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While still preliminary, the findings from this study have provided two important novel observations 1. an overall elevation of TSH levels is a biological concomitant of the 'jet lag syndrome"; 2. exposure to dark/sleep is capable of exerting immediate phase-shifting effects of human rhythms.

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AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
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Phase-Shifting Effects of Light and Exercise on the Human Circadian Clock
Principal Investigator: Eve Van Cauter, Ph. D.
Department of Medicine
University of Chicago.

FINAL TECHNICAL REPORT

STATEMENT OF WORK

There were two major objectives of this research: 1. to examine the immediate effects of single light pulses on human circadian rhythms; 2. to examine the immediate effects of single exposure to non-photic stimuli which have demonstrated zeitgeber potency in other mammals, i.e. increased physical activity at usual rest/sleep times and forced inactivity/sleep during usual active periods. Both objectives have been reached and the results are described below.

STATUS OF RESEARCH EFFORTS

1. Immediate phase-shifting effects of single pulse of light

A major objective of the studies proposed in the original application was to determine the magnitude and direction of immediate phase-shifts of human rhythms following a single exposure to a 3-hour pulse of bright light presented at various circadian times. Our study design attempted to overcome some of the limitations of previous studies by using multiple endocrine markers, in addition to the rhythm of body temperature, to derive circadian phase and amplitude, and obtain more accurate estimations of the actual timing of the stimulus relative to endogenous circadian phase position. The pulse of light was presented under "constant routine" conditions, i.e. a regimen of constant wakefulness in recumbent position, constant dim light and constant caloric intake (under the form of an intravenous glucose infusion at a constant rate) following a period of entrainment to a fixed light-dark and sleep-wake cycle. Measurement of the resultant phase-shifts was performed under "constant routine" conditions on the first day following pulse presentation. Four overt rhythms which are strongly dependent on circadian timing, i.e. the rhythms of plasma TSH, plasma melatonin, plasma cortisol and body temperature, were simultaneously monitored for 38 consecutive hours. Seventeen subjects each participated in three separate studies, one baseline study with measurements of circadian phase positions in the absence of zeitgeber stimulus, one study with exposure to a 3-hour pulse of light and one study with exposure to a 3-hour pulse of exercise. The results from the study with exposure to exercise are described in the next section. The studies were separated by at least two weeks. The baseline study was performed first in order to obtain an estimation of the endogenous circadian phase of the individual. The order of the two other studies was randomized.

The timing of light exposure was referenced to the time of occurrence of the body temperature minimum as observed during the baseline study. In 8 subjects (# 1-8), the center of the stimulus was timed to coincide with the timing of the body temperature minimum, in 3 other subjects (# 9-11), the center of the stimulus was timed to follow the timing of the temperature minimum by 2 hours and in the remaining 6 subjects (#12-17), the center of the stimulus was timed to precede the temperature minimum by 3 hours. Because the baseline study preceded the study with light exposure by at least two weeks, it was expected that the timing of the body temperature minimum would not remain

constant and that the exact timing of stimulus exposure relative to endogenous circadian phase would have to be inferred *a posteriori*. In 11 out of 17 studies with light exposure, the temperature minimum was masked by a temperature rise induced by light and therefore hormonal markers of circadian phase had to be used to infer *a posteriori* the timing of pulse presentation. Estimations of circadian phase could however be derived from the TSH profiles and, in 13 out of 17 studies, could be derived from the melatonin profiles. In the four other subjects, the beginning of light exposure preceded the onset of the nocturnal melatonin rise and thus prevented the estimation of this marker of circadian phase. Masking effects of recovery sleep prevented the use of the cortisol profiles to estimate changes in endogenous circadian phase.

The three panels of Figure 1 show the relationships between the timing of the circadian temperature minimum (in baseline studies and in studies in which light exposure did not raise body temperature), the timing of the onset of the TSH rise and the timing of the onset of the melatonin rise as observed on day 1 of the constant routine. These three markers of circadian phase were in good concordance, indicating that subjects who have an early TSH rise also tend to have an early melatonin rise and an early minimum of body temperature.

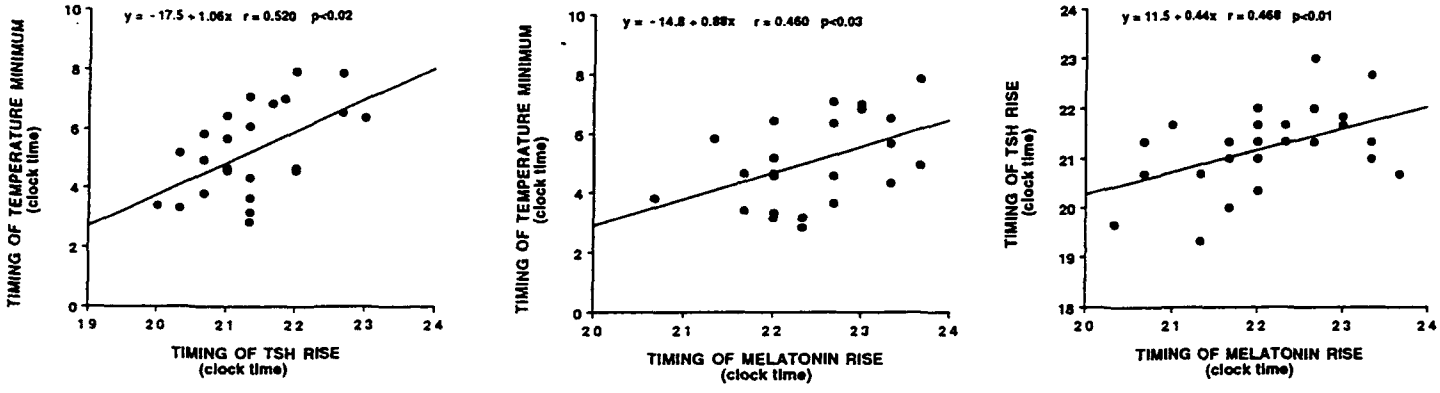

Figure 1: Relationships between markers of circadian phase in baseline conditions

The regression line between the timing of the TSH rise and the timing of the temperature minimum shown in the left panel of Figure 1 was used to estimate the timing of the temperature minimum in studies in which the minimum was masked by a temperature rise induced by light exposure. A second estimation of the timing of the temperature minimum was obtained from the regression line between the timing of the melatonin rise and the timing of the temperature minimum shown in the central panel of Figure 1. In the calculation of the phase-response curves, the average of these two estimations of the timing of the body temperature minimum was used. The phase-response curves for the TSH and melatonin rises were in excellent agreement. Indeed, as shown in Figure 2, there was an excellent correlation between the phase-shift of the melatonin rise and the phase-shift of the TSH rise in studies with light exposure (center), but not in baseline studies (left).

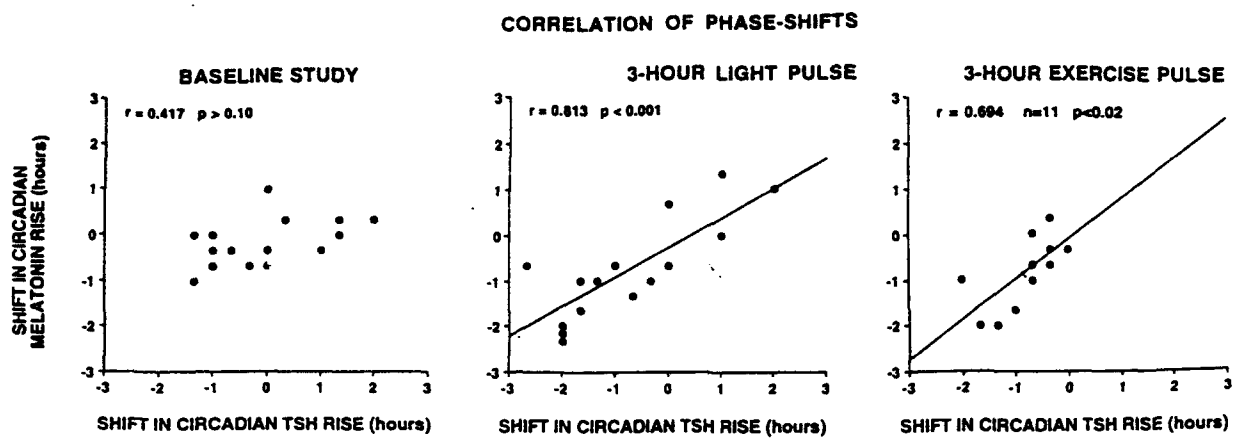

Figure 2: Correlations between the phase-shifts of the circadian melatonin rise and of the circadian TSH rise in the baseline study , in the study with light exposure and in the study with exercise exposure

Figure 3 shows the phase-response curves in the baseline studies (left) and in the studies with light exposure (center). The cross-over between the phase delay and the phase advance regions occurred more than one hour after the temperature minimum, i.e. more than 7 hours after the onset of the melatonin rise. The phase delay region encompassed all times of pulse presentation from 5 hours before the temperature minimum. i.e. around midnight, until approximately one hour after the temperature minimum, i.e. around 06:00. In the phase-delay region, phase-shifts of the melatonin and TSH rises in response to light averaged -74 min ± 44 min and -67 min ± 59 min, respectively, in contrast to phase-shifts of -9 min ± 30 min ($p<0.001$) and -17 min ± 71 min ($p<0.07$), respectively, in the baseline studies. The mean shift derived from both phase markers was -72 min ± 45 min in the light study versus -10 min ± 47 min in the baseline study. Phase advances were only observed in studies where the light pulse was centered at least one hour after the temperature minimum and were both less consistent and of smaller magnitude than the phase delays. On average, phase-shifts of the melatonin and TSH rises were 28 min ± 48 min and 56 min ± 67 min, respectively, in the advance region, in contrast to -8 min ± 33 min ($p<0.20$) and -12 min ± 69 min ($p=0.11$) in the baseline studies. The mean shift derived from both phase markers averaged 42 min ± 35 min in the studies with light exposure, versus -16 min ± 45 min in the baseline studies. For both the melatonin and the TSH rises, as well as for their mean shift, phase-shifts in the baseline study were not statistically significant from zero (all $p>0.25$) in either the phase advance region or the phase delay region.

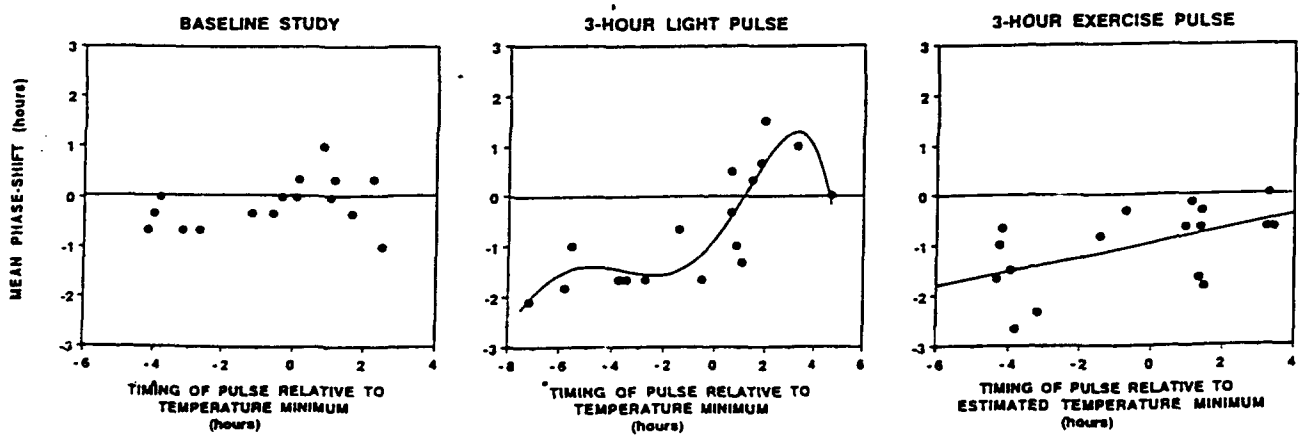

Figure 3: Phase-shifts observed in the baseline study (i.e. in the absence of stimulus), in the study with light exposure and in the study with exercise exposure.

This study has provided unambiguous evidence that a single exposure to bright light presented over a background of dim light without an intervening dark period may cause shifts of human circadian rhythms within less than 24 hours, with the direction and magnitude of the shifts depending on the timing of pulse presentation relative to endogenous circadian phase. This is the first demonstration of immediate phase-shifting effects of light *per se* since all previous studies on the response of the human circadian system to light pulses have involved protocols where the phase-shifts presumably caused by bright light exposure were measured after the subjects were exposed to one or more episodes of sleep and/or darkness. The phase-response curve obtained in the present study demonstrates that the response to a single light pulse on the first day after exposure lies in the delaying direction for most of the nocturnal period, with the crossover point occurring approximately one hour after the temperature minimum. Overall, the magnitude of the phase-shifts averages one hour, with delays being larger than advances. The phase-shifts are not accompanied by significant alterations in measures of circadian amplitude. The immediate response of the human circadian clock to a single 3-hour light pulse is thus characteristic of "type 1" resetting.

Other details of this study may be found in publications #1 and #2 (Appendices 1 and 2).

2. Immediate phase-shifting effects of single pulse of exercise

A second major objective of the previous application was to determine whether a single bout of physical activity is capable of phase-shifting human rhythms within one day after exposure. Following a period of entrainment to a fixed light-dark and sleep-wake cycle, the subjects were studied under constant routine conditions once in the absence of stimulus and once with a 3-hour pulse of exercise interrupting the constant routine conditions.

In the absence of any published observations on possible effects of exercise on the phase of human rhythms, the timing and duration of exercise exposure were selected based on extrapolations from observations in nocturnal rodents. The exercise period was chosen to be 3 hours in duration, i.e. long enough to be consistently associated with phase-shifting effects when appropriately timed with respect to the circadian cycle in rodents, but short enough to be compatible with the expected level of endurance of young healthy volunteers who were not competitive athletes. Since the phase-advance and phase-delay regions to activity-inducing stimuli are separated by only a few hours in rodents, the 3-hour period of exercise was also chosen to be short enough so as to not overlap the putative phase advance and phase delay regions in humans. In nocturnal rodents, activity induces shifts in the circadian clock when given during the usual rest period or during the period of low activity. We thus hypothesized that the most likely time when exercise may have zeitgeber effects on human rhythms would be during the night. A design parallel to that used for examining the immediate phase-shifting effects of light (see section 1. above) was thus used to investigate putative zeitgeber effects of physical exercise.

The timing of the exercise period was referenced relative to the time of occurrence of the body temperature minimum as observed during the baseline study. In 8 subjects (# 1-8), the center of the exercise period was timed to coincide with the timing of the body temperature minimum, in another 3 subjects (# 9-11), the center of the exercise period was timed to follow the timing of the temperature minimum by 2 hours and in the remaining 6 subjects (#12-17), the center of the exercise period was timed to precede the temperature minimum by 3 hours. Because the baseline study preceded the study with exercise by at least two weeks, it was expected that the timing of the body temperature minimum would not remain constant and that the precise timing of stimulus exposure relative to endogenous circadian phase would have to be inferred *a posteriori*. In the exercise studies, the temperature minimum was masked by the exercise-induced temperature rise and therefore hormonal phase markers had to be used to infer *a posteriori* the timing of pulse presentation.

Work loads during the periods of exercise were individually tailored to the subject's exercise capacity. During the week preceding the exercise study, each subject was studied twice as an outpatient in the University of Chicago Cardiac Exercise Physiology Laboratory using the same exercise equipment (Schwinn Airdyne arm and leg exerciser) as used during the study itself, once to determine his peak oxygen uptake (VO_2 max) for the arm exercise and once to determine his peak oxygen uptake for the cycling exercise. A symptom-limited maximal upright ergometry test was used for both the arm and the leg exercise with, respectively, a 15 or 30 watts/min incremental protocol. A "high" (i.e. 60 % of VO_2 max) and "low" (i.e. 40% of VO_2max) workloads were defined for the cycling exercise, based on the individual VO_2max levels. A "high" and "low" workload were similarly defined for the arm exercises. The VO_2max was on average 2872 ± 377 ml/min for the cycling exercise and 1935 ± 285 ml/min for the arm exercise. During the 3-hour exercise period, the workload and type of exercise were varied in 5 cycles of 36 minutes each, with alternating cycles of high (60% of VO_2max) and low (40% of VO_2max) workloads (starting with high workload). Each 36-min cycle included 15 min of arm exercise, 15 min of leg exercise and 6 min of rest. All subjects completed the 3-hour exercise period without difficulty.

As indicated above, the timing of the minimum of body temperature was masked by the temperature rise induced by exercise in all studies. Therefore, estimations of the timing of the exercise pulse relative to the minimum of body temperature had to be derived from the TSH and melatonin profiles using the demonstrated relationships between the timing of the circadian temperature minimum, the onset of the TSH rise and the onset of the melatonin rise illustrated in Figure 1. Whenever two estimations of the timing of the body temperature minimum were available, the average of the two was used in the calculation of the phase-response curve.

The right panel of Figure 3 shows the phase-response curve of the timing of the melatonin rise in the studies with exercise exposure. Phase-delays following exercise exposure were observed in 15 of 17 studies as compared to 8 of 17 studies under baseline conditions. On average, the phase shift was -55 min ± 38 min, i.e. highly significantly different from zero ($p<0.001$), and highly significantly different from the average phase-shift in baseline studies (- 9 min ± 32 min, $p<0.001$). Linear regression analysis suggested that the phase-delays tended to be larger when the exercise pulse occurred earlier in the nighttime ($r=0.414$, $p<0.10$). As illustrated in Figure 2 (right panel), there was an excellent overall concordance between the shift of the TSH rhythm and that of the melatonin rhythm in those studies with exercise exposure where the amplitude of the TSH rhythm after exercise exposure was sufficiently large (> 30% of daytime levels) to allow for a reliable estimation of the onset of the rise.

This study clearly demonstrates that a single nighttime period of physical exercise presented under dim light conditions is capable of phase delaying the circadian rhythm of human melatonin secretion within one day. In a majority of individual studies, a simultaneous delay of the TSH rhythm could be observed. The finding of concordant phase-shifts of two independent overt rhythms strongly supports the hypothesis that, in humans as in other mammalian species, physical activity is capable of exerting zeitgeber effects on the human circadian clock. The comparison between the phase-response curves to light and to exercise shown in Figure 3 indicates that the absolute magnitude of the phase-shifts in response to a 3-hour pulse of exercise is similar to that in response to a 3-hour pulse of bright light. However, in contrast to the phase-response curve to light, the phase-response curve to exercise did not include a phase-advance region in the range of timings of stimulus presentation covered in the present study. Thus, nighttime exercise, starting as early as 22:30 or ending as late as 10:30, was consistently associated with phase delays. A trend towards smaller phase-delays towards the morning hours raises the possibility that a cross-over point may occur in the morning and that if the timing of the exercise period had been later in the subjective day (e.g., around noon), phase advances may have been observed.

Other details of this study may be found in publication #3 (Appendix 3).

3. Effects of light and exercise on circadian variations in alertness and performance

In our studies on the immediate effects of exposure to light and exercise, measures of alterness (Stanford Sleepiness Scale) and performance were obtained at hourly intervals throughout the 43 hours of constant routine included in this protocol. Figure 4 shows the mean hourly variations of the scores on the Stanford Sleepiness Scale (SSS; top), the scores on the Digit Symbol Substitution test (DS; center) and the scores on the Symbol Copying task (SC; bottom) during the 17 baseline studies (i.e. in the absence of light or exercise exposure).

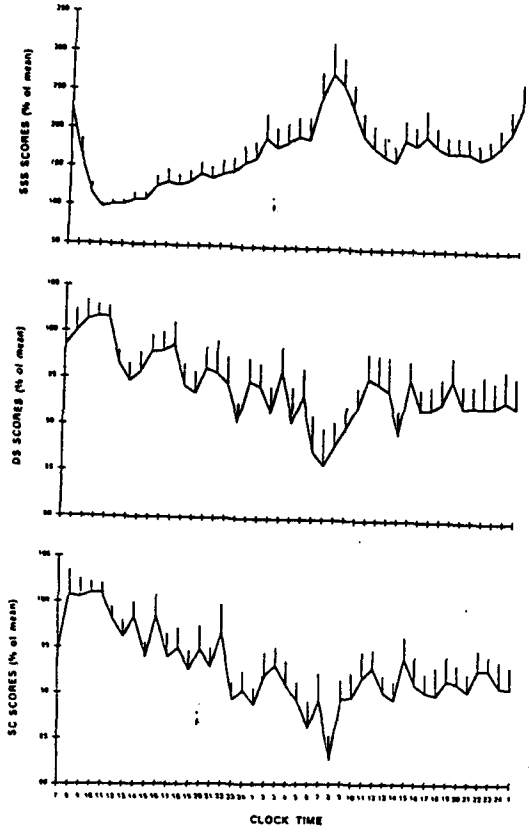

Figure 4: variations in measures of sleepiness and performance during 43 hours of baseline constant routine.

All scores were expressed as a percentage of the mean "basal" score, i.e. the mean score between 10:00 and 12:00 on the first day of the constant routine, to take into account inter-individual variations in baseline assessment of sleepiness and performance. Sleepiness was not minimal upon morning awakening, as scores continued to decrease until the late morning (10:00-12:00), reflecting an increase in alertness which was paralleled by an elevation of DS and SC scores. Starting in the the early afternoon, SSS scores increased steadily in an essentially linear fashion until an abrupt rise which started between 05:00 and 06:00, i.e. around the time of the minimum of body temperature, in a majority of volunteers. Peak sleepiness was observed between 07:00 and 08:00, and was followed, despite the persistence of the sleep deprivation condition, by a remarkable return to pre-

dawn levels. Sleepiness/alertness then remained at relatively high stable levels throughout the second day of the constant routine, until the late evening when scores rose again. The scores on the DS and SC tasks faithfully mirrored the variations in SSS scores, with optimal performance in the late morning of the first day, and lowest performance in the early morning hours of the second day. The coefficients of correlation between the profiles of, respectively, SSS score and DS score, SSS score and SC score, and DS and SC score, were all statistically significant ($p<0.01$).

Figure 5 shows the variations in SSS scores in the studies with light exposure (left) and exercise exposure (right) as compared to the baseline study (center). The range of timing of stimulus exposure is indicated by an arrow. There were no significant differences in SSS scores between the baseline study and the study with light exposure during any time interval of the constant routine. In contrast, there was a significant decrease in early morning peak SSS score (i.e. mean of scores obtained at 07:00, 08:00 and 09:00) in the exercise study as compared to the baseline study ($p<0.02$) indicating that the extreme early morning sleepiness experienced following an entire night of sleep deprivation was somewhat alleviated by exercise. This is further demonstrated in Figure 6 where the profiles of SSS scores are referenced to the timing of exercise exposure, rather than clock time. Exercise exposure in the late evening and early morning resulted in an immediate improvement in subjective sleepiness. Exercise exposure in the middle of the night, i.e. before the major rise in sleepiness around the time of the minimum of body temperature, did not have significant effects on SSS scores. Note that the end of the exercise period was generally followed by an abrupt rise in sleepiness.

These studies indicate that the physical activation induced by exercise was also accompanied by a certain level of "mental arousal". These aspects of our study were the object of an oral communication at the 4rth Meeting of the Society for Research on Biological Rhythms.

Figures 5 and 6 are on page 8.

4. Direct neuroendocrine effects of exercise

It is well established that aerobic exercise of sufficient intensity (> 50% VO_2max) produces acute hypothalamic-pituitary-adrenal stimulation. A small stimulatory effect of exercise on plasma cortisol levels was indeed observed in the present study, even though the workload (i.e. 40-60% VO_2max) was at the threshold for eliciting such a response. The area under the cortisol curve was at least 10% larger during exercise than during recumbency in 9 of 17 subjects (with the increase averaging 67.8 ± 48.2 %), was similar (i.e. within $\pm$ 10%) in 4 of 17 subjects and was at least 10% lower during exercise than during recumbency in the remaining 4 subjects (with the decrease averaging 26.8 ± 9.5 %).

Aerobic exercise is also known to acutely stimulate GH release. To verify that our exercise stimulus was indeed associated with this well-described neuroendocrine activation, plasma GH levels were measured in all samples collected during the first 24 hours of the baseline study, and during the first 24 hours of the study with exercise exposure. A significant pulse of plasma GH started withi 40 min after the beginning of the exercise period in 16/17 subjects. On average, the amount of GH secreted during the exercise period was 272 ± 239 μg as compared to 47 ± 95 μg during the same period of time when the subject was in recumbent position ($p<0.01$ by paired t test).

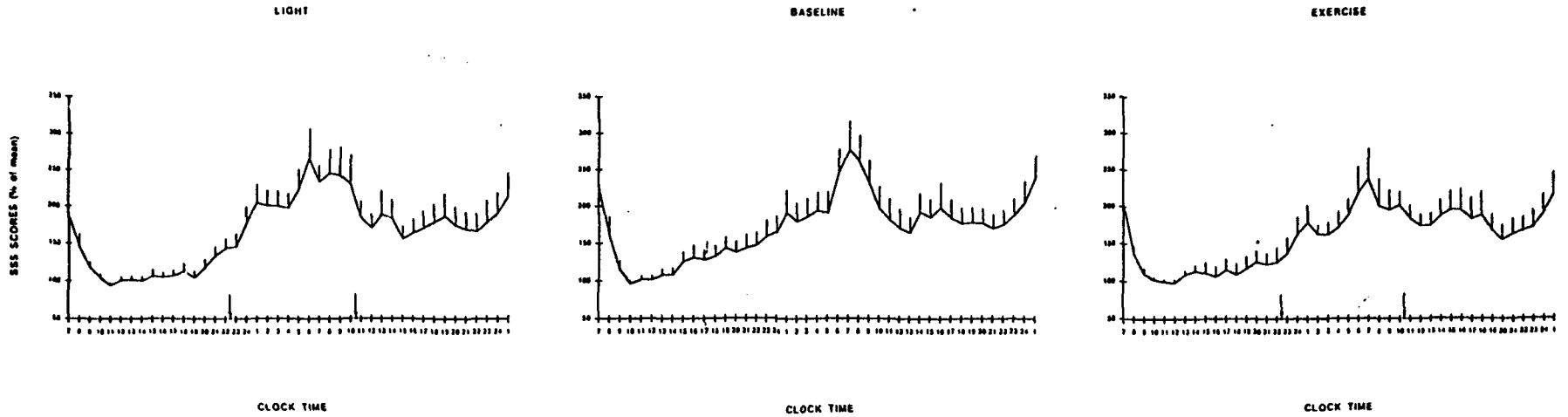

Figure 5: mean (+SEM) scores on the Stanford Sleepiness Scale (SSS) during 43 hours of baseline constant routine (center) as compared to constant routine conditions interrupted by 3-hour of exposure to bright light (left) or 3 hours of exposure to exercise (right). The arrows indicate the range of timings of stimulus exposure. Scores are expressed as percentage of basal level.

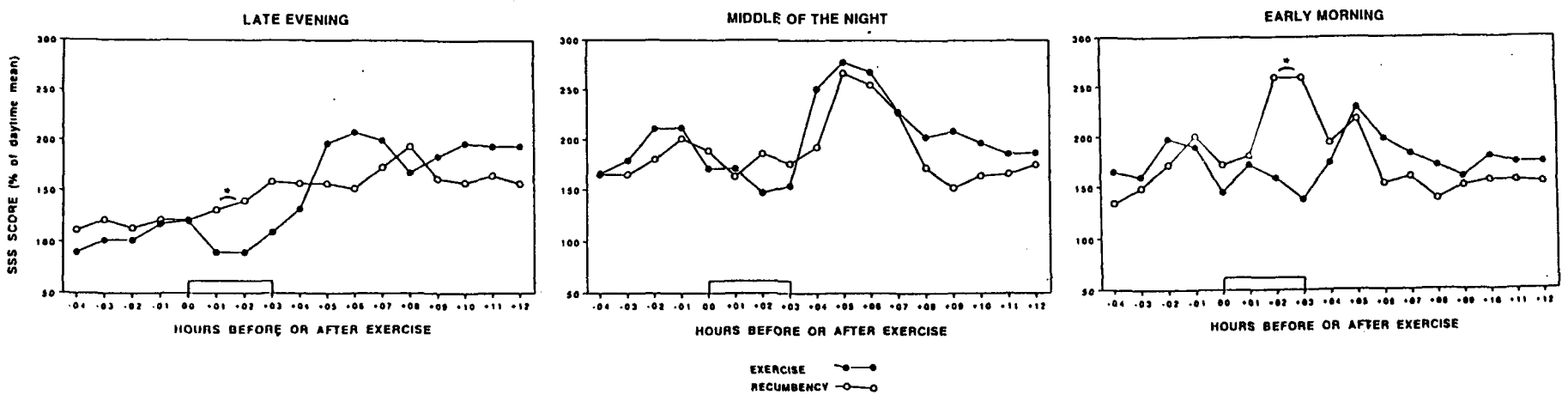

Figure 6: mean (+SEM) scores on the Stanford Sleepiness Scale (SSS) before and after exercise exposure in the late evening (left), middle of the night (center) and early morning (right). The open bars indicate the period of exercise. Asterisks indicate significant differences between the exercise and baseline conditions ($p<0.05$).

Unexpectedly, exercise had robust stimulatory effects on plasma TSH levels. When given early in the night, exercise resulted in a higher nocturnal TSH acrophase (left panel of Figure 7). When given in the middle of the night, exercise resulted in a higher TSH acrophase (center panel of Figure 7). When given late in the night, exercise delayed the early morning decline in TSH concentrations (right panel of Figure 7). Overall, the area under the TSH curve was 24.6 ± 15.3 % larger during the exercise period than during the same period of time when the subject was in recumbent position ($p=0.0001$). These immediate effects of exercise thus increased TSH amplitude during the first day of the study (229 ± 91 % of daytime nadir versus 175 ± 55 % in the baseline study, $p<0.05$) and were also associated with a lower TSH amplitude during the second day of the study (50 ± 28% of daytime nadir versus 79 ± 22 % in the baseline study, $p<0.05$). Ongoing analyses of concomitant triiodothyronine (T3) profiles in our laboratory suggest that this greater inhibition in circadian TSH rise on the second day of the constant routine in the exercise, as compared to the baseline, studies is related to an enhanced negative feedback effect resulting from the exercise-induced elevation of thyroid hormone levels. An example is shown in Figure 8. Despite the prolonged half-life of T3, the concentration profile of this hormone shows a remarkable parallelism with that of TSH. Effects of exercise on the thyroid axis have never been previously recognized. The failure to previously recognize exercise as a physiological stimulus of TSH secretion probably reflects the fact that sensitive radioimmunoassays, capable of reliably detecting variations in physiological TSH levels, have only become available during the past 6-8 years. Our novel finding on the effects of exercise on TSH secretion has been presented as a communication at the 1993 Annual Meeting of the Endocrine Society (abstract #1653). These results are presently being prepared for full-length publication.

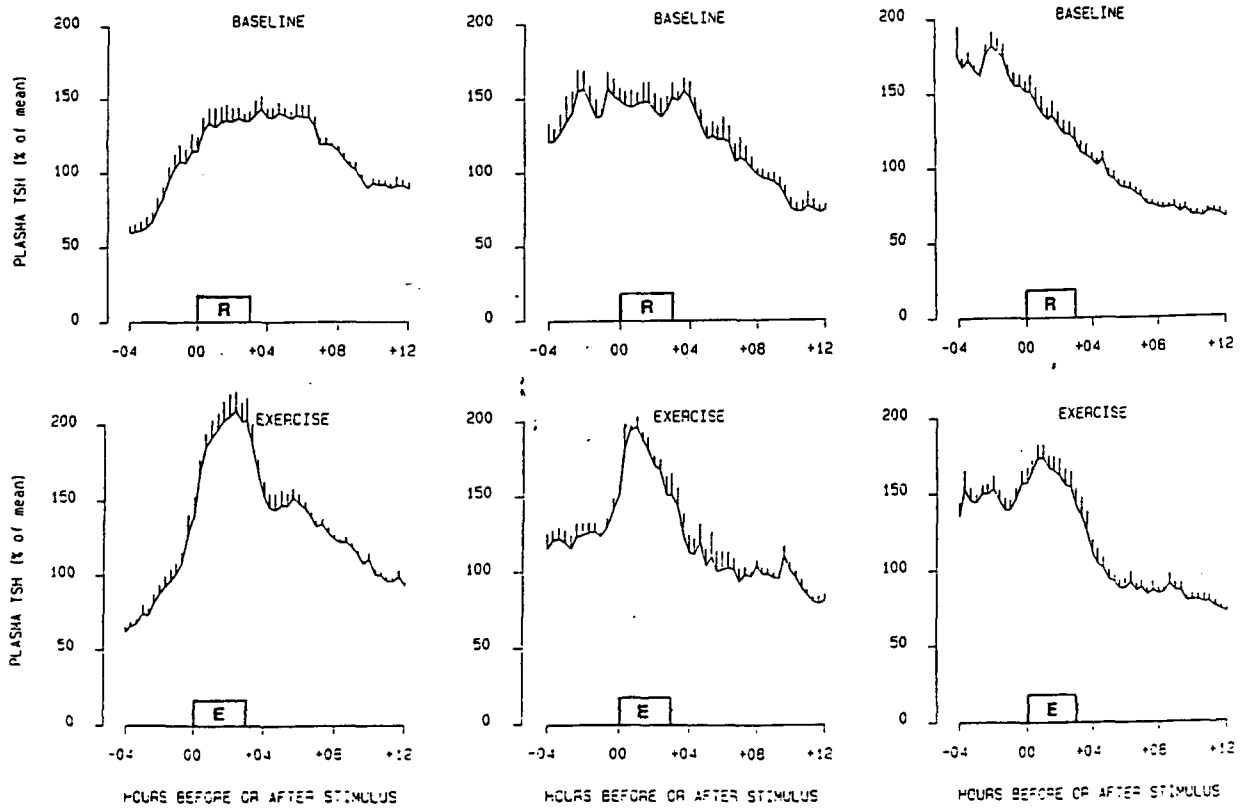

Figure 7: mean (+SEM) profiles of plasma TSH during a 3-hour period of recumbency (R) or exercise (E) in the late evening (i.e. when TSH levels are normally rising; left), middle of the night (i.e. when TSH levels are normally at their peak; center) and later part of the night (i.e. when TSH levels are normally declining; right). To account for inter-subject variability , the data are expressed as % of the individual mean.

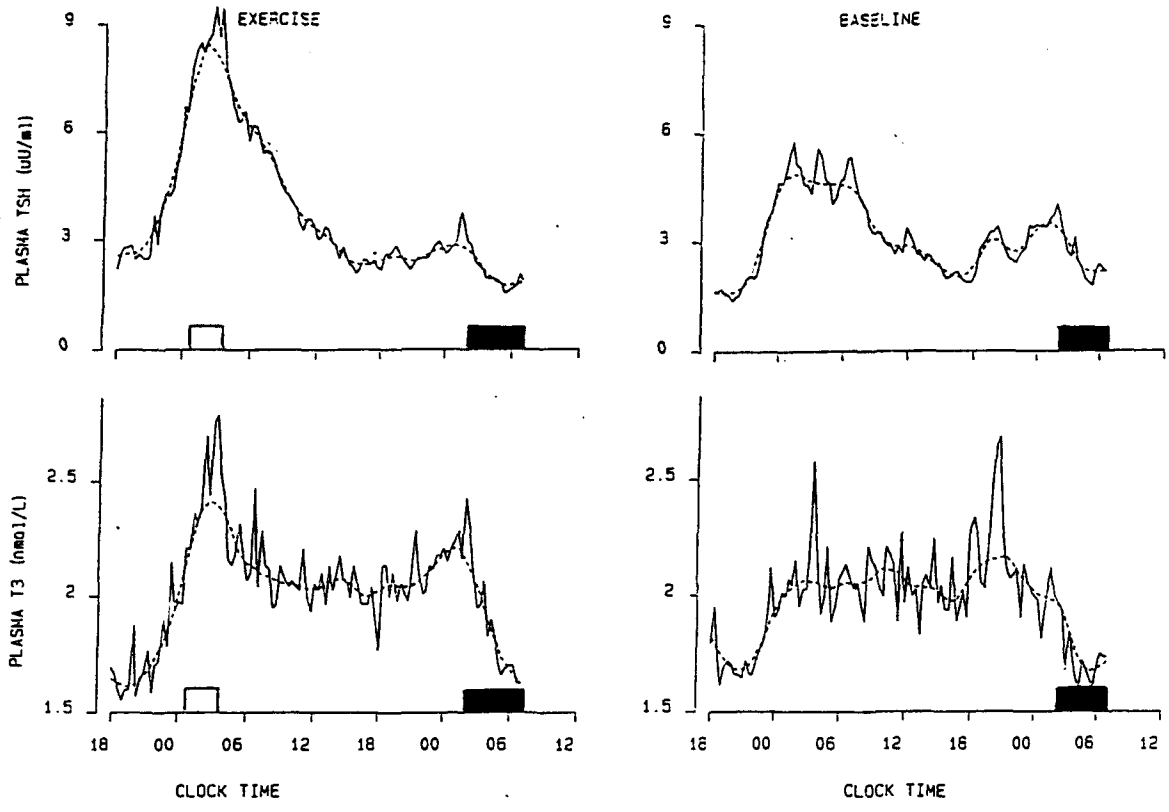

Figure 8: profiles of plasma TSH levels (top) and plasam triiodothyronine (T3) levels in the same subject studied under constant routine conditions with (left) or without (right) interruption by a 3-hour exercise period (shown as an open rectangle). Black bars represetn the periods of recovery sleep. Best-fit curves are shown in dashed lines.

Several reports in both animals and humans have indicated that the effects of exercise on melatonin secretion may be phase-dependent. In the rat, forced swimming during the dark phase, when melatonin levels are elevated, induces a rapid drop in pineal and blood melatonin levels. In the human, brief periods of high intensity exercise administered in the late evening (22:00-23:00) appear to be associated with an immediate blunting of the nocturnal rise, persisting until the early morning hours. In our study, the immediate effects of exercise on melatonin concentration were inconsistent and were not significant at the group level. It is likely that the intensity of exercise in our study was not sufficient to elicit robust effects on melatonin secretion. The area under the melatonin curve, expressed as a percentage of the total area under the curve for the first 24-hour of the study, was at least 10% less during exercise than at the same clock time during continuous recumbency in 10 of 17 subjects, with the reduction averaging 42.0 ± 22.5 %. In 5 of 17 subjects, the standardized area under the melatonin curve was at least 10% larger during the exercise study, with the increase averaging 25.4 ± 18.4 %. In the last two subjects, differences between the exercise condition and the recumbency condition were less than 2%. We failed to identify significant correlations between the timing of the exercise pulse, the direction and magnitude of changes in melatonin levels and the direction and magnitude of phase-shifts of the melatonin rise.

5. Effects of exposure to bright light and exposure to dark/sleep in facilitating adaptation to an 8-hour advance shift

The purpose of this study was to determine whether adaptation to an 8-hour advance of the sleep-wake and light-dark cycles could be facilitated by adequately timed exposure to bright light or by administration of a hypnotic agent which facilitates sleep at a time of normal activity.

Eight subjects participated each in three trials: one baseline trial with dim light exposure during waking hours and administration of a placebo at the advanced bedtimes, one trial with bright light exposure during waking hours and administration of a placebo at the advanced bedtimes and one trial with dim light exposure during waking hours and administration of a hypnotic agent (Zolpidem, Stilnoct™) at the advanced bedtimes to facilitate sleep during the normal active period. The trials were performed in random order and were separated by one month. Following one week of entrainment to fixed bedtimes (23:00-07:00) including two nights of habituation in the sleep laboratory, blood sampling at 20-min intervals for the measurement of TSH, melatonin, cortisol and GH levels was started and continued without interruption for 72 hours. This 72-hour span included one night of sleep at the normal bedtimes (23:00-07:00), and two periods of recumbency in total darkness (i.e. dark/sleep) scheduled 8 hours earlier than the usual bedtimes, i.e. at 15:00-23:00. Sleep was polygraphically recorded during all dark periods. Wakefulness was monitored at all other times. Body temperature and wrist activity were recorded continuously.

The experimental work involved in this study has been completed as planned. The TSH assays have been completed for all subjects and all trials. The cortisol assays have been completed for 4 subjects. Limited melatonin data from four trials are currently available. Preliminary analyses of the available data have already evidenced important findings which are briefly described below.

Figure 9 shows the mean profiles of plasma TSH for the baseline (placebo) trial with dim light exposure (top), the trial with bright light exposure and placebo administration (center) and the trial with dim light exposure and zolpidem administration. The timings of placebo administration are indicated by thin arrows, whereas the timings of zolpidem administration are indicated by bold arrows. Black bars represent the periods of dark/sleep. Open bars represent the periods of exposure to bright light. To account for inter-individual variations in basal TSH levels, the data were expressed as a percentage of the mean. As in our previous studies, there was a remarkable reproducibility of the TSH profile on the first study day. In all three trials, the onset of the nocturnal TSH rise occurred between 20:30 and 21:30 and the nocturnal acrophase represented an approximate twofold rise above the daytime levels. As expected, sleep onset at the normal nocturnal time inhibited further rise of TSH concentrations. In all three trials, daytime levels resumed in the morning. The next circadian rise of TSH occurred during the daytime dark period and was observed 1 to 2 hours earlier than on the previous day, suggesting that exposure to dark, even without pharmacological facilitation of sleep, caused a phase-advance of the TSH rhythm. As will be shown in Figures 10 and 11 below, this advance was also observed for cortisol and melatonin, supporting the concept that exposure to dark/sleep *per se* has resulted in an immediate 1 to 2 hours advance phase-shift of the circadian clock. Unexpectedly, the ends of the scheduled periods of daytime dark/sleep were associated in all three trials with a sharp, immediate rebound of TSH secretion. This rebound was significantly larger under the dim light placebo conditions (top) than under the bright light (center) and dim light-zolpidem (bottom) conditions. These data thus demonstrate an acute effect of bright light exposure on TSH levels in man. Pharmacological sleep induction also significantly limited the rebound of TSH secretion at the end of the dark periods. As a result of these rebound TSH secretions, overall morning TSH levels (e.g. at 07:00) at the end of the study averaged approximately 250% of baseline levels in the dim light-placebo condition (top), 150% of baseline levels in the bright light-placebo condition (center) and 220% in the dim light-zolpidem condition. This is the first demonstration of cumulative effects of an abrupt phase-shift of sleep-wake and light-dark cycles on a blood constituent. Previous studies of endocrine alterations during real or simulated "jet lag" have

examined the profiles of cortisol, prolactin, melatonin and/or GH and have identified abnormalities in the timing, but not in the overall levels, of hormonal secretions.

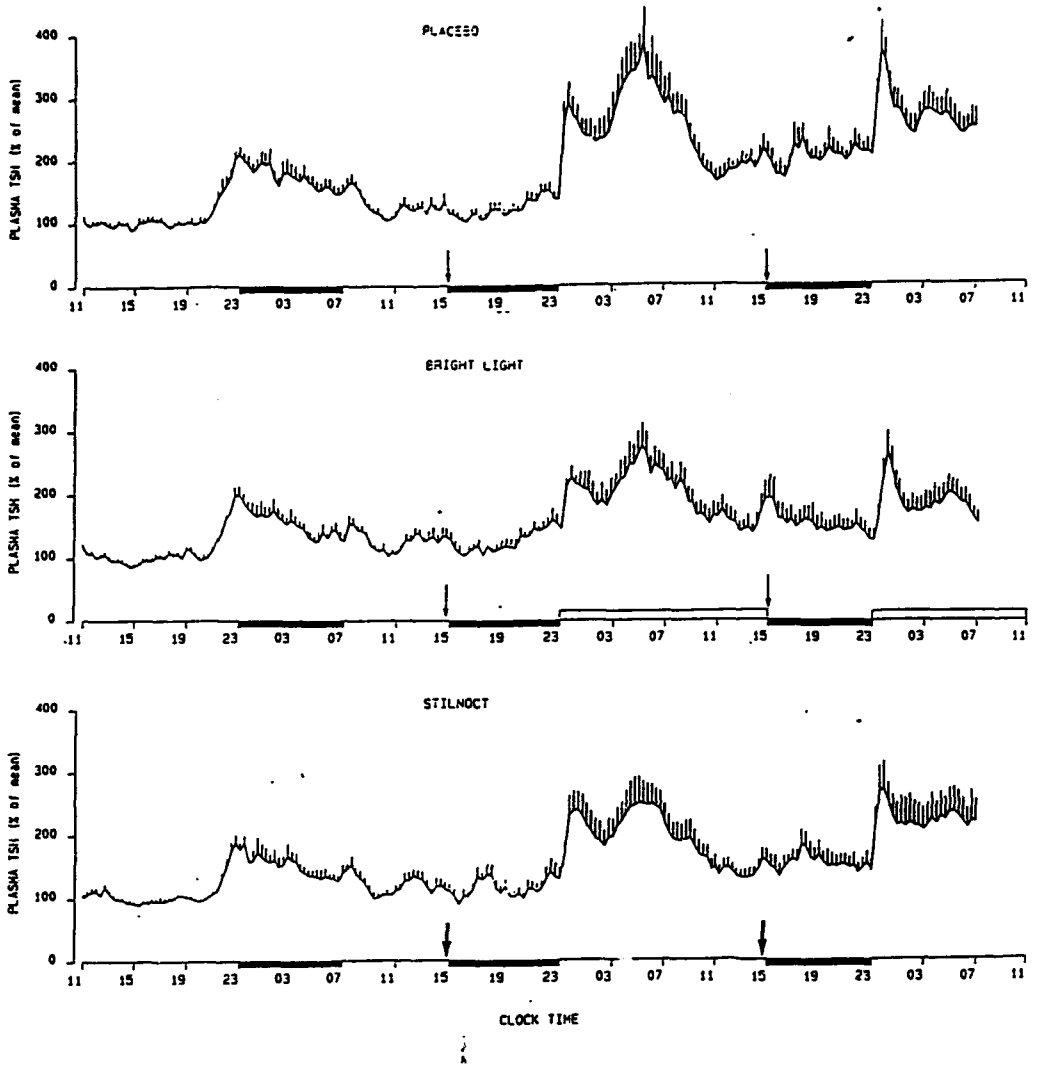

Figure 9: mean (+SEM) profiles of plasma TSH levels from 8 subjects studied during adaptation to an 8-hour advance of the sleep-wake/dark-light cycle treated with oral administration of a placebo and dim light conditions during waking hours (top), oral administration of a placebo and bright light conditions during waking hours (center), or administration of a hypnotic drug and dim light conditions durig waking hours (bottom). Black bars show the sleep/dark periods. Open bars show the periods of exposure to bright light. Bold arrows show the times of administration of the hypnotic drug. Thin arrows show the times of placebo administration.

Figure 10 shows the mean cortisol profiles from 4 subjects during the three trials. Symbols are as in Figure 9. Although the waveshapes of the profiles is severely altered following the advance of the sleep-wake and light-dark cycles, the mean 24-hour cortisol levels did not vary significantly across the study period. This is in contrast to the TSH profiles, where an overall elevation of TSH secretion was observed in the course of adaptation to the simulated "jet lag". On the first day, the onset of the circadian cortisol rise occurred between 02:00 and 03:00 in all three trials. On the second day, this onset appeared to have advanced by several hours (i.e. occurring between 22:00 and 23:00). A more detailed analysis awaits the availability of a more complete hormonal data set as well as of measures of sleep duration and quality.

Figure 11 illustrates individual melatonin profiles from two subjects during the first 48 hours of the study in the trials without pharmacological sleep induction. On the second day of the study, the onset of the melatonin rise (indicated by an arrow) occurred one to two hours earlier than on the first day in both subjects and both trials, indicating that exposure to dark/sleep is capable of causing an immediate advance of the melatonin rise. In view of the similar findings in the TSH and cortisol profiles, these observations provide evidence that exposure to dark/sleep presented over a background of dim light exert zeitgeber effects on the human circadian clock.

While still preliminary, the findings from this study have provided two important novel observations 1. an overall elevation of TSH levels is a biological concomitant of the "jet lag syndrome"; 2. exposure to dark/sleep is capable of exerting immediate phase-shifting effects of human rhythms.

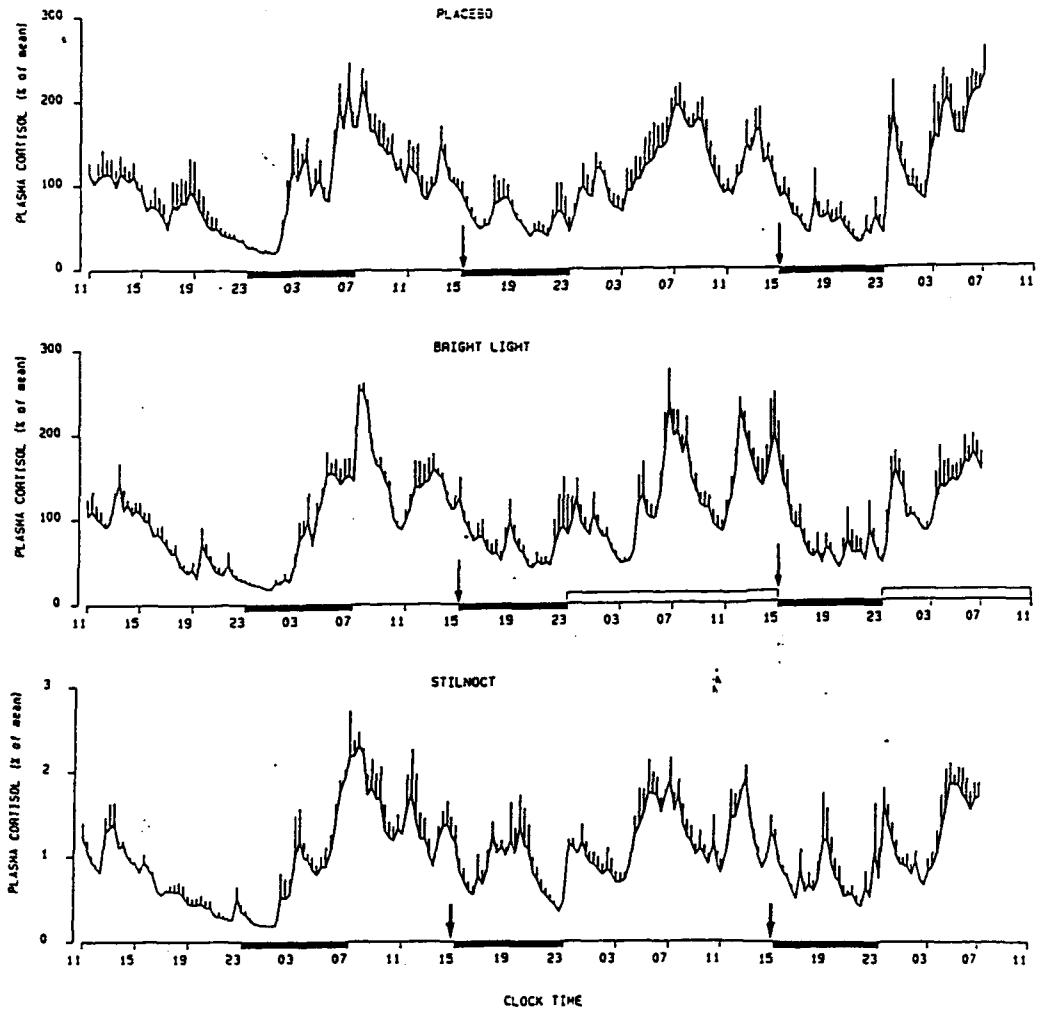

Figure 10: mean profiles of plasma cortisol levels from 4 subjects studied during adaptation to an 8-hour advance of the sleep-wake/dark-light cycle treated with oral administration of a placebo and dim light conditions during waking hours (top), oral administration of a placebo and bright light conditions during waking hours (center), or administration of a hypnotic drug and dim light conditions durig waking hours (bottom). Symbols are as in Figure 9.

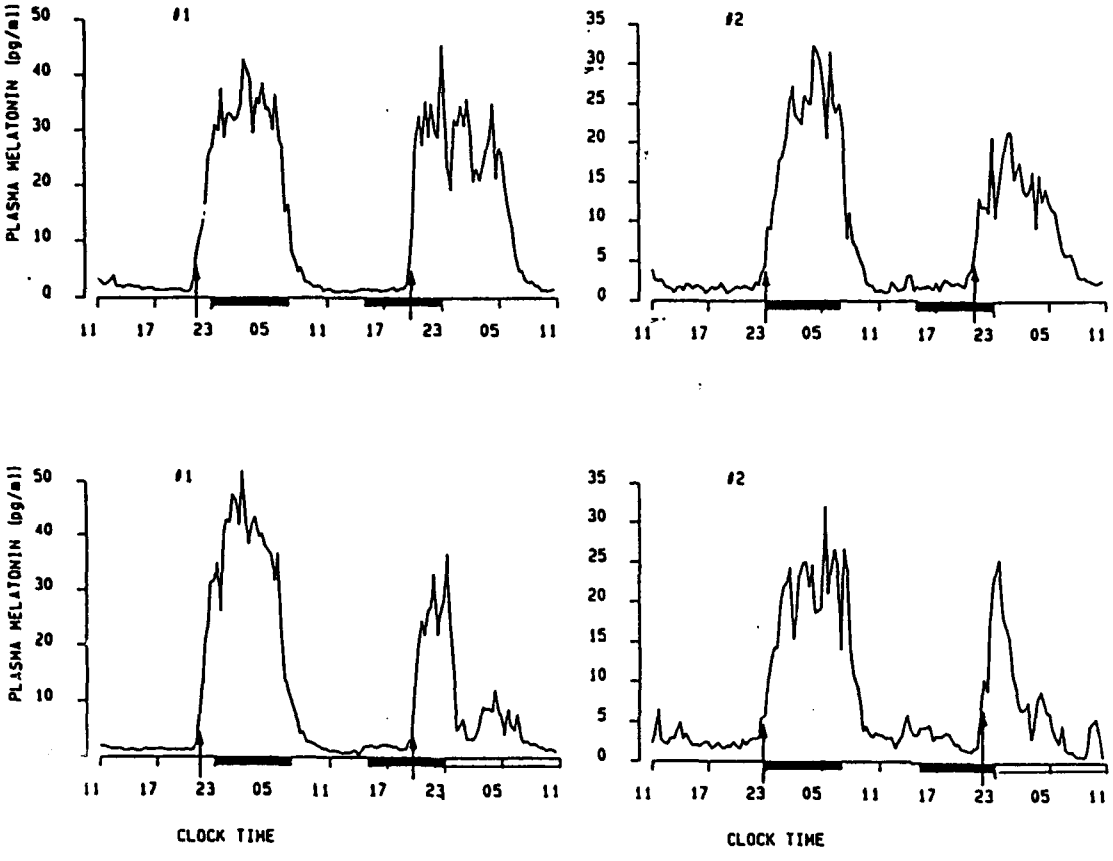

Figure 11: individual profiles of plasma melatonin levels in two subjects (# 1 and #2) studied during adaptation to an 8-hour advance of the sleep/dark period with either dim light (top) or bright light (bottom) exposure during waking hours. The arrows indicate the timing of the onset of the melatonin rise. The black bars show the sleep/dark periods. The open bars show the periods of bright light exposure.

PUBLICATIONS 1993-1994

1. Van Cauter E, Sturis J, Byrne MM, Blackman JD, Scherberg NH, Leproult R, Refetoff S and Van Reeth O. Preliminary studies on the immediate phase-shifting effects of light and exercise on the human circadian clock. J Biol Rhythms, 8 (suppl): S99-S108, 1993. **Appendix 1**

2. Van Cauter E, Sturis J, Byrne MM, Blackman JD, Leproult R, Ofek G, L'Hermite-Balériaux M, Refetoff S, Turek FW and Van Reeth O. Immediate phase-shifting effects of light on the human circadian clock: demonstration of a large phase delay using hormonal phase markers. in press in the Am J Physiol (Endocrinology and Metabolism), 1994. **Appendix 2**

3. Van Reeth O, Sturis J, Byrne MM, Blackman JD, , L'Hermite-Balériaux M, Leproult R, Oliner C, Refetoff S, Turek FW and Van Cauter E. Nocturnal exercise phase-delays the circadian rhythms of melatonin and thyrotropin in normal men. in press in the Am J Physiol (Endocrinology and Metabolism), 1994. **Appendix 3**

4. Byrne MM, Van Reeth O, Blackman JD, Sturis J, Van Cauter E. Nighttime exercise increases TSH secretion in normal men. Proceedings of the 75th Annual Meeting of the Endocrine Society, # 1653, p 464, 1993 (Abstract)

5. Linkowski P, Van Onderbergen A, Kerkhofs M, Bosson D, Mendlewicz J, Van Cauter E. A twin study of the circadian and pulsatile variations of plasma cortisol: evidence for genetic control of the human circadian clock. Am J Physiol (Endocrinology/Metabolism) 264: E173-E181, 1993.

6. Linkowski P, Kerkhofs M, Van Onderbergen A, Hubain P, Copinschi G, L'Hermite-Balériaux M., R. Leclercq, M. Brasseur, J. Mendlewicz, E. Van Cauter. The 24-hour profiles of cortisol, prolactin and growth hormone in mania. in press in Arch Gen Psychiatry, 1994.

7. Kerkhofs M, Van Cauter E, Van Onderbergen A, Caufriez A, Thorner MO, Copinschi G. Sleep promoting effects of growth hormone-releasing hormone in normal young men. Am J Physiol (Endocrinology/Metabolism), 264: E594-E598, 1993.

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10. Sturis J, O'Meara NM, Shapiro ET, Blackman JD, Tillil H, Polonsky KS, Van Cauter E. Differential effects of glucose stimulation upon rapid pulses and ultradian oscillations of insulin secretion. J Clin Endocrinol Metab, 76: 895-901, 1993.

11. O'Meara NM, Sturis J, Blackman JD, Roland DC, Van Cauter E, Polonsky KS. Analytical problems in the detection of rapid oscillations of peripheral insulin and C-peptide in normal man. Am J Physiology (Endocrinology/Metabolism) 264: E231-E238, 1993.

12. O'Meara NM, Sturis J, Van Cauter , Polonsky KS. Lack of control by glucose of ultradian insulin secretory oscillations in impaired glucose tolerance and NIDDM. J Clin Invest 92: 262-271, 1993.

13. Degaute JP, Van Cauter E, Van De Borne P, Linkowski P. Twenty-four--hour blood pressure and heart rate profiles in humans: a twin study. Hypertension 23: 244-253, 1994.

14. Van Cauter E. Hormones and Sleep. In: The Pharmacology of Sleep, A. Kales (ed), Handbook of Experimental Pharmacology , Springer-Verlag, Berlin, 1994, in press.

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16. Leproult R, Van Reeth O, Byrne MM, Sturis J, Van Cauter E. Effects of exposure to light or exercise on sleepiness and performance during constant routine conditions. Abstract # 109, 4rth Meeting of the Society for Research on Biological Rhythms, Jacksonville FL, May 1994. **Appendix 4**

17. Van Cauter E, Leproult R. A meta-analysis of the 24-hour profile of plasma cortisol in normal man: evidence for age-related alterations in circadian phase and amplitude. Abstract # 141, Abstract # 109, 4rth Meeting of the Society for Research on Biological Rhythms, Jacksonville FL, May 1994.

PROFESSIONAL PERSONNEL 1993-1994

Byrne, Maria, MD	Fellow in Endocrinology until July 1993 (funded by Training Program), Instructor in the Dept of medicine as of July 1993.
Rachel Leproult	BS in mathematical statistics, funded by this award in Jan-Feb 1994
Laurence Plat, MD	Fellow in Endocrinology, funded by the Belgian Diabetes Association
Orfeu Buxton	Ph.D. student at Northwestern University; funded by AASERT award starting September 93
Isidore Hochner, MD	50% effort from March 1, 1993, until November 30, 1993.

INTERACTIONS

Speaker, Advances in Sleep Medicine Winter Meeting, Breckenridge, CO, February 14-17, 1993.

Speaker, Workshop on "Circadian Rhythms and Sleep Disorders: Role of Melatonin", organized by the Institut de Recherches Internationales Servier, New York City, NY, September 2-5, 1993.

Speaker, Puberty Meeting, MacArthur Foundation Planning Initiative on Psychopathology and Development, September 26-27, 1993, Chicago, Il.

Invited participant, Workshop on Night Operations/Human Chronobiology, Air Force Office of Scientific Research, Brooks Air Force Base, Texas, Jan 6-7, 1994.

Speaker, Plenary Session, 38th Annual Meeting of the German Endocrine Society, Würzburg, Germany, March 2-5, 1994.

Speaker, Symposium on Implications of Circadian Rhythm Abnormalities in Depression, 4th Meeting of the Society for Research on Biological Rhythms, Amelia Island, Florida, May 4-8, 1994.

Speaker, Plenary Session, 8th Annual Meeting of the Association of Professional Sleep Societies, June 4-9, 1994, Boston, MA

Chairperson of the Scientifi Program Committee of the 4rth Meeting of the Society for Research on Biological Rhythms