J Formos Med Assoc 2010;109(10):694–701

Contents lists available at ScienceDirect

Journal of the Formosan Medical Association

Journal homepage: http://www.jfma-online.com

Original Article

Detection of Nighttime Melatonin Level in Chinese Original Quiet Sitting

Chien-Hui Liou,[1] Chang-Wei Hsieh,[2,3] Chao-Hsien Hsieh,[1,4] Der-Yow Chen,[1,5] Chi-Hong Wang,[6] Jyh-Horng Chen,[1†] Si-Chen Lee[7*†]*

Background/Purpose: Some research has shown that melatonin levels increase after meditation practices, but other research has shown that they do not. In our previous functional magnetic resonance imaging study, we found positive activation of the pineal body during Chinese Original Quiet Sitting (COQS). To find other supporting evidence for pineal activation, the aim of this study was to evaluate the effect of COQS on nighttime melatonin levels.

Methods: Twenty subjects (11 women and 9 men, aged 29–64 years) who had regularly practiced daily meditation for 5–24 years participated in this study. All subjects served alternately as participants in the meditation and control groups. COQS was adopted in this study. Tests were performed during two nighttime sessions. Saliva was sampled at 0, 10, 20, 30, 45, 60 and 90 minutes after COQS and tested for level of melantonin. Time period effect analysis and mixed effect model analysis were preceded by paired *t* test analysis.

Results: In the meditation group ($n = 20$), the mean level of melatonin was significantly higher than the baseline level at various times post-meditation ($p < 0.001$). Within the control group ($n = 20$), the mean level of melatonin at various times was not significantly different compared with baseline ($p > 0.05$). These results suggested that the melatonin level was statistically elevated in the meditation group and almost unchanged in the control group after nighttime meditation. The urine serotonin levels detected by measuring 5-hydroxyindole-3-acetic acid levels were also studied, but no detectable difference between the groups was found.

Conclusion: Our results support the hypothesis that meditation might elevate the nighttime salivary melatonin levels. It suggests that COQS can be used as a psychophysiological stimulus to increase endogenous secretion of melatonin, which in turn, might contribute to an improved sense of well-being.

Key Words: meditation, melatonin, pineal body, saliva

[1]Interdisciplinary MRI/MRS Laboratory, [7]Department of Electrical Engineering, [5]Department of Psychology, and [4]Neurobiology and Cognitive Science Center, National Taiwan University, Taipei, [2]Department of Photonic and Communication Engineering, Asia University, Taichung County, [3]Anthro-Celestial Research Institute, The Tienti Teachings, Nantou County; [6]Department of Neurology, Cardinal Tien Hospital Yung Ho Branch, Yung Ho City, Taipei County, Taiwan.

Received: July 17, 2009
Revised: October 18, 2009
Accepted: January 1, 2010

***Correspondence to:** Professor Si-Chen Lee, Department of Electrical Engineering, National Taiwan University, 1 Section 4, Roosevelt Road, Taipei 10617, Taiwan.
E-mail: sclee@cc.ee.ntu.edu.tw
OR
***Correspondence to:** Professor Jyh-Horng Chen, Interdisciplinary MRI/MRS Laboratory, Department of Electrical Engineering, National Taiwan University, 1 Section 4, Roosevelt Road, Taipei 10617, Taiwan.
E-mail: jhchen@ntu.edu.tw
[†]*Si-Chen Lee and Jyh-Horng Chen contributed equally to this work.*

Chinese Original Quiet Sitting (COQS) is a type of traditional Chinese meditation. In ancient China, meditation was the principal practice used by Taoists to temper the spirit and body. In our previous functional magnetic resonance imaging (fMRI) study, we found positive activation of the pineal body during this meditation practice.[1] The pineal body produces many substances, including melatonin,[2] and melatonin levels during COQS have never been studied, therefore, we hypothesized that COQS might also cause an increase in melatonin levels. Furthermore, there have been many studies on the physiological effects of melatonin. As has been reported, melatonin is effective in reducing cancer development or the risk of cancer mortality.[3–5] It also has functional effects on cellular bioenergetics[6] and cellular regulation.[7] In addition, it possesses antioxidant effects,[8–10] anti-aging properties,[11] and locally regulates human placental function.[12] Circadian rhythm corresponds to the secretion of melatonin.[13–17] Melatonin might also influence immune function and seasonal fertility in some animals.[18,19] The potential interaction between COQS, pineal activation and melatonin levels becomes more interesting and important due to these special physical effects of melatonin.

In recent years, meditation has become an increasingly popular form of exercise worldwide. Some religious people practice meditation on a daily basis to improve their body, mind and spirit. There are many different styles of meditation. However, the mechanism by which meditation improves health remains unclear. Massion et al tested the hypothesis that the regular practice of mindfulness meditation is associated with increased physiological levels of melatonin, and have obtained some positive results.[20] Tooley et al studied whether a period of meditation can influence melatonin levels. Transcendental Sidhi and yoga meditation have also been studied and their practice has been shown to result in significantly higher plasma melatonin levels.[21] Harinath et al evaluated the effects of Hatha yoga and Omkar meditation on cardiorespiratory performance and melatonin secretion. They found that plasma melatonin levels increase after 3 months of yoga.[22] In contrast, Solberg et al studied melatonin secretion during ACEM meditation (ACEM is a meditation organization that originated in Oslo, Norway), and although they have found that advanced meditators have higher melatonin levels than non-meditators, melatonin actually decreases during long-term meditation.[23] Carlson et al investigated the benefits of a mindfulness-based stress reduction meditation program for early-stage breast and prostate cancer patients, and have found no overall changes in melatonin levels.[24] It appears that the secretion of melatonin might depend on the style of meditation. Therefore, it is necessary to determine whether nighttime salivary melatonin levels are elevated or depressed after COQS.

Melatonin is synthesized endogenously from the amino acid tryptophan (derived from serotonin) by the enzyme 5-hydroxyindole-O-methyltransferase. Serotonin is a monoamine neurotransmitter that is synthesized in serotonergic neurons in the central nervous system and enterochromaffin cells in the gastrointestinal tract. Bujatti et al found a highly significant increase in 5-hydroxyindole-3-acetic acid (5-HIAA; the main breakdown product of serotonin) in urine after transcendental meditation.[25] In contrast, Solberg et al found that serotonin concentrations decreased in meditation and reference groups after 1 hour of meditation ($p < 0.01$).[23] These contrasting results need to be examined further.

Urine serotonin levels detected by 5-HIAA were measured in our study. The aim of this study was to investigate the nighttime salivary melatonin levels after COQS.

Materials and Methods

Meditation style

The overall COQS process is separated into two distinct parts: (1) several minutes of silent recitation of specific religious mantra and mental imagination of receiving spiritual energy (which is named "Invitation of Primordial Qi": IPQ); and

(2) a longer period of relaxation with no further action of the mind and letting Qi do its work (referred to as "Allow its Natural Workings": ANW).[26,27] Chen et al studied COQS using electroencephalography and have found a marked increase in brain θ-waves and decreased α- and β-waves.[28] We also carried out an fMRI study.[1] Nighttime salivary melatonin levels during this meditation practice were measured.

Salivary melatonin analysis

A number of biological fluids can be sampled as sources of melatonin, including saliva, serum, plasma, urine, sweat and tissue extracts. The most direct and convenient way to measure melatonin levels is through saliva analysis. For subject comfort and to minimize influencing the levels during sampling, we performed the saliva sampling as described below.

Experimental design

The test periods chosen were at night. The overall sampling period was set to 90 minutes, including 10 minutes of rest for subjects to calm down, 5 minutes of IPQ, 30 minutes of ANW, and then a resting state of 45 minutes. The sampling points were set at 0, 10, 20, 30, 45, 60 and 90 minutes from the beginning of the experiment (23:00) (Figure 1). The salivary samples of the meditation group members were obtained by the control group members or other helpers.

Subjects and regulations

Twenty subjects (11 women and 9 men) participated in the meditation and control groups. All subjects were in good health; exclusion criteria included epilepsy, psychosis, diseases of the nervous system, and a history of head trauma. Before our meditation experiments, all subjects were first asked to read the experimental instructions and were given an explanation of the test. Each subject filled out the "Subject Information and Agreement form". After each test, a short discussion was held to allow us to collect information on each subject's situation and conditions. The mean age of the subjects was 52.17 ± 2.16 years [mean ± standard error of the mean (SEM); range, 29–64 years], with meditation experience of 15.83 ± 1.42 (5–24) years. They practiced meditation every day, 2.03 ± 0.16 (1–4) times, with a mean practice duration of 57.5 ± 3.98 (30–90) minutes each time. Their average bedtime was 23.58 ± 0.14 (22.5–24.5) o'clock. Thirteen subjects were members of religious groups (age, 29–63 years) and lived in the Taoist Sanctuary. Seven subjects were retired (age, 57–64 years). All subjects were well-trained meditators (Table 1).

Experiments were carried out over 2 days. Different sleep/wake schedules might have different melatonin secretion profiles. Maintenance of normal daily activities of every subject was taken

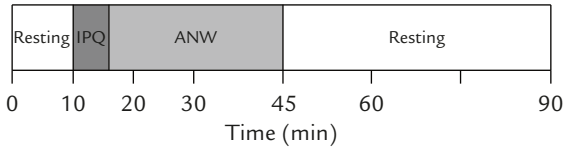

Figure 1. The test periods adopted in the salivary melatonin study of meditation practice. IPQ = Invitation of Primordial Qi; ANW = Allow its Natural Workings.

Table 1. Information on the subjects and self-evaluation of the effect of saliva sampling operation during the meditation process (n = 20)

	Mean ± SEM (range)
What is your age?	52.17 ± 2.16 (29–64)
How many years have you practiced meditation?	15.83 ± 1.42 (5–24)
How many times do you practice every day?	2.03 ± 0.16 (1–4)
How long do you practice each time? (min)	57.50 ± 3.98 (30–90)
When do you go to bed each night? (o'clock)	23.58 ± 0.14 (22.5–24.5)
Does the saliva sampling operation have any adverse effects on your meditation? (1–10 scale)*	2.25 ± 0.19 (1–5)

**The scale numbers are set from 1 to 10, with a 1 meaning no effect at all and a 10 meaning a very seriously adverse effect. Every subject was asked to give a score subjectively right at the end of the experiment. SEM = Standard error mean.*

into consideration during the test to avoid diurnal changes in hormone levels, and to eliminate misleading results from differences in subjects' endogenous circadian phases. A proper period of experiment was carefully chosen. As the average bedtime of the subjects was around 23:35 hours, all experimental procedures and apparatus were prepared at 22:30 hours and performed between 23:00 and 00:30 hours. Furthermore, all subjects participated in the meditation and control groups during these 2 research days. Half of the subjects who participated in the meditation group on the first day were assigned to the control group on the second day, and *vice versa*. Before the test, all subjects were instructed to maintain their normal daily activities, but not to meditate or eat in the 3 hours before the experiment. Subjects were instructed to refrain from coffee, tea, and smoking for at least 4 hours before the test, and to refrain from alcohol and bananas for at least 24 hours before the experiment. During the experimental period, the meditation group was advised to stay alert and not to fall asleep during this process. The control group was advised to sit and rest in the manner in which they preferred, but to avoid talking, sleeping, or walking. Furthermore, when the meditation group was in the IPQ stage, the control group was asked to recite one short poem slowly. During the sampling period, there was no verbal communication. Subjects refrained from eating and avoided beverages that contained artificial colorants, as well as coffee or alcoholic beverages.

Light exposure control

All subjects were asked to reach the test room at 22:30 hours for preparation on the 2 experimental days. All subjects were in the same room; therefore, the light exposure of each individual subject was controlled at the same level on these two experimental nights. During the period 22:50–23:00 hours, all subjects sat in the test room and verbal communication was allowed with the light on. The light was switched off in the test room at exactly 23:00 hours and remained off until the end of the experiment, which allowed only the light of the corridor to pass through the windows. The first 10 minutes was a rest period for subjects to enable them to calm down. No verbal communication was allowed. The subsequent periods were COQS-IPQ for 5 minutes, COQS-ANW for 30 minutes, and 45 minutes for further rest. Light intensity was carefully controlled and maintained at the same level during these two dates. Light levels were monitored throughout the experiment and did not exceed 5 lux.

Sampling and data analysis

To test whether post-meditation melatonin levels differed from baseline levels, saliva samples were obtained using saliva collection tubes (Salivette, Sarstedt Inc., Rommelsdorf, Germany) and analyzed with competitive enzyme-linked immunoassay (Direct Saliva Melatonin ELISA kit; Buhlmann Laboratories, Schonenbuch, Switzerland). Results were analyzed using CODA Automated EIA Analyzer (Bio-Rad, Hercules, USA). The means and SEM of each value of the sampling data were first calculated. As a result of the repeated measurement design of our study, within-subject data were assumed to be correlated. To test the time period effect, a paired *t* test was performed to compare the mean difference between various time points [Post I: $10 < t \leq 45$ min (with 3 time points); Post: $10 < t \leq 90$ min (5 time points); Post II: $45 < t \leq 90$ min (2 time points)] and the baseline [Pre: $0 \leq t \leq 10$ min (2 time points); the average of data on 0 and 10 min] by treatment. One-sided analysis of variance was used for hypothesis testing for the treatment and time effects, and the *p* values reported were one-sided.

Urine serotonin analysis

The main breakdown product of serotonin, 5-HIAA, was analyzed in the urine samples of the 20 subjects. Each subject collected the urine in a 1.5-L bottle with 3 mL 6 N HCl within 6 hours of the experiment on their mediation and control dates. Samples were stored in a cold chamber kept at $< 5^{\circ}$C before being analyzed. The analytical instrument adopted was the high-performance liquid chromatography, which contained the solvent and sample manager, column chamber and

Table 2. Results of the time point effect analysis on melatonin levels

Treatment	Time period*	Mean	SEM	Paired *t* test	
				One-sided *p*	95% CI
Meditation	Pre	8.37	2.13	—	—
	Post I	9.42	2.44	0.043	(0.05, ∝)
	Post	9.93	2.39	<0.001	(0.86, ∝)
	Post II	10.70	2.42	<0.001	(1.26, ∝)
Control	Pre	5.65	1.11	—	—
	Post I	6.51	1.50	0.152	(–0.55, ∝)
	Post	6.70	1.48	0.106	(–0.35, ∝)
	Post II	6.97	1.48	0.064	(–0.12, ∝)

** Pre: $0 \le t \le 10$ min; Post I: $10 < t \le 45$ min; Post: $10 < t \le 90$ min; Post II: $45 < t \le 90$ min. SEM = Standard Error of the Mean; CI = confidence interval.*

detector. The concentrations of the sample constituents were identified and quantified. Analytical results of raw data and chromatography were also obtained. Means and SEM of the data were calculated as the final results.

Results

Subject self-evaluation

All subjects were asked to perform a self-evaluation task after the experiments to ascertain whether the saliva sampling operation had adverse effects on their meditation. The scale numbers were set from 1 to 10, with 1 meaning no effect and 10 meaning a very strong effect. Every subject was asked to give a score immediately after the end of the experiment. Although these scores might have been arbitrary or subjective, they did provide certain information on what happened among the subjects during the sampling operation. The mean self-evaluation score was 2.25 ± 0.19 (range, 1–5; Table 1), which suggested that the saliva sampling operation had a slight effect on the subjects during the meditation process. Most subjects claimed that they were immediately able to return to the good and positive meditating situation after each saliva sampling operation.

Salivary melatonin analysis

Upon processing the data from the melatonin analysis, we first dealt with the baseline homogeneity analysis. The baseline values (average of data at 0 and 10 min) in the meditation/control treatment were 8.37 ± 2.13/5.65 ± 1.11, and the mean baseline difference between meditation and control treatment was 2.72, with a paired *t* test *p* value of 0.060, and the 95% confidence interval of –0.12 to 5.56. We also applied a time point effect analysis. Within the mediation treatment group, the mean level of melatonin at various post-meditation time points (Post I: 9.42 ± 2.44 pg/mL; Post: 9.93 ± 2.39 pg/mL; Post II: 10.70 ± 2.42 pg/mL) was significantly higher than baseline levels (Pre: 8.37 ± 2.13 pg/mL), with $p < 0.05$ for "Post I" and $p < 0.001$ for both "Post" and "Post II" periods. However, within the control treatment group, the mean level of melatonin at various post time points (Post I: 6.51 ± 1.50 pg/mL; Post: 6.70 ± 1.48 pg/mL; Post II: 6.97 ± 1.48 pg/mL) was not significant ($p > 0.05$) in comparison to baseline levels (Pre: 5.65 ± 1.11 pg/mL) (Table 2). The results suggested that the melatonin level was significantly elevated after meditation in the meditation group and almost unchanged in the control group. Figure 2 shows the results of analysis of melatonin levels of the meditation group and the control group. The *p* values of the meditation and control groups between "Pre" and "Post I", "Post", "Post II" are also shown.

Analysis of urine serotonin

The serotonin levels (urine 5-HIAA) of the meditation and control groups for the 20 subjects were

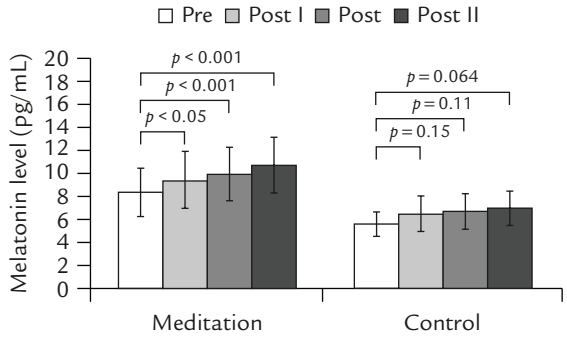

Figure 2. Analysis of melatonin levels in the meditation and control groups. The time periods were Pre: $0 \le t \le 10$ minutes; Post I: $10 < t \le 45$ minutes; Post: $10 < t \le 90$ minutes; Post II: $45 < t \le 90$ minutes. Error bars represent standard error of the mean.

Table 3. Serotonin level in different groups*

Outcome	Meditation group	Control group	*p*
Serotonin (mg/6 hr)†	0.86 ± 0.10	0.96 ± 0.15	0.22

**Data presented as mean ± standard error mean; †urine serotonin levels were analyzed using the samples of the 20 subjects. Each subject collected all urine from the onset point until 6 hours later, on both their mediation and control experiment days.*

analyzed (Table 3). The results showed no statistical difference ($p = 0.22$) between the 5-HIAA levels in the meditation group (0.86 ± 0.10 mg/6 hr) and the control group (0.96 ± 0.15 mg/6 hr).

Discussion

During the experimental process, we carefully controlled the timing and light exposure and each subject participated in the meditation and control groups. Thus any misleading and negative effect due to light-induced suppression and differences in individual circadian cycles should have been reduced. Our results exhibited statistical significance (with $p < 0.05$ for Post I and $p < 0.001$ for Post and Post II periods) and support the hypothesis that COQS can elevate nighttime melatonin levels. These results also support our previous fMRI observation of pineal activation during COQS.[1] All the information suggests that COQS, pineal activation and melatonin levels have a positive interaction, which implies that COQS activates the pineal body and results in nighttime secretion of melatonin. Massion et al,[20] Tooley et al[21] and Harinath et al[22] also found elevation of nighttime melatonin levels. Massion et al measured urine melatonin, whereas Tooley et al and Harinath et al measured plasma melatonin. In our study, although the variation in salivary melatonin levels was not as sensitive as in plasma, salivary melatonin was still significantly increased in COQS for well-trained meditators. As the nighttime melatonin levels are elevated, meditation might influence the effects of melatonin; these include cancer prevention,[3–5] influencing cellular bioenergetics functions[6] and cellular regulation,[7] antioxidant effects,[8–10] anti-aging properties,[11] regulation of human placental function,[12] circadian rhythm,[13–17] immune function[18,19] and any other function that might relate to melatonin.

Revell et al published melatonin daily profiles from their research of circadian phase determination.[29] From these profiles, we see that the daily melatonin levels are higher at midnight and lower in the daytime. As we have already mentioned, Solberg studied melatonin secretion during the meditation process and found that melatonin is decreased after long meditation.[23] Perhaps this discrepancy is due to the fact that they set the experimental period in the daytime (starting from 09:00 hours and lasting for 3 hours). The results of samples taken during in a 3-hour daytime mediation period might also need to be clarified. The ultimate explanation of the decline in melatonin levels, whether temporal or sampling, needs further investigation. Carlson et al investigated a mindfulness-based stress reduction meditation program for early-stage breast and prostate cancer patients, and found no overall changes in melatonin.[24] It is possible that the following two reasons explain their findings: (1) the daytime (14:00 hours) samples used in the melatonin assay could have led to insignificant change; and (2) their subjects were patients who attended a short meditation training course (8 weeks), and the duration of training could have been insufficient. The combined effect of these two or other

reasons might have produced the similarities between the control and test groups. Further testing of these hypotheses is now required.

We also dealt with the mixed effect model analysis. The results showed that the main effect of treatment/time was not significant [$F(1,171) = 1.09$, $p = 0.300$/$F(4,171) = 1.41$, $p = 0.233$]. The interaction between treatment and time was also not significant [$F(4,171) = 0.29$, $p = 0.883$]. All 20 subjects participated in the meditation and control groups, therefore, the "self-anticipating effect" of the meditators (during the "Pre" state and waiting for the meditation period) and the "regulated routine effect" (the daily course and custom of a well-trained meditator to participate in the control group) might have played a role in our results. Neither of these effects is believed to have led to the significant difference between the meditation and control groups.

In this study, we examined the influence of COQS on nighttime salivary melatonin levels. Our results suggest that nighttime melatonin level was significantly elevated for the meditation group but almost unchanged for the control group. This supports the hypothesis that this meditation practice could elevate nighttime melatonin levels. These results also support our previous fMRI observation of positive activation of the pineal body during meditation practices. All the information obtained implies that meditation causes activation of the pineal body, which results in secretion of nighttime melatonin, and which might also have certain physiological effects on the human body. Further studies could be needed to establish the circadian phase as a baseline for each individual, and to determine more conclusively detailed melatonin secretion profiles and daytime melatonin levels under the influence of COQS.

Acknowledgments

Thanks are due to the Tienti Teachings for introducing us to the subjects. We also thank Dr Min-Long Lai (Union Clinical Laboratory, Taipei, Taiwan) for his analytic assistance with the sampling of melatonin and serotonin. In addition, we would like to thank Professor Jen-Pei Liu and Dr Tzu-Chi Lee (Consulting Center for Statistics and Bioinformatics, National Taiwan University, Taipei, Taiwan) for their statistical analysis of the experimental data.

References

1. Liou CH, Hsieh CW, Hsieh CH, et al. Studies of Chinese Original Quiet Sitting by using functional magnetic resonance imaging: exploring the brain activation area of "Invitation of Primordial Qi" stage. *Neuroimage* 2005;26(Suppl 1):S61.
2. Guyton AC, Hall JE. Reproductive and Hormonal Functions of the Male (and Function of the Pineal Gland). In: *Textbook of Medical Physiology*, 11th edition. Philadelphia: W.B. Saunders, 2005:1009–10.
3. Sánchez-Barceló EJ, Cos S, Mediavilla D, et al. Melatonin–estrogen interactions in breast cancer. *J Pineal Res* 2005; 38:217–22.
4. Srinivasan V, Spence DW, Pandi-Perumal SR, et al. Melatonin, environmental light, and breast cancer. *Breast Cancer Res Treat* 2007;103:331–41.
5. Mills E, Wu P, Seely D, et al. Melatonin in the treatment of cancer: a systematic review of randomized controlled trials and meta-analysis, *J Pineal Res* 2005;39:360–6.
6. Acuña-Castroviejo D, Martín M, Macías M, et al. Melatonin, mitochondria, and cellular bioenergetics. *J Pineal Res* 2001;30:65–74.
7. Natarajan M, Reiter RJ, Meltz ML, et al. Effect of melatonin on cell growth, metabolic activity, and cell cycle distribution. *J Pineal Res* 2001;31:228–33.
8. Tan DX, Manchester LC, Hardeland R, et al. Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. *J Pineal Res* 2003;34:75–8.
9. Kaptanoglu E, Palaoglu S, Demirpence E, et al. Different responsiveness of central nervous system tissues to oxidative conditions and to the antioxidant effect of melatonin. *J Pineal Res* 2003;34:32–5.
10. Rodriguez C, Mayo JC, Sainz RM, et al. Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 2004;36:1–9.
11. Magri F, Sarra S, Cinchetti W, et al. Qualitative and quantitative changes of melatonin levels in physiological and pathological aging and in centenarians. *J Pineal Res* 2004; 36:256–61.
12. Iwasaki S, Nakazawa K, Sakai J, et al. Melatonin as a local regulator of human placental function. *J Pineal Res* 2005; 39:261–5.
13. Snyder SH, Zweig M, Axelrod J, et al. Control of the circadian rhythm in serotonin content of the rat pineal gland. *Proc Natl Acad Sci USA* 1965;53:301–5.

14. Zimmerman NH, Menaker M. The pineal gland: a pacemaker within the circadian system of the house sparrow. *Proc Natl Acad Sci USA* 1979;76:999–1003.
15. Takahashi JS, Hamm H, Menaker M. Circadian rhythms of melatonin release from individual superfused chicken pineal glands in vitro. *Proc Natl Acad Sci USA* 1980; 77:2319–22.
16. Nogueira Pires ML, Benedito-Silva AA, Pinto L, et al. Acute effects of low doses of melatonin on the sleep of young healthy subjects. *J Pineal Res* 2001;31:326–32.
17. Arendt J, Skene DJ. Melatonin as a chronobiotic. *Sleep Med Rev* 2005;9:25–39.
18. Nelson RJ, Drazen DL. Melatonin mediates seasonal changes in immune function. *Ann NY Acad Sci* 2000;917:404–15.
19. Majewski P, Adamska I, Pawlak J, et al. Seasonality of pineal gland activity and immune functions in chickens. *J Pineal Res* 2005;39:66–72.
20. Massion AO, Tears J, Hebert JR, et al. Meditation, melatonin and breast/prostate cancer: hypothesis and preliminary data. *Med Hypotheses* 1995;44:39–46.
21. Tooley GA, Armstrong SM, Norman TR, et al. Acute increases in night-time plasma melatonin levels following a period of meditation. *Biol Psychol* 2000;53:69–78.
22. Harinath K, Malhotra AS, Pal K, et al. Effects of Hatha Yoga and Omkar Meditation on cardiorespiratory performance, psychologic profile, and melatonin secretion. *J Altern Comp Med* 2004;10:261–8.
23. Solberg EE, Holen A, Ekeberg O, et al. The effects of long meditation on plasma melatonin and blood serotonin. *Med Sci Monit* 2004;10:CR96–101.
24. Carlson LE, Speca M, Patel KD, et al. Mindfulness-based stress reduction in relation to quality of life, mood, symptoms of stress and levels of cortisol, dehydroepiandrosterone sulfate (DHEAS) and melatonin in breast and prostate cancer outpatients. *Psychoneuroendocrinology* 2004;29: 448–74.
25. Bujatti M, Biederer P. Serotonin, noradrenaline, dopamine metabolites in transcendental meditation-technique. *J Neural Transm* 1976;39:257–67.
26. The T'ienti Teachings. *Introducing 'Chinese Original Quiet Sitting' And 'Serene Quiet Sitting'*. Available at: http://www.tienti.org/about/qsit.php/ [Date accessed: January 10, 2008]
27. Lee YC (Han-ching Lao-jen), Mair DC. Refuge for the Human Spirt. In: *The Ultimate Realm – a New Understanding of Cosmos and Life* 1st edition: *Tempering of the spirit*. T'ienti Teachings Publishing Co., 1994:84–91.
28. Chen JC, Tsai HY, Lee TC, et al. The effect of orthodox celestial emperor (Tian-Di) meditative qigong on electroencephalogram. *J Chinese Med* 1997;8:137–54.
29. Revell VL, Kim H, Tseng CY, et al. Circadian phase determined from melatonin profiles is reproducible after 1 wk in subjects who sleep later on weekends**.** *J Pineal Res* 2005; 39:195–200.