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The Effects of Melatonin on Menstrual Characteristics, Prolactin and Premenstrual Syndrome-Like Symptoms During a Simulated Eastward Deployment

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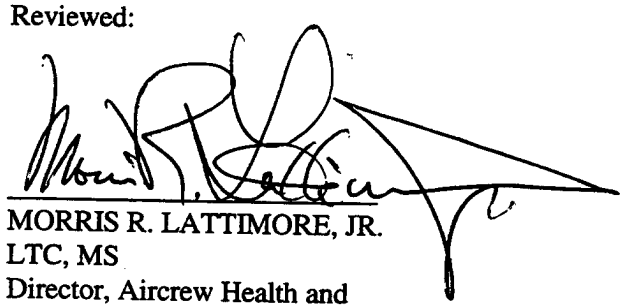

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In a previous study, we investigated the effect of exogenous melatonin (10 mg) given at bedtime (2300) for 7 consecutive days during the late follicular and early luteal phase of the monthly cycle, on menstrual characteristics. Our results showed that under our specific conditions, exogenous melatonin has little effect on menstrual characteristics. Since melatonin receptors likely up- and down-regulate depending on the availability of melatonin, 2300 would be during the nightly endogenous increase and receptors should be at a low level. The study reported here was double blind and placebo controlled and was undertaken to determine whether exogenous melatonin (10 mg), given earlier in the day when melatonin receptors are at a high level (1300), would have a greater effect on menstrual characteristics. This study also involved the use of bright lights to shift activity rhythms and simulate an eastward deployment across six time zones. As in the previous study, dose days occurred during the late follicular and early luteal phases of the menstrual cycle. We observed several apparently minor effects, including shorter overall cycle length during the dose cycle and longer cycle length during the following cycle,
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shorter menses during the month following melatonin administration, and decreased LH surge during the post-dose month. Additionally, melatonin administration apparently results in a reduction of study-related stress. This is apparent when comparing the melatonin and placebo groups in this study. Because of the relatively small number of participants in each group and the wide variability between subjects, statistical significance can not be demonstrated for most variables. We do not feel that any of these changes should be a concern during deployment conditions, and in fact, melatonin is likely to be beneficial because of its ability to resynchronize circadian rhythms, function as an antioxidant, and reduce or alleviate stress. It would seem that, logically, the next step would be to increase the sample size, and include males to determine if the antistress properties can be confirmed and are gender independent.

Acknowledgments

We would like to acknowledge the excellent technical advice and assistance of Mr. Richard Conley of American Laboratory Products Company. Without his knowledgeable suggestions at various stages of the study, the melatonin assays would have been doomed to failure. Also, thanks to MAJ Jack Brown for his critical reading of the manuscript and many helpful suggestions.

Table of contents

List of figures

Table of contents (continued)

List of figures (continued)

Page

List of tables

List of tables (continued)

Page

Introduction

Melatonin (N-acetyl-5-methoxytryptamine), a natural hormone which has been shown to resynchronize circadian rhythms and induce sleep in humans (Arendt et al., 1987; Dawson and Encel, 1993; Reiter, 1991; Wurtman, 1986), is currently being marketed widely as a dietary supplement to alleviate desynchronosis (desynchronization of physiological and behavioral rhythms) and assist in obtaining quality sleep. Desynchronosis often results from rapid shifts in work schedules from day to night, or from shifts in the light-dark cycle due to time zone crossing. Symptoms resulting from desynchronosis include fatigue, sleepiness, lethargy, insomnia, gastrointestinal tract disorders, and poor mental performance (for review see Comperatore and Krueger, 1990). Melatonin therapy has been demonstrated to be effective in preventing sleep loss and in maintaining alertness following travel across multiple time zones (Arendt and Broadway, 1987; Comperatore et al., 1996; Petrie et al., 1989). Thus, melatonin can be a potentially effective chronobiotic and ameliorate desynchronosis during travel.

Melatonin is produced by the pineal gland in the absence of bright light. In humans, melatonin synthesis reaches peak levels during the night and lowest levels during the day. Known side effects of melatonin chronobiotic doses (5-10 mg) are limited to sleepiness, fatigue, and reduced alertness shortly after administration, but not upon awakening (Arendt et al., 1987; Comperatore et al., 1996; Petrie et al., 1989). However, in females, due to a potential inhibitory influence of melatonin over the hypothalamo-pituitary-ovarian axis (Aleem et al., 1984; Nordlund and Lerner, 1977), melatonin use may be associated with secondary disruptions of the menstrual cycle. Therefore, one must ask whether this nonprescription hormone can be used safely to reduce desynchronosis resulting from travel, or might short-term use result in disruption of the menstrual cycle?

Melatonin and the menstrual cycle

Although the exact relationship between melatonin and the monthly cycle in females has not been clearly established, there is considerable evidence for interaction between melatonin and luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Cagnacci et al., 1991; Diaz et al., 1993; Nordlund & Lerner, 1977; Voordouw et al., 1992). There may be a relation between the early-morning onset of the LH surge, which occurs in a majority of women (Testart et al., 1982), and the concurrent decline in melatonin secretion (Brzezinski et al., 1987; Brzezinski et al., 1988; Zimmerman et al., 1990). In a study involving the daily administration of 300 mg of melatonin for up to 4 months to 12 women, there was a significant decrease in mean LH levels compared to controls (Voordouw et al., 1992).

Following 1 g/day melatonin to 2 normal cycling and 2 non-cycling females, decreases in serum LH on days 14 and 21 of the cycle were reported (Nordlund and Lerner, 1977). Findings on serum FSH were not consistent. The two normal cycling females reported normal periods at this dose of melatonin. Mixed results were reported following the administration of 2 mg of melatonin given at 1600 and 2000 to seven cycling females (Terzolo et al., 1993). During the mid-follicular phase, two subjects demonstrated increased release of LH, and three subjects

demonstrated decreased release of LH. As a group, there was no significant variation. Their conclusion was that the effect of melatonin on LH most likely depends upon individual sensitivity. Exogenous melatonin (100 mg, n=6 and 2.5 mg, n=5) at 0800 during the early follicular phase (days 2-5) was reported to enhance the release of LH without modifying FSH (Cagnacci et al., 1991).

Finally, indirect evidence for a melatonin effect on menstrual hormone patterns arose from a recent study (Diaz et al., 1993). They investigated levels of LH, FSH, and melatonin in young women in regular physical training and compared them to controls. Higher daytime levels of melatonin, lower basal levels of LH in the early follicular phase or luteal phase, and higher levels of FSH in the luteal phase but no change during the early follicular phase were reported. They suggested that melatonin plays an inhibitory role on menstrual cycle hormone patterns in young women in training. Supporting this notion is evidence showing that endogenous nocturnal melatonin levels, in women experiencing amenorrhea, are more than double the normal levels observed in cycling women (Berga et al., 1988; Brzezinski et al., 1988; Laughlin et al., 1991).

Summarizing, a specific and definitive role of melatonin in the regulation of the menstrual cycle has not been unequivocally demonstrated. However, taken together, evidence supports the notion of a role for melatonin in the control of normal hypothalamo-pituitary-gonadal function. Moreover, abnormal nocturnal elevations in melatonin concentration and delays in its morning offset may be directly associated with the induction of amenorrhea.

In females with normal menstrual cycles, a tenuous relationship between endogenous melatonin and basal LH has been reported (Wetterberg et al., 1976). Higher than normal levels were reported during menses and lower basal levels were reported prior to the day of the LH surge. However, overwhelming evidence contradicts this finding (Berga and Yen, 1990; Brun et al., 1987; Fellenberg et al., 1982; McIntyre and Morse, 1990; Webley and Leidenberger, 1986; Zimmerman et al., 1990). While this inconsistency is disturbing, seasonal changes in basal melatonin levels may complicate the interpretation of these findings and account for inconsistent results.

Effects of exogenous melatonin on daily variations in LH serum concentrations consistently suggest a functional relationship between melatonin and the hypothalamo-pituitary-gonadal axis. Several studies suggest that the daily decrease in melatonin serum concentrations may be part of the mechanism associated with the circadian change in LH concentration. Melatonin was consistently shown to decrease prior to the morning increase in LH levels (Brzezinski et al., 1987; Brzezinski et al., 1988; Voordouw et al., 1992; Zimmerman et al., 1990).

However, mixed results with melatonin (2 mg) administrations at 1600 and 2000 were reported (Terzolo et al., 1993). Considering that a timing relationship may exist between the early morning reduction in melatonin concentration and the rise in LH, further studies on melatonin-LH relationships may require strict control of melatonin administration time. Results reported in most studies are limited to the time of dose administration, and can not be generalized to other administration regimens.

In previous work in our laboratory we investigated the effect of melatonin (10 mg) when given at bedtime (2300) for 7 consecutive nights to normally cycling healthy females during the late follicular and early luteal phase of the monthly cycle on LH, FSH, menstrual characteristics, and cognitive performance. We demonstrated little effect of melatonin on menstrual cycle length, length of menses, and timing of the LH and FSH monthly rhythms (Kirby et al., 1996). However, there was a tendency for the monthly LH surge to be decreased in amplitude for members of the melatonin group. We concluded from that study that any effect of melatonin (under those specific test conditions) should not be a concern during military deployments. There were no reported side effects from melatonin administration, and melatonin volunteers were unable to determine whether they were receiving melatonin or placebo. In addition to hormone levels and menstrual characteristics, cognitive testing was done on the volunteers, both upon waking in the morning and throughout the day following melatonin administration. Melatonin volunteers performed better than placebo on some tasks, but not on others. Our impression was that based upon hormone changes and menstrual characteristics, 10 mg of melatonin given at 2300 for 7 days would not preclude female soldiers from using melatonin during a deployment. We still have concerns about the deficits recorded on tasks in the cognitive test battery, but the regimen needs to be tested in an operational scenario before definitive statements can be made. It is important to emphasize that melatonin given at 2300 is not necessarily the same as melatonin administered at any other time.

Melatonin and prolactin

Prolactin (PRL) is produced by the anterior lobe of the pituitary gland (adenohypophysis). Secretion of PRL varies predictably during the day, with lowest levels at midday and highest levels at night. Its secretion is regulated by the inhibitory effect of dopamine. Factors affecting PRL secretion include physiological stimuli such as pregnancy, nipple stimulation, coitus, exercise, sleep, and stress. The traditionally accepted primary role for prolactin in the female is the promotion of mammary gland development, and initiation and maintenance of lactation.

In addition to its possible influence on LH and FSH, melatonin has been implicated by some, but not by others, in the control of PRL secretion. Controversial findings could result from gender differences, monitoring of basal versus stimulated PRL release, different experimental approaches, different doses of melatonin or time of administration, and with women, different phases of the menstrual cycle.

Plasma PRL was reported to exhibit a daily rhythm showing a nocturnal peak 1-2 hours after that of melatonin, and remaining consistent throughout the menstrual cycle (Brzezinski et al., 1988). Evening administration of melatonin (2 mg) has been reported to stimulate the thyrotropin releasing hormone induced PRL secretion, especially during the follicular phase of the menstrual cycle (Terzolo et al., 1991). The same laboratory administered 4 mg of melatonin to women in the evening and reported a stimulatory effect on PRL release (Terzolo et al., 1993). Strongly supporting the interaction between melatonin and PRL, nighttime exposure to bright light, sufficient to induce a decrease in nocturnal melatonin secretion, resulted in a decrease in PRL secretion in women (Bispink et al., 1990). Daytime administration of melatonin, when

levels of endogenous melatonin are extremely low, stimulated the release of PRL in women. As little as 1 mg melatonin, given to young women at 1300, was enough to induce a significant increase in serum PRL (Okatani and Sagara, 1993; Okatani et al., 1994).

Melatonin receptors and their control

Melatonin receptors are found at many locations both within the central nervous system and in other regions of the body. If these receptors behave as many other receptor populations, their numbers would decrease with increased availability of melatonin (down regulation) and would increase when melatonin availability is low (up regulation). There is ample evidence for this in the animal literature (Gauer et al., 1994; Tenn and Niles, 1993; Piketty and Pelletier, 1993; Poon et al., 1994). Similar regulation of receptor density by the endogenous ligand is well known for other G-protein coupled receptors (Sibley and Lefkowitz, 1985). The rise in endogenous melatonin in circulation begins after sunset and reaches maximum levels about 0200-0300 (Brown et al., 1985). Melatonin production then is inhibited by bright light, and there is little available melatonin throughout the day.

Since the nightly rise in endogenous melatonin was well underway at the time selected for administration in our previous study (2300), it is likely that fewer receptor sites were available on which exogenous melatonin could act. We hypothesize that the administration of melatonin could have altogether different effects on LH, menstrual characteristics, or other parameters such as prolactin or cognitive testing if it occurred at a time when there was little available melatonin and available receptor sites were at a maximum. During daylight hours, there is little available melatonin and receptors should be up-regulated. This, of course, would be the situation encountered during an Eastward deployment across multiple time zones.

Studies elucidating the human phase response curve (PRC) for exogenous melatonin indicate that its administration before the endogenous rise of melatonin and the fall of core body temperature (e.g., from early afternoon to sunset) results in advances of the sleep wake cycle. Therefore, when investigating the effect of exogenous melatonin on up-regulated receptors (afternoon administration), the sleep-wake cycle of the volunteers will be altered.

The primary objective of this study was to examine the effects of exogenous melatonin, during the phase advance region of the PRC, on LH, prolactin, menstrual characteristics, and to a lesser extent, cognitive ability. We hypothesized that maintaining high levels of melatonin in serum during the advance region of the PRC (when there is little endogenous melatonin) might have a much more robust effect on hormones and menstrual characteristics than melatonin when given during the PRC dead zone (2300 in our previous study).

Methods

The design of the study was double blind, between subjects, and placebo controlled. Participants were 20 female volunteers between the ages of 18 and 39, meeting specific criteria to

assure regular menstrual cycle history and health status (e.g., negative chorionic gonadotropin (β-hCG), no oral contraceptive use for the previous 3 months, regular menstrual rhythms, good general health). Pregnancy tests were done periodically throughout the study. Also, volunteers were asked to refrain from consuming alcohol, caffeinated beverages, or any type of medication with known central nervous system effects throughout the in-house days during cycle 4.

The total duration of participation consisted of five consecutive menstrual cycles. The first, second, third, and fifth months of participation involved collection of information on timing of menses and ovulation, menstrual regularity, mood, and LH levels. Menstrual regularity data were used to document the timing of menses and to approximate 7 days comprising the pre-ovulatory LH surge for menstrual cycle 4. In addition to simply accessing mood, a daily menstrual questionnaire provided information on the incidence of premenstrual syndrome (PMS)-like symptoms.

For 7 days during cycle 4, volunteers lived in the sleep laboratory at the USAARL. The 7 days were scheduled, based upon the first 3 cycles, to include the LH surge. During the 7 days, participants remained at the U.S. Army Aeromedical Research Laboratory (USAARL) for testing and shifting to a new light-dark cycle. A light exposure regimen was used to mimic the changes in the light-dark cycle corresponding to traveling eastwardly across five time zones. On in-house day 1, volunteers trained at the laboratory on a cognitive test battery (see below). On days 3-6, participants were exposed to bright lights (3000 lux at eye level, 0130-0720 CDT), asked to remain indoors after 1330 CDT, and wear dark sunglasses after 1400, thus mimicking the light-dark cycle after a six time zone eastward shift.

Within 10 days of their scheduled in-house stay during cycle 4, and again on day 6 of their in-house stay, volunteers spent 24 hours in the hospital where they provided hourly saliva and blood samples, a urine sample every 3 hours, and performance data from five cognitive sessions. On each of these days, an intravenous catheter was used for collection of hourly blood samples.

Melatonin (10 mg) or placebo was administered daily for 5 consecutive days (days 2-6) at 1300 during the advance region of the human melatonin PRC. Blood pressure and pulse were recorded throughout the in-house stay just before dose administration, at bedtime, and upon waking. The potential benefit of testing the 10 mg melatonin dose is the lack of toxicity, short half-life, lack of side effects, sleep induction effects, and already demonstrated efficacy in maintaining sleep and alertness during a military deployment (Comperatore et al., 1996). Body temperature data were recorded on days 1-7 of cycle 4 using both tympanic and oral temperature.

The last dose of melatonin/placebo was given at 1300 on in-house day 6. That also was the in-house day the volunteers were in the hospital providing hourly samples of blood and saliva. They returned to the USAARL at about 0800 on day 7 and completed that day as scheduled; test sessions at 1330 and 1500, and bedtime at 1630. Volunteers were awakened at 0030 on day 8, completed test sessions at 0130, 0300, and 0610 without bright light exposure, and were released from the USAARL facility at approximately 0800 after a brief post-study medical evaluation.

For the next 7 days while at home, volunteers were asked to collect hourly saliva samples from 1200 until bedtime to be analyzed for melatonin content.

Biochemical assays

Urine samples were collected daily beginning on the seventh day of each cycle until 4 days after the LH surge. Also, urine samples were collected every 3 hours while awake during the in-house days of cycle 4, and just prior to dosing during drug administration days. Hormone levels in urine were determined using the Abbott IMx* automated bench top immunochemistry analyzer. These were used to identify the monthly surge in LH, as well as to determine whether pharmacological levels of melatonin inhibit LH release or alter its timing. Blood levels of β-hCG, determined utilizing the IMx, were used as a test for pregnancy. Prolactin levels were determined from blood samples drawn on the pre-in-house day and on in-house day 6/7 using the IMx. Urinary levels of 6-sulphatoxymelatonin (aMT6s) and both salivary and blood levels of melatonin were measured by direct radioimmunoassay (RIA) (ALPCO, Inc.*, Windham, NH; Stockgrand*, Guilford, Surrey) from specimens collected during the pre-in-house hospital day and the in-house days of cycle 4. Sensitivity for the aMT6s RIA is 2.0 pg/ml with an intra-assay coefficient of variation (CV) of 7.8% and an inter-assay CV of 8%. The melatonin RIA had a sensitivity of 0.3 pg/ml with an intra-assay CV of 6.6% with serum and an inter-assay CV of 7.7%. Daily determinations of melatonin or metabolite concentration provided evidence of changes in melatonin rhythms as a function of daytime dosing. Twenty-four hour melatonin and prolactin rhythms were determined from samples collected on the two hospital days during cycle 4. The IMx immunoassays have a sensitivity of 0.5 mIU/ml for LH, 0.6 ng/ml for PRL, and 2 mIU/ml for total β-hCG.

LH analysis by urine immunoassay

The accepted method for determining levels of LH is to perform assays on blood samples. Since we were interested in determining LH levels from day 7 of each monthly cycle until 4 days after the LH surge, and in multiple daily samples while in-house, we felt that subjecting volunteers to multiple blood draws was unacceptable. In a previous study, we utilized the Abbott IMx* analyzer to determine levels of LH in urine samples. In that study, eight volunteers were asked to check their first void samples with the Clearplan Easy* one-step ovulation predictor (Whitehall Robbins), which works by measuring LH in the urine through the use of monoclonal antibodies. Six of the eight had positive results on the ovulation predictor on the same day that immunoassay results showed the LH peak. The other two volunteers had positive results on the ovulation predictor within 2 days of the IMx determined LH peak. This is not surprising since LH levels during the mid-cycle surge often remain elevated for 2 days or more.

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* See manufactures' list at Appendix A

Cognitive testing

A cognitive assessment battery including a dual task vigilance task (modified version of the Bakan vigilance test - Dollins et al., 1993), a four-choice reaction task which resembles the Wilkinson four choice reaction time task, an auditory vigilance task, and a profile of mood states (POMS) questionnaire consisting of 65 adjectives, each of which is rated on a 5-point scale, was used to determine the time course of the effects of the melatonin regimen. Each test session took about 70 minutes to complete. Training for the cognitive tests was done on the pre in-house hospital day and the first in-house day. This was sufficient training to allow performance to stabilize prior to testing on in-house days 2-7, when volunteers completed five testing sessions each day.

The POMS questionnaires were administered each morning upon waking during the in-house stay, and are the only cognitive results to be discussed in this report. Factor analysis of the rating for each of the adjectives contained on the questionnaire yields the following six factors: tension-anxiety; depression-dejection; anger-hostility; vigor-activity; fatigue-inertia; confusion-bewilderment. The higher the cumulative score in each factor, the more the subject identified with the mood associated with that factor. Each morning during the in-house stay, volunteers also completed a post-sleep questionnaire upon waking. Together with data from activity monitors, this enabled us to evaluate sleep patterns.

Activity monitors (Precision Control Design, Inc*) were used to study the rest/activity cycles of participants during 14 days just prior to reporting to the USAARL, throughout 7 days at the USAARL, and for 14 days after leaving the laboratory. Consistent sleep disruption may result in stress and influence menstrual regularity. Monitoring for disrupted sleep patterns prior to the in-house stay prevented inclusion of participants experiencing sleep-related menstrual anomalies. Activity data provided information on the stability of sleep duration prior to implementation of the drug regimen.

Typical in-house dose day

During a typical dose administration day, volunteers were awakened at 0030, and started their first cognitive test session at 0130. The other morning test sessions were at 0300 and 0610. As a minimum, each volunteer received 70 minutes of bright light exposure during each test session. The kitchen and break room also were illuminated with the same bright lights until 0720. After completing the last morning test session, volunteers had free time to exercise, read, or just relax with music or television. At 1300, following measurement of vital signs, doses were given. Volunteers were not allowed to be outside after 1330, and wore dark glasses to limit daylight exposure while moving about in the building after that time. The fourth cognitive session of the day began at 1330, and the final session at 1500. Bedtime was at 1630.

Results

Melatonin concentrations

Levels of melatonin were assayed from samples of saliva and serum collected both on the pre-in-house hospital day and while volunteers were in-house during cycle 4. Additionally, urine samples collected every 3 hours while volunteers were awake during their in-house stay were assayed for aMT6s. Because we had both saliva and limited serum samples in addition to urine, we placed much less emphasis on the results of urine assays for aMT6s. Since aMT6s is a metabolite of melatonin, it lags behind the actual melatonin changes and is likely to be more dependent on metabolic differences between volunteers. Concentration of aMT6s also varies with frequency of urination. Although we tried to control for this, we met with little success.

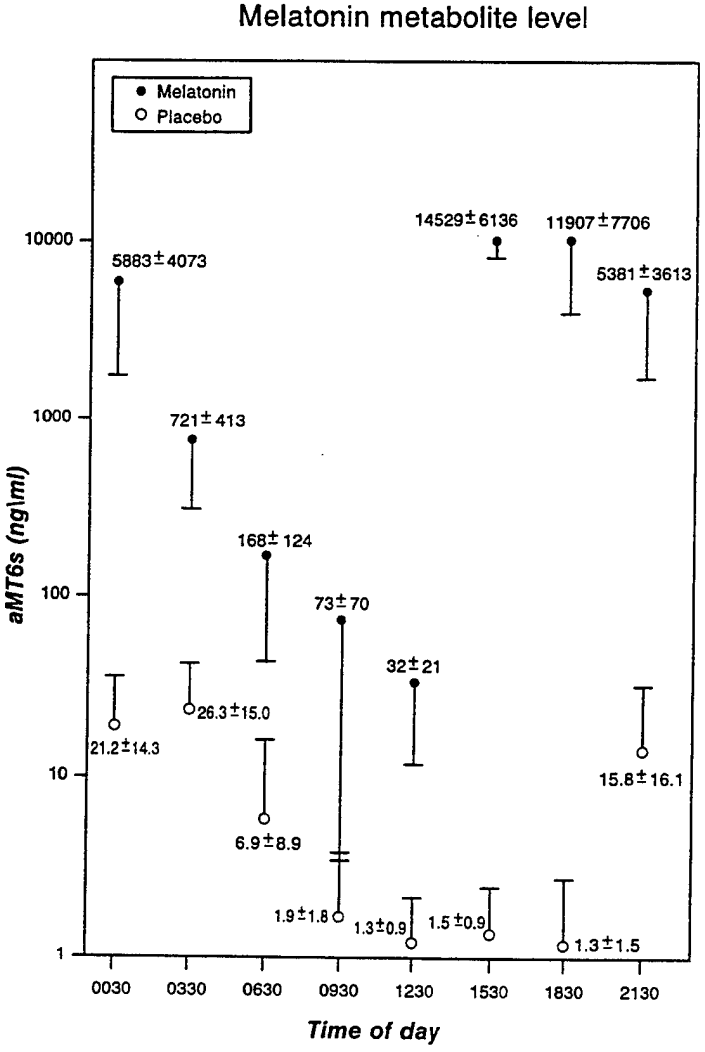

Figure 1. Melatonin metabolite levels in urine. Mean aMT6s concentration plotted against time of day both the placebo and melatonin groups during in-house days 3-6. Error bars indicate ± the standard deviation of the mean.

Because of these reasons, there is considerable variation in aMT6s levels. Figure 1 shows mean aMT6s levels for the members of both the placebo and melatonin groups plotted against time of day for the last 4 in-house dose days (3-6). The first dose day was not included because the timing of events on that day differed from the other four. In general, values for each volunteer at each time point across the 4 dose days agree quite well. However, when we combine the data into melatonin and placebo groups, we incorporate variability from one subject to the next, especially for the melatonin group after dose. Note the large standard deviation associated with most of the means. An important point to emphasize from Figure 1 is the fact that the lowest value for aMT6s (1230) in the melatonin group during the dose days is higher than the highest value for the placebo group (0330). Although volunteers went to bed on these days at 1630, we had a number of urine samples at 1830 and 2130 so they were added to Figure 1 as well. Figure 2 shows levels of salivary melatonin for the melatonin and placebo groups during in-house dose days 3-5. Days 2 and 6, the first and last dose days, were not included because volunteer

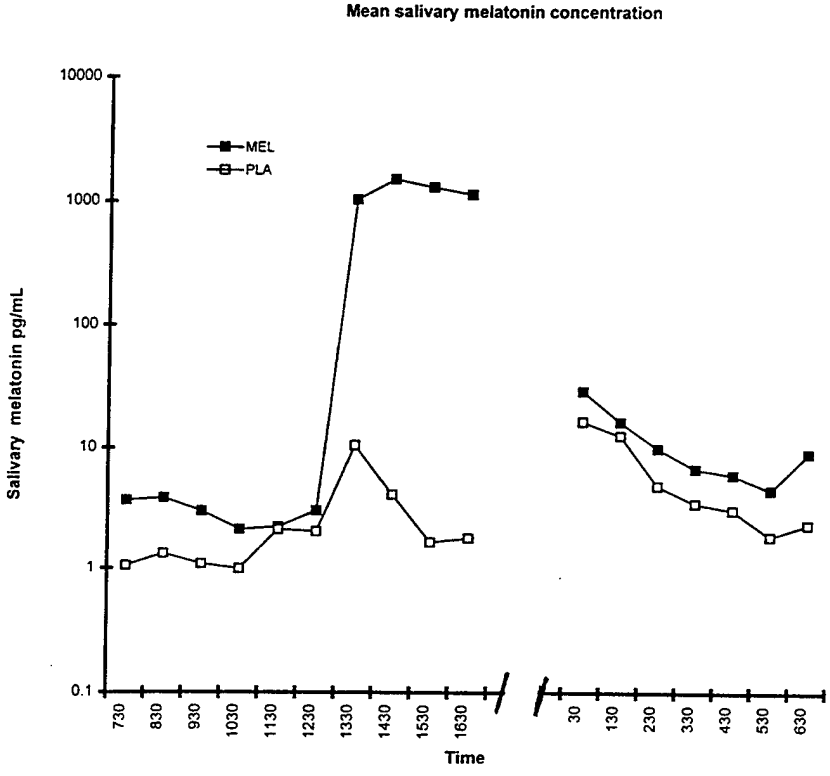

Figure 2. Saliva melatonin for the melatonin and placebo groups during dose days 3-5. Bedtime was from 1630-0030.

schedules were slightly different on those days and samples were collected at different times than on days 3-5. Of interest is the fact that the baseline melatonin activity (0800-1300) has been reset to a higher level during the dose days for the melatonin group, and the melatonin levels

during the morning test sessions remain elevated above the pre-in-house baseline. Melatonin levels during the two afternoon cognitive sessions (1330 and 1500) are within the peak area of the post-administration melatonin curve. Since blood was drawn only on the pre-in-house day and on the sixth day in-house, we can not create a similar plot for serum levels of melatonin. Figure 3 shows levels of salivary and serum melatonin for the melatonin group on the pre-in-house day, and Figure 4 shows the same information for day 6 (last dose day). Notice in Figure 3 that the endogenous serum melatonin begins its nightly increase (2000) before the salivary melatonin begins to increase (2100). Usually we find the serum melatonin to be three to four times higher than the salivary melatonin, however for the melatonin group in Figure 3, the serum peak is about five times greater than the salivary peak. Remembering that samples are collected

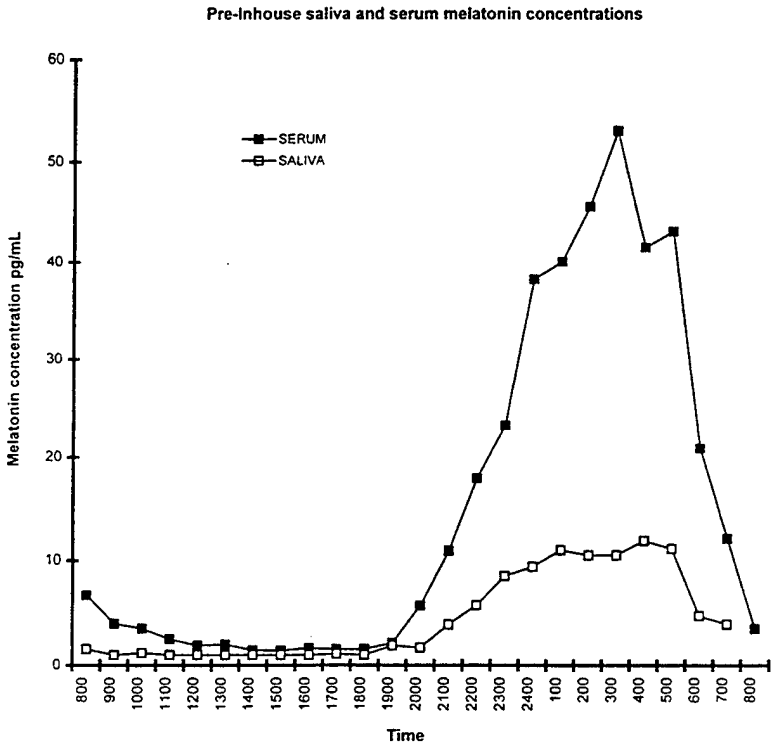

Figure 3. Melatonin group saliva and serum melatonin levels on the pre-in-house day.

hourly and melatonin is given at 1300, both the serum and saliva curves in Figure 4 are close to their peak levels by 1400, although there are individual differences.

To determine how much the bright light treatment shifted the endogenous melatonin release, Figure 5 shows both pre-in-house (PINH) and day 6 in-house (INH) salivary melatonin levels plotted against time of day for the placebo group. Since they did not receive melatonin, the difference between the time of melatonin increase from the pre-in-house collection and the day 6

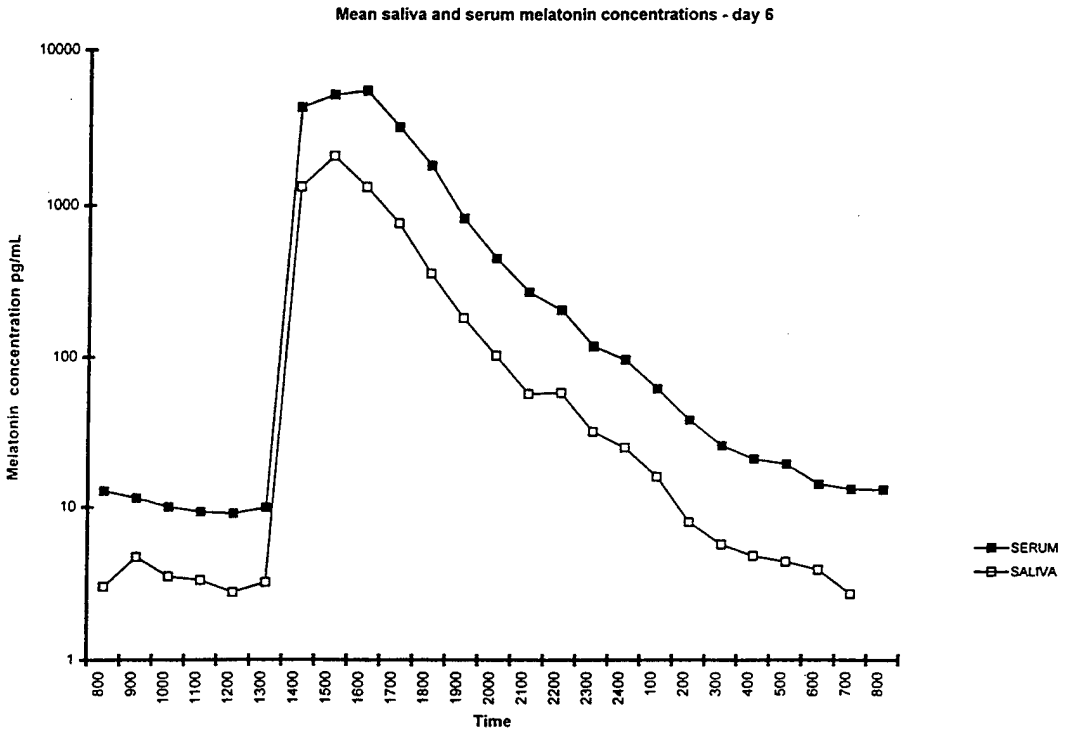

Figure 4. Melatonin group saliva and serum melatonin levels on the last dose day, in-house day 6.

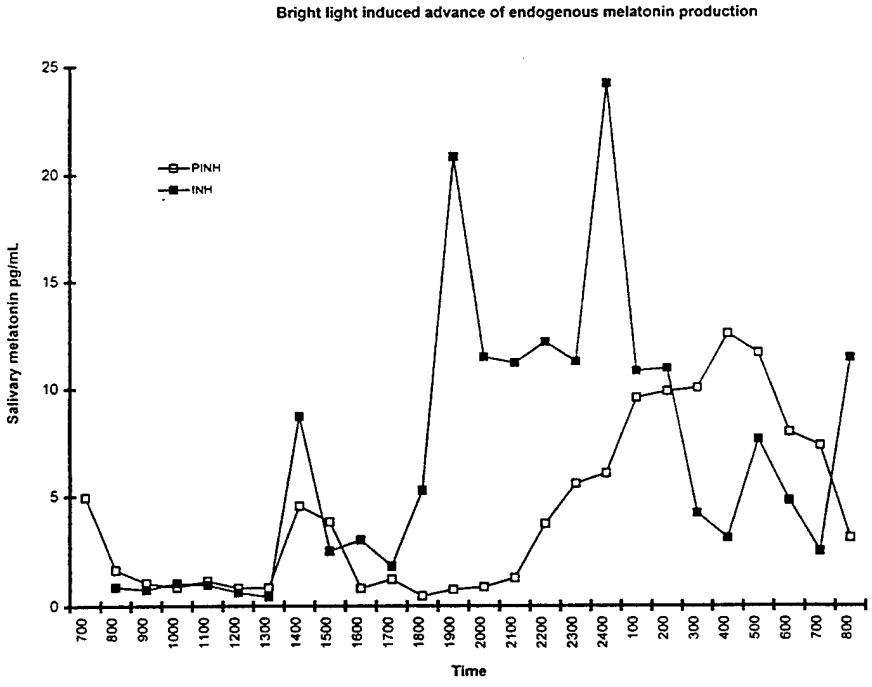

Figure 5. Pre-in-house and day 6 saliva melatonin levels for the placebo group. Note the shift in the day 6 curve (INH) as a result of bright light exposure.

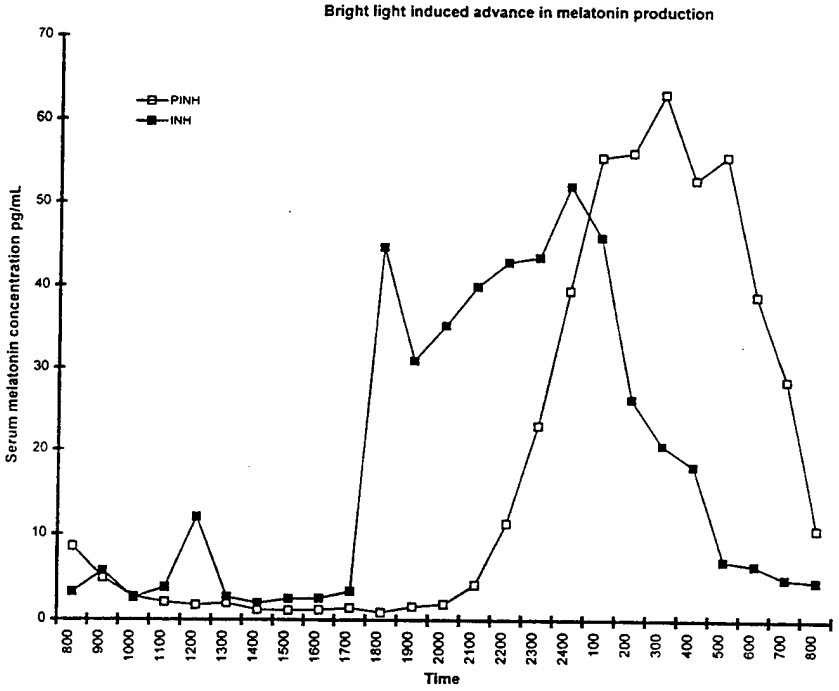

Figure 6. Pre-in-house and day 6 serum melatonin levels for the placebo group. Note the shift in the day 6 curve (INH) as a result of bright light exposure.

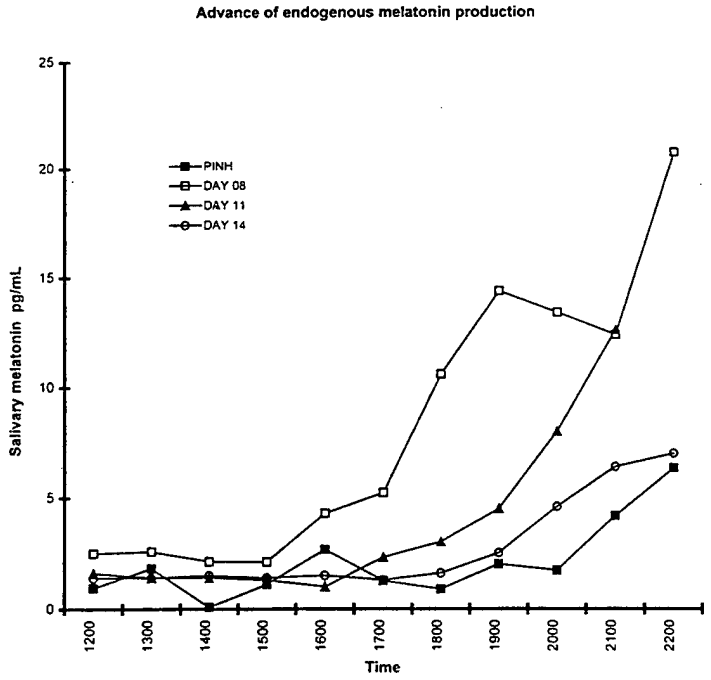

Figure 7. Pre-in-house, day 8, 11, and 14 saliva melatonin levels for 7 members of the melatonin group. Days 8, 11, and 14 are 3, 5, and 8 days after the last dose administration and bright light exposure.

collection was caused by the light exposure. That difference is between 4 and 5 hours. Figure 6 is a similar plot for serum melatonin in the placebo group. In good agreement with Figure 5, the shift due to the bright light treatment is between 4 and 5 hours. Since the melatonin group received 10 mg of melatonin at 1300 each day for 5 days (last dose day being the in-house 24 hour sample day), we were not able to determine the shift in production of endogenous melatonin from plots of serum melatonin. Although samples were collected on day 7, the last full in-house day and the first day following five daily melatonin administrations, we were unable to determine the melatonin onset because bedtime was at 1630. For the melatonin group it was necessary to rely on saliva samples collected at home on the days following the in-house stay to get an indication of the shift resulting from 5 days of melatonin treatment. Unfortunately, most volunteers did not reliably provide saliva samples after leaving the lab. Figure 7 shows pre-in-house, day 8, 11, and 14 salivary melatonin for seven members of the melatonin group. Remember that the last dose was given on day 5 and the volunteers were on normal lighting after that time. So based upon data collected 2 days after the last dose, Figure 7 shows a shift of at least 5 hours still remaining. As a population, the onset of the endogenous production of melatonin is returning toward pre-in-house timing on days 11 and 14 (5 and 8 days after the last dose of melatonin).

Awareness of symptoms following melatonin

Following the in-house stay during cycle 4, all volunteers were asked if they thought they had received melatonin. Of the 10 melatonin volunteers, 5 correctly identified that they were taking melatonin, 4 had no idea, and 1 felt sure she was taking placebo. Four of those correctly identifying melatonin did so because they were quite tired during one or both of the test sessions following the dose. The other volunteer correctly identifying that she was taking melatonin could not identify any specific reason. Not all members of the melatonin group felt sleepy in the 3.5 hours between the dose and bedtime. Of the 10 placebo volunteers, 4 thought they were taking melatonin, 3 correctly identified that they were receiving placebo, and 3 had no idea. Three of the four who thought they were taking melatonin felt that way for the same reason reported by four members of the melatonin group, they were very drowsy during the post-dose test sessions. The other volunteer who thought she was taking melatonin claimed she was sleeping through the night which was very unusual for her, and she had a hard time waking in the morning. Vital signs were checked for all volunteers upon waking in the morning before getting out of bed, and these signs were never outside the normal physiological range. One member of the melatonin group felt her alertness was enhanced above normal upon waking. One member of the placebo group complained of a queasy stomach each day after the dose, and was sure she was receiving melatonin. There apparently are no consistent symptoms following the 10 mg dose of melatonin, and volunteers are unable to consistently distinguish between melatonin and placebo. It also is of interest that with our particular paradigm both melatonin and placebo volunteers felt drowsy during the two afternoon cognitive sessions following dose administration.

Menstrual cycle length after melatonin

Since each volunteer kept a menstrual cycle diary, cycle length was counted as the number of days from the start of menses (day 1) during one cycle until the first day of menses during the next cycle. Table 1 shows cycle length (CL) and length of menses (ML) for each volunteer over all the cycles in which they participated. All volunteers participated in at least five cycles, although some had up to three additional cycles because of scheduling conflicts of various types that delayed their in-house phase.

Table 1.
Menses length and total cycle length.

		CYCLE 1		CYCLE 2		CYCLE 3a		CYCLE 3b		CYCLE 3c		CYCLE 3d		CYCLE 4		CYCLE 5	
Vol #	Drug	CL	ML	CL	ML	CL	ML	CL	ML	CL	ML	CL	ML	CL	ML	CL	ML
05	P	34	6	26	6	34	4	32	4					30	4	36	5
07	M	24	4	23	4	25	4	25	4					23	3	26	4
08	P	33	6	30	6	30	6							31	5	30	6
09	M	27	6	28	4	28	4							25	3	31	4
10	M	28	4	30	4	30	3							27	3	30	3
13	M	27	5	26	5	29	7	25	6					27	5	27	4
14	P	31	4	32	5	27	6							33	6	35	
17	P	29	7	25	6	30	8	26	6	26	7			27	7	27	7
19	P	27	5	23	4	24	4							24	5	31	5
20	M	27	5	28	5	28	5							27	5	31	5
21	P	27	4	28	7	30	4							27	4	43	4
22	M	23	6	22	5	32	6							22	6	29	5
25	P	36	7	30	6	33	5							27	5	32	7
27	M	25	5	25	5	26	4							27	9	25	4
29	P	30	6	27	10	26	5	26	6					29	4	27	7
31	P	26	4	29	4	27	5							27	5	27	4
32	P	28	6	30	6	26	4	26	4	26	5	26	6	28	5	25	7
33	M	29	4	29	4	27	4	27	4					27	4	28	4
36	M	26	4	28	6	24	5							26	6	28	6
37	M	26	6	25	6	25	4							25	5	23	5

Since we collected menstrual cycle data for at least three cycles for each volunteer prior to the in-house stay and dose administration, changes in cycle length for the dose and post-dose cycles are compared to the mean length of the previous cycles, and the results are shown in Table 2. The numbers in parenthesis are the mean change in cycle length in days for that group.

Table 2.
Change in cycle length.

DOSE CYCLE

	Shorter	Longer	Same
Melatonin	7(1.7)	2(1.0)	1
Placebo	6(1.7)	3(1.9)	1

POST DOSE CYCLE

	Shorter	Longer	Same
Melatonin	2(1.3)	7(2.1)	1
Placebo	6(0.8)	4(7.6)	

To determine what variation in cycle length might be normal, we determined the mean of the variation present in all of the pre-dose cycles for both the melatonin and placebo groups. The overall mean was 2.2 days. For the dose cycle data above, three of the seven melatonin and one of the six placebo volunteers with shorter cycles and only one of the three placebo volunteers with a longer cycle exceeded the 2.2 days. For the post-dose cycle data above, the 2.2 day limit was exceeded by one of the two melatonin members with shorter cycles, three of the seven melatonin members with longer cycles, and all four of the placebo members with longer cycles.

Length of menses after melatonin

Cycle 5 was the only period of menses that occurred after the dose. The length of menses for each volunteer for each cycle in which they participated is listed in Table 1. As was the case

Table 3.
Change in length of menses.

Post-Dose Month

	Shorter	Longer	Same
Melatonin	6(0.9)	2(0.5)	2
Placebo	2(0.7)	7(0.7)	

with overall cycle length, we determined the mean for the previous cycles and compared cycle 5 menses length to that mean value for each individual volunteer. Those results are presented in Table 3.

To determine the normal variation in menses each month for our population, we determined the mean of the monthly variation for each volunteer for each cycle through cycle 4. The mean was 0.9 day. Two of the six members of the melatonin group with shorter menses and two of the seven members of the placebo group with longer menses showed change greater than a day. Numbers in parenthesis in Table 3 are the mean change in menses length in days for that group.

Table 4.
Cycle day of luteinizing hormone peak.

Vol #	Dose	Cyc 1	Cyc 2	Cyc 3a	Cyc 3b	Cyc 3c	Cyc 3d	Cyc 4	Cyc 4*	Cyc 5
05	P	20	15	23	20			20	19	18
07	M	11	10	12	12			13	13	12
08	P	8	16	16				18	18	22
09	M	16	15	14				13	12	17
10	M	16	18	17				17	16	19
13	M	18	17	16	15			15	14	15
14	P	19	21	19				22	22	24
17	P	15	12	17	--	13		15	14	14
19	P	16	11	15				16	16	19
20	M	17	17	16				16	15	20
21	P	15	16	14				14	14	--
22	M	15	19	20				19	18	18
25	P	21	16	19				14	14	19
27	M	13	12	14				13	13	12
29	P	20	16	13	15			18	18	19
31	P	12	11	11	14	13		12	12	12
32	P	16	16	11	14	13	13	17	17	15
33	M	15	14	15	14			14	13	14
36	M	12	12	11				12	12	13
37	M	12	12	12				12	12	11

Cyc 4* is the cycle day of the LH peak based upon the highest value obtained any time of day while in-house. All other peak days are based on values from first voids.

Timing and amplitude of the LH surge after melatonin

LH levels were determined from first void urine samples while not in-house, and from both first void and multiple daily samples while in-house. Results from urine samples agree quite well with accepted LH changes over the course of the monthly cycle. Baseline activity for LH is usually quite low, and there is a strong elevation during the preovulatory surge. Often the surge persists for 2 or 3 days. Since the LH surge is an accurate predictor of ovulation, altered timing of the LH surge following melatonin treatment would indicate altered timing for ovulation. Table 4 shows the timing of the LH surge for each cycle of participation for all members of both the melatonin and placebo groups. All LH values were determined from first void samples except the column marked Cyc 4*. Values in that column were determined from the highest LH value any time of the day while in-house.

Table 5.
Changes in LH peak day.

Dose Month to Pre-Dose Month

	Earlier	Later	Same
Melatonin	3(7)	2(2)	5(1)
Placebo	2(3)	6(6)	2(1)

Post-Dose Month Compared to Dose Month

	Earlier	Later	Same
Melatonin	4(3)	4(6)	2(1)
Placebo	3(2)	5(5)	1(2)

To investigate possible changes in the timing of the LH surge because of melatonin, the peak day of the month in question was compared to the peak day of the previous month. This comparison is listed in Table 5. The LH surge was based upon first void values only. When comparing anything to the dose month, numbers in parenthesis are based upon the highest value anytime of the day while in-house.

Table 6 shows the actual LH peak values for the surge during each month of participation. Again, in the column marked Cyc 4*, the LH surge values were determined from the highest value obtained anytime of the day while in-house during cycle 4. All other values are obtained from analysis of first void samples. To investigate whether or not melatonin treatment might

change the actual amplitude of the LH surge, we compared the surge amplitude during cycles 4 and 5 (dose month and post-dose month). That comparison is shown in Table 7.

Table 6.
Luteinizing hormone peak values (mIU/ml).

Vol #	Dose	Cyc 1	Cyc 2	Cyc 3a	Cyc 3b	Cyc 3c	Cyc 3d	Cyc 4	Cyc 4*	Cyc 5
05	P	55.65	26.84	94.86	54.84			7.14	12.30	17.91
07	M	39.12	54.39	18.53	19.03			24.93	44.72	8.38
08	P	19.51	37.39	32.84				31.78	31.78	33.24
09	M	21.96	27.29	23.50				40.91	202.7	16.30
10	M	28.55	18.87	46.18				33.68	40.67	21.71
13	M	24.42	8.71	35.37	9.94			16.32	43.19	23.05
14	P	27.87	52.66	28.39				35.15	35.15	20.16
17	P	32.61	41.67	81.91	--	44.31		12.52	36.17	35.11
19	P	38.71	18.48	42.23				8.77	18.79	19.90
20	M	22.20	34.42	14.20				13.38	28.70	44.51
21	P	29.37	50.77	22.81				33.28	94.98	--
22	M	16.52	38.99	64.50				23.15	64.43	63.61
25	P	109.1	65.54	32.83				68.71	68.71	40.04
27	M	64.44	47.02	60.30				54.20	152.1	48.91
29	P	14.69	31.29	59.14	16.71			26.76	44.96	43.61
31	P	43.46	73.01	19.57	11.82	47.55		14.48	144.6	40.87
32	P	49.47	27.79	18.95	29.98	20.66	20.12	24.91	63.62	36.74
33	M	35.00	8.52	14.17	16.10			16.10	44.32	34.25
36	M	41.59	47.38	61.95				65.22	65.22	43.19
37	M	54.07	51.77	27.75				27.96	158.8	26.44

Cyc 4* is the peak based upon the highest value obtained any time of the day while in-house. All other values are from first void samples.

Prolactin levels and PMS-like symptoms after melatonin

Prolactin levels were determined by immunoassay from hourly blood samples collected during the pre-in-house day and on the last in-house day of dose administration (day 6). Hourly PRL levels for all members of the melatonin and placebo groups, both pre-in-house and on day 6, are shown in Tables B1-B4 of Appendix B. Using the values from these tables, mean hourly PRL levels for pre-in-house and day 6 were determined and plotted against time of day for members of the placebo (Figure 8) and melatonin groups (Figure 9). Note that the nighttime

Table 7.
LH peak amplitude.

Dose Month to Pre-Dose Month

	Increase	Decrease	Same
Melatonin	5(8)	4(2)	1
Placebo	5(6)	5(4)	

Post-Dose Month Compared to Dose Month

	Increase	Decrease	Same
Melatonin	4(1)	6(9)	
Placebo	7(3)	2(6)	

PRL increase begins about 2400 for both groups during the pre-in-house sampling, and advances significantly during the in-house stay. Additional information is revealed clearly if we plot the in-house (day 6) PRL means for the melatonin and placebo groups together (Figure 10). The

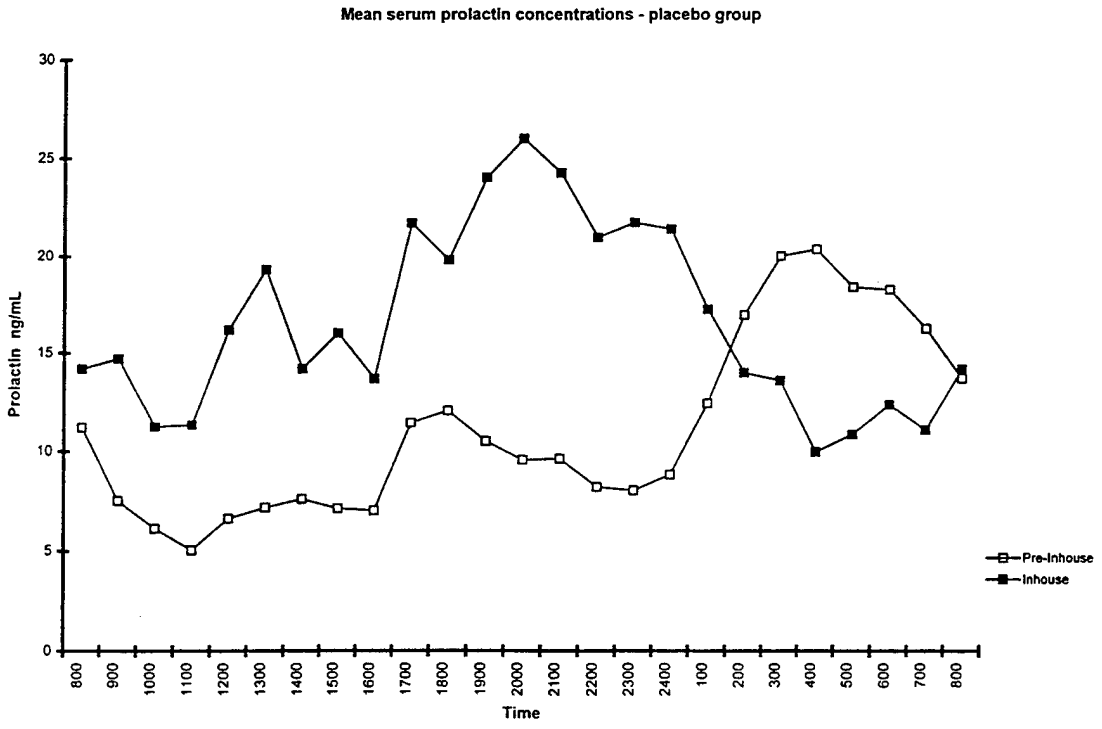

Figure 8. Mean hourly pre-in-house and day 6 serum prolactin levels for the placebo group.

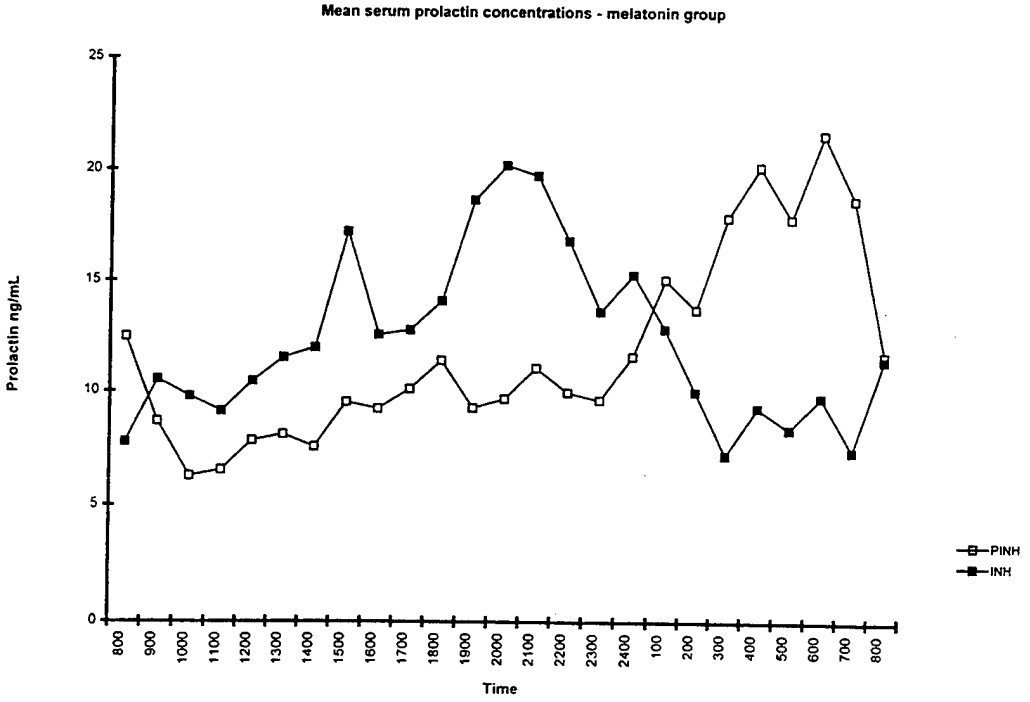

Figure 9. Mean hourly pre-in-house and day 6 serum prolactin levels for the melatonin group.

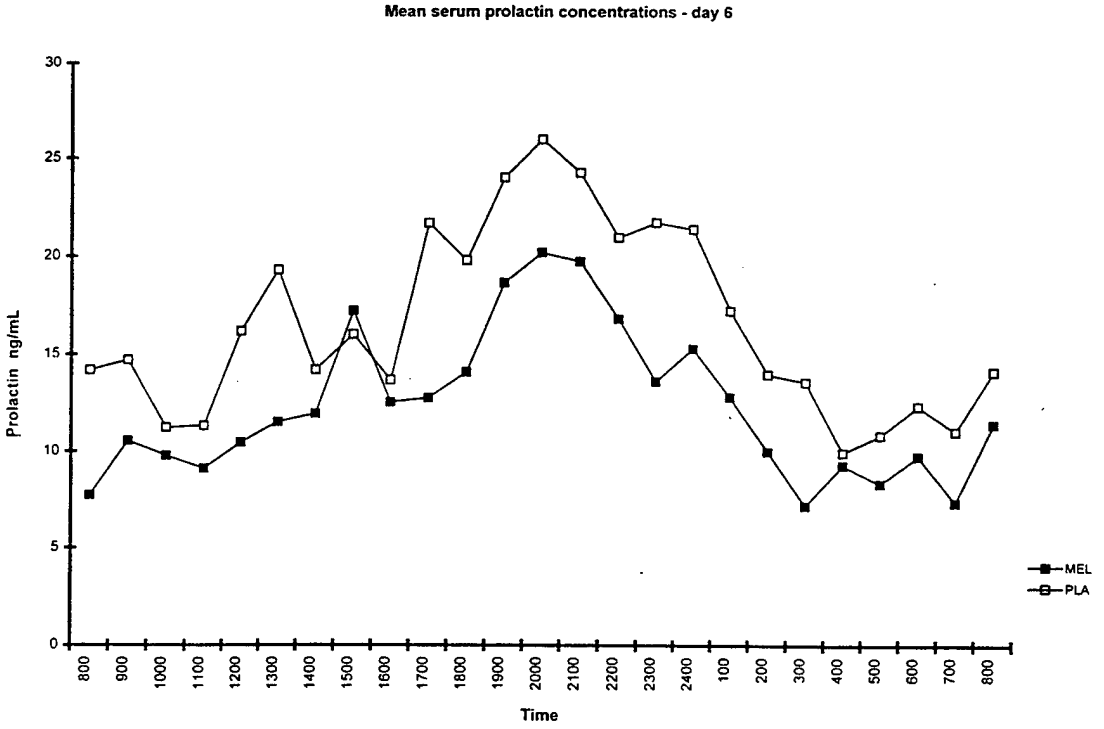

Figure 10. Mean hourly serum prolactin levels for both groups on day 6. Note the increase at 1300 for the placebo group and at 1500 for the melatonin group.

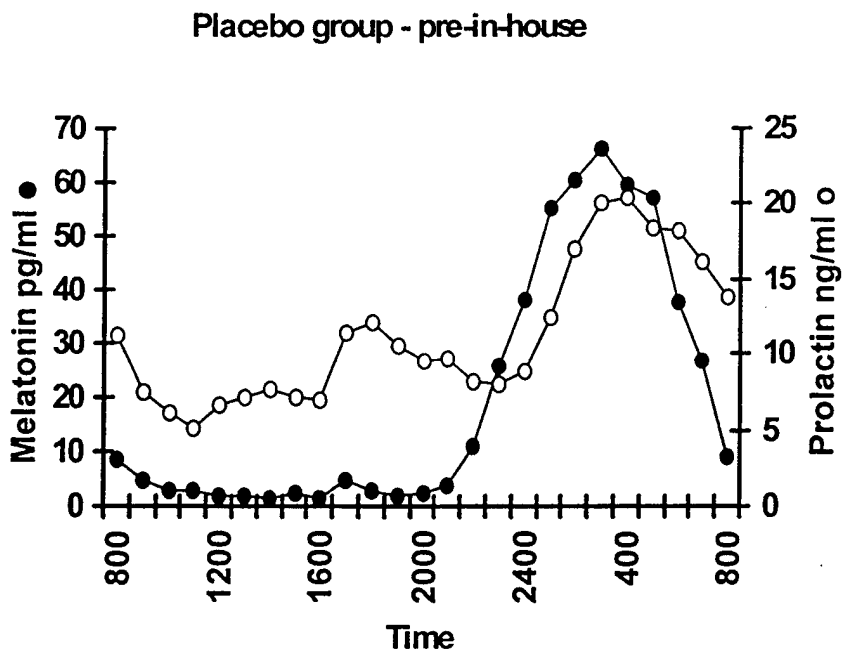

Figure 11. Relationship between nightly increase in melatonin and prolactin. Hourly pre-in-house serum melatonin and prolactin for the placebo group.

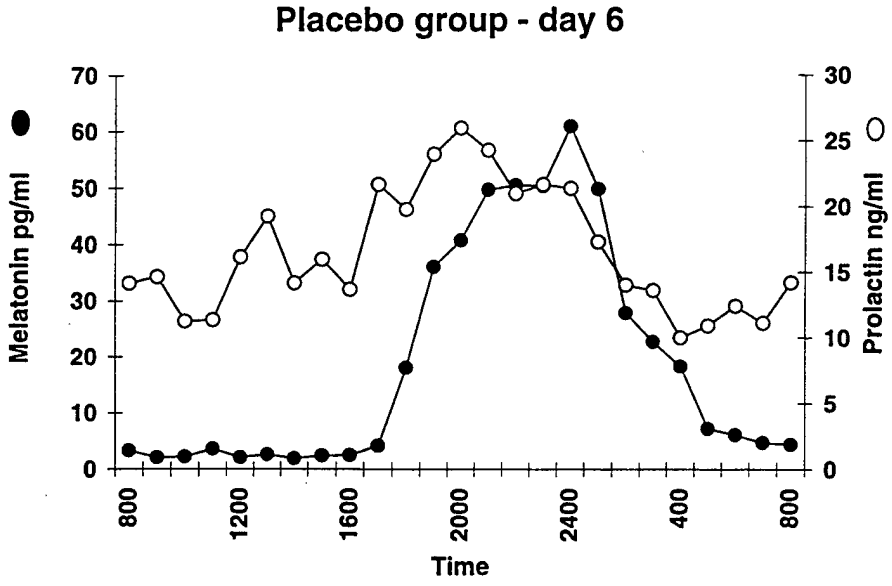

Figure 12. Relationship between nightly increase in melatonin and prolactin. Hourly in-house day 6 serum melatonin and prolactin for the placebo group.

placebo group shows an early increase peaking at 1300 (dose time), a decrease, and a gradual climb to the daily peak value by 2000. The melatonin group shows a similar pattern, however the initial peak is at 1500. It is interesting that the daily peak values (after the 1300 and 1500 increases) for the two groups occur at the same time, and the decrease in the daily PRL surge is quite similar in both groups. Figure 11 shows the pre-in-house PRL and serum melatonin values plotted together against time for the placebo group. Note that the nightly melatonin increase precedes the nightly increase in PRL. The early evening (1700) PRL peak also is prominent in this figure. Figure 12 shows PRL and melatonin values obtained from blood samples collected on day 6 plotted against time for the placebo group. Note that the nightly (bedtime now 1630) PRL increase now precedes the nightly increase in melatonin.

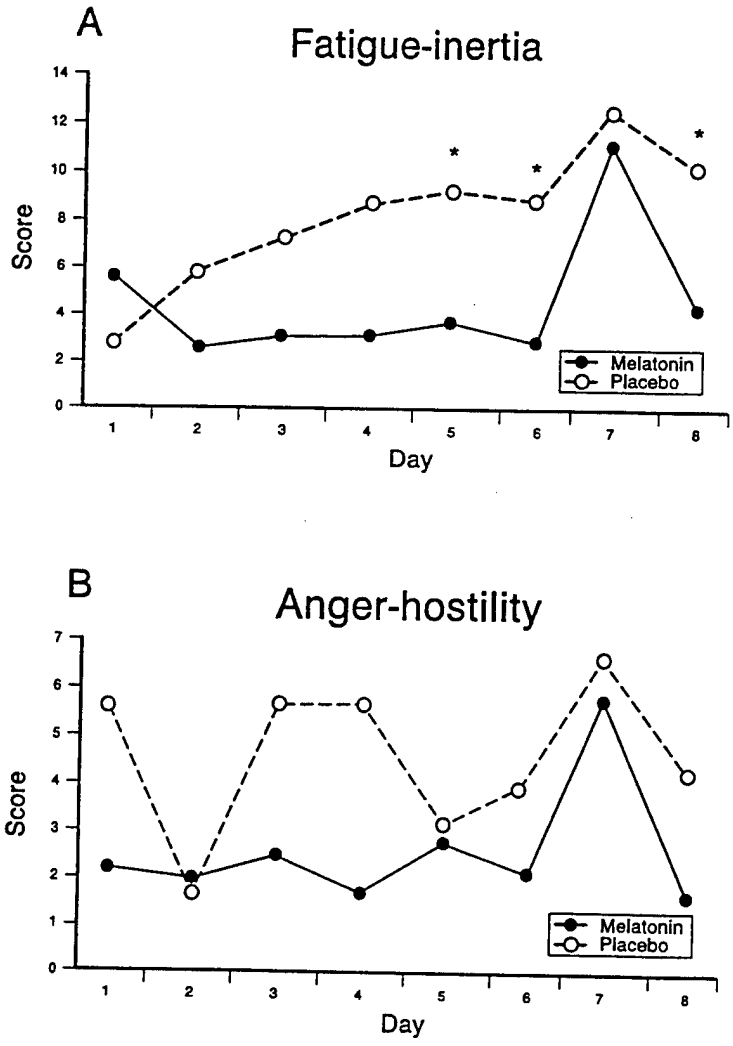

Figure 13. Results from POMS for categories of fatigue-inertia (A) and anger-hostility (B) for the melatonin and placebo group. Statistical significance ($p<0.05$) indicated by *.

POMS, menstrual questionnaires, and sleep

Results gathered from the POMS questionnaires showed that the members of the melatonin group consistently showed lower scores than the placebo group in all factors suggesting adverse moods (Figures 13A&B, 14A&B, 15A), and higher scores on vigor and activity (Figure 15B), indicating better rest and an overall better sense of well-being. The melatonin group was less fatigued and in better "spirits" than members of the placebo group. Even on day 7 when both groups were sleep deprived following their last 24 hour blood draw, the melatonin group showed

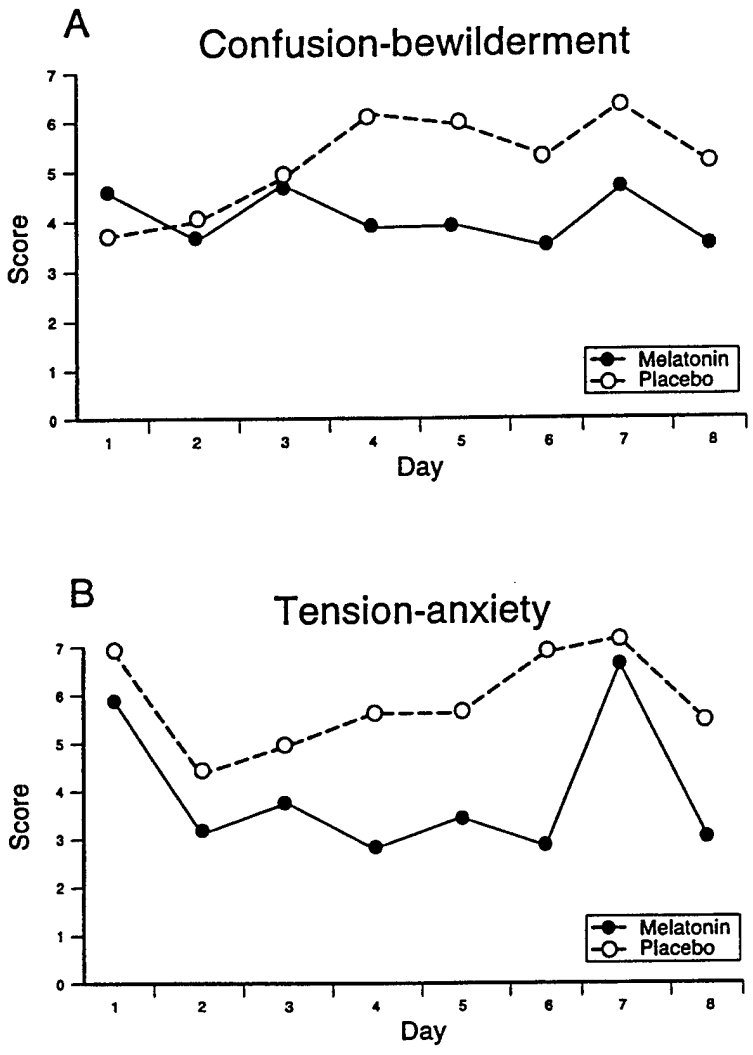

Figure 14. Results from POMS for categories of confusion-bewilderment (A) and tension-anxiety (B) for the melatonin and placebo group. No differences were significant.

less fatigue and inertia, anger and hostility, confusion and bewilderment, and tension and anxiety; more vigor and activity; and the same amount of depression and dejection as their placebo

counterparts. Although the melatonin group consistently demonstrated scores indicative of less stress and anxiety, statistical significance ($p<0.05$) was demonstrated only for the factors of fatigue-inertia (Figure 13A, Day 5, $p<0.04$; Day 6, $p<0.04$; Day 8, $p<0.05$) and vigor-activity (Figure 15B, Day 8, $p<0.01$).

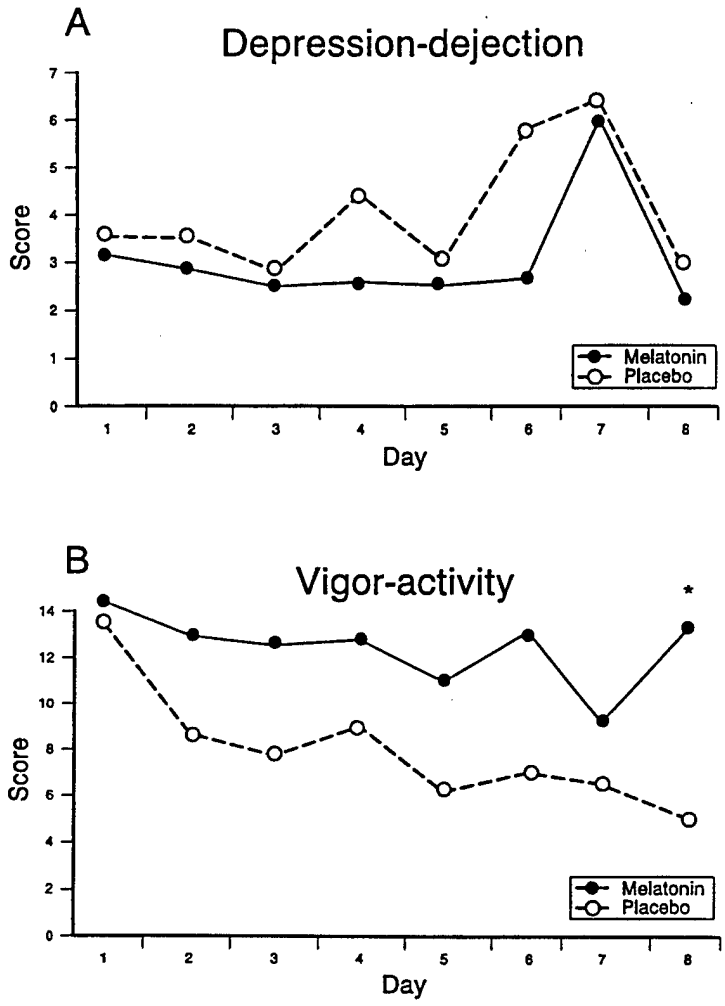

Figure 15. Results from POMS for categories of depression-dejection (A) and vigor-activity (B) for the melatonin and placebo group. Statistical significance ($p<0.05$) indicated by *.

The daily menstrual questionnaire did not reveal a consistent pattern. Two of the 10 members of the melatonin group showed increased incidence of water retention and breast tenderness beginning at the end of the in-house stay, but this was not found in the other 8 volunteers. Analysis of results from the activity monitors and the post-sleep questionnaires revealed no significant difference between members of the melatonin and placebo groups.

Discussion

In this report we have demonstrated several apparently minor effects on the menstrual cycle and menstrual characteristics resulting from the administration of melatonin (10 mg) to healthy females at 1300 for 5 days during the late follicular and early luteal phases of the menstrual cycle. Observed effects include shorter overall cycle length during the dose cycle and longer cycle length during the following cycle, shorter menses during the month following melatonin administration, and decreased LH surge during the post-dose month. Additionally, we believe we have demonstrated that melatonin administration results in a reduction of study-related stress. Each of these changes will be discussed in the following paragraphs.

Melatonin

Before any discussion of changes in menstrual characteristics, it is important to discuss the underlying changes in melatonin upon which any menstrual changes are dependent. Members of the melatonin group received daily administration of 10 mg of melatonin during their in-house stay. Examination of Figures 4 and 5 show that peak levels of melatonin in serum (mean 7000 pg/ml) and saliva (mean 2500 pg/ml) are reached within 1-2 hours after the 1300 administration. It is not surprising to have such high levels in blood and saliva, since 10 mg of melatonin is the same as 10,000,000,000 pg. Since melatonin is highly lipophilic, it readily is absorbed and distributed into every anatomical compartment. The major metabolism for melatonin occurs in the liver. Melatonin is hydroxylated, and the hydroxylated metabolites then are excreted in the urine as sulphate or glucuronide conjugates with the major metabolite being the sulphate conjugate. Published values for melatonin half-life are 60 minutes or less (Lane and Moss, 1985; Waldhauser et al., 1990; Waldhauser et al., 1984), and a recent study of melatonin pharmacokinetics in man revealed a half-life in blood of 28.4 minutes (Mallo et al., 1990). Since some of the 10 mg dose likely is not absorbed by the digestive tract and up to 90% of that entering the blood is lost through marked first pass hepatic metabolism, we are not sure of the proportion of the 10 mg available to affect menstrual characteristics or cognitive function. However, if we assume the entire dose is absorbed and available, simple half life calculations using a 30 minute half-life tell us that by 0200, during the first cognitive session, the 10 billion pg dose has been reduced to 150 pg. When this is divided by an estimate of the amount of serum in the average adult female body, we arrive at an approximation of 0.4 pg/ml of serum. This of course does not take into account the endogenous production of melatonin, and is far less than our measured values. The mean serum melatonin value measured in the melatonin volunteers at 0200 on day 7, 13 hours after the fifth and final daily dose of melatonin, was 40.8 pg/ml.

Although the volunteers did not receive bright light treatment on day 7, 0200 on that day was into the daylight period of their simulated deployment. At 0200, serum melatonin values were elevated above normal baseline/daylight levels, and were in fact about the level of the normal endogenous nighttime melatonin peak (61.0 pg/ml for the melatonin group). For a comparison, pre-in-house melatonin levels for all study participants at 0700 (roughly equivalent to 0200 of the simulated deployment) was 19.5 pg/ml. In spite of the elevated melatonin levels, volunteers performed well on the cognitive tasks and did not show decrements as might have been expected.

These results will be discussed in a separate report (Comperatore and Kirby, in preparation). It is important to emphasize here that members of the melatonin group maintained levels of melatonin throughout the dose days which were elevated above normal levels. Even ignoring absolute levels, the shape of the melatonin curve resulting from exogenous administration is not at all like the endogenous melatonin curve. Instead of a late night increase in melatonin which maintains some plateau level until the early morning melatonin decrease, members of the melatonin group experienced greatly elevated melatonin levels which peaked rapidly by 1500 and continuously fell throughout the remainder of the day. By the first cognitive testing session (0130), melatonin had fallen to levels which approximated the pre-in-house nightly surge. In terms of melatonin levels, the body was experiencing an extended night. Because of the shape difference in the serum melatonin curve between endogenous and exogenous melatonin, a small pharmacological dose resulting in levels similar to endogenous production might produce entirely different effects. It also has been suggested that younger people are more sensitive to melatonin than older (Webb and Puig-Domingo, 1995). Because of our relatively limited sample size, we can not address that issue from our results.

The dose of melatonin required to produce a particular effect is a question that we can not address from our study, since we tested only one dose (10 mg). Cagnacci et al. (1994) reported that the effect of melatonin on core body temperature is "all or nothing." They claimed that levels of melatonin in the pharmacological range were just as effective as levels that were minimally detectable. In contrast to those results, Deacon and Arendt (1995) reported a significant correlation between the dose of melatonin and the magnitude of temperature suppression, and the degree of phase shift of both the temperature rhythm and the plasma melatonin onset time. While questions therefore remain regarding the optimal dose of melatonin for a particular situation, the best solution is likely to be the smallest dose with the least side effects resulting in the desired change.

Menstrual characteristics and LH

Because of a potential inhibitory influence of melatonin over the hypothalamo-pituitary-ovarian axis, melatonin use could be associated with secondary disruptions of the menstrual cycle. Other than evidence for interaction between melatonin and LH (Cagnacci, 1996; Cagnacci and Volpe, 1996), the exact relationship between melatonin and the monthly cycle is unclear. Although our sample size was limited in our previous study (Kirby et al., 1996), we saw no consistent changes in the length of the menstrual cycle following melatonin (10 mg) at 2300. We report here that 7 of the 10 volunteers in the melatonin group had a shorter cycle during the dose month, however, the same trend was observed in the placebo group (6 of 10) (see Table 2). Of those with shorter cycles, only three of the melatonin and one of the placebo volunteers exceeded the 2.2 day mean variation present in all the pre-dose cycles. This could suggest that the shorter cycle during the dose month is within normal variation. It is, however, intriguing that the melatonin and placebo groups separate during the post-dose cycle with 7 of the 10 members of the melatonin group showing a longer cycle compared to only 4 of the 10 members of the placebo group. Because of individual variation and limited group size, it is difficult to say with any certainty whether or not the changes in cycle length result from the melatonin administration.

It is our feeling that this change is more consistent than anything we saw after melatonin at 2300 in our previous study, and likely results from the different time of administration.

There was a trend for shorter menses (five of eight volunteers) compared to the mean of the previous cycles for volunteers in the melatonin group when dose time was 2300 (Kirby et al., 1996). We were uncertain as to the significance of that observation, however, we now see a similar change in this study following melatonin at 1300. We showed in Table 3 that 6 of the 10 members of the melatonin group had shorter menses the post-dose month. Of the other four members of the melatonin group, two had longer menses and two had no change in their menses during the post-dose month. On the other hand, seven of the nine members of the placebo group demonstrated longer menses. Since changes in the melatonin group agree well with the melatonin group results from the previous study and are opposite to the changes in the placebo group, we feel that shorter menses may well be linked to the 10 mg melatonin dose. Unlike the finding with cycle length, these changes do not appear to be dependent on time of day of administration.

A link between melatonin and shorter menses is not totally unexpected. Women with hypothalamic amenorrhea (Brzezinski et al., 1988) and amenorrheic athletes (Laughlin et al., 1991; Diaz et al., 1993) have been reported to have elevated levels of serum melatonin. It is therefore not surprising that administration of exogenous melatonin, sufficient to maintain melatonin above normal levels, might affect length of menses.

There is a good deal of evidence in the recent literature that melatonin influences LH in the human female. Melatonin, either 100 mg in a single dose or 2.5 mg in three divided doses, was shown to augment overall LH secretion during the early follicular phase of normally cycling women (Cagnacci et al., 1991). A well controlled experiment on normally cycling women compared changes in LH following stimulation with gonadotrophic releasing hormone (GnRH) after administration of 3 mg melatonin during the follicular (days 4-6) and the luteal (days 18-21) phases of the menstrual cycle (Cagnacci et al., 1995). They reported that melatonin enhanced the release of LH to GnRH stimulation during the follicular but not the luteal phase of the cycle. The stimulatory effect of melatonin on LH during the follicular phase of the menstrual cycle also is stressed in recent review articles (Cagnacci, 1996; Cagnacci and Volpe, 1996). In contrast, Voordouw et al. (1992) reported that daily administration of 300 mg melatonin for up to 4 months to normally cycling females resulted in significantly decreased mean LH levels. In a separate study, normally cycling females received 10 mg melatonin every 4 hours for 7 days during the follicular phase of the cycle (Zimmerman et al., 1990). The amount of LH secreted and the timing of the LH surge was essentially unchanged when compared with spontaneous cycles in the same volunteers before melatonin. In another study, normally cycling females received two doses of 2 mg melatonin 4 hours apart during the mid-follicular phase (Terzolo et al., 1993). They reported several different LH profiles following melatonin, and concluded that any effect of melatonin on LH may depend on individual sensitivity. They also stressed that differences between their results and those obtained by Cagnacci et al. (1991) may depend upon such factors as seasonal differences and time of day of administration.

Instead of drawing blood samples to determine LH levels, our LH results are based upon assays performed on urine samples. This was done because of the number of blood samples required to follow LH levels for several months. Also, we showed previously that urine-based LH assays agree well with blood assays when used to identify the monthly LH surge (Kirby et al., 1996). Absolute values will of course differ between the two methods. Our urine-based assays were performed on first void samples except during the in-house stay. During that time we collected samples every 3 hours while the volunteers were awake and performed LH assays on every sample. Although the first void assay did a good job at identifying the LH surge, it did not always correctly identify the maximum surge amplitude. On our multiple daily samples, we noticed that the absolute LH peak amplitude often occurred at another time of the day and could therefore shift the identification of the LH peak day either a day earlier or later. This complicated our analysis of the LH data as will be discussed in the following paragraph.

In our previous study with melatonin administration at 2300 during the late follicular and early luteal phase of the cycle, there were no consistent changes in the timing of the LH peak day. However, when the dose month was compared to the pre-dose month, our initial observation was that all eight members of the melatonin group exhibited decreased LH peak amplitude. This is completely opposite to the melatonin effect in recent reports by Cagnacci that melatonin increases release of LH when given during the follicular phase (Cagnacci, 1996; Cagnacci et al., 1995; Cagnacci and Volpe, 1996). Our results are entirely different if we reevaluate the same data using the highest LH value of the multiple daily samples collected while in-house instead of the first void. Six of the eight members of the melatonin group now show increased LH peak amplitude during the dose month. If, however, we were consistently underestimating the LH peak using levels obtained from first void samples in the pre-dose month, we would expect that an increase would be seen when comparing pre-dose month values to the true absolute LH peak amplitude during the dose month. Since multiple daily urine samples were collected only while in-house and we likely captured true LH peak amplitudes only from multiple daily samples, we decided to compare only LH values obtained from first void samples. If the LH peak amplitude was decreased during the dose month, we might expect to see an increase when the post-dose month is compared to the dose month. As expected, six of the eight members of the melatonin group showed an increase during the post-dose month.

In our current study, results addressing LH peak day or amplitude (see Tables 5 and 7) also vary depending upon whether or not LH values were obtained from first void samples or from multiple daily samples while in-house. For the reasons mentioned in the previous paragraph, we feel that results obtained from first void samples are our only option. As in the previous study, there are no consistent differences in the timing of the LH peak day. We do, however, see a decrease in LH peak amplitude in 7 of the 10 members of the melatonin group when comparing post-dose month to pre-dose month, and in 6 of the 10 when comparing post-dose month to dose month. That all members of the melatonin group did not show similar changes may reflect individual variations in sensitivity to melatonin. As in our previous study, this is completely opposite to the results reported by Cagnacci (Cagnacci, 1996; Cagnacci et al., 1995; Cagnacci and Volpe, 1996). We are unsure why our results differ, but it could be that Cagnacci administered melatonin earlier in the follicular phase. Another difference between the two

paradigms is that Cagnacci limited participation to women whose body weight was within 10% of ideal. We were much less selective, and our participants had a much wider range of body weights. This might suggest variations in metabolism which could alter the availability of orally administered melatonin. However, we feel it unlikely that body weight differences could explain an LH increase in one study and a decrease in another. If there truly is a difference between melatonin effects when administered at different times of the menstrual cycle, it suggests that sensitivity to exogenous melatonin may depend upon the endocrine environment at the time of administration.

There was considerable variability in melatonin and other hormone levels (LH or PRL) between volunteers both prior to and following melatonin administration. While results of hormone assays were quite reliable within a given volunteer during the dose days, group analysis led to large standard errors. In our attempts to demonstrate differences between the melatonin and placebo groups, high interindividual variability hampered statistical validation.

Prolactin and PMS

Premenstrual syndrome is a group of disorders, characterized by mood and behavioral disturbances during the post-ovulatory phase of the menstrual cycle, which resolve at or near the onset of menses. The symptoms generally included in PMS can be divided into two groups: somatic and psychologic. Somatic symptoms include bloating, breast swelling and pain, pelvic pain, headache, skin disorders, and changes in bowel habits. Common psychologic symptoms include irritability, aggressiveness, depression, anxiety, inability to concentrate, tension, lethargy, insomnia, change in appetite, and mood swings (O'Brien, 1985). The incidence of PMS is estimated to be at 30-40%, with severe PMS occurring in less than 10% of women (Johnson, 1987).

Prolactin has been associated with PMS for several reasons: it has a direct effect on the breast and therefore may be responsible for reported breast symptoms; it is a hormone related to stress; it promotes retention of sodium, potassium, and water (O'Brien, 1985). One of the secondary objectives of this study was to determine whether or not there was a melatonin-induced increase in PRL secretion, and if so, might the PRL increase be accompanied by secondary symptoms such as improvement or exacerbation of PMS-like symptoms. There is some suggestion for this in the literature. Halbreich et al. (1976) reported that mean serum PRL was significantly higher in women with PMS symptoms than in matched controls. However, O'Brien and Symonds (1982) did a similar study and reported no consistent changes in serum PRL in either the PMS or control groups during the menstrual cycle. They concluded that there was no correlation between mood changes and levels of PRL.

Melatonin, PRL and stress

There is considerable evidence in the literature that melatonin has a facilitory effect on the secretion of PRL. Most women have a small early evening peak of PRL, followed by a larger nocturnal peak later in the night. The early evening rise in PRL is likely not linked to melatonin,

since the evening increase in melatonin concentrations occurs 2-3 hours after that of PRL. The nocturnal plasma peak of PRL is reported to occur 1-2 hours after the nightly peak for melatonin (Brzezinski et al., 1988; Lisoni et al., 1986; Okatani and Sagara, 1993), although others report the PRL peak to occur 2-4 hours after the melatonin peak (Okatani et al., 1994; Webley and Lenton, 1987). Allowing for variation among individuals, the consistency of the phase delay between the nocturnal melatonin peak and that of PRL suggests a physiological relationship between the two. Many studies conclude that melatonin stimulates the release of PRL in both males (Waldhauser et al., 1987; Webley et al., 1988; Mallo et al., 1988) and females (Bispink et al., 1990; Terzolo et al., 1993; Webley and Lenton, 1987; Okatani and Sagara, 1993; Lisoni et al., 1986; Terzolo et al., 1991). Although the mechanism by which melatonin affects the release of PRL is not well defined, there seems to be little question of the link between the two. Plasma melatonin has been reported to be high in patients with hyperprolactinemia (Wetterberg, 1979). This is expected if melatonin controls the release of PRL. Finally, the facilitory role of melatonin on the release of PRL was reported to be statistically significant only in the follicular phase of the menstrual cycle (Terzolo et al., 1991). Although there was a similar trend in the luteal phase, it was not significant. This agrees well with the reported effect of melatonin on LH during the follicular but not luteal phase of the menstrual cycle (Cagnacci, 1996), which we were unable to confirm.

Since melatonin apparently facilitates the release of PRL which itself may be linked to PMS-like symptoms (water retention and breast tenderness), we were surprised that the melatonin group did not consistently demonstrate increased PMS-like symptoms. This was seen in only 2 of the 10 volunteers. Perhaps water retention and breast tenderness are dependent upon some specific aspect of the release of PRL which was not met by the stimulation resulting from our 10 mg melatonin dose. In a study designed to explain the hormonal changes underlying PMS, Rubinow et al. (1988), obtained multiple blood samples across the menstrual cycle in women with well-characterized menstrually related mood disorders and reported no diagnosis-related differences in a number of hormones, including PRL. They concluded that PMS is not caused by a simple hormonal deficiency. Whatever the reason, it would appear that an afternoon melatonin regimen requiring the 10 mg dose will not result in female soldiers being burdened with increased symptoms of PMS.

It has been known for some time that human plasma PRL shows a significant rise under various stressful situations. Prolactin was reported to increase as much as five times during major surgery with general anesthesia, during gastroscopy, during proctoscopy, and exercise (Noel et al., 1972). They concluded that PRL release was induced by stress. Our results showed that the nightly increase in PRL was shifted earlier in both groups (Figures 8 and 9). Figures 5 and 6, showing melatonin levels in the placebo group while in-house, show that the nightly production of melatonin also was shifted earlier. Presumably, the bright light treatment inhibited the normal endogenous nightly production of melatonin and advanced it to an earlier time. Earlier production of melatonin would then advance the production of PRL. In addition to the overall shift to an earlier time of the PRL curve, we find the in-house peaks at 1300 in the placebo group (Figure 8) and 1500 in the melatonin group (Figure 9) extremely interesting. Since dose administration was at 1300, the PRL peak at 1300 in the placebo group was obviously too early to be caused by melatonin and very well could be a result of the stress or anxiety of the

impending dose. Various studies have demonstrated that stress is capable of eliciting the release of PR (Dongyun and Yumin, 1990; Noel et al., 1972; Schedlowski et al., 1992). Increased stress in the placebo group also might be indicated by the fact that the nightly PRL increase precedes rather than follows the nightly melatonin increase on day 6 (see Figure 12). Instead of a peak at 1300, the melatonin group demonstrated a PRL peak at 1500. This was 2 hours after administration of melatonin, and agrees well with reports of a 1-2 hour delay between the administration of melatonin and increased release of PRL (Brzezinski et al., 1988; Lisoni et al., 1986; Okatani and Sagara, 1993). That the melatonin group does not demonstrate a peak at 1300 could indicate that they are experiencing much less stress than the placebo group under similar conditions. This also is demonstrated by results of the POMS questionnaire completed by each volunteer. The melatonin group consistently demonstrated scores indicative of less stress and anxiety than their placebo counterparts. Taken together, these results suggest that melatonin relieves or lessens anxiety and stress.

An alternative explanation for the difference between PRL release in the two groups could be sleep differences between the placebo and melatonin groups while in-house. Since melatonin is marketed as a sleep aid, it would be a reasonable assumption that the melatonin group was sleeping better than the placebo group. Since we did not record sleep, we can not address this directly. However, the assumption was not supported by results from activity monitors or the post-sleep questionnaires completed upon waking each morning while in-house. Both groups were limited to eight hours of bedtime, and it was closely monitored by technicians. Although members of the melatonin group slept somewhat longer than the placebo group, especially on the transition nights, the difference was not significant. It would appear that the melatonin group was under less stress than the placebo group, and the difference was because of the presence of melatonin, and not sleep differences.

Although we have reported various changes in menstrual characteristics and hormone levels, we saw no side effects during this study that should preclude the use of a melatonin regimen utilizing a 10 mg dose at 1300 during deployment conditions. Questions continuously arise about a seasonal effect in melatonin studies. Seasonal time of in-house stay and dose administration for our volunteers ranged from spring through winter, but there was no observable difference in their response to melatonin based upon season. This is not surprising because of the supraphysiological dose of melatonin used in this study. It also is reassuring, since relatively large doses are likely to be used in deployment conditions to resynchronize circadian rhythms as quickly as possible. Also, because the dose was so large, we might have predicted significant differences in dose recognition between melatonin and placebo volunteers. However, neither group was able reliably to identify whether they were receiving melatonin or placebo, and both groups felt drowsy during the afternoon cognitive sessions following dose administration at 1300.

It seems quite clear that melatonin, like any other receptor agonist, should have different effects depending upon whether or not the receptors are up- or down-regulated. If they are up-regulated, there simply are more receptors available to receive the agonist and initiate some physiological or behavioral change. We feel certain that this is the reason for different effects

following 1300 or 2300 administration times for a 10 mg dose of melatonin. Since there is almost no endogenous melatonin at 1300, melatonin receptors should be maximally up-regulated resulting in a maximal effect. Therefore, it is encouraging that any melatonin-induced effects reported here were minimal and should not preclude its use in field situations.

The next logical step is to investigate even larger doses of melatonin. Since a 10 mg dose results in high supraphysiological levels of melatonin, larger doses may result in faster resetting of circadian rhythms without additional side effects. Also, it is important to establish whether or not the anti-stress effect is independent of gender.

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Appendix A.

List of manufacturers.

Abbott Diagnostics
A Division of Abbott Laboratories
One Abbott Park Road
Abbott Park, IL 60064

ALPCO, Inc.
P.O. Box 451
Windham, NH 03087

Precision Control Design, Inc.
135 Eglin Parkway, S.E.
Ft. Walton Beach, FL 32540

Stockgrand Ltd.
School of Biological Sciences
University of Surrey
Guilford, Surrey
GU2 5XH, UK

Whitehall-Robbins Healthcare
American Home Products Corporation
Five Giralda Farms
Madison, NJ 07940

Appendix B.

Prolactin levels for melatonin and placebo groups.

Table B-1.
Melatonin volunteers - pre-in-house prolactin levels (ng/ml)

MPIN	07	09	10	13	20	22	27	33	36	37
0800	11.1	3.9	9.9	25.2	15.0	17.8	12.6	-	4.2	-
0900	8.5	2.8	8.3	20.6	11.2	13.5	6.6	5.4	4.3	5.3
1000	8.9	2.6	7.0	-	10.6	10.9	5.0	4.4	3.0	3.9
1100	8.2	3.3	-	13.7	-	11.6	4.5	4.2	3.3	3.3
1200	6.8	3.7	6.8	12.5	13.1	13.6	6.4	6.5	5.0	3.5
1300	7.9	3.9	-	14.0	9.7	17.3	6.5	5.1	4.1	4.1
1400	7.2	4.8	8.4	9.5	-	15.6	7.1	4.0	4.8	6.2
1500	9.5	4.8	8.1	15.5	10.9	21.3	9.2	6.3	5.2	4.4
1600	8.8	4.8	5.5	21.8	11.7	15.5	6.7	7.5	4.4	5.5
1700	8.1	4.1	10.7	23.0	12.8	16.8	9.0	7.1	5.0	4.5
1800	7.4	-	13.7	20.7	13.1	15.9	8.1	10.5	6.7	6.6
1900	8.1	4.9	12.4	15.7	10.9	16.4	8.9	6.5	4.4	4.5
2000	8.1	4.3	13.5	16.9	13.1	18.2	6.6	5.8	3.8	6.5
2100	10.1	5.0	12.5	19.6	10.3	23.3	-	7.0	4.8	7.1
2200	11.9	5.2	10.7	16.8	10.5	17.5	9.9	6.6	4.8	5.8
2300	9.3	5.8	9.0	17.2	15.2	14.1	8.0	6.5	4.9	6.3
2400	8.4	15.6	9.1	15.0	-	14.0	-	9.9	8.5	12.4
0100	8.3	15.9	9.1	20.1	14.4	17.2	-	13.6	10.5	26.6
0200	8.4	10.3	9.6	20.7	-	18.3	-	12.0	12.2	18.3
0300	10.2	11.7	12.8	29.0	13.2	34.6	-	16.0	11.5	22.1
0400	14.6	13.9	17.3	29.9	-	22.9	-	13.1	12.2	37.5
0500	16.3	7.7	17.7	26.4	23.1	26.2	-	12.9	9.4	20.9
0600	26.7	8.9	42.6	23.1	-	22.1	-	16.0	10.2	23.2
0700	19.7	6.3	23.7	26.6	25.2	22.0	-	11.3	10.2	23.5
0800	16.1	4.2	15.3	19.0	-	13.5	-	9.3	6.0	10.1

Table B-2.
Melatonin volunteers - in-house prolactin levels (ng/ml)

MIN	07	09	10	13	20	22	27	33	36	37
0800	9.6	4.3	7.0	-	-	10.0	-	-	-	-
0900	15.7	3.8	7.2	17.1	17.5	13.9	5.6	10.0	-	4.2
1000	13.4	4.8	8.1	15.9	10.4	14.0	5.2	7.5	-	8.8
1100	13.5	6.2	7.3	16.5	13.3	8.9	5.9	7.0	6.6	6.0
1200	12.9	5.6	16.9	19.0	11.0	11.5	5.9	7.0	8.7	6.2
1300	17.2	9.3	9.4	18.2	17.3	14.0	5.0	7.0	8.3	9.7
1400	17.5	12.4	9.5	20.9	13.5	14.5	5.6	11.2	7.0	7.6
1500	28.2	15.9	15.3	27.7	15.7	23.4	7.7	11.3	7.7	19.3
1600	17.3	13.5	11.6	17.6	12.9	18.4	5.1	10.3	6.2	12.7
1700	18.1	9.7	10.2	22.3	10.1	12.8	5.5	11.7	7.3	20.0
1800	19.0	11.6	9.4	36.1	-	9.5	6.1	-	8.2	12.8
1900	15.6	6.3	32.8	34.4	-	19.4	7.5	17.2	9.2	25.5
2000	21.9	5.9	20.7	40.9	-	21.0	13.5	29.6	11.2	17.0
2100	25.3	9.1	33.1	34.1	29.3	13.8	12.3	15.7	10.6	14.1
2200	21.4	12.6	21.2	19.8	-	21.5	13.7	12.5	-	11.9
2300	18.1	8.9	25.3	-	-	13.2	9.0	9.6	-	11.3
2400	18.9	12.5	16.3	23.4	21.9	23.6	8.2	10.0	12.2	6.0
0100	10.2	11.9	15.5	-	-	22.6	14.7	9.9	8.7	9.3
0200	13.5	6.4	10.3	16.1	13.2	17.3	5.9	6.0	5.7	5.8
0300	10.3	5.4	8.2	-	-	12.1	5.0	5.9	5.2	5.5
0400	12.5	4.6	7.8	15.1	12.6	11.0	-	6.1	5.1	9.0
0500	12.5	4.6	7.7	-	-	15.4	7.7	6.3	6.0	6.6
0600	9.2	3.8	9.7	20.1	11.6	15.8	-	6.4	4.8	6.7
0700	10.4	3.9	7.8	-	-	13.1	5.1	6.1	5.0	7.7
0800	13.1	5.3	12.3	19.0	19.9	10.6	7.4	7.8	6.6	12.7

Table B-3.
Placebo group - pre-in-house prolactin levels (ng/ml)

PPIN	05	08	14	17	19	21	25	29	31	32
0800	10.5	7.4	16.1	-	-	16.4	-	5.7	16.4	-
0900	10.0	5.7	11.7	3.8	-	11.3	5.2	4.9	11.3	15.6
1000	6.9	5.4	7.7	3.5	-	10.4	5.6	3.3	10.4	-
1100	6.0	4.7	5.2	2.9	-	7.7	5.3	3.5	7.7	12.4
1200	7.2	5.6	5.5	3.6	-	13.6	7.9	3.0	13.6	15.9
1300	8.5	4.8	4.3	5.9	9.3	13.0	7.5	4.1	13.0	13.6
1400	7.1	6.7	12.1	3.9	8.2	12.6	6.3	3.9	12.6	16.1
1500	8.2	5.3	9.3	4.7	5.6	11.5	7.7	4.7	11.5	16.7
1600	7.3	6.4	5.3	3.3	8.4	11.6	8.9	5.0	11.6	15.3
1700	11.6	11.5	8.9	4.4	7.8	18.9	23.0	5.4	18.9	19.7
1800	-	13.2	5.8	6.4	8.5	14.3	30.5	5.7	14.3	18.6
1900	16.2	8.8	5.5	5.1	10.4	13.5	20.1	4.4	13.5	19.6
2000	10.9	8.6	13.4	4.7	11.4	12.1	10.8	4.6	12.1	23.3
2100	12.1	13.2	13.6	4.1	9.2	12.5	8.3	4.0	12.5	20.9
2200	9.4	9.8	10.2	3.2	6.8	10.7	7.4	-	10.7	15.6
2300	8.9	8.7	8.2	2.9	7.1	13.2	7.2	-	13.2	15.5
2400	10.7	8.2	12.8	2.5	9.6	13.7	6.2	6.9	13.7	20.2
0100	11.2	16.1	17.0	6.8	15.6	12.6	7.8	-	12.6	17.6
0200	10.4	11.5	15.6	15.4	13.3	31.6	9.1	28.8	31.6	21.0
0300	13.3	15.4	12.9	39.3	16.0	21.0	22.1	-	21.0	27.0
0400	13.2	14.2	12.4	21.8	25.7	20.2	26.1	29.2	20.2	25.3
0500	14.0	13.6	18.9	18.2	21.9	21.2	21.0	-	21.2	20.7
0600	10.6	16.1	15.1	16.4	18.3	26.7	22.6	20.4	26.7	22.0
0700	9.5	16.7	17.5	12.6	15.9	18.3	23.2	-	18.3	20.1
0800	9.6	27.6	16.5	6.7	10.9	17.8	11.7	8.9	17.8	18.1

Table B-4.
Placebo group - in-house prolactin levels (ng/ml)

PIN	05	08	14	17	19	21	25	29	31	32
0800	17.1	16.1	13.7	-	15.0	-	-	9.1	-	-
0900	14.6	15.5	17.7	-	16.6	15.6	13.5	9.5	15.6	16.3
1000	13.5	12.9	12.7	3.5	15.4	14.7	9.2	8.2	14.7	-
1100	11.7	14.9	7.7	3.3	18.2	16.2	10.1	8.7	16.2	16.0
1200	12.4	18.6	21.7	3.9	31.3	18.2	12.9	10.5	18.2	14.6
1300	11.9	24.9	31.0	3.8	24.2	23.0	12.2	23.5	23.0	15.4
1400	10.8	18.6	14.1	3.9	25.2	17.8	9.1	14.1	17.8	12.4
1500	12.7	25.7	13.6	4.3	25.8	20.3	10.2	15.5	20.3	-
1600	10.5	17.0	14.1	3.6	24.3	14.9	8.5	16.6	14.9	-
1700	12.1	23.6	31.5	4.2	30.2	19.9	27.4	22.9	19.9	-
1800	8.7	24.5	25.1	3.5	33.9	19.9	15.0	27.7	19.9	-
1900	21.6	37.8	29.2	4.8	22.3	20.8	17.4	38.2	20.8	-
2000	15.1	31.4	20.3	11.2	22.3	31.1	17.1	59.3	31.1	-
2100	18.8	28.7	20.4	11.6	29.1	28.9	18.2	38.4	28.9	-
2200	17.8	22.3	23.5	5.7	34.2	29.6	13.8	20.8	29.6	-
2300	28.2	20.0	24.6	10.7	30.1	18.0	17.7	24.5	18.0	-
2400	17.1	19.6	21.0	10.9	27.3	17.0	24.6	33.6	17.0	-
0100	16.3	14.9	23.6	5.8	23.4	18.1	17.2	18.7	18.1	-
0200	10.4	9.9	34.1	5.2	14.9	15.5	10.7	11.3	15.5	-
0300	9.8	16.7	23.3	3.9	13.8	22.2	9.0	10.1	22.2	-
0400	8.6	8.6	15.5	3.1	11.4	14.6	9.1	8.9	14.6	-
0500	9.3	8.2	15.1	3.1	14.8	15.3	9.5	11.7	15.3	-
0600	10.4	6.2	13.1	6.3	21.2	14.3	10.1	17.5	14.3	-
0700	8.3	6.3	15.7	4.4	11.1	13.6	11.5	17.8	13.6	-
0800	12.1	13.3	17.7	4.8	10.5	13.7	16.5	24.8	13.7	-