

# Evening intake of $\alpha$ -lactalbumin increases plasma tryptophan availability and improves morning alertness and brain measures of attention<sup>1–3</sup>

C Rob Markus, Lisa M Jonkman, Jan HCM Lammers, Nicolaas EP Deutz, Marielle H Messer, and Nienke Rigtering

## ABSTRACT

**Background:** Brain serotonin function is thought to promote sleep regulation and cognitive processes, whereas sleep abnormalities and subsequent behavioral decline are often attributed to deficient brain serotonin activity. Brain uptake of the serotonin precursor tryptophan is dependent on nutrients that influence the availability of tryptophan via a change in the ratio of plasma tryptophan to the sum of the other large neutral amino acids (Trp:LNAA).

**Objective:** We tested whether evening consumption of  $\alpha$ -lactalbumin protein with an enriched tryptophan content of 4.8 g/100 g increases plasma Trp:LNAA and improves alertness and performance on the morning after sleep, particularly in subjects with sleep complaints.

**Design:** Healthy subjects with ( $n = 14$ ) or without ( $n = 14$ ) mild sleep complaints participated in a double-blind, placebo-controlled study. The subjects slept at the laboratory for 2 separate nights so that morning performance could be evaluated after an evening diet containing either tryptophan-rich  $\alpha$ -lactalbumin or tryptophan-low placebo protein. Evening dietary changes in plasma Trp:LNAA were measured. Behavioral (reaction time and errors) and brain measures of attention were recorded during a continuous performance task.

**Results:** Evening  $\alpha$ -lactalbumin intake caused a 130% increase in Trp:LNAA before bedtime ( $P = 0.0001$ ) and modestly but significantly reduced sleepiness ( $P = 0.013$ ) and improved brain-sustained attention processes ( $P = 0.002$ ) the following morning. Only in poor sleepers was this accompanied by improved behavioral performance ( $P = 0.05$ ).

**Conclusion:** Evening dietary increases in plasma tryptophan availability for uptake into the brain enhance sustained alertness early in the morning after an overnight sleep, most likely because of improved sleep. *Am J Clin Nutr* 2005;81:1026–33.

**KEY WORDS** Sleep complaints, continuous performance task, attention,  $\alpha$ -lactalbumin, tryptophan, event-related brain activity

## INTRODUCTION

Reduced alertness after poor sleep often deteriorates cognitive-behavioral functioning (1). Most of the observed negative effects are on performance that requires sustained attention, which are recognized by vigilance tasks that are repetitive, simple, and of long duration (2, 3). Vigilance studies indicate that sleepiness progressively reduces attention and efficient stimulus detection (4), as evidenced by lower behavioral responsiveness and by reduced parietal event-related brain activity that occurs

$\approx 300$  ms after stimulus onset (P300), which indicates reduced attention processing (5). Consequently, reduced vigilance after poor sleep often leads to dramatic catastrophes and traffic accidents (6). Moreover, sleep disturbances often precede affective complaints and depressive symptoms (7). From a prevention perspective, it is important to search for mechanisms that mediate the effect of poor sleep on cognitive performance.

The effects of poor sleep on performance may be partly mediated by a biochemical imbalance of brain serotonin (5-hydroxytryptamine, or 5-HT). Brain 5-HT seems to be involved in the regulation of sleep and cognitive processes (8, 9), and sleep abnormalities and cognitive decline in clinical populations are partly attributable to deficient brain 5-HT activity (10, 11). Accordingly, reduced 5-HT concentrations resulting from the exhaustion of its plasma precursor tryptophan was found to provoke sleep abnormalities seen in depression (10, 12), whereas increases in available plasma tryptophan for uptake into the brain improved sleep in different subjects (9, 13, 14). These sleep-promoting effects of tryptophan may be stronger in subjects with sleep complaints, because they are vulnerable to the sleep-reducing effects of tryptophan depletion (12). In further support, sleep complaints are often accompanied by stress and emotional decline (15, 16) that, in turn, may alter 5-HT receptor sensitization (17, 18) and subsequently increase tryptophan and 5-HT vulnerability (18). Even though these findings suggest positive relations between tryptophan availability and sleep, beneficial effects of increases in evening tryptophan on morning alertness have not yet been investigated. Instead, studies exclusively focused on the effects of tryptophan alterations on electroencephalographic (EEG) sleep recordings without exploring its relevance to morning performance.

Available brain tryptophan and 5-HT in humans is thought to rise with the intake of L-tryptophan or carbohydrates, which raise

<sup>1</sup> From the Departments of Experimental Psychology (CRM, MHM, and NR) and Neurocognition (LMJ) and the Biomedical Center (NEPD), University of Maastricht, Maastricht, Netherlands, and TNO Quality of Life, Zeist, Netherlands (JHCML).

<sup>2</sup> The  $\alpha$ -lactalbumin protein was provided by Davisco Foods International (Eden Prairie, Minnesota).

<sup>3</sup> Reprints not available. Address correspondence to CR Markus, University of Maastricht, Department of Experimental Psychology, PO Box 6200 MD Maastricht, Netherlands. E-mail: r.markus@psychology.unimaas.nl.

Received July 1, 2004.

Accepted for publication January 14, 2005.

the ratio of plasma tryptophan to large neutral amino acids (Trp:LNAA) (19, 20). These methods, however, may have physical side effects, and carbohydrate ingestion showed no or only small behavioral effects due to modest tryptophan alterations (21, 22). An alternative method for raising tryptophan availability, and probably 5-HT, may be the intake of  $\alpha$ -lactalbumin (A-LAC) protein, which contains the highest tryptophan content of all food-protein sources (23). Recently, it was shown that A-LAC containing 1.3 g/100 g tryptophan causes a 48% increase in plasma Trp:LNAA (18). In addition, A-LAC was found to increase brain 5-HT concentrations (24) and to reduce sleep disturbances in rats (25). The present study investigated whether the evening intake of A-LAC may improve morning performance in subjects with sleep complaints. To enhance the effects of A-LAC on Trp:LNAA, A-LAC protein was used with a greater tryptophan content (4.8 g/100 g) than that used in previous studies (18).

## SUBJECTS AND METHODS

### Subjects

Dutch University students ( $n = 235$ ) filled out the Dutch Groningen Sleep Questionnaire (GSQ; 26) and a questionnaire package concerning personal details. From the highest quartile of the GSQ score ( $\bar{x} \pm \text{SD}$ :  $22 \pm 2$ ; range: 20–27), 14 subjects (7 men and 7 women aged  $22 \pm 2$  y) were selected for the poor-sleepers group; from the lowest quartile of the GSQ score ( $16 \pm 1$ ; range: 15–17), 14 subjects (7 men and 7 women aged  $22 \pm 3$  y) were selected for the “good sleepers” group. Exclusion criteria for participation were chronic and current illness; a history of psychiatric or medical illness; medication use; metabolic, hormonal, or intestinal diseases; irregular diets or deviant eating habits; excessive use of alcohol, cigarettes, coffee, or drugs; allergy to milk products; and pregnancy as assessed by health and lifestyle questionnaires. All subjects who participated in the experiment had a body mass index (BMI; in  $\text{kg}/\text{m}^2$ ) in the normal range (BMI: 19–26), were nonsmokers, and were not allowed to drink alcohol or take any drug for 2 d before and during the experiment. The study was approved by the Medical Ethics Committee (MEC 03-0625) of the Academic Hospital Maastricht (Maastricht, Netherlands) and complied with the requirements of the European Council of Good Clinical Practice adopted by the 52nd World Medical Association General Assembly, Edinburgh, United Kingdom (October 2000). All subjects gave their informed consent to participate in the experiment.

### Methods

The experimental procedure was conducted according to a double-blind, placebo-controlled design, and the data analysis was conducted without knowledge of the subject’s assignment and dietary condition.

During 2 experimental sessions, subjects with and without sleep complaints stayed at the laboratory for an overnight sleep for the monitoring of their early morning alertness and mental functioning under task-related EEG-ERP registration, after either an evening diet containing tryptophan-enriched A-LAC or standard protein (placebo). The diets were isoenergetic and contained equal amounts of protein, carbohydrate, and fat. The order of presentation of the A-LAC and placebo diets was counterbalanced between subjects. Both experimental sessions were separated by a 3- or 4-wk period to allow for the control of the

women’s menstrual cycles, whereas women using oral contraceptives participated during the time when they actually consumed the contraception pill. Two weeks before the start of the experiment, all subjects were invited to the laboratory to familiarize themselves with the sleep conditions and experimental procedures.

For each experimental session, 2 subjects arrived at the laboratory at 1800 (see **Figure 1** for an overview). The subjects were instructed to consume a standardized lunch at 1230, for which they received precise descriptions and a food diary, and to fast from 1300 onward (only water or tea without sugar was permitted). After their arrival, the subjects consumed an evening meal together with an A-LAC or placebo milk shake at 1830, which was followed by a second milk shake 1 h later (at 1930). Two hours after intake of the second drink, a blood sample was taken and collected in a tube. Then, subjects were allowed to watch television or to read in a private guest room and also conducted a computer test as part of another study. At 2250, the subjects filled in a computerized Stanford Sleepiness Scale (27) and then went to bed at 2300 (lights off) in a private bedroom until 0700 in the morning (lights on). After showering, the subjects filled in a second sleepiness scale and were brought into the laboratory. After the EEG electrodes were connected, the subjects were seated in front of a computer screen in an electrically shielded and soundproof cabin and conducted a continuous performance task (CPT). Before the CPT started, the subjects first had to run through a practice session until a predetermined criterion was reached ( $>80\%$  hits and  $<500$  ms). After completion of the CPT, the electrodes were disconnected and the subjects were allowed to go home.

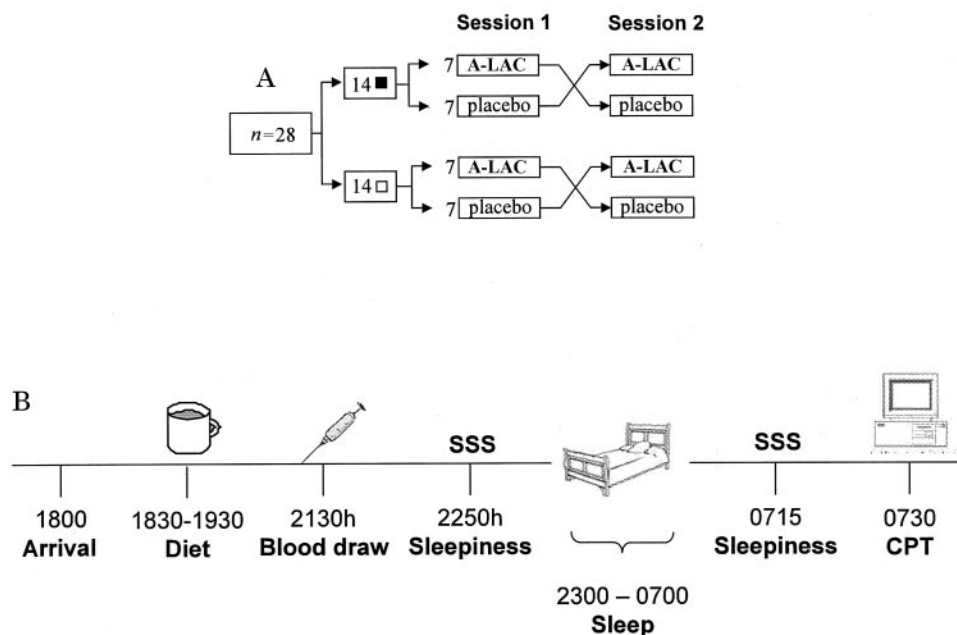
### Diets

On both experimental evenings, an isoenergetic diet (Knorr Orienty Nasi; Unilever Bestfoods, Rotterdam, Netherlands) providing 325 kcal (13% of energy as protein, 86% of energy as carbohydrate, and 1% of energy as fat) was provided to the subjects. The 2 dietary conditions were similar, with the exception of an additional milk shake in which the protein sources differed. The milk shake for the experimental diet contained 20 g tryptophan-enriched (4.8 g/100 g tryptophan) A-LAC protein (Davisco Foods International, Eden Prairie, MN) and the milk shake of the placebo diet contained 20 g (1.4 g/100 g tryptophan) sodium caseinate (DMV International, Veghel, Netherlands). The milk shakes were prepared by mixing the A-LAC or placebo protein powder with 7 g strawberry milk shake mix (Nesquik; Nestlé, Vevey, Switzerland) and 200 mL water. The first milk shake was served with the meal (1830), and the second milk shake was served 1 h later (at 1930). A research assistant blind to the dietary conditions supervised the dietary intake to make sure that all foods were consumed within 20 min. The amino acid profile of the protein sources and the nutrient composition of the milk shakes are given in **Table 1**.

### Measurements

#### Sleepiness

Changes in sleepiness were measured by using the Stanford Sleepiness Scale (27), which was offered on a computer screen and has a 7-point interval scale ranging from “strongly disagree” to “strongly agree.” The first scale was offered 10 min before the subjects went to bed (at 2250), and a second scale was offered the



**FIGURE 1.** General design and time schedule of the experiment. A: During 2 experimental sessions, the subjects with ( $n = 14$ ; ■) or without ( $n = 14$ ; □) mild sleep complaints slept overnight in the study laboratory; their performance was monitored the morning after the evening intake of a tryptophan-rich  $\alpha$ -lactalbumin (A-LAC) diet or a casein (placebo) diet. B: During each experimental session, the subjects consumed the diets after their arrival at the study laboratory; a blood sample was collected 2 h after dietary intake (at 2130). After the first measurement of sleepiness, the subjects slept from 2300 to 0700. After the subjects awoke and a second measure of sleepiness was made, behavioral and brain measures were assessed during a continuous performance task (CPT). SSS, Stanford Sleepiness Scale.

following morning 10 min after the subjects awoke (at 0710). The sleepiness scale comprises 7 statements concerning feelings of alertness ranging from “feeling active and vital” (score of 1) to “almost dreaming, sleep onset will be soon” (score of 7). The Stanford Sleepiness Scale is particularly sensitive to changes in alertness that are caused by poor sleep and sleep loss (28).

#### Continuous performance task

The CPT was used to measure changes in vigilance (29). A series of randomly presented letters (A, E, H, K, L, and X) was presented at the center of the computer screen. The subjects were instructed to push a button as quickly as possible when the letter X appeared on the screen, but only when it was preceded by the

letter A (A-X sequence, target condition). When an A was followed by another letter (A-not-X, nontarget condition), the subjects were instructed to not respond. The CPT was administered in 3 time blocks of  $\approx 4$ -min duration, consisting of 264 trials each, to measure sustained attention. Each block contained 24 A-X sequences and 24 A-other sequences, and 24 X, K, E, H, and L letters were presented randomly alone without a preceding A. Each trial lasted 950 ms, including stimulus duration of 150 ms and a fixed interstimulus interval of 800 ms, and the total task duration was  $\approx 13$  min ( $3 \times 4.18$  min). Before the task onset, the subjects had to practice to reach sufficient performance (minimal 80% hits and a reaction time (RT) of  $< 500$  ms), which was generally accomplished within 3–4 min. Stimulus presentation and acquisition of behavioral data were controlled by Experimental Run Time System software (ERTS, version 3.18; Berisoft Corp, Frankfurt, Germany). For each subject, the number of hits (correct A-X responses), misses (missed A-X responses), and false alarms (A-other) as well as the mean RTs during the hits were computed as behavioral measures of (sustained) attention efficiency.

#### Electroencephalographic recordings

During the CPT, EEG activity was registered continuously from tin electrodes by means of an electro cap from the midline scalp positions, of which only the signals at the parietal electrode (Pz) were analyzed for the purpose of this study. Both horizontal and vertical electro-oculographic (EOG) activity was recorded with the use of 2 tin electrodes attached to the outer canthi of both eyes (horizontal EOG) and from 2 infraorbital and supraorbital tin electrodes placed in line with the pupil of the left eye (vertical EOG). A ground electrode was attached to the middle of the

**TABLE 1**

Amino acid profile of the milk shake containing tryptophan-rich  $\alpha$ -lactalbumin (A-LAC) and of the tryptophan-poor milk shake (placebo)<sup>1</sup>

	A-LAC	Placebo
Nutrient (g)		
A-LAC protein	20	0
Sodium caseinate protein	0	20
Fruit aroma powder	7	7
Water	200	200
Amino acid profile (g/100 g)		
Isoleucine	6.0	5.8
Leucine	10.8	10.1
Phenylalanine	4.1	5.4
Tyrosine	4.4	5.8
Valine	4.3	7.5
Tryptophan	4.8	1.4
Trp:LNAA	1.7	0.4

<sup>1</sup> LNAA, large neutral amino acids.

forehead, and a reference electrode was placed on the left mastoid. Electrode impedance was kept below 5 K- $\Omega$ . Signals were recorded at 250 Hz, with an online band pass filter of 0.05–50 Hz, with the use of NEUROSCAN software and NEUROSCAN amplifiers (NEUROSCAN 4.2; Tefa Portanje, Woerden, Netherlands).

Offline, the continuous EEG was divided into epochs from 200 ms prestimulus to 952 ms poststimulus, filtered with a 30-Hz (24 dB/Oct) digital low-pass filter and baseline corrected with a 200-ms prestimulus interval. EEG signals were corrected for vertical EOG artifacts by means of an eye-movement correction algorithm (30), and all EEG epochs containing artifacts exceeding  $\pm 75 \mu\text{V}$  were removed. Averaged parietal ERPs were computed for individual subjects in each of the 3 CPT blocks, only for target trials to which subjects responded correctly. In the ERP, peak amplitudes and latencies of the P300 component were scored relative to baseline at Pz in a 300–550 ms window.

### Biochemical analyses

Blood samples were collected in 5-mL evacuated tubes containing sodium heparin and centrifuged at  $2850 \times g$  (5000 rpm) for 5 min at 4 °C. Subsequently, the supernatant fluid (100  $\mu\text{L}$ ) was mixed with 4 mg sulfasalicylic acid and stored at  $-80 \text{ }^\circ\text{C}$  until analyzed. Amino acid analysis in plasma was conducted with HPLC with the use of a 2–3- $\mu\text{m}$  Bischof Spherisorb ODS II column (31). Plasma Trp:LNAA was calculated by dividing the plasma tryptophan concentration by the sum of the other large neutral amino acids, ie, valine, isoleucine, leucine, tyrosine, and phenylalanine.

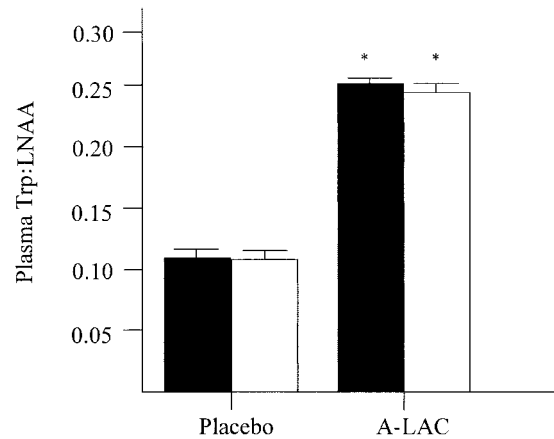
### Experimental design and statistical analysis

The main research question formulated in the introduction was analyzed by means of univariate repeated-measures analysis of variance (ANOVA) by using the general linear model (SPSS 7.5 for WINDOWS; SPSS Inc, Chicago) with one between-subjects factor “group” (poor sleepers compared with good sleepers, as independent variables) and one within-subjects factor “diet” (A-LAC compared with placebo, as independent variables) on changes in plasma Trp:LNAA and sleepiness and behavioral (RT and errors) and ERP changes during the CPT as dependent variables. For the effect on behavioral and ERP changes during the CPT across the 3 time blocks, multivariate ANOVA also included “block” as a within-subjects factor with first- and second-order polynomial contrasts. Although we counterbalanced for the sequence of dietary condition (order of diet), this factor was incorporated as a covariant when a significant main or interaction effect was found. This was the case for the ERP P300 amplitude and latency. Significant results shown with these procedures were further examined by post hoc tests. Huynh-Feldt- or Greenhouse-Geisser-corrected  $P$  values, their corresponding epsilons, and the original (ie, uncorrected) df are reported when the sphericity assumption was not met. All statistics were evaluated at a significance level of 5%. Data are reported as means  $\pm$  SDs.

## RESULTS

### Plasma Trp:LNAA

Repeated-measures ANOVA with “diet” as the within-subjects factor and “group” as the between-subjects factor showed a significant effect of diet ( $P < 0.0001$ ), which indicated



**FIGURE 2.** Mean ( $\pm$ SEM) ratio of total plasma tryptophan to other large neutral amino acids (Trp:LNAA) 2 h after the evening intake of a tryptophan-rich  $\alpha$ -lactalbumin diet (A-LAC) or a casein (placebo) diet in healthy subjects with ( $n = 14$ ; ■) or without ( $n = 14$ ; □) mild sleep complaints. Repeated-measures ANOVA showed a significant main effect of diet ( $P < 0.0001$ ). \*Significantly different from placebo,  $P < 0.05$ . The mean difference between diets was significant ( $P < 0.0001$ ) when both groups were pooled. There were no significant differences between the subjects with or without sleep complaints.

a significant (130%) increase in plasma Trp:LNAA after A-LAC as compared with placebo. As shown in **Figure 2**, the mean plasma Trp:LNAA was higher after the A-LAC diet condition ( $0.25 \pm 0.02$ ) than after the placebo diet condition ( $0.11 \pm 0.03$ ). There was no significant effect of group or an interaction between diet and group.

### Sleepiness

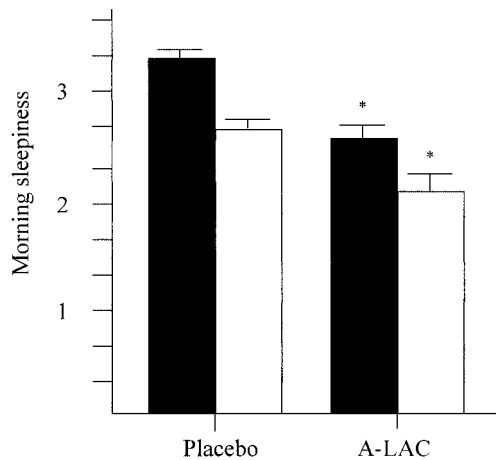
Repeated-measures ANOVA with diet as the within-subjects factor and group as the between-subjects factors on evening sleepiness showed no significant effect ( $P > 0.2$ ). A second analysis with diet as the within-subjects factor and group as the between-subjects factor on morning sleepiness showed a significant effect of diet ( $P = 0.013$ ). As shown in **Figure 3**, all subjects were less sleepy in the morning after evening intake of A-LAC ( $2 \pm 0.1$ ) than after placebo intake ( $3 \pm 0.1$ ). There was no significant effect of group or an interaction between diet and group.

### Continuous performance task

#### Behavioral findings

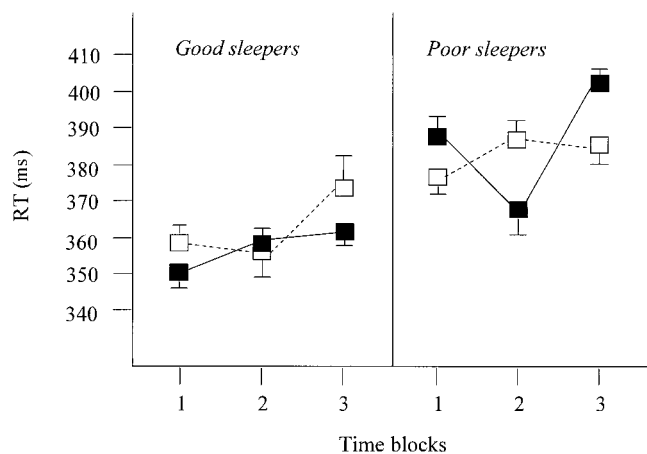
Repeated-measures ANOVA with diet and block as within-subjects factors and group as the between-subjects factor on RT showed a significant effect of block ( $P = 0.045$ ) and a significant interaction effect of diet  $\times$  block  $\times$  group ( $P = 0.05$ ), which originated from a significant quadratic polynomial contrast ( $P = 0.014$ ). As indicated in **Figure 4**, RT was lower in the poor-sleepers group during the second block (from  $369 \pm 51$  to  $379 \pm 53$  ms) and higher at the end of the task during the third block ( $401 \pm 40$  ms;  $P = 0.014$ ) after the A-LAC diet, whereas there were no significant differences after the placebo diet ( $P > 0.5$ ). This effect or any other effect was not found in the good-sleepers group.

To see whether this interaction effect on RT was accompanied by alterations in the number of errors, we also conducted an

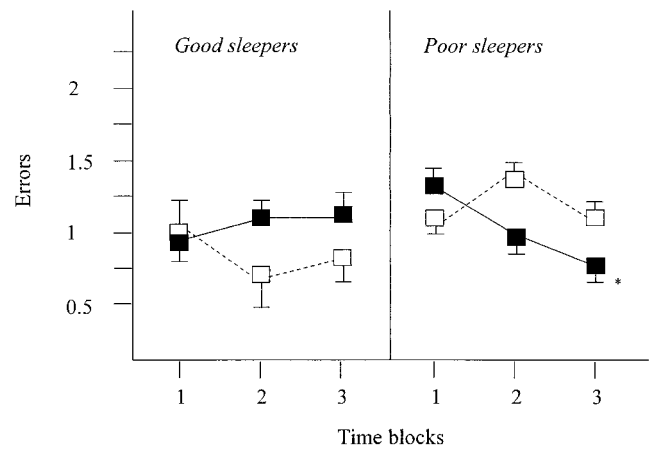


**FIGURE 3.** Mean ( $\pm$ SEM) changes in sleepiness scores after an overnight sleep following the evening intake of a tryptophan-rich  $\alpha$ -lactalbumin (A-LAC) diet or a casein (placebo) diet in healthy subjects with ( $n = 14$ ; ■) or without ( $n = 14$ ; □) mild sleep complaints. Repeated-measures ANOVA showed a significant main effect of diet ( $P = 0.013$ ). \*Significantly different from placebo,  $P < 0.05$ . The mean difference between diets was significant ( $P = 0.013$ ) when both groups were pooled. There were no significant differences between the subjects with or without sleep complaints.

ANOVA on the number of missed A-X responses. The analysis showed no significant interaction effect of diet  $\times$  block  $\times$  group ( $P > 0.2$ ). However, as indicated in **Figure 5**, the analysis on the number of false alarms (A-other) did show a significant interaction of diet  $\times$  block  $\times$  group ( $P = 0.008$ ), which indicated a significant linear reduction in the number of false alarms across blocks after the A-LAC diet in poor sleepers ( $P = 0.048$ ) but not in good sleepers ( $P > 0.7$ ).



**FIGURE 4.** Mean ( $\pm$ SEM) changes in reaction time (RT) during a continuous performance task after an overnight sleep following the evening intake of a tryptophan-rich  $\alpha$ -lactalbumin diet (■) or a casein (placebo) diet (□) in healthy subjects with ( $n = 14$ ) or without ( $n = 14$ ) mild sleep complaints (poor and good sleepers, respectively). Repeated-measures ANOVA showed a significant effect of block ( $P = 0.045$ ) and a significant interaction effect of diet  $\times$  block  $\times$  group ( $P = 0.05$ ). In the poor sleepers, morning RT was significantly lower halfway through the task (block 2) and was higher at the end of the task (block 3) after the evening intake of  $\alpha$ -lactalbumin than after the intake of placebo. The quadratic trends were significantly different between treatments ( $P = 0.05$ ) but not in the good sleepers.

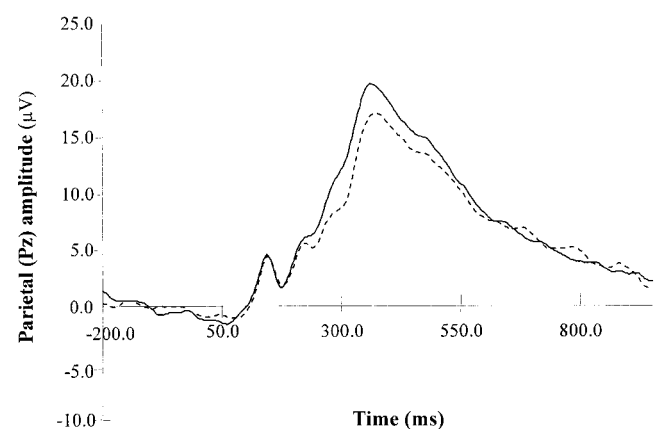


**FIGURE 5.** Mean ( $\pm$ SEM) changes in the number of errors during a continuous performance task after an overnight sleep following the evening intake of a tryptophan-rich  $\alpha$ -lactalbumin diet (■) or a casein (placebo) diet (□) in healthy subjects with ( $n = 14$ ) or without ( $n = 14$ ) mild sleep complaints (poor and good sleepers, respectively). Repeated-measures ANOVA showed a significant interaction effect of diet  $\times$  block  $\times$  group ( $P = 0.008$ ). In the poor sleepers, morning reaction time was significantly lower halfway through the task (block 2) and was higher at the end of the task (block 3) after the evening intake of  $\alpha$ -lactalbumin than after the intake of placebo. The quadratic trends were significantly different between treatments ( $P = 0.05$ ) but not in the good sleepers.

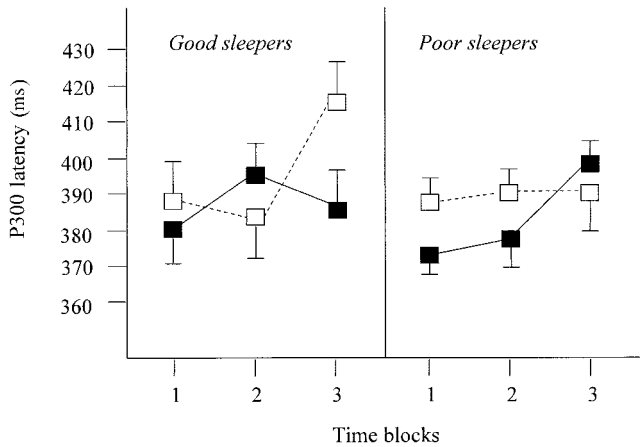
#### ERP findings

Repeated-measures ANOVA with diet and block as within-subjects factors and group as the between-subjects factor on parietal P300 amplitudes showed a significant effect of diet ( $P < 0.002$ ), which indicated that morning P300 amplitude was significantly higher after the A-LAC diet ( $23 \pm 6 \mu\text{V}$ ) than after the placebo diet ( $19 \pm 6 \mu\text{V}$ ) (**Figure 6**). No other main or interaction effects were found.

Repeated-measures ANOVA on parietal P300 latency showed a significant effect of diet ( $P = 0.002$ ) and a significant interaction of diet  $\times$  block  $\times$  group ( $P < 0.04$ ). Further post hoc testing



**FIGURE 6.** Mean amplitudes of event-related brain activity  $\approx 300$  ms after stimulus onset (P300 amplitude) during a continuous performance task that was completed after an overnight sleep following the evening intake of a tryptophan-rich  $\alpha$ -lactalbumin diet (solid line) or a casein diet (broken line); the data for all subjects ( $n = 28$ ) were pooled. Repeated-measures ANOVA showed a significant main effect of diet ( $P = 0.002$ ). The early morning P300 amplitude was significantly higher after the evening intake of  $\alpha$ -lactalbumin than after the intake of placebo ( $P = 0.005$ ). There were no significant differences between the subjects with or without sleep complaints.



**FIGURE 7.** Mean ( $\pm$ SEM) latency of event-related brain activity  $\approx$ 300 ms after stimulus onset (P300 amplitude) during a continuous performance task completed after an overnight sleep following the evening intake of a tryptophan-rich  $\alpha$ -lactalbumin diet (■) or a casein (placebo) diet (□) in healthy subjects with ( $n = 14$ ) or without ( $n = 14$ ) mild sleep complaints (poor and good sleepers, respectively). Repeated-measures ANOVA showed a significant effect of diet ( $P = 0.002$ ) and a significant interaction of diet  $\times$  block  $\times$  group ( $P = 0.04$ ). Further ANOVA testing within each group showed a near-significant effect of diet  $\times$  block in the poor sleepers ( $P = 0.07$ ) but not in the good sleepers. In the poor sleepers, morning P300 latency appeared to increase as a function of time-on-task after the evening intake of  $\alpha$ -lactalbumin was compared with the intake of placebo (post hoc dependent  $t$  test with time block as a within-subjects factor;  $P = 0.05$ ); this was not found in the good sleepers.

in separate groups showed a near-significant effect of diet  $\times$  block in poor sleepers ( $P = 0.07$ ) and no significant effect in good sleepers. As shown in **Figure 7**, poor sleepers showed higher latency from blocks 1 + 2 to the last block (as a function of time-on-task) after the A-LAC diet as compared with after the placebo diet; this effect was not found in good sleepers.

## DISCUSSION

The present study investigated whether evening consumption of A-LAC increases plasma tryptophan availability for uptake into the brain and improves morning alertness and attention in subjects with sleep complaints. The results showed a large increase in tryptophan availability after A-LAC intake. Morning alertness improved modestly but significantly in all subjects and was accompanied by improved vigilance performance in subjects with mild sleep complaints.

### Evening dietary effect on Trp:LNAA

Evening consumption of A-LAC containing 4.8 g/100 g Trp resulted in a 130% increase in the plasma Trp:LNAA as compared with placebo. This indicated that, after the A-LAC diet, more tryptophan was available for uptake into the brain (32). This rise in Trp:LNAA is considerably higher than the 48% increase that was previously found after consumption of A-LAC containing 1.7 g/100 g tryptophan (18) and the previously reported 20–45% increases after the consumption of foods such as carbohydrates (22). Although a 40–50% variation in plasma Trp:LNAA is thought to be sufficient to change available brain tryptophan and 5-HT synthesis and release (18, 20, 32–34), a 130% increase may cause an even larger increase in available brain tryptophan (and probably 5-HT) and, therefore may also result in

a greater release of functionally active brain 5-HT and related behavior as previously suggested (18, 35). This certainly merits further investigation.

### Dietary effect on early morning sleepiness

It was assumed that A-LAC would especially induce feelings of sleepiness in the evening in poor sleepers, leading, in turn, to improved sleep and reduced feelings of sleepiness in the morning. Contrary to our expectations, there was no effect on evening sleepiness. We found that poor sleepers were sleepier in the evening, regardless of dietary condition. In contrast, there was a significant dietary effect on sleepiness in the morning. Poor sleepers and good sleepers felt less sleepy and more alert in the morning after the consumption of A-LAC than after the consumption of placebo in the evening. This apparent contradictory effect of diet on evening compared with morning sleepiness may, however, not be surprising after all. The subjects had just finished a computer test before evening sleepiness was measured as part of another study. In addition, evening changes in feelings of sleepiness do not necessarily reflect the transitional phase between wakefulness and sleep onset and subsequent sleep progress, whereas early morning changes in sleepiness do. Hence, changes in morning scores on the Stanford's Sleepiness Scale most likely indicate higher alertness due to improved sleep (28).

### Dietary effects on early morning performance

There was a significant increase in RT as a function of task duration, which indicated successful task manipulation of vigilance. Generally, increased sleepiness after poor sleep significantly reduces vigilance performance as evidenced by an increase in RTs and an increase in the number of errors (4). Only in poor sleepers was RT significantly reduced halfway through the task and became higher at the end of the task after evening A-LAC consumption. Moreover, only in these subjects was there a modest but significant reduction in the number of false alarms across task performance.

The results also showed higher parietal P300 amplitudes during the morning task performance in all subjects after evening intake of A-LAC. Together with the reduction in morning sleepiness after A-LAC intake, this finding indicates a general increase in attention (5). Hence, an increase in parietal P300 amplitude is thought to reflect a rise in neural activity involved in attention that is controlled by the activation or arousal state of the subjects and its consequent drive to perform (5). Together with the behavioral findings, it appears as if such an increase in cognitive resources modestly improved task performance in poor sleepers. In addition, in these subjects, P300 latency increased across task performance after evening intake of A-LAC; this is thought to reflect slower cognitive evaluation processes and may suggest that subjects take more time to perform well.

### Are the dietary effects of A-LAC intake due to improved sleep?

The remaining question is whether the effects of evening A-LAC intake on subsequent morning sleepiness and performance are mediated by improved sleep. This appears to be likely. First, the effects cannot be caused by higher plasma tryptophan availability in the morning. On the basis of previous studies, it is expected that food-induced increases in plasma Trp:LNAA in

humans remain for  $\approx 4$ –5 h after intake (with peak scores occurring  $\approx 3$  h after intake), followed by a fast gradual return to baseline values (33, 36, 37). In addition, the highest increases in the present study were expected around bedtime and should have been sustained for only a few hours after midnight. Second, previous findings showed reduced sleep after evening tryptophan depletion and improved sleep after evening increases in tryptophan availability (9, 10, 13, 14). In addition, it was shown by Minet-Ringuet et al (25) that even the intake of A-LAC containing lower tryptophan concentrations than used in the present study was capable of reducing sleep disturbances after food deprivation in rats. Third, current dietary findings on reduced morning scores on the Stanford Sleepiness Scale further support the assumption that morning alertness improves as a result of better sleep and reduced sleep loss (28). Finally, the increases in parietal (P300) event-related brain activity indicate enhanced arousal and attention processing (5), which have been found to decrease particularly after poor sleep (4). In addition, Smulders (38) reported lower morning P300 amplitudes and reduced performance accuracy after sleep deprivation, as indicated by increased RTs in combination with a higher amount of errors.

## Conclusion

The present study showed a significant 130% increase in the plasma Trp:LNAAs 2 h after the evening intake of an A-LAC-enriched standard diet as compared with a placebo diet. This was accompanied by reduced sleepiness and higher task-related brain activity the following morning, which suggests improved alertness due to better sleep. Only in subjects with mild sleep complaints was this accompanied by modest but significantly improved vigilance performance. The current findings support the assumption that evening consumption of tryptophan-rich A-LAC may improve early morning performance indirectly by enhancing available brain tryptophan and subsequent sleep improvement. However, further evidence is needed, including EEG sleep measures, in subjects with more severe sleep complaints. For instance, beneficial effects on morning performance may be mediated by reduced sleep onset latency or changes in rapid eye movement latency, because these are particularly affected by acute tryptophan depletion (10) or by L-tryptophan (10).



We express our appreciation to Cees van Leeuwen for his medical consulting assistance.

CRM designed the study and was responsible for the overall data analysis and the writing of the manuscript. LMJ participated in the study design and was responsible for the EEG-ERP methodology and data acquisition. LMJ, JHCML, MHM, and NR participated in conducting the study and in collecting and analyzing the data. NEPD was responsible for the plasma amino acid analysis. All authors reviewed the study protocol and the manuscript. None of the authors had any conflicts of interest.

## REFERENCES

- Pilcher JJ, Huffcutt AJ. Effects of sleep deprivation on performance: a meta-analysis. *Sleep* 1996;19(4):318–26.
- Johnson LC. In: Webb WB, ed. *Biological rhythms, sleep and performance*. New York, NY: Wiley, 1982.
- Wilkinson RT. Sleep deprivation: performance tests for partial and selective sleep deprivation. In: *Progress in clinical psychology*. Vol 3. Dreams and dreaming. London, United Kingdom: Grune and Stratton, 1986.
- Dinges DF, Kribbs NB. Performing while sleepy: effects of experimentally-induced sleepiness. In: Monk TH, ed. *Sleep, sleepiness and performance*. Chichester, United Kingdom: Wiley & Sons, 1991: 97–128.
- Polich J, Kok A. Cognitive and biological determinants of P300: an integrative review. *Biol Psychol* 1995;41:103–146.
- Monk TH. *Sleep, sleepiness and performance*. Chichester, United Kingdom: Wiley & Sons Ltd, 1991.
- Morin CM, Ware JC. Sleep and psychopathology. *Appl Prev Psychol* 1996;5(4):211–24.
- Jouvet M. Sleep and serotonin: an unfinished story. *Neuropsychopharmacology* 1999;21(2S):24S–7S.
- Hartmann E, Greenwald D. Tryptophan and human sleep: an analysis of 43 studies. In: Schlossberger HG, Kochen W, Linzen B, Steinhart H, eds. *Progress in tryptophan and serotonin research*. New York, NY: DeGruyter, 1984:297–304.
- Bhatti TJ, Gillin C, Seifritz E, et al. Effects of a tryptophan-free amino acid drink challenge on normal human sleep electroencephalogram and mood. *Biol Psychiatry* 1998;43:52–9.
- Maes M, Meltzer H. The serotonin hypothesis of major depression. In: Bloom FE, Kupfer DJ, eds. *Psychopharmacology: the fourth generation of progress*. New York, NY: Raven Press, 1995:933–44.
- Riemann D, Feige B, Hornyak M, Koch S, Hohagen F, Volderhozer U. The tryptophan depletion test: impact on sleep in primary insomnia: a pilot study. *Psychiatry Res* 2002;109:129–35.
- Hartmann E. Effects of L-tryptophan on sleepiness and sleep. *J Psychiatr Res* 1983;17(2):107–13.
- Schnieder-Helmert D, Spinweber CL. Evaluation of L-tryptophan for treatment of insomnia: a review. *Psychopharmacology* 1986;89:1–7.
- Waters WF, Adams SG, Binks P, Vernado P. Attention, stress and negative emotion in persistent sleep-onset and sleep-maintenance insomnia. *Sleep* 1993;16(2):128–36.
- Harvey AG. Pre-sleep cognitive activity: a comparison of sleep-onset insomniacs and good sleepers. *Br J Clin Psychol* 2000;39:275–86.
- Kennett GA, Dickinson SL, Curzon G. Enhancement of some 5-HT-dependent behavioral responses following repeated immobilisation in rats. *Brain Res* 1985;330:253–63.
- Markus CR, Olivier B, Panhuysen G, et al. The bovine protein  $\alpha$ -lactalbumin increases the plasma ratio of tryptophan to the sum of the other neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. *Am J Clin Nutr* 2000;71:1536–44.
- Fernstrom JD, Wurtman RJ. Brain serotonin content: physiological dependence on plasma tryptophan levels. *Science* 1971;173:149–52.
- Curzon G. Effects of food intake on brain transmitter amine precursors and amine synthesis. In: Sandler M, Silverstone T, eds. *Psychopharmacology and food*. Oxford, United Kingdom: Oxford University Press, 1985:59–70.
- Spring BJ, Chiodo J, Bowen DJ. Carbohydrates, tryptophan, and behaviour: a methodological review. *Psychol Bull* 1987;102(2):234–56.
- Markus CR. Food, stress and mood. In: Wadson D, Dodds F, eds. *Performance functional foods*. Cambridge, United Kingdom: Woodhead Publishing Limited, 2003:5–20.
- Heine W, Radke M, Wutzke KD, Peters E, Kundt G.  $\alpha$ -Lactalbumin-enriched low-protein infant formulas: a comparison to breast milk feeding. *Acta Paediatr* 1996;85:1024–8.
- Orosco M, Rouch C, Beslot F, Feurte S, Regnault A, Dauge V. Alpha-lactalbumin-enriched diets enhance serotonin release and induce anxiolytic and rewarding effects in the rat. *Behav Brain Res* 2004;148:1–10.
- Minet-Ringuet J, Le Ruyet PM, Tomé D, Even PC. A tryptophan-rich protein diet efficiently restores sleep after food deprivation in the rat. *Behav Brain Res* 2004;152:335–40.
- Mulder-Hajonides van der Meulen WREH. Measurement of subjective sleep quality. *Proceedings of the International European Sleep Conference*. Amsterdam, Netherlands: Elsevier, 1981.
- Hoddes E, Zarcone V, Smythe H, Phillips R, Dement WC. Quantification of sleepiness: a new approach. *Psychophysiology* 1973;10:431–6.
- Babkoff H, Caspy T, Mikulincer M. Subjective sleepiness ratings: the effect of sleep deprivation, circadian rhythmicity and cognitive performance. *Sleep* 1973;14(6):534–9.
- Rosvold HE, Mirsky AF, Sarason J, Bransome ED, Beck LH. A continuous performance test of brain damage. *J Consult Psychol* 1956;20:343–50.
- Semlitsch HV, Anderer P, Schuster P, Presslich O. A solution for reliable and valid reduction of ocular artefacts, applied to the P300 ERP. *Psychophysiology* 1986;23:695–703.

31. Van Eijk HMH, Rooyackers DR, Deutz NEP. Rapid routine determination of amino acids in plasma by high-performance liquid chromatography with a 2–3  $\mu\text{m}$  Spherisorb ODS II column. *J Chromatogr* 1993; 620:143–8.
32. Fernstrom JD, Wurtman RJ. Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science* 1972;178:414–6.
33. Lyons PM, Truswell AS. Serotonin precursor influenced by type of carbohydrate meal in healthy adults. *Am J Clin Nutr* 1988;47:433–9.
34. Kaye WH, Gwirtsman HE, Brewerton TD, George DT, Wurtman RJ. Bingeing behavior and plasma amino acids: a possible involvement of brain serotonin in bulimia nervosa. *Psychiatry Res* 1988;23:31–43.
35. Markus CR, Olivier B, de Haan EHF. Whey protein rich in  $\alpha$ -lactalbumin increases the plasma Trp/LNAA ratio, and improves cognitive performance in stress-vulnerable subjects. *Am J Clin Nutr* 2002;75:1051–6.
36. Markus CR, Klöpping-Ketelaars WI, Pasman W, Klarenbeek B, van de Berg H. Dose-dependent effect of alpha-lactalbumin in combination with two different doses of glucose on the plasma ratio Trp/LNAA. *Nutr Neurosci* 2000b;3:345–55.
37. Wurtman RJ, Wurtman JJ, Regan MM, McDermott JM, Tsay RH, Breu JJ. Effects of normal meals rich in carbohydrates or proteins on plasma tryptophan and tyrosine ratios. *Am J Clin Nutr* 2003;77:128–32.
38. Smulders F. The selectivity of age effects on information processing. PhD thesis. University of Amsterdam, Netherlands, 1993.
39. Hartmann E, Spinweber CL. Sleep induced by L-tryptophan. *J Nerv Ment Dis* 1979;167:497–9.
40. Wurtman RJ, Fernstrom JD. L-Tryptophan, L-tyrosine and the control of brain monoamine synthesis. In: Snyder SH, ed. *Perspectives in neuropharmacology*. New York, NY: Oxford University Press, 1972:143–93.
41. Delgado PL, Price LH, Miller HL, et al. Serotonin and the neurobiology of depression; effects of tryptophan depletion in drug-free depressed patients. *Arch Gen Psychiatr* 1994;51:865–74.
42. Heninger GR, Delgado PL, Charney DS. The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. *Pharmacopsychiatry* 1996;29:2–11.