American Journal of Therapeutics 0, 000–000 (2009)

A Randomized, Placebo-Controlled Trial of an Amino Acid Preparation on Timing and Quality of Sleep

William Shell, MD,[1]* Debbie Bullias, BS,[2] Elizabeth Charuvastra, RN,[1] Lawrence A. May, MD,[3] and David S. Silver, MD[4]

This study was an outpatient, randomized, double-blind, placebo-controlled trial of a combination amino acid formula (Gabadone) in patients with sleep disorders. Eighteen patients with sleep disorders were randomized to either placebo or active treatment group. Sleep latency and duration of sleep were measured by daily questionnaires. Sleep quality was measured using a visual analog scale. Autonomic nervous system function was measured by heart rate variability analysis using 24-hour electrocardiographic recordings. In the active group, the baseline time to fall asleep was 32.3 minutes, which was reduced to 19.1 after Gabadone administration ($P = 0.01$, n = 9). In the placebo group, the baseline latency time was 34.8 minutes compared with 33.1 minutes after placebo (P = nonsignificant, n = 9). The difference was statistically significant ($P = 0.02$). In the active group, the baseline duration of sleep was 5.0 hours (mean), whereas after Gabadone, the duration of sleep increased to 6.83 ($P = 0.01$, n = 9). In the placebo group, the baseline sleep duration was 7.17 $\pm$ 7.6 compared with 7.11 $\pm$ 3.67 after placebo (P = nonsignificant, n = 9). The difference between the active and placebo groups was significant ($P = 0.01$). Ease of falling asleep, awakenings, and am grogginess improved. Objective measurement of parasympathetic function as measured by 24-hour heart rate variability improved in the active group compared with placebo. An amino acid preparation containing both GABA and 5-hydroxytryptophan reduced time to fall asleep, decreased sleep latency, increased the duration of sleep, and improved quality of sleep.

Keywords: amino acid, medical food, sleep, trial

INTRODUCTION

The management of sleep disorders continues to be a problem, particularly because hypnotic drugs have side effects that are dose-related.[1] The problems associated with hypnotic drugs include abolishment of stage IV sleep, reduction of REM sleep, continued morning grogginess, residual cognitive disorders, tolerance, and long-term dependency.[2,3] The inherent difficulties with these drugs are magnified by the large number of people that manifest sleep disorders. Alternative medications such as tricyclic antidepressants and antiepileptics are not complicated with dependency issues, but otherwise have a similar side effect profile. Even a small incidence of side effects is important when the population at risk is large.

Certain amino acids are known to influence sleep cycles, including sleep latency and duration of sleep.[4–7] The use of amino acids to influence sleep cycles has been handicapped by a large dose requirement for individual amino acids, attenuation of response, and potential side effects of large dose amino acids. Accordingly, there is a need for a low-dose amino acid system to modify sleep cycles and provide an alternative to hypnotic and other medications.

[1]*Targeted Medical Pharma, Inc.;* [2]*Physician Therapeutics;* [3]*UCLA School of Medicine; and* [4]*Cedars Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA.*
Support provided by Targeted Medical Pharma, Inc.
**Address for correspondence and reprint requests to: William Shell, MD, 2980 Beverly Glen Circle, Suite 301, Los Angeles, CA 90077. E-mail: weshell@ptlcentral.com*

We have developed an amino acid-based formula that contains 5-hydroxytryptophan, GABA and choline in low dose (termed Gabadone). 5-hydroxytryptophan and GABA acids are known to influence sleep cycles. 5-hydoroxytryptophan, a tryptophan analog, is believed to influence both sleep latency and apneic events. GABA is implicated in improving sleep latency,[8] the time to fall asleep.[9] Choline was added to the preparation because there are indications that choline, like acetylcholine, potentiates bursts of REM sleep while facilitating stage IV sleep.[10–14] Previous studies have examined the use of these amino acids alone and not in combination.

In this pilot study, we performed a randomized, double-blind, placebo-controlled trial of Gabadone in patients with altered sleep. The primary end points included ability to fall asleep and assessment of sleep quality.[15,16] In addition, a 24-hour electrocardiographic measurement of heart rate variability was used to objectively assess sleep cycles.[17]

MATERIALS AND METHODS

Study design

Eighteen subjects were randomized to treatment and placebo. Each of the 18 subjects underwent baseline examination to include a sleep study questionnaires and 24-hour electrocardiographic recording. Nine subjects were randomized to a 1-week ingestion of Gabadone, two capsules at bedtime, and nine subjects were randomized to a 1-week ingestion of placebo, two capsules at bedtime. Each morning after ingestion of either active or placebo, the subject filled out sleep questionnaires, including both PSEQ and Leeds Sleep Evaluation Visual Analog formats. On the seventh day, a repeat 24-hour electrocardiographic examination was performed in all 18 subjects. Baseline and day 7 data were analyzed.

Patient selection

Subjects were identified by solicitation of persons interested in taking a formula that may support restful sleep. Inclusion criteria were male and females older than age 18 and younger than age 65 with a history of intermittent nonrestorative sleep.

Patient exclusions were subjects currently taking prescription sleeping medication, subjects who have previously taken Gabadone, patients with known endocrine disease, pregnant or lactating females, and subjects with implanted pacemakers or other implanted electrical devices. Patients were asked to maintain their regular sleep regimen.

Data collection

At baseline, the following measurements were taken: weight, percent body fat, age, sex, Pittsburgh Sleep Quality Index (PSQI-B), and Leeds Sleep Evaluation Visual Analog Scale (LSEQ-B). On each of day one through six, the PSQI and LSEQ were obtained in the morning after awakening. On day seven, the LSEQ and PSQI were filled out and the patient returned to the clinic. The forms were obtained and baseline measurements were repeated.

The day before baseline measurements were taken, a 24-hour electrocardiogram was recorded. On day six, a second 24-hour electrocardiographic recording was begun and completed on day seven.

The Gabadone or placebo was taken at bedtime on days one through seven.

Visual analog scale measurement of sleep quality the leeds sleep evaluation questionnaire

The LSEQ Sleep Valuation Questionnaire has been specifically designed to monitor subjectively perceived changes in sleep duration. The LSEQ consists of a 100-mm visual analog scale. Four factors were measured on each LSEQ, including: 1) falling asleep; 2) quality of sleep; 3) ease of waking from sleep; and 4) behavior after awakening. LSEQ was compared with the subjects' usual sleep pattern. A lower score on the LSEQ indicates better quality of sleep.

Pittsburgh sleep quality index

The PSQI is a validated self-report questionnaire that provides specific sleep-related information and a rating of sleep related factor on a Likert scale. The specific sleep information includes bedtime, rising time, minutes to fall asleep, and actual hours slept.

We also collected the number of awakenings per night. The subjective sleep quality indices included quality of sleep, daytime alertness, nighttime awakenings, respiratory distress, physical discomfort, frequency of dreams, disturbing dreams, perception of snoring, and daytime dysfunction. For each patient, a total Likert score was generated.

The data analyzed from the PSQI included duration of sleep total amount of sleep, sleep efficiency, and subjective sleep quality. On day seven, daytime dysfunction was analyzed.

The subjects were blinded to the nature of the capsule at the time the LSEQ and PSQI questionnaires are administered. The data were entered before the codes were broken.

Holter monitoring

Patients underwent 24-hour electrocardiographic monitoring using a three-lead system. The data were transferred to a Reynold's Medical System Analyzer (Pathfinder). That digitized data were analyzed for arrhythmia and heart rate variability. The heart rate variability was analyzed in both the time and frequency domain. We used methods described in the *American Journal of Medicine*. Both 24-hour and circadian activation of parasympathetic function were measured.

Gabadone

The amino acid preparation contained choline bitartrate, 5-hydroxytryptophan as griffonia extract, GABA, grape seed extract, hydrolyzed whey protein, valerian extract, ginkgo biloba, glutamic acid, and cocoa. An evening dose of two capsules contained 2000 mg.

Primary study end points were the difference in sleep latency time, duration of sleep, and perceived am grogginess.

RESULTS

Time to fall asleep—sleep latency

The results are tabulated in Table 1. In the active group, the baseline time to fall asleep was 32.3 minutes, whereas after Gabadone, the time was 19.1 (t = 2.91, $P < 0.01$, n = 9, Fig. 1). In the placebo group, the baseline latency time was 34.8 minutes compared with 33.1 1 week later (P = nonsignificant, n = 9). The difference at day 7 between Gabadone and placebo was statistically significant ($P < 0.01$).

Hours slept

In the active group, the duration of sleep was 5.00 hours at baseline and increased by day 7 after administration of Gabadone to 6.83 hours (t = –3.14, $P < 0.01$, n = 9). In the placebo group, the baseline sleep duration was 7.16 compared with 7.11 at day 7 (P = nonsignificant, n = 9). The difference was between groups was statistically significant ($P < 0.01$).

Ease of falling asleep

The ease of falling asleep was measured using a 100-point visual analog scale. In the active group, the baseline perceived ease of falling asleep 44.4 on a 100-point scale, whereas after taking Gabadone, it was 64.8 ($P = 0.02$, n = 9). In the placebo group, the baseline was 43 and at day 7 was 54 ($P = 0.11$, n = 9). The differences were not statistically significant.

Awakenings

Perceived awakenings are associated with apneic events. In the active group, the baseline number of perceived awakenings was 4.3 compared with 2.6 after Gabadone administration ($P < 0.01$, n = 9). In the placebo group, the baseline awakenings were 2.8 and at day 7 was 3.1 (P = nonsignificant). The difference was statistically significant ($P = 0.02$, n = 18).

Minutes awake during awakenings

In the active group, the number of minutes during the awakenings was perceived as 21.1 minutes during baseline and 8.3 after Gabadone ($P = 0.02$, n = 9). In the placebo group, the baseline was 46.9 minutes and at day 7 was 36.7 (P = nonsignificant).

Restorative sleep and AM grogginess

As a measure of restorative sleep, we assessed perceived am grogginess. In the active group, the baseline was 30.6 on a 100-point scale and was 11.1 after Gabadone ($P < 0.01$, n = 9). In the placebo group, the baseline was 67.8 and 65 after placebo (P = nonsignificant, n = 9). The difference between the two groups was statistically significant.

Table 1. Study results.

Variable	Value Baseline Active	Value Day 7 Active	*P* Value	Value Baseline Placebo	Value Day 7 Placebo	*P* Value	Difference Active	Difference Placebo	*P* Value
Time to sleep (minutes)	32.33	19.11	0.01	34.83	33.11	0.89	13.22	1.72	0.02
Hours slept	5.00	6.83	0.01	7.17	7.11	0.46	1.83	−0.06	0.01
Ease of falling asleep	44.44	64.78	0.02	43.00	54.11	0.11	20.33	11.11	0.23
Number of awakenings	4.33	2.56	<0.01	2.78	3.11	0.36	−1.78	0.33	0.03
Minutes awake	21.11	8.33	0.02	46.89	36.67	0.15	12.78	10.22	0.41
AM grogginess (minutes)	30.56	11.11	0.01	67.78	65.00	0.32	19.44	2.78	0.03
24-hour heart rate	182.37	346.94	0.04	194.01	213.40	0.25	164.56	19.39	0.05
Circadian index	−392.89	735.89	0.01	197.43	−402.78	0.05	1100.49	−599.00	<0.01

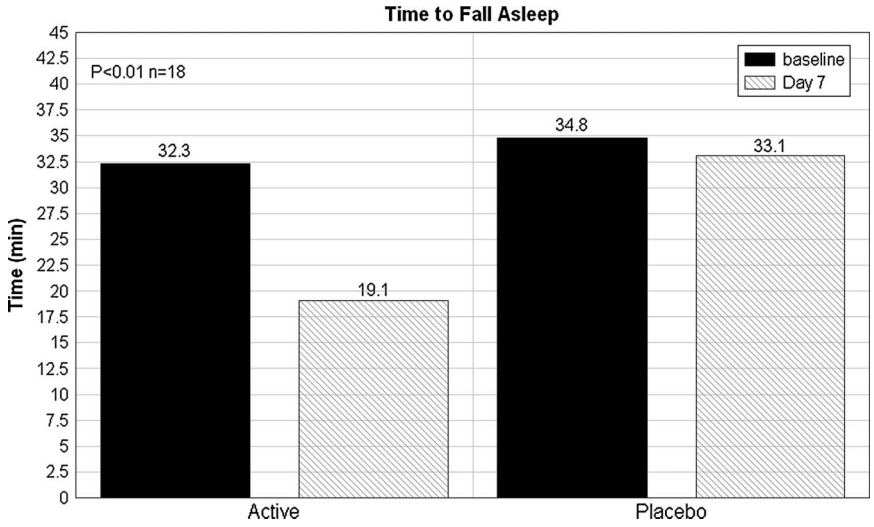

FIGURE 1. Sleep latency. The time to fall asleep was assessed at baseline and day 7 by questionnaire and measured in minutes.

Perceived snoring

Perceived snoring is another measure of improved sleep quality. Patients on Gabadone had a 25% reduction in perceived snoring, which was a statistically significant reduction, whereas patients taking placebo noted no significant difference.

Autonomic nervous system function

Assessment of parasympathetic autonomic nervous system function, an objective measure of nighttime autonomic function, showed substantial improvement in patients taking Gabadone when compared with placebo.

There were no adverse events reported in either the treatment or placebo groups.

DISCUSSION

The carefully timed activation of the three neurotransmitters—serotonin, acetylcholine, and GABA—is required to initiate sleep, maintain sleep, induce restorative sleep, and increase REM sleep time.[18–25] If the timing and secretion of these three neurotransmitters are altered, normal sleep cycles and restorative sleep do not occur. For example, the benzodiazepine drugs, including Ambien, reduce sleep latency but abolish phase IV sleep and REM sleep.[26–30]

Serotonin initiates sleep and reduces measured sleep latency (time to fall asleep).[31–36] The timing of serotonin release is critical to initiation of sleep. The amount of serotonin release is also critical. At the initiation of sleep, a small amount of serotonin is released. The peak concentration of serotonin occurs within several hours after sleep initiation. The failure to produce serotonin, the production of insufficient serotonin, or the production of excessive amounts of serotonin will result in the failure to initiate sleep.

Serotonin function and deficiency is intimately involved in sleep apnea, snoring, REM sleep, and depression associated with sleep disorders. An alteration of the tryptophan/serotonin axis will result in altered sleep patterns. Appropriate production and release of serotonin will ameliorate sleep disorders.

Production of acetylcholine after initiation of sleep results in restorative delta IV sleep.[37–49] After the burst of serotonin that initiates sleep, acetylcholine release increases the duration of phase IV restorative sleep. Acetylcholine production in the sleep centers also increases the frequency and duration of REM episodes. The commonly used hypnotics, including Ambien, abolish phase IV sleep and inhibit REM sleep.

GABA is the main inhibitory neurotransmitter.[50–52] The initiation and maintenance of sleep depends on the availability of GABA to the GABA receptors. The most commonly used drug hypnotics act by sensitizing the GABA receptors. GABA provides general inhibition of the nervous system to allow sleep.

Gabadone contains a formula blend of selected GRAS (generally regarded as safe by the US Food and Drug Administration) ingredients that are derived from the normal human food chain. The primary ingredients are key amino acids, the building blocks of proteins. The Gabadone formula is designed to increase the function of the neurotransmitters serotonin, acetylcholine, and GABA in patients with sleep disorders.

The Gabadone formula is based on a five-component patent pending process to provide for the conversion of a neurotransmitter precursor into a neurotransmitter. The five-component system includes: 1) each neurotransmitter is synthesized from an amino acid precursor; 2) stimulation of the uptake of the neurotransmitter precursor is required to initiate the conversion of a precursor to a neurotransmitter; 3) because most neurons are inhibited from firing, an adenosine antagonist such as and cocoa powder is added to disinhibit the neuron; 4) stimulation of neurons to release a specific neurotransmitter is required; and 5) a system must be used to prevent attenuation of the precursor response, a well-known precursor phenomena. Gabadone has been formulated to encompass this five-component system.

Gabadone is designed to produce neurotransmitters related to physiological functions, including initiation of sleep, maintenance of sleep, and reinduction of sleep if awakening occurs during the night. In the Gabadone formulation, choline is used as a precursor to acetylcholine.[53–55] 5-hydroxytryptophan, derived from the naturally occurring form known as Griffonia Seed Extract, is used as a precursor to serotonin[31,56,57]; and GABA is directly administered as an inhibitory neurotransmitter. Ginkgo biloba is used as an uptake stimulator. Glutamic acid is used to produce glutamate, a neuronal stimulator. Cocoa is used to disinhibit the adenosine brake. Grape seed extract, containing polyphenols,[58–61] is used to avoid the attenuation usually associated with neurotransmitter precursor administration. GABA is administered as an inhibitory neurotransmitter.[62]

Many sleep disorders are associated with inadequate availability of the key neurotransmitter precursors choline, 5-hydroxytryptophan, and GABA. Determination of the Recommended Dietary Allowance of a dietary ingredient in normal subjects is frequently accomplished by analyzing blood levels of the nutrient in normal subjects. Assessing the nutritional deficiency in the presence of a disease such as a sleep disorder is more complex. US Food and Drug Administration scientists have proposed a physiological methodology to determine the nutritional deficiency during a disease. For example, if a physiological parameter such as sleep latency is measured, administration of the nutrient such as 5-hydroytryptophan resulting in the improvement of the physiological parameter improves such as reduction of sleep latency, you have established the presence of the nutrient deficiency in the disease. These studies indicate that sleep disorders are associated with the nutrient deficiencies of choline, 5-hydroxytryptophan, and GABA.

This study primarily used questionnaire data to assess primary and secondary end points. In addition, analysis of 24-hour electrocardiogram-derived analysis of heart rate variability was also performed. Heart rate variability measured over 24 hours is not altered by placebo. The improvement observed suggests that neurotransmitter function associated with the parasympathetic autonomic nervous system improved.

This pilot study supports the concept that combining neurotransmitter precursors may help in the management of sleep disorders. Larger, randomized trials would appear to be indicated.

REFERENCES

1. Ancoli-Israel S. Insomnia in the elderly: a review for the primary care practitioner. *Sleep*. 2000;23(Suppl 1):S23–S30.
2. Bastien CH, LeBlanc M, Carrier J, et al. Sleep EEG power spectra, insomnia, and chronic use of benzodiazepines. *Sleep*. 2003;26 3:313–317.
3. Johns M, Hocking B. Daytime sleepiness and sleep habits of Australian workers. *Sleep*. 1997;20:844–849.
4. Curzon G. The role and control of brain 5-hydroxytryptamine. *Acta Neurol (Napoli)*. 1977;32:121–131.
5. George CF, Millar TW, Hanly PJ, et al. The effect of L-tryptophan on daytime sleep latency in normals: correlation with blood levels. *Sleep*. 1989;12:345–353.
6. Gottesmann C. GABA mechanisms and sleep. *Neuroscience*. 2002;111:231–239.
7. Bowers MB Jr, Hartmann EL, Freedman DX. Sleep deprivation and brain acetylcholine. *Science*. 1966;153: 1416–1417.
8. Maurizi CP. The therapeutic potential for tryptophan and melatonin: possible roles in depression, sleep, Alzheimer's disease and abnormal aging. *Med Hypotheses*. 1990;31:233–242.
9. Nakajima T, Sasaki T, Nakagome K, et al. Comparison of the effects of zolpidem and zopiclone on nocturnal sleep and sleep latency in the morning: a cross-over study in healthy young volunteers. *Life Sci*. 2000;67:81–90.
10. Calvo JM, Datta S, Quattrochi J, et al. Cholinergic microstimulation of the peribrachial nucleus in the cat. II. Delayed and prolonged increases in REM sleep. *Arch Ital Biol*. 1992;130:285–301.
11. Jasper HH, Tessier J. Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. *Science*. 1971;172:601–602.
12. Koella WP. The organization and regulation of sleep. A review of the experimental evidence and a novel integrated model of the organizing and regulating apparatus. *Experientia*. 1984;40:309–338.
13. McGinty DJ, Drucker-Colin RR. Sleep mechanisms: biology and control of REM sleep. *Int Rev Neurobiol*. 1982;23:391–436.
14. Mendelson WB. Neurotransmitters and sleep. *J Clin Psychiatry*. 2001;62(Suppl 10):5–8.

15. Abetz L, Arbuckle R, Allen RP, et al. The reliability, validity and responsiveness of the Restless Legs Syndrome Quality of Life questionnaire (RLSQoL) in a trial population. *Health Qual Life Outcomes*. 2005;3:79.
16. Ancolio C, Tardieu S, Soubrouillard C, et al. A randomized clinical trial comparing doses and efficacy of lormetazepam tablets or oral solution for insomnia in a general practice setting. *Hum Psychopharmacol*. 2004;19:129–134.
17. Haley RW, Vongpatanasin W, Wolfe GI, et al. Blunted circadian variation in autonomic regulation of sinus node function in veterans with Gulf War syndrome. *Am J Med*. 2004;117:469–478.
18. Wurtman RJ, Lieberman HR. Melatonin secretion as a mediator of circadian variations in sleep and sleepiness. *J Pineal Res*. 1985;2:301–303.
19. Giedke H. On the role of serotonin in sleep regulation and depression. A commentary on 'Neurobiological bases for the relation between sleep and depression' (J Adrien). *Sleep Med Rev*. 2003;7:101–102.
20. Grazia de SM, Imeri L, De MW, et al. Sleep regulation: interactions among cytokines and classical neurotransmitters. *Adv Neuroimmunol*. 1995;5:189–200.
21. Kayama Y, Koyama Y. Control of sleep and wakefulness by brainstem monoaminergic and cholinergic neurons. *Acta Neurochir Suppl*. 2003;87:3–6.
22. Mendelson WB. Neurotransmitters and sleep. *J Clin Psychiatry*. 2001;62(Suppl 10):5–8.
23. Morrison JL, Sood S, Liu H, et al. GABAA receptor antagonism at the hypoglossal motor nucleus increases genioglossus muscle activity in NREM but not REM sleep. *J Physiol*. 2003;548:569–583.
24. Penalva RG, Lancel M, Flachskamm C, et al. Effect of sleep and sleep deprivation on serotonergic neurotransmission in the hippocampus: a combined in vivo microdialysis/EEG study in rats. *Eur J Neurosci*. 2003;17:1896–1906.
25. Seigneur J, Kroeger D, Nita DA, et al. Cholinergic action on cortical glial cells in vivo. *Cereb Cortex*. 2006;16: 655–668.
26. Massironi G, Galluzzi S, Frisoni GB. Drug treatment of REM sleep behavior disorders in dementia with Lewy bodies. *Int Psychogeriatr*. 2003;15:377–383.
27. Casey DE. The relationship of pharmacology to side effects. *J Clin Psychiatry*. 1997;58(Suppl 10):55–62.
28. Jobert M, Poiseau E, Jahnig P, et al. ECG activity in the sleep of insomniac patients under the influence of lormetazepam and zopiclone. *Neuropsychobiology*. 1995; 31:204–209.
29. Lemoine P, Allain H. Induction of sleep. *Sleep*. 1996; 19(Suppl):S1–S6.
30. Wagner J, Wagner ML, Hening WA. Beyond benzodiazepines: alternative pharmacologic agents for the treatment of insomnia. *Ann Pharmacother*. 1998;32:680–691.
31. Bender DA. Biochemistry of tryptophan in health and disease. *Mol Aspects Med*. 1983;6:101–197.
32. Blois R, Feinberg I, Gaillard JM, et al. Sleep in normal and pathological aging. *Experientia*. 1983;39:551–558.
33. Buguet A. Is sleeping sickness a circadian disorder? The serotonergic hypothesis. *Chronobiol Int*. 1999;16:477–489.
34. Curzon G. The role and control of brain 5-hydroxytryptamine. *Acta Neurol (Napoli)*. 1977;32:121–231.
35. Drucker-Colin RR, Spanis CW. Is there a sleep transmitter? *Prog Neurobiol*. 1976;6:1–22.
36. Espana RA, Scammell TE. Sleep neurobiology for the clinician. *Sleep*. 2004;27:811–820.
37. Carchedi F, Di CM, Casacchia M, et al. Sleep: biochemical correlations and pharmacological considerations. *Clin Ter*. 1979;91:3–10.
38. Celesia GG, Jasper HH. Acetylcholine released from cerebral cortex in relation to state of activation. *Neurology*. 1966;16:1053–2063.
39. Crouzier D, Baubichon D, Bourbon F, et al. Acetylcholine release, EEG spectral analysis, sleep staging and body temperature studies: a multiparametric approach on freely moving rats. *J Neurosci Methods*. 2006;151:159–167.
40. Domino EF, Yamamoto K, Dren AT. Role of cholinergic mechanisms in states of wakefulness and sleep. *Prog Brain Res*. 1968;28:113–133.
41. Gaillard JM. Neurochemical regulation of the states of alertness. *Ann Clin Res*. 1985;17:175–184.
42. Gillin JC, Sitaram N, Mendelson WB. Acetylcholine, sleep, and depression. *Hum Neurobiol*. 1982;1:211–219.
43. Gillin JC, Mendelson WB, Sitaram N, et al. The neuropharmacology of sleep and wakefulness. *Annu Rev Pharmacol Toxicol*. 1978;18:563–579.
44. Graves L, Pack A, Abel T. Sleep and memory: a molecular perspective. *Trends Neurosci*. 2001;24:237–243.
45. Hilakivi I. Biogenic amines in the regulation of wakefulness and sleep. *Med Biol*. 1987;65:97–104.
46. Holman RB, Elliott GR, Barchas JD. Neuroregulators and sleep mechanisms. *Annu Rev Med*. 1975;26:499–520.
47. Jones BE. From waking to sleeping: neuronal and chemical substrates. *Trends Pharmacol Sci*. 2005;26:578–586.
48. Jones BE. The organization of central cholinergic systems and their functional importance in sleep–waking states. *Prog Brain Res*. 1993;98:61–71.
49. Kalia M. Neurobiology of sleep. *Metabolism*. 2006; 55(Suppl 2):S2–S6.
50. Manfridi A, Brambilla D, Mancia M. Sleep is differently modulated by basal forebrain GABA(A) and GABA(B) receptors. *Am J Physiol Regul Integr Comp Physiol*. 2001; 281:R170–R175.
51. Monti JM, Jantos H, Monti D. Increase of waking and reduction of NREM and REM sleep after nitric oxide synthase inhibition: prevention with GABA(A) or adenosine A(1) receptor agonists. *Behav Brain Res*. 2001;123:23–35.
52. Swainston HT, Keating GM. Zolpidem: a review of its use in the management of insomnia. *CNS Drugs*. 2005;19: 65–89.
53. Babb SM, Ke Y, Lange N, et al. Oral choline increases choline metabolites in human brain. *Psychiatry Res*. 2004; 130:1–9.
54. Blusztajn JK, Wurtman RJ. Choline and cholinergic neurons. *Science*. 1983;221:614–620.
55. Erickson CK, Matchett JA. Correlation of brain amine changes with ethanol-induced sleep-time in mice. *Adv Exp Med Biol*. 1975;59:419–430.

56. Fernstrom JD, Wurtman RJ. Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science*. 1972;178:414–416.
57. Fernstrom JD, Wurtman RJ. Nutrition and the brain. *Sci Am*. 1974;230:84–91.
58. Dulloo AG, Seydoux J, Girardier L, et al. Green tea and thermogenesis: interactions between catechin–polyphenols, caffeine and sympathetic activity. *Int J Obes Relat Metab Disord*. 2000;24:252–258.
59. Luceri C, Caderni G, Sanna A, et al. Red wine and black tea polyphenols modulate the expression of cycloxygenase-2, inducible nitric oxide synthase and glutathione-related enzymes in azoxymethane-induced f344 rat colon tumors. *J Nutr*. 2002;132:1376–1379.
60. Richelle M, Tavazzi I, Offord E. Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa, and tea) prepared per cup serving. *J Agric Food Chem*. 2001;49:3438–3442.
61. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. *J Nutr*. 2000;130(Suppl):2073S–2085S.
62. Shi J, Yu J, Pohorly JE, et al. Polyphenolics in grape seeds—biochemistry and functionality. *J Med Food*. 2003;6: 291–299.