistent with current diagnostic criteria for impaired glucose tolerance (publication # 6, 7).

![Figure 3. A: Mean (+SEM) profiles of blood glucose and serum insulin during the IVGTT. In the sleep debt condition, the duration of the glucose response was longer (shaded area) and the acute insulin response to glucose (shaded area) was lower than under fully rested conditions. B: Mean (+SEM) profiles of blood glucose and serum insulin in response to breakfast.](image)

Other measurements taken during the study suggest possible mechanisms underlying the decrease in
glucose tolerance associated with sleep loss. The reduction in AIRG could be related to an alteration in the relative importance of sympathetic (inhibitory) and para-sympathetic (stimulatory) control of pancreatic function. Indeed, estimations of sympa-tho-vagal balance derived from recordings of heart rate variability were 15 to 20% higher in the sleep restriction than in the sleep recovery condition (p<0.02). The brain is a major site of non-insulin dependent glucose uptake. In normal subjects studied at bed rest after an overnight fast, a decrease in glucose effectiveness (SG) likely represents a decrease in brain glucose utilization, consistent with PET studies of sleep-deprived subjects.

When compared to the fully rested condition, the state of sleep debt was also associated with alterations of the 24-hour profile of plasma cortisol, including a shorter quiescent period (537±44min versus 634±24min; p<0.03) and elevated levels in the afternoon and early evening (p=0.0001). We have previously observed elevated evening cortisol levels in conditions of acute total and partial sleep loss, which may reflect decreased efficacy of the negative feedback regulation of the HPA axis. The rate of decrease of free cortisol concentrations in saliva between 16:00 and 21:00 was approximately 6-fold slower during the sleep debt condition than after full sleep recovery (0.07±0.13 versus 0.43±0.13 nmol/L/h; p<0.01; abstract #5).

In the sleep debt condition, a major proportion of the 24-hour GH output occurred before the onset of sleep, while the subjects were maintained actively awake in the laboratory. A robust increase of GH secretion also occurred after sleep onset. As a result of the splitting of the normal single sleep-onset GH secretory pulse into two pulses, the total duration of elevated GH secretion was significantly prolonged in the state of sleep debt and correlative evidence suggested that there was a relationship between the duration of active, nocturnal GH secretion and the impairment of morning glucose tolerance (publication #8, abstract #6, 8).

Taken together, these results demonstrate that the profiles of the counter-regulatory hormones cortisol and GH are altered in ways that could contribute to a reduction of glucose tolerance.

Figure 4: mean (+SEM) 24-hour profiles of plasma leptin levels at the end of sleep curtailment and at the end of sleep recovery.

The state of sleep debt was also associated with a major alteration of leptin profiles (Figure 4). In normal lean adults, plasma leptin levels exhibit a circadian variation with minimum values during the daytime, a nocturnal rise, and maximal values during early to mid sleep. It has been speculated that the nocturnal rise in leptin serves to suppress appetite during the overnight period of fast and sleep. The 24-h mean was 4.2±0.8 ng/ml in the fully rested state
versus 3.0±0.5 ng/ml in the state of sleep debt (p<0.005) and the amplitude of the rhythm was approximately 50% larger (p<0.03). These findings suggest that a state of sleep debt may be associated with appetite stimulation and carbohydrate craving.

Semi-chronic sleep restriction resulted in a more than 50% reduction in stages 1+2 and REM sleep and SWS was reduced by 27%. Alertness, mood and performance were all markedly diminished. These alterations were reversed by sleep extension. A detailed analysis of the waking EEG is currently ongoing, with the intent of identifying alterations in waking EEG patterns that can be temporally associated with diminished cognitive function. These results were presented at two professional society meetings (see abstracts #5, 6, 7, 8, 9). A manuscript describing these alterations in EEG patterns, mood, and cognitive function is currently in preparation.

The response to influenza vaccination in subjects who were immunized on the 4th day of sleep restriction (total sleep loss: 16 hours) as compared to subjects who had maintained normal sleep patterns with bedtimes averaging 8 hours. Mean antibody titers were significantly lower 10 days post-vaccination in the sleep-deprived subjects as compared to controls. Trends for negative correlations between increases in evening cortisol concentrations and differences between antibody titers from baseline to day 10 suggest that immune function was adversely impacted by sleep loss via an alteration in glucocorticoid regulation. A manuscript describing these effects of sleep loss on immune function is currently under review (publication #11).
C. PERSONNEL SUPPORTED

Eve Van Cauter, Ph.D.  Professor
Samuel Refetoff, M.D.  Professor
Orfeu M. Buxton  graduate student
Rachel Leproult, B.S.  Data manager
Georges Copinschi, M.D.  Professor
George Haake  Technician
Zhiqun Nie  Technician

D. PUBLICATIONS

Original Articles


Reviews, Book Chapters


16) Van Cauter E, Spiegel K. Sleep as a mediator of the relationship between socio-economic status and health: A hypothesis. In: Socioeconomic status and


Abstracts


4) Leproult R., Colecchia E.F., Van Cauter E. Exposure to bright light results in a rapid stimulation of the corticotropic axis in the early morning but not in the afternoon. 6th Meeting of the Society for Research on Biological Rhythms, Amelia Island, Florida, USA, abstract #237, May 1998. (Oral presentation.)


11) Spiegel K., L’Hermite-Baleriaux M., Leproult R., Van Cauter E. 24-hour melatonin and body temperature rhythms are affected by sleep restriction and sleep extension. 8th Meeting of the European Pineal Society, Tours, France, July 1999.

TRANSITIONS


5) Eve Van Cauter, Invited participant at the “Allostatic Load Meeting” of the MacArthur Foundation (Prof. B. McEwen), Rockefeller University, December 9-


8) Eve Van Cauter, Invited speaker at the Workshop on “Glucocorticoids and Stress Vulnerability”, Center for Psychobiological and Psychomotor Research, University of Trier, Germany, June 25, 1999.


