Manipulating serotonin function in depression

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Diet rich in alpha-lactalbumin improves memory in unmedicated recovered depressed patients and matched controls

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Journal of Psychopharmacology, 20, 526-535

Abstract

Depression is associated with reduced brain serotonin (5-hydroxytryptamine; 5-HT) function and with cognitive dysfunctions. A diet rich in alpha-lactalbumin protein has been found to increase the ratio tryptophan /large neutral amino acids (tryptophan/ LNAA), and to improve cognitive functioning in individuals with high neuroticism scores. Since cognitive dysfunctions sometimes persist after remission of depression, the present study investigated the effects of alpha-lactalbumin-enriched diet on cognition in recovered depressed patients. Twenty-three recovered depressed patients and 20 healthy matched controls without a history of depression consumed meals rich in alpha- lactalbumin or casein protein in a double-blind crossover design. Mood, cognitive function and plasma amino-acids were assessed at both sessions before and after dietary intake. Alpha-lactalbumin protein had no effect on mood, but improved abstract visual memory and impaired simple motor performance. These effects were independent of history of depression. Supplements of alpha-lactalbumin may be useful for nutrition research in relation to age- or disease-related memory decline. The present findings should be further examined in different (e.g. medicated) samples. The long-term effects of alpha-lactalbumin should also be investigated.

Introduction

Depression is associated with impaired cognitive functioning (Austin et al., 2001). Impaired spatial and verbal memory has most frequently been reported (Burt et al., 1995: review). However, deficits in other domains are also common, including psychomotor skills, attention and executive functioning (Austin et al., 2001). Mild deficits often persist after remission (Paradiso et al., 1997), irrespective of residual symptoms (Weiland-Fiedler et al., 2004) or medication status (Paradiso et al., 1997).

The serotonin (5-hydroxytryptamine; 5-HT) system is important in the regulation of mood as well as cognitive functions. Selective serotonin reuptake inhibitors (SSRIs) are found to relieve depressive symptoms and to enhance memory function in humans and rats (McEntee & Crook, 1991). Conversely, experimental depletion of L-tryptophan (the precursor of serotonin) induces depressive symptoms in depression-vulnerable individuals (see for reviews: Booij et al., 2003; Van der Does, 2001a) and impairs long-term memory in healthy volunteers (Riedel et al., 1999; Schmitt et al., 2000). The effects of enhanced 5-HT activity on cognitive processes have frequently been investigated by using low protein diets that consist almost entirely of carbohydrates. These diets increase the amount of plasma tryptophan as compared to the other large neutral amino acids (ratio tryptophan/LNAA) that competes with tryptophan for uptake into the brain (Fernstrom & Wurtman, 1972). High-carbohydrate meals have been found to improve cognitive performance in both clinical and healthy populations, but the results are not consistent (see for reviews: Benton & Nabb, 2003; Dye et al., 2000; Gibson & Green, 2002). These inconsistent results may be related to the timing of intervention or the amount of carbohydrates consumed. Furthermore, individual differences in stress-vulnerability may be involved, as carbohydrates

improved mood and information processing in high stress-prone but not in low stress-prone healthy individuals (Markus et al., 1998). The use of carbohydrate-rich meals poses methodological difficulties, including lack of a placebo condition and expectancy effects (Dye et al., 2000; Gibson & Green, 2002; Spring et al., 1987). Besides, a very large amount of carbohydrates, and consequently high-caloric meals may be needed to produce biochemical and behavioural changes, making implementation in a regular diet undesirable.

A different method to enhance tryptophan availability involves using alpha-lactalbumin protein (Markus et al., 2000; 2002). Alpha-lactalbumin protein has the highest L-tryptophan concentration of all protein fractions (Heine et al., 1996). A diet enriched with alpha-lactalbumin increased the ratio plasma tryptophan/LNAA by 46–48% in healthy volunteers, as compared to casein (placebo). This effect is two times higher than the effect generally found after a carbohydrate- rich diet (Markus et al., 2000), or after 7 days of daily treatment with L-tryptophan (Chouinard et al., 1985). Alpha-lactalbumin improved mood and information processing, and attenuated stress-induced cortisol-responses in stress-vulnerable subjects (non-patients with high neuroticism scores) but not in controls (low neuroticism scores) (Markus et al., 2000; 2002). These data suggest that enhancing 5-HT function through diet may be particularly beneficial for vulnerable individuals under high levels of stress and may improve stress coping.

In the present study we were particularly interested in investigating the cognitive effects of alpha-lactalbumin in recovered depressed patients and matched controls. Since cognitive dysfunctions may persist after remission, we expected to find residual cognitive impairments at baseline in recovered depressed patients relative to controls. Furthermore, we expected that alpha-lactalbumin would improve cognitive function compared to a casein diet, and

that the effects of alpha-lactalbumin would be more pronounced in recovered depressed patients than in controls. We have previously reported that alpha-lactalbumin did not change mood or cortisol response following laboratory stress in recovered depressed patients or controls (Merens et al., 2005). However, cognitive effects in the absence of mood effects have regularly been reported after manipulations of neurotransmitters, e.g. tryptophan depletion (Booij et al., 2005a; 2005c) or a single dose of an antidepressant (Harmer et al., 2002; 2003a).

Methods and materials

Participants

Twenty-three recovered depressed patients (21 females and two males) and 20 controls (17 females and three males) participated in the study. Some of the patients were former participants of a randomized psychotherapy trial, whereas additional patients and all controls were recruited via advertisements at Leiden University or in local newspapers. Inclusion criteria were: age between 18 and 65; meeting DSM-IV criteria for history of depression (patient group only); free of antidepressant medication for at least 3 months; no history of psychiatric disorders and having no first-degree relative of major depressive disorder (control group only); no current psychiatric disorder; Montgomery-Asberg Depression Rating Scale lower than eight (Montgomery & Asberg, 1979) and a Body Mass Index (BMI in kg/m^2) above 18. Exclusion criteria were: substance abuse within last 3 months, psychosis (lifetime), major physical illness, lactation, pregnancy and excessive dieting or binge eating. Diagnoses, demographic and clinical background variables were verified by means of the Structured Clinical Interview for DSM-IV (SCID-I) (First et al., 1995).

Composition of the diet

At each session, participants received a carbohydrate-rich breakfast and lunch. The energy intake per session totalled 294 kcal (1229 kJ), of which 21% was from fat, 75% as carbohydrates and 5% as protein. Each breakfast and lunch consisted of one slice of bread, 10 g butter, 15 g fruit jelly, grape juice (200 ml) and a chocolate drink. The two diets were identical with the exception of the composition of a chocolate drink in which the protein sources differed. The chocolate drink in the alpha-lactalbumin diet contained a whey-protein fraction rich in alpha-lactalbumin (containing 12.3 g/kg tryptophan; tryptophan/LNAA ratio of 8.7) and the chocolate drink in the control diet contained sodium caseinate (containing 9.5 g/kg tryptophan, tryptophan/LNAA ratio of 4.7). The composition and preparation of the chocolate drinks were similar in appearance and macronutrient composition as in Markus et al. (2000; 2002). Other nutrients or drinks were not allowed until the end of session, except for water during the whole day and one cup of coffee or tea without milk +1.25 hours after breakfast.

Instruments

Mood: Changes in mood were measured using the Dutch shortened paper and pencil version of the Profile of Mood States questionnaire (POMS) (McNair et al., 1971; Wald & Mellenbergh, 1990). The POMS comprises five different subscales for mood. The subscale Anger (range: 0–28), Depression (range: 0–32), Fatigue (range: 0–24) and Tension (range: 0–24) refer to a negative mood state, whereas the subscale Vigour (range: 0–20) concerns a positive mood.

Personality: Neuroticism (N) was measured with the shortened Eysenck Personality Questionnaire (EPQ-RSS)(Eysenck & Eysenck, 1991). The Dutch translation (Sanderman et al., 1995) was used, which has different norms from the original (about 1.5 points lower). According to the manual, the mean N score of the general population is around four. Norms for psychiatric patients are not available; however we found a mean N score of 6.4 (S.E. 0.55) in a recent study of 39 (partially) remitted depressed outpatients (who were in treatment and who had a mean of 4.4 past episodes of depression) (Van der Does & Booij, 2005).

Cognition:

Sternberg Memory Scanning Task

The computerized Memory Scanning Task is based on the information processing model of Sternberg, who distinguishes scanning and non-scanning stages of information processing (Sternberg, 1969). The Memory Scanning Task consisted of three trials, corresponding to a set of two, three or six consonants respectively. In each trial, the set of letters is presented in the middle of the screen, and the participant was instructed to memorize them ('memory' set). After memorization, 90 letters in each trial are presented on the screen in a random order one by one for 1500 ms each at an interval range of 500 ms. Fifty percent of the letters presented belonged to the memory set and 50% did not. Participants were instructed to push on the 'yes' button if the letter presented belonged to the memory set of that condition and on the 'no' button when it did not. Reaction times and number of errors for each condition were the outcome measures of this test.

Abstract Patterns Recognition Task (APRT)

The APRT modelled after Rubinsztein et al. (2001) measures (speed of) retrieval of non-verbal abstract information from short-term memory (STM) and long-term memory (LTM). Sixteen abstract patterns were presented consecutively for 3000 ms, with 500 ms intervals. Participants were instructed to memorize the patterns. After three presentations of the complete series, two patterns were presented simultaneously; one that had been learned and a new pattern. Participants had to indicate as fast as possible which one had been previously presented. The recognition procedure was repeated after 35 minutes, during which verbal tasks were administered. Sensitivity measures (A') were calculated for the proportion of correctly recognized patterns, corrected for response tendency by the formula: $A' = 1-1/4(fr/cr + (1-cr)/(1-fr))$, in which fr = the proportion of falsely recognized patterns and cr = proportion of correctly recognized patterns (signal detection theory; Pollack & Norman, 1964).

Stroop Colour Word Task (SCWT)

The Stroop test measures focused attention and response inhibition. Names of colours (red, yellow, blue, green) printed in black were presented one by one for a maximum of 1500 ms on a computer screen. Participants were instructed to read these words as fast as possible (Condition I). Next, coloured patches were presented (Condition II) of which the name of the colour had to be named. Finally, names of colours printed in an incongruent colour were presented and participants were instructed to name the colour of the ink (Condition III). Median reaction times (RTs) and errors were recorded. Interference was defined as the percentage of extra time needed for condition III relative to the average of conditions I and II.

Left/right choice reaction time

This task was used to assess motor speed and response inhibition as a function of task difficulty. The word 'left' or 'right' was presented in randomized order either at the left or the right side of the screen. Participants were instructed to respond to the meaning of the word while ignoring its location, as fast as possible. The task consisted of two consecutive subtasks in which the stimulus interval differed (1000 ms fixed vs. 500–1500 ms variable). Correct responses and RTs were registered.

Tower of London (TOL)

The TOL modelled after Owen et al. (1995b) is a planning task consisting of three coloured balls (red, yellow and blue) placed on three sticks in various arrangements. Two arrangements were presented on the upper and lower half of the screen. The patient was instructed to indicate the minimal number of moves necessary to change the first arrangement into the second (two to five moves). Correct responses and RTs were registered.

Blood plasma

A blood sample was obtained (10 ml) using EDTA tubes to determine total plasma tryptophan and the other large neutral amino acids (tyrosine, phenylalanine, isoleucine, leucine and valine). Immediately after sampling, the blood was centrifuged for 20 minutes at 2650 gmax and the plasma was stored at -20 °C. Quantitative amino acid analysis was performed by an ion-exchange chromatography on a Biochrom 20 automated amino acid analyser (Pharmacia) as described elsewhere (Merens et al., 2005).

Design and procedure

The study was conducted according to a randomized double-blind crossover design with two experimental sessions. One week before the first experimental session, after receiving oral and written information about the study and providing written informed consent, potential participants were invited to a screening interview that included the Structured Clinical Interview for DSM-IV (SCID-I) (First et al., 1995), Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery & Asberg, 1979), the Beck Depression Inventory-II (BDI-II) (Beck et al., 1996) and a medical examination. The SCID and the MADRS interviews were conducted by trained clinical psychologists. In addition, the cognitive test battery was administered.

During the experimental sessions, participants came to the laboratory at 8 or 9 am (-1h) after an overnight fast. Baseline mood was measured with the POMS and a first blood sample was obtained, followed by administration of cognitive tasks. The cognitive test battery took 45 minutes. Next, participants ate breakfast, including a drink containing either tryptophan-rich alpha-lactalbumin or casein (0 h). Lunch, at +2 h, also contained either an alpha-lactalbumin or casein drink. A second blood sample was taken at +3.5 h, followed by the POMS and cognitive tests at +3.75 h. Participants were tested individually, and remained in a private research room between assessments.

The order of presentation of the alpha-lactalbumin and casein diets was counterbalanced. Both experimental sessions were separated by exactly four weeks. Pre-menopausal women not taking a contraceptive pill were tested during their mid-to-late follicular phase (days 4–10), whereas pre-menopausal women taking the contraceptive pill participated during the period in which they actually took the contraceptive pill. All participants were paid €80 for

participation. The study was approved by the Medical Ethics Committee of Leiden University Medical Centre.

Statistical analysis

Prior to analysis, all variables were examined for accuracy of data-entry, missing values and fit between their distributions and the assumptions of the statistical analyses. Group differences in demographic characteristics, baseline mood ratings and cognitive performance were examined by means of chi-square statistics and multivariate analyses of variance (MANOVA) by using the General Linear Model (GLM: SPSS 11.5 for Windows, SPSS Inc, Chicago). RTs of the TOL and Left/Right task were $\log^{10}$ transformed prior to analysis. POMS scores were analysed with nonparametric statistics because transformations were unsuccessful, as shown by visual inspection and Shapiro Wilk statistics (Stevens, 1996). The effects of the interventions on cognitive tests and biochemical outcome measures were analysed by separate repeated measure multivariate analyses of variance, using Intervention (alpha-lactalbumin vs. casein) and Time (before vs. after intervention) as within-subjects factors and Group (recovered depressed patients vs. controls) as between-subjects factor. Thus Intervention x Time and Intervention x Time x Group interactions reflected the main effects of interest. Level of difficulty was added as a within-subjects factor for the Memory Scanning Task (three levels) and Tower of London (four levels); whereas the Left/Right tasks consisted of an additional level 'condition' (congruent vs. incongruent). Significant results revealed by these procedures were further examined by post hoc tests. Although we counterbalanced for order of intervention, we first conducted analyses with Order of intervention as a covariate. However, Order of

intervention did not contribute to any of the effects. All statistics were evaluated at a significance level of 5%.

Results

Data screening/ drop-outs

Of a total of 49 participants who were included, 43 (23 recovered depressed patients; 20 controls) completed the study. Three recovered depressed patients were included but decided not to participate. Three patients dropped out after the first session; the first case due to nausea (after alpha-lactalbumin), the second case dropped out because of feeling uncomfortable with venapunction during the first session (casein); the third one could not be contacted to schedule the second session on time (casein). These patients were left out of all analyses. Due to a computer failure, data of the cognitive tasks during the screening session for one control patient were lost. For one patient, data of the Memory Scanning task during the screening session were missing. TOL data during the screening session were missing for another patient. Morning assessments of the Left/Right task in the casein condition were unavailable for one patient. Twenty-two of the 172 blood samples (13%) were missing because of difficulties with the venapunction. Cases with missing data were omitted separately by analysis.

Participants, baseline cognitive performance

Clinical and demographic characteristics are shown in Table 1. Recovered depressed patients ('recovered MDD') did not differ from the participants with no history of depression ('controls') in terms of gender distribution, age and education level, indicating that matching was successful. BDI-II scores were higher in the patient group than in controls ($F(1,41) = 7.31$; $p = .01$); however,

BDI-II scores are low and well within the normal range in both groups. There were no group differences on any of the cognitive tasks conducted during the screening session or in the morning sessions of both conditions. Controlling data for group differences in BDI-II scores did not change these results.

Dietary effects on amino acids

Repeated measures analyses for tryptophan/LNAA with Intervention and Time as within-subjects factors and Group as between-subjects factors revealed a main effect of Intervention ($F(1,29) = 84.39$; $p < .001$), Time ($F(1,29) = 7.08$; $p = .01$) and a significant interaction between Intervention and Time ($F(1,29) = 193.28$; $p < .001$). Tryptophan/ LNAA increased significantly by 20.9% compared to baseline after the alpha-lactalbumin-diet and decreased by 30.0% after the casein diet. After alpha-lactalbumin, the tryptophan/LNAA ratio was 71.5% higher than after casein. After alpha-lactalbumin, tryptophan levels increased 77.5% relative to baseline and were 54.0% higher than after casein ($p < 0.001$). There were no group or baseline differences in plasma tryptophan or ratio tryptophan/LNAA. Repeated measures analyses for tyrosine/LNAA revealed a main effect of Intervention ($F(1,29) = 22.51$; $p < .001$), Time ($F(1,29) = 8.14$; $p = .01$) and a significant interaction between Intervention and Time ($F(1,29) = 123.59$; $p < .001$). Compared to baseline levels, tyrosine/ LNAA decreased 11.5% in the alpha-lactalbumin condition and increased 28.1 % in the casein condition. After casein, the tyrosine/LNAA ratio was 35.8% higher than after alpha-lactalbumin.

Table 1. Characteristics of the investigated sample

	Recovered MDD (n = 23)	**Controls** (n = 20)
Mean age in years ± SD	29.96 ± 9.7	26.95 ± 10.1
Female	n = 21	n = 17
Body Mass Index (BMI) (kg/m^2 ± SD)	22.84 ± 2.5	21.69 ± 2.1
Eduction level High[1] / Medium[2] / Low [3]	1 / 14 / 7	1 / 12 / 7
MADRS	1.30 ± 1.6	0.80 ± 1.6
BDI-II	4.43 ± 4.5	1.45 ± 2.2
Number of previous episodes	2.00 ± 0.9	
- Single episode	n = 7	
- Multiple episodes	n = 15	
Age of onset first episode ± SD	19.91 ± 7.7	
History of treatment:		
- SSRI	n = 1	
- Psychotherapy	n = 7	
- SSRI + Psychotherapy	n = 5	
- Alternative treatment	n = 3	
- Spontaneous recovery	n = 7	

[1] Higher vocational education, university; [2] Secondary education, medium and higher level or senior secondary vocational education; [3] Primary education, secondary education lower level. MADRS = Montgomery Asberg Depression Rating Scale; BDI-II = Beck Depression Inventory – 2nd edition; SSRI = selective serotonin reuptake inhibitor.

Mood

POMS depression scores (mean ± SE) in the alpha-lactalbumin session changed from 0.74 ± 0.29 to 0.17 ± 0.14 for the recovered MDD group. The mean ± SE for the control group in the alpha-lactalbumin session on that scale was 0.00 ± 0.00 both before and after intervention. POMS depression scores in the casein condition changed from 1.00 ± 0.47 to 0.17 ± 0.10 for the recovered MDD group and from 0.30 ± 0.16 to 0.00 ± 0.00 for the control group. Non-parametric tests did not reveal a significant Group or Intervention effect on any POMS subscale.

Cognition

Multivariate analysis of variance revealed an intervention x time interaction for the outcome measures of the APRT ($F(4,38) = 3.06$; $p = .03$). Further univariate tests revealed significant Intervention x Time interactions for the STM measures A' ($F(1,41) = 5.99$; $p = .02$) and RT ($F(1,41) = 4.07$; $p = .05$) and also for LTM–RT ($F(1,41) = 4.49$; $p = .04$), but not for LTM–A' ($F(1,41) = 0.08$; $p = .78$) (Figures 1 and 2). Alpha-lactalbumin diet improved the number of correctly recognized abstract pictures and improved speed of recognition from STM and LTM, but there were no group differences, as shown by nonsignificant Intervention x Time x Group interactions for STM–A' ($F(1,41) = 2.04$; $p = .16$), STM–RT ($F(1,41) = 0.53$; $p = .47$) and LTM–RT ($F(1,41) = 2.26$; $p = .14$). The interaction for LTM–A' was statistically a trend ($F(1,41) = 3.08$; $p = .09$). To further explore the effects as shown in Figures 1 and 2, separate analyses were conducted for the recovered MDD and control group. These should be interpreted with caution however because of an absence of an interaction with group in the primary analyses of interest. In the recovered MDD group, significant Intervention x Time interactions were

found for STM–A' ($F(1,22) = 9.09$; $p = .006$), LTM–RT ($F(1,22) = 4.64$; $p = .04$), and a trend for LTM–A' ($F(1,22) = 2.89$; $p = .10$). The Intervention x Time interaction for STM–RT was not significant ($F(1,22) = 0.70$; $p = .41$), whereas this interaction was the only significant interaction in the control group ($F(1,19) = 5.07$; $p = .04$).

For the Left/Right task, there was a multivariate significant Intervention x Time x Group effect ($F(2,39) = 4.12$; $p = .02$), due to a group difference in the subtask with variable stimulus intervals ($F(1,40) = 7.78$; $p = .01$). Separate analysis for the recovered MDD group and the control group showed that controls became faster after alpha-lactalbumin condition relative to casein condition, whereas the reverse pattern occurred in the recovered MDD group. Using a similar double multivariate repeated measures design, the number of errors increased in the alpha-lactalbumin condition relative to casein condition, as shown by an Intervention x Time interaction effect ($F(2,39) = 3.47$; $p = .02$). Univariate tests showed that this was true for the fixed interval subtask ($F(1,40) = 6.87$; $p = .01$). There were no higher order interactions with group.

On the Memory Scanning Task, there was an overall multivariate main effect of memory set on total reaction time ($F(2,40) = 81.34$; $p < .001$) and number of errors ($F(2,40) = 18.33$; $p < .001$), indicating that the time to respond and the number of errors increased when the memory set becomes larger. A similar pattern was found for the TOL, with slower responses ($F(3,36) = 173.53$; $p < .001$) and a higher number of errors ($F(3,36) = 43.85$; $p < .001$) as the task became more difficult.

There were no Intervention x Time, Intervention x Time x Group on the Memory Scanning Task, Stroop Task and TOL (Table 2).

Figures 1 and 2. Mean change (SE) in percentage of correctly recognized patterns (Figure 1, above) and median RT (ms) for correctly recognized patterns (Figure 2, below) for the Abstract Patterns Recognition Task as a function of group and intervention. Scores are changes relative to baseline performance (post – pre-intervention).

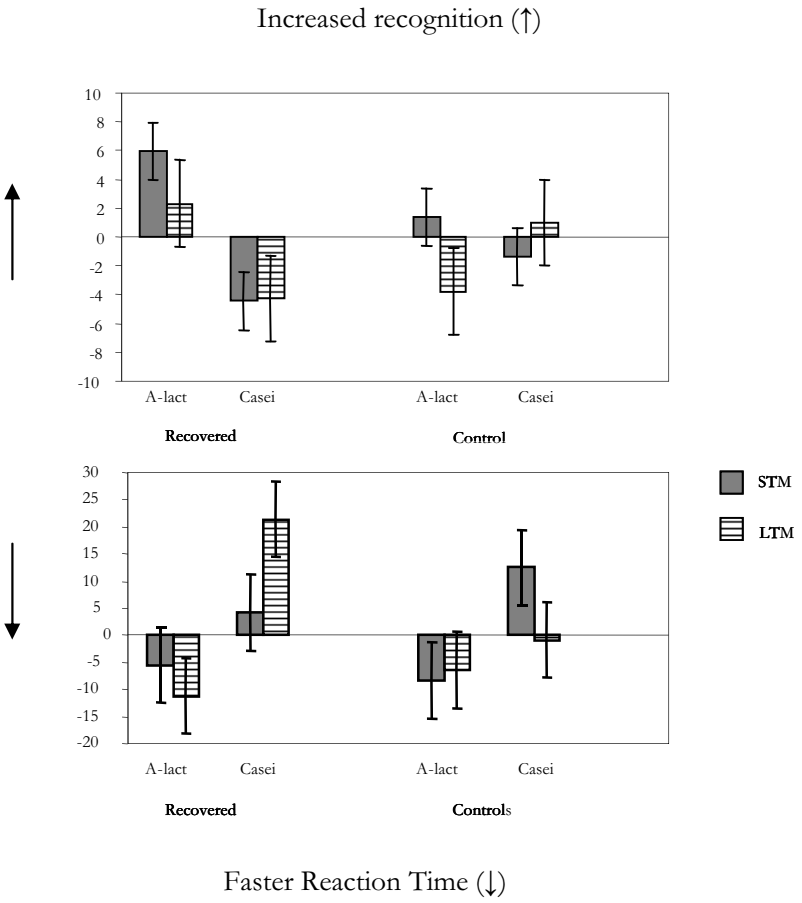

Table 2. The means (SE) of the cognitive tasks as a function of group, time and intervention

		Recovered MDD (*n* = 23)		**Controls** (*n* = 20)	
Cognitive test	Diet	before diet	after diet	before diet	after diet
Memory Scanning					
- % correct					
2 letters	α-lac	97.6 (0.5)	98.0 (0.4)	97.8 (0.3)	97.1 (0.4)
	casein	98.1 (0.4)	98.4 (0.3)	96.9 (0.5)	97.2 (0.3)
3 letters	α-lac	98.1 (0.5)	97.9 (0.5)	97.3 (0.4)	96.8 (0.5)
	casein	97.9 (0.5)	98.0 (0.4)	96.3 (0.5)	97.2 (0.5)
6 letters	α-lac	95.9 (0.8)	94.3 (1.3)	94.2 (1.1)	92.8 (1.2)
	casein	95.7 (0.8)	94.4 (1.1)	93.0 (0.8)	91.4 (2.1)
- RT(ms)					
2 letters	α-lac	464 (13)	465 (13)	468 (13)	456 (14)
	casein	456 (10)	463 (13)	465 (15)	467 (15)
3 letters	α-lac	489 (11)	489 (13)	503 (12)	492 (13)
	casein	491 (15)	480 (14)	516 (15)	496 (15)
6 letters	α-lac	560 (18)	567 (21)	580 (19)	599 (29)
	casein	570 (21)	566 (20)	583 (19)	594 (25)
SCWT					
Condition I (ms)	α-lac	476 (10)	488 (15)	478 (14)	463 (13)
	casein	473 (9)	489 (12)	468 (13)	454 (12)
Condition II (ms)	α-lac	563 (15)	557 (15)	529 (15)	515 (13)
	casein	557 (13)	554 (14)	537 (14)	523 (13)
Condition III	α-lac	726 (21)	696 (23)	699 (21)	660 (18)
(ms)	casein	720 (21)	710 (22)	701 (24)	652 (20)
Interference (%)	α-lac	39.5 (1.9)	32.9 (2.5)	38.6 (2.2)	35.0 (2.0)
	casein	40.0 (2.9)	36.1 (2.8)	39.6 (3.6)	33.3 (2.7)
Left/Right Task					
Congruent (ms)	α-lac	600 (19)	573 (21)	607 (21)	567 (18)
	casein	604 (18)	573 (13)	597 (21)	564 (16)
Incongruent (ms)	α-lac	602 (18)	603 (19)	624 (25)	588 (16)
	casein	599 (17)	584 (17)	608 (23)	586 (20)
Congruent	α-lac	619 (17)	613 (15)	629 (17)	588 (17)
(variable)	casein	625 (14)	607 (17)	618 (20)	609 (18)
Incongruent	α-lac	622 (16)	624 (18)	627 (20)	594 (14)
(variable)	casein	637 (16)	604 (16)	617 (19)	606 (18)

Table 2. *(continued)*

Cognitive test	Diet	**Recovered MDD** (*n* = 23)		**Controls** (*n* = 20)	
		before diet	after diet	before diet	after diet
TOL % correct					
2 step	α-lac	88.6 (2.9)	89.1 (3.0)	92.2 (2.7)	93.3 (2.9)
	casein	86.8 (3.9)	88.6 (3.2)	90.5 (2.3)	92.2 (2.5)
3 step	α-lac	89.5 (2.6)	89.1 (3.3)	89.4 (1.7)	88.9 (3.5)
	casein	91.4 (2.6)	92.7 (1.6)	89.4 (2.2)	91.7 (2.6)
4 step	α-lac	77.3 (4.2)	78.6 (3.8)	86.1 (3.3)	83.9 (3.1)
	casein	81.8 (3.3)	79.5 (3.7)	81.7 (3.2)	84.4 (2.4)
5 step	α-lac	74.1 (4.2)	74.5 (3.9)	72.8 (3.9)	80.0 (3.7)
	casein	75.0 (3.3)	75.5 (3.7)	68.3 (4.1)	72.2 (3.6)
TOL RT (ms)					
2 step	α-lac	4113 (280)	3513 (158)	4444 (465)	3784 (234)
	casein	3861 (235)	3627 (251)	4273 (320)	3710 (244)
3 step	α-lac	4766 (290)	4621 (1299)	5457 (325)	5235 (299)
	casein	4931 (323)	5007 (326)	5649 (608)	4834 (310)
4 step	α-lac	6936 (492)	6690 (407)	8095 (490)	7210 (431)
	casein	6670 (343)	6372 (327)	8008 (609)	7006 (442)
5 step	α-lac	10819 (786)	10010 (864)	13384 (1305)	12365 (1139)
	casein	10915 (703)	10584 (855)	11547 (922)	11398 (874)

α -lac = alpha-lactalbumin condition; SCWT = Stroop Colour Word Task; TOL = Tower of London; RT = reaction time

Discussion

The aim of this study was to investigate whether increased tryptophan availability after alpha-lactalbumin diet affects cognitive function, particularly in recovered depressed patients. The alpha-lactalbumin diet increased plasma tryptophan/LNAA ratio (21% increase from morning to afternoon; afternoon ratio 71.5% higher than in the placebo condition). Memory performance after alpha-lactalbumin improved in both groups, and no other reliable effects were found.

Baseline cognitive performance in recovered depressed patients vs. controls

Unexpectedly, no baseline differences in cognitive performance were found between recovered depressed patients and controls. Using the same cognitive tests, our previous study in remitted depressed patients showed impaired abstract long-term visual memory at baseline relative to controls (Booij et al., 2005a). This result is also in contrast with other studies that found residual cognitive impairments in short-term memory, attention and executive functioning in recovered depressed patients (e.g. Paradiso et al., 1997). One likely explanation for this difference might be that the recovered MDD group in the present study was much younger (30.0 years) than in our previous study (48.7 years) or in Paradiso et al. (55.9 years), with about one third of the individuals having experienced a single episode during late adolescence rather than multiple episodes. Cognitive impairments in the recovered phase may be more severe in patients with recurrent episodes than in those with a single episode (Kessing, 1998).

Dietary effects on cognitive performance

The alpha-lactalbumin diet improved abstract visual memory in both recovered depressed patients and controls. More specifically, alpha-lactalbumin improved recognition and speed of retrieval from short- and long-term abstract visual memory. Overall, there was no interaction with group. There were no effects on the TOL, Memory Scanning Task and Stroop Task, indicating that alpha-lactalbumin did not change the encoding phase, working memory, perception or general motor speed. Thus, alpha-lactalbumin may specifically affect memory consolidation in an early phase.

This effect mirrors the consolidation deficit that was found after lowering 5-HT function by tryptophan depletion in healthy volunteers (Riedel et al., 1999; Rubinsztein et al., 2001) and is in line with improved memory after a single dose of citalopram in healthy volunteers (Harmer et al., 2002). Alpha-lactalbumin also improved abstract visual memory in females with premenstrual symptoms (Schmitt et al., 2005). However, it is important to mention that the latter study did not include a control group. Hence, the present study showed that the beneficial effects on memory are not limited to individuals vulnerable to 5-HT related mental disorders. This is of interest because of the fact that the mean age of the participants in the present study was 28.5 years, while cognitive processes usually start to decline around 45–50 years (Hedden & Gabrieli, 2004: review). Thus abstract visual memory improved even though performance was uncompromised by aging or psychiatric symptoms.

Results might also be (partly) explained by impaired memory in the casein condition. Without a non-intervention control group, these possibilities cannot be separated. Nevertheless, the change of tryptophan/LNAA ratio in the casein condition was comparable to what is usually observed after a

balanced meal (Fischer et al., 2003; Spring et al., 1987), which justifies the use of casein as a placebo procedure (Schmitt et al., 2005).

Alpha-lactalbumin impaired motor performance, as shown by an increased number of errors in the fixed interval condition of the Left/Right task, irrespective of group. These results are consistent with previous studies finding decreased performance on simple RT tasks after 5-HT stimulation in patients and in healthy volunteers (Riedel et al., 2002; Sobczak et al., 2003). As intervention had no effect on the number of errors in the more difficult versions of the Left/Right task (variable time intervals) or on working memory tasks (Tower of London/Memory Scanning Task), this suggests that alpha-lactalbumin impaired cognitive performance when the task was relatively easy and monotonous, possibly due to the sleep-inducing properties of alpha-lactalbumin (Markus et al., 2005; Minet-Ringuet et al., 2004).

Complex interactions were found on the variable time interval condition of the Left/Right task. RTs improved in the control group after alpha-lactalbumin and remained unchanged after casein. Conversely, in the recovered MDD group, RTs improved after casein but did not change after alpha-lactalbumin. We have no clear explanation for this finding. In the Left/Right task, both task uncertainty (incongruent vs. congruent trials) and time uncertainty (fixed vs. variable stimulus time intervals) were manipulated. Alpha-lactalbumin had no differential effect on the congruent and incongruent trials, a finding consistent with the lack of effect on interference levels on the Stroop Colour Word Task in the present study. Thus, 5-HT did not affect performance in conditions of task uncertainty. No previous studies investigated the effects of 5-HT and/or depression on motor speed as a function of time uncertainty.

The lack of effect on the Memory Scanning Task found in the present study contrasts with the results of Markus et al. (1998; 2002), who reported improved information processing in individuals with high neuroticism scores. Life events and neuroticism are risk factors for major depression, and individuals with high neuroticism have a greater risk of major depression in response to a stressful life event (Kendler et al., 2004). Individuals with high neuroticism may benefit more from an alpha-lactalbumin diet because a high amount of 5-HT activity is required to cope with stress, thereby increasing the risk of a shortage in brain 5-HT concentrations, which increases the risk of depression (Markus et al., 2002). However, about 60% of the individuals with high neuroticism scores actually became depressed (Ormel et al., 2001). In addition, neuroticism has been shown to be stable over time in adulthood (Kendler et al., 2004). In the present study, most recovered depressed patients had been treated. They may also have coping mechanisms developed through effective treatment, which may reduce the detrimental effects of stress. The neuroticism (N) scores of the recovered depressed group in the present study were above average to high (mean ± SD: 5.64 ± 2.3, range: 2–10), while the control group had low-average scores (mean ± SD: 2.60 ± 2.0, range 0–8). These group differences are statistically and clinically significant but seem smaller than in Markus et al. (2002), who used a different measure and selected students with N scores in the lowest and highest quartile of a large subject pool. The results of the present study suggest that high neuroticism and history of depression are different concepts in terms of 5-HT vulnerability.

Mood remained unaffected, which is consistent with the finding that single administration of SSRIs ameliorated emotional processing in healthy volunteers, without changing mood (e.g. Harmer et al., 2003a). These findings support our suggestion that cognitive markers may be more sensitive makers

for changes in 5- HT function than mood or symptom scales (Booij et al., 2005a).

Effect of diet on plasma amino acids and serotonin

The increases in tryptophan and ratio tryptophan/LNAA were within similar range as in Schmitt et al. (2005) and Markus et al. (2000; 2002). However, the composition of breakfast and lunch used in Markus et al. (2000; 2002) and Schmitt et al. (2005) contained about three times more calories and two times more carbohydrates than in the present study, whereas the composition of the alpha-lactalbumin or sodium caseinate containing chocolate drink were identical. Thus, the present study demonstrated that alpha-lactalbumin is able to raise tryptophan levels without necessarily ingesting an excessive amount of carbohydrates, suggesting that alpha-lactalbumin might be relatively easy to implement within a regular diet. A study, reporting an increase of 16% relative to baseline using an amount of 12g alpha-lactalbumin combined with a regular meal, supports this notion (Beulens et al., 2004).

However, as we assessed total tryptophan concentration and not free tryptophan, it must be acknowledged that we do not know how much tryptophan following alpha-lactalbumin actually reaches the brain. Plasma tryptophan, however, circulates in two forms: either bound to plasma albumin proteins (80–90%) or free (10–20%). It has been argued that only free-circulating tryptophan controls the uptake of plasma tryptophan into the brain, whereas others have suggested that total tryptophan (plasma free and bound levels) is the most decisive factor. Separate studies have shown that both increases in total plasma tryptophan (initiated, for instance, by immobilization stress or carbohydrate consumption) and increases in free tryptophan (initiated, for instance, by physical stress or fasting) may lead to an increase in brain

tryptophan and serotonin activity (Chaouloff, 1993). Moreover, total- and free tryptophan are very closely related, also following an alpha-lactalbumin diet (Attenburrow et al., 2003). In animals, administration of alpha-lactalbumin increased baseline extracellular 5-HT in the hypothalamus, indicating that alpha-lactalbumin not only enhance 5-HT synthesis but also its release (Orosco et al., 2004). In humans, increases of 20–40% in ratio tryptophan/LNAA led to significant increases in peripheral markers of 5-HT activity, including cortisol and prolactin (Anderson et al., 1990b; Kaye et al., 1988; Markus et al., 2000).

Changes in mood and cognitive performance might also be related to raised plasma tyrosine levels, as catecholamines are involved in mood and cognitive processes as well (Booij et al., 2003: review). The effects on catecholamine precursor levels in the alpha-lactalbumin condition are probably negligible – in fact, the tyrosine/LNAA ratio decreased slightly. The rise in the casein condition was higher, which is not surprising as this diet contained less tryptophan and twice the amount of tyrosine (Markus et al., 2000; Markus et al., 2002). No other alpha-lactalbumin experiments have reported tyrosine/LNAA ratios. In healthy samples, tyrosine administration improved Stroop performance and working memory (Deijen & Orlebeke, 1994), however we found no intervention effect on working memory tasks. Similarly, a memory consolidation deficit was induced by ATD and not by Acute Phenylalanine Tyrosine Depletion (APTD), whereas a working memory deficit was induced by APTD and not by ATD (Harrison et al., 2004). High-dose ATD (100 g) markedly decreases tryptophan levels and 5-hydroxyindoleacetic acid (5-HIAA), but also induces an increase (50%) of tyrosine/LNAA levels – however, homovanillic acid (HVA) remains unaffected, measured either in cerebrospinal fluid (Carpenter et al., 1998) or plasma (Van der Does & Booij,

2005). Nevertheless, it is recommended to investigate the biochemical specificity further by combining alpha-lactalbumin- enriched diets with monoaminergic depletion paradigms and to develop alternative placebo procedures.

In conclusion, diet enriched with alpha-lactalbumin enhanced memory, irrespective of history of depression. Mood and other cognitive functions remained unaffected. As 5-HT activity is reported to decline with aging (McEntee & Crook, 1991), the present findings could be further examined in older samples. The long-term effects of alpha-lactalbumin should also be investigated.