

Whey protein rich in α -lactalbumin increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids and improves cognitive performance in stress-vulnerable subjects¹⁻³

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ABSTRACT

Background: Cognitive performance often declines under chronic stress exposure. The negative effect of chronic stress on performance may be mediated by reduced brain serotonin function. The uptake of the serotonin precursor tryptophan into the brain depends on nutrients that influence the availability of tryptophan by changing the ratio of plasma tryptophan to the sum of the other large neutral amino acids (Trp-LNAA ratio). In addition, a diet-induced increase in tryptophan may increase brain serotonergic activity levels and improve cognitive performance, particularly in high stress-vulnerable subjects.

Objective: We tested whether α -lactalbumin, a whey protein with a high tryptophan content, would increase the plasma Trp-LNAA ratio and improve cognitive performance in high stress-vulnerable subjects.

Design: Twenty-three high stress-vulnerable subjects and 29 low stress-vulnerable subjects participated in a double-blind, placebo-controlled, crossover study. All subjects conducted a memory-scanning task after the intake of a diet enriched with either α -lactalbumin (α -lactalbumin diet) or sodium caseinate (control diet). Blood samples were taken to measure the effect of dietary manipulation on the plasma Trp-LNAA ratio.

Results: A significantly greater increase in the plasma Trp-LNAA ratio after consumption of the α -lactalbumin diet than after the control diet ($P = 0.0001$) was observed; memory scanning improved significantly only in the high stress-vulnerable subjects ($P = 0.019$).

Conclusion: Because an increase in the plasma Trp-LNAA ratio is considered to be an indirect indication of increased brain serotonin function, the results suggest that dietary protein rich in α -lactalbumin improves cognitive performance in stress-vulnerable subjects via increased brain tryptophan and serotonin activities. *Am J Clin Nutr* 2002;75:1051-6.

KEY WORDS α -Lactalbumin, tryptophan, serotonin, stress, cognitive performance, whey protein, large neutral amino acids, Netherlands

INTRODUCTION

It has frequently been shown that chronic stress may have a detrimental effect on cognitive performance (1-3), eg, after traumatic stress experiences (3) and chronic life events (1). In

the past 2 decades, interest in the study of the mediating effects of different neurotransmitters on cognitive performance has increased, although the mechanisms involved in chronic stress-induced cognitive decline are still far from being understood.

A possible factor mediating the negative effect of chronic stress on cognitive performance is an imbalance in brain serotonin (5-hydroxytryptamine) function. Increased brain serotonin activity appears to be a prerequisite for maintaining control over cognitive information processes (4) and is involved in learning and memory (5). As brain serotonin secretion increases under stress (6, 7), chronic stress may result in frequently elevated concentrations of cerebral serotonin. As a consequence, serotonin release may be exhausted under chronic stress exposure, resulting in the depletion of available concentrations of brain serotonin and tryptophan, the precursor of serotonin, and causing serotonin activity to fall below functional needs (8-10).

Different dietary methods have been introduced to investigate the effect of alterations in brain serotonin activity on performance. In one method, plasma tryptophan availability for uptake into the brain is manipulated by feeding a diet with a greater amount of carbohydrate than protein. Because serotonin is synthesized from the dietary amino acid L-tryptophan, brain serotonin concentrations may increase after the intake of a carbohydrate-rich, protein-poor (CRPP) diet. A CRPP diet increases the ratio of plasma tryptophan to the sum of the other large neutral amino acids (Trp-LNAA ratio), giving tryptophan an advantage in the competition for access into the brain (8, 11-16).

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Because of the relation between serotonin function and learning and memory and between a CRPP diet and serotonin availability, the effects of carbohydrates on cognitive performance have been studied (17). The results of these studies, however, have been inconsistent, showing that carbohydrates improve performance (18), deteriorate performance (19), or show no effect at all (15, 20).

These inconsistencies can be explained by individual differences in the vulnerability of subjects to experience stress (8, 9). As serotonin function increases under acute stress, brain serotonin concentrations may be exhausted under continuous stress exposure. As a consequence, the serotonergic system of subjects prone to stress (high stress–vulnerable subjects) may become more sensitive to dietary-induced alterations in tryptophan availability because of compensatory receptor sensitization (21, 22).

The hypothesis that increases in the plasma Trp-LNAA ratio improve cognitive performance only in high stress–vulnerable subjects has recently received some support (9). In that study, a CRPP diet improved memory scanning after laboratory stress only in high stress–vulnerable subjects. However, because the authors did not measure changes in the plasma Trp-LNAA ratio and a placebo-controlled design was not used, it remained questionable whether a change in the plasma Trp-LNAA ratio was the mechanism involved.

A promising method for studying the cognitive-enhancing effects of increases in available brain tryptophan is to enrich the dietary protein component with α -lactalbumin. α -Lactalbumin is a whey protein with the highest tryptophan content (6%) of all food protein sources (23). Recently, α -lactalbumin was shown to increase the plasma Trp-LNAA ratio by 46–48% (24, 25) and to have mood-improving effects in high stress–vulnerable subjects under acute stress (25). The aim of the current study was to investigate whether a whey-protein fraction rich in α -lactalbumin can improve cognitive performance in high stress–vulnerable subjects by increasing the plasma Trp-LNAA ratio. Subjects prone to high or low stress participated in a double-blind, placebo-controlled experiment to compare the effects of diets containing either α -lactalbumin–enriched whey protein or casein protein.

SUBJECTS AND METHODS

Subjects

Dutch University students ($n = 425$) recruited through newspaper advertisements completed the Inadequacy Scale of the Dutch Personality Inventory (26), which measures neuroticism, and a questionnaire concerning personal details. Neuroticism is defined as the disposition to see events as alarming and to experience aversive emotional states (27). Thus, neurotic subjects are high stress–vulnerable (28) and frequently experience stress (29). From the highest quartile of the inventory scores ($\bar{x} \pm \text{SD}$: 26 ± 3 ; range: 18–35), 23 subjects (10 men and 13 women) were selected for the high stress–vulnerable (HS) group and from the lowest quartile of inventory scores (4.5 ± 1.2 ; range: 2–6), 29 subjects (13 men and 16 women) were selected for the low stress–vulnerable (LS) group. The subjects ranged in age from 17 to 33 y (mean age: 20 ± 2 y in the HS group and 21 ± 3 y in the LS group). Exclusion criteria for participation were chronic and current illnesses, psychiatric history, medication use, or irregular diets. All subjects selected for the experiment had a body mass index (BMI; in kg/m^2) in the normal range (BMI of 20–25): a mean of 23 ± 2 in the HS group and of 24 ± 2 in the LS group.

The study was approved by the Institutional Review Board of the experimental unit of the TNO Institute and complied with the European Council principles of Good Clinical Practice established in the Helsinki Declaration at the 18th World Medical Association.

Methods

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

On 2 experimental days, subjects performed a memory-scanning task after consuming breakfast and brunch containing either α -lactalbumin–enriched whey protein (α -lactalbumin diet) or casein protein (control diet). The order of presentation of the α -lactalbumin diet or the control diet was counterbalanced between subjects. Both experimental days were separated by a 4-wk period to allow for the control of the female subjects' menstrual cycle. Women not using contraception participated during their mid-late follicular phase (day 4–10), whereas women using contraception participated during the time when they actually consumed the contraception pill.

On each experimental day, 2 subjects arrived at the institute at 0900 and 1000, respectively. Subjects were sedentary, and only reading was allowed in the study room. Subjects fasted overnight before both experimental days; only water or tea without sugar was permitted. The subjects were fed breakfast on arrival, a between-meal snack at 1015 or 1115 (coffee or tea with a candy bar), and brunch at 1100 or 1200. One and one-half hours after brunch, a blood sample was taken and transferred to a 10-mL tube containing EDTA. The subjects were then brought into a laboratory room, seated in front of a computer screen, and instructed about the experiment. After receiving instruction, the subjects were exposed to a computerized Sternberg memory-scanning task (30, 31).

Diets

On both experimental days, subjects received an isoenergetic diet containing 7995 kJ with 9.9% of energy as protein, 61.4% of energy as carbohydrate, and 28.7% of energy as fat (Table 1). On both days, the diet foods were consumed over the breakfast, snack, and brunch periods. The 2 diets were similar except for the composition of a chocolate drink in which the protein sources

TABLE 1
Nutrient composition of the α -lactalbumin and control diets¹

Nutrient	α -Lactalbumin diet ²	Control diet ³
Carbohydrate		
(g)	289	289
(% of energy)	61.4	61.4
Protein		
(g)	47	47
(% of energy)	9.9	9.9
Fat		
(g)	62	62
(% of energy)	28.7	28.7
Energy (kJ)	7995	7996

¹Both diets contained bread, margarine, fruit jelly, tea, coffee, candy bar, grape juice, and a chocolate drink.

²The chocolate drink contained whey protein rich in α -lactalbumin.

³The chocolate drink contained sodium caseinate.

TABLE 2Composition and amino acid profile of the chocolate drink used in the α -lactalbumin and control diets¹

	α -Lactalbumin diet	Control diet
Composition (g)		
α -Lactalbumin-enriched whey protein	20	0
Sodium caseinate	0	15.5
Cacao	3.5	3.5
Granulated sugar	10	10
Dehydrated butter	0	3.25
Water	200	200
Amino acid profile		
Isoleucine (g/kg)	27.61	31.80
Leucine (g/kg)	47.56	59.31
Phenylalanine (g/kg)	20.80	32.24
Tyrosine (g/kg)	16.82	33.13
Valine (g/kg)	29.52	44.09
Tryptophan (g/kg)	12.32	9.51
Trp:LNAA	8.7	4.7

¹LNAA, large neutral amino acids.

differed. The chocolate drink in the α -lactalbumin diet contained a whey-protein fraction rich in α -lactalbumin (Proxime Alpha; Borculo Domo Ingredients, Borculo, Netherlands), and the chocolate drink in the control diet contained sodium caseinate (DMV International, Veghel, Netherlands). The chocolate drink was prepared within 15 min before breakfast (first drink) and 15 min before brunch (second drink) by mixing the manipulated chocolate powder with 200 mL water (75–80°C). Both chocolate drinks were isoenergetic and contained equal amounts of protein, carbohydrate, and fat. Rum aroma was added to mask the taste differences between the chocolate drinks. The subjects were supervised during the consumption of the diets to ensure that all foods were consumed. The nutrient composition and amino acid profile of the chocolate drinks were analyzed by HPLC (Ansynth Service BV, Roosendaal, Netherlands) and are shown in **Table 2**. As shown, the chocolate drink in the α -lactalbumin diet contained 12.32 g tryptophan/kg (plasma Trp-LNAA ratio = 8.7), whereas the chocolate drink in the control diet contained 9.51 g tryptophan/kg (plasma Trp-LNAA ratio = 4.7).

Cognitive performance

A computerized Sternberg memory-scanning task was used as a cognitive performance test because this task allows for subtracting different levels of mental processing (30, 31). This task consisted of 4 subtasks, corresponding to memory sets of 3, 4, 5, and 6 different consonants. Each subtask started with a presentation on the computer screen of the particular set to be memorized for as long as each subject needed, after which 60 trials followed. The presented letter belonged to the memorized set in 30 of the trials but not in the other 30 trials. Every first 10 trials in each subtask were used for practice and, therefore, the results were not used in the analysis. In each trial the probe letter was presented at the center of the screen for 1 s (machine paced with an interval range of 1 s). The order of presentation of the 60 letters was random. The subjects were instructed to decide as quickly as possible whether the presented letter did or did not belong to the memory set by pressing a red (no) or green (yes) button with the preferred hand. Specific button boards were used for both left- and right-handed subjects. Both the reaction time (RT) and

the amount of errors across the different subtasks were taken as a measure of accuracy of cognitive performance.

Biochemical analyses

The blood samples were collected in 10-mL evacuated tubes containing EDTA and centrifuged at $2650 \times g_{\max}$ for 20 min at 20°C. The supernatant fluid was stored at -70°C until analyzed. For the measurement of plasma amino acids, a sensitive, reproducible, and fully automated method was used as previously described (32). This method is based on reversed-phase HPLC and *o*-phthaldialdehyde precolumn derivitization with the use of a 5-mm Spherisorb octadecylsilane 2 column (125 \times 3 mm internal diameter; Phase Separations, Queenspenny, United Kingdom) for routine determination. The plasma Trp-LNAA ratio was ultimately calculated by dividing the plasma tryptophan concentration by the sum of the concentrations of other LNAAs, ie, valine, isoleucine, leucine, tyrosine, and phenylalanine.

Statistical analysis

The main research questions formulated in the introduction were analyzed by means of repeated-measures multivariate and univariate analyses of variance with the use of the general linear model (SPSS version 7.5 for WINDOWS; SPSS Inc, Chicago). The study had one between-subjects factor [stress-vulnerability (HS or LS group as independent variables)] and 2 within-subjects factors [diet (α -lactalbumin diet or control diet as independent variables) and memory load (memory sets of 3, 4, 5, or 6 consonants)]. For the effect of diet manipulation on RT and the amount of errors as a function of memory load, a multivariate analysis of variance was performed with first- and second-order polynomial contrasts (linear and quadratic effects). Only significant results from the multivariate analysis were further examined by univariate tests. Huynh-Feldt- or Greenhouse-Geisser-corrected *P* values, their corresponding epsilons, and the original (ie, uncorrected) degrees of freedom were reported when the sphericity assumption was not met. All statistics were evaluated at a significance level of 5% (two-tailed). Data are reported as means \pm SDs.

RESULTS

Plasma Trp-LNAA ratio

Repeated-measures analysis of variance with stress vulnerability as the between-subjects factor and diet as the within-subjects factor showed a significant effect of diet ($P < 0.0001$) on the plasma Trp-LNAA ratio, the increase in which was 43% greater after the α -lactalbumin diet than after the control diet. The mean plasma Trp-LNAA ratio was 0.073 ± 0.012 after the control diet and 0.104 ± 0.013 after the α -lactalbumin diet. No significant effects of stress vulnerability or any interaction effect were found.

Cognitive performance

Multivariate analysis of variance with stress vulnerability as the between-subjects factor and diet as the within-subjects factor showed a significant interaction effect of diet \times stress vulnerability ($P = 0.016$), indicating that the mean RT differed significantly between the HS and LS groups depending on the dietary condition. Further testing showed that the RT in the HS group was significantly lower ($P = 0.05$) after the α -lactalbumin diet (758 ± 137 ms) than after the control diet (800 ± 173 ms), whereas in the LS group the RT tended to be higher, although not



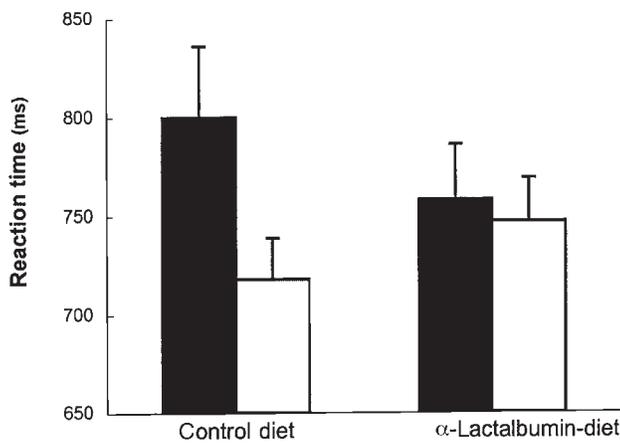


FIGURE 1. Mean (\pm SD) reaction time in the high stress-vulnerable (■) and low stress-vulnerable (□) groups after consumption of a diet containing protein as sodium casein (control diet) and a diet containing α -lactalbumin-enriched whey protein (α -lactalbumin diet). The reaction time improved significantly after the α -lactalbumin diet only in the high stress-vulnerable group ($P = 0.016$).

significantly so, after the α -lactalbumin diet (747 ± 118 ms) than after the control diet (718 ± 113 ms) (**Figure 1**).

It was further explored whether in the HS group the increase in RT as a function of memory load was less after the α -lactalbumin diet than after the control diet. This slope—the rate at which the RT increases with memory load—is interpreted by Sternberg (30, 31) as an indicator of the time needed for scanning an item in short-term memory. Multivariate analysis of variance with stress vulnerability as the between-subjects factor and diet and memory load as the within-subjects factors showed a significant effect only of memory load on RT ($P < 0.0001$), indicating a mean increase in RT as a function of memory load across memory sets of 3 (688 ± 142 ms), 4 (670 ± 120 ms), 5 (763 ± 146 ms), and 6 (894 ± 214 ms) items in all subjects, regardless of dietary condition ($P = 0.0001$). Further univariate analysis showed that this effect originated from the second- and third-polynomial contrast ($P < 0.0001$ and $P = 0.017$, respectively), even though the first linear contrast was also highly significant ($P = 0.0001$). This second- and third-polynomial effect seems to be caused by a learning effect during the first memory set. In addition, a second multivariate analysis performed without the first memory set again showed a significant effect of memory load ($P = 0.004$) originating from a linear increase in the RT ($\epsilon = 0.692$, $P < 0.0001$).

It was investigated whether subjects in the HS group would also make fewer errors on memory scanning after the α -lactalbumin diet than after the control diet. Multivariate analysis of variance with stress vulnerability as the between-subjects factor and diet as the within-subjects factor showed no significant effect of diet \times stress vulnerability or any other interaction effect.

A second multivariate analysis of variance with stress vulnerability as the between-subjects factor and memory load as the within-subjects factor showed a significant effect of memory load ($P < 0.0001$). Further polynomial analysis showed that this effect originated from a linear increase in the amount of errors as a function of memory load ($P = 0.04$), indicating that all subjects made more errors when memory load increased from a memory set of 3 (0.6 ± 1.15 errors), to one of 4 (0.7 ± 1.1 errors), 5 (1.22 ± 1.7 errors), or 6 (1.10 ± 1.6 error). No other effects were found.

DISCUSSION

In the current study we showed that α -lactalbumin-enriched whey protein increased the plasma Trp-LNAA ratio in both the HS and LS groups, but only in the HS group was the RT better (faster) after consumption of the α -lactalbumin diet than after the control diet.

Dietary effect on the plasma Trp-LNAA ratio

The plasma Trp-LNAA ratio was significantly higher after the α -lactalbumin diet than after the control diet, indicating that more tryptophan was available for uptake into the brain during the α -lactalbumin diet. This is expected to lead to an increase in central serotonin synthesis (11–14). The 43% increase in the plasma Trp-LNAA ratio observed in this experiment is comparable with the 42% increase previously found in stress-prone subjects after consumption of a genuine CRPP diet (8). The current results confirm our recent findings that α -lactalbumin-enriched whey protein prevents the usual reducing effect of standard protein on the plasma Trp-LNAA ratio and constitutes an alternative dietary method to increase brain tryptophan and serotonin concentrations in double-blind, placebo-controlled designs (24, 25).

Dietary effect on cognitive performance

Multivariate analysis showed a significant 2-way interaction effect of diet \times stress vulnerability on the mean RT across all 4 memory sets. Further testing indicated that this interaction effect particularly originated from faster memory scanning in the HS group after the α -lactalbumin diet than after the control diet. This effect was not found in the LS group. In all subjects, there was a significant linear increase in the RT as a function of memory load, particularly across the last 3 memory sets. The slope of the RT is regarded as an index of a cognitive comparison process involved in scanning an item in short-term memory, whereas the intercept is a measure of both the input and output stages of information processing regardless of memory load (30, 31). Because there were no significant effects of dietary manipulation on the slope of the RT, the 2-way interaction effect on the mean RT indicates that the input and output stages of information processing improved after the α -lactalbumin diet only in the HS group. Comparable effects were found in a previous study of the effects of a CRPP diet in stress-prone subjects (9). In that study, input and output stages of information processing after a CRPP diet improved only in high-stress subjects after controllable stress (9). The authors suggested that the memory-enhancing effect of the CRPP diet may have been caused by increases in tryptophan available for uptake into the brain. Results from the current study support this hypothesis because we showed that increases in plasma tryptophan available for uptake into the brain in a placebo-controlled design improved memory scanning only in the HS group.

Effect of dietary manipulation on brain serotonin

Although it is generally known that changes in the plasma Trp-LNAA ratio can significantly affect central serotonin function, in humans it is not clear to what extent the ratio must increase to cause meaningful changes in brain serotonin synthesis and function. For instance, in one study it was argued that an increase in the plasma Trp-LNAA ratio of $\geq 50\%$ is necessary to cause a meaningful enhancement of brain serotonin in humans (33). In that study it was shown that even an impressive 47% increase in the plasma Trp-LNAA ratio after consumption of an orange juice drink did not significantly increase the concentration of the



serotonin metabolite 5-hydroxyindoleacetic acid in cerebrospinal fluid, which is thought to reflect serotonergic activity in the brain. The increase in the plasma Trp-LNAA ratio was not significant because of a small group of neurologic patients ($n = 5$; mean age: 71 y) diagnosed with normal pressure hydrocephalus. Others found that a 49.5% decrease in the plasma Trp-LNAA ratio was accompanied by significantly lower 5-hydroxyindoleacetic acid concentrations in the cerebrospinal fluid (34), and still others reported that increases of 20–40% in the plasma Trp-LNAA ratio led to brain serotonin-mediated neuroendocrine alterations (35, 36). These different findings and perspectives emphasize the need to search for conclusive evidence concerning the extent to which the plasma Trp-LNAA ratio must increase to cause a meaningful change in brain serotonin concentrations. Our results suggest that enhancing the tryptophan availability in the brain leads to a clear effect on performance, although only in stress-vulnerable individuals. It is probable that enhancement of tryptophan leads to an enhanced function of the serotonin system in the brain, which is apparently necessary to counteract the effects of stress on performance.

Conclusion

The results of the current study showed a significantly greater increase in the plasma Trp-LNAA ratio after consumption of the α -lactalbumin diet than after consumption of the control diet; cognitive performance improved only in the HS group. These effects of dietary manipulation did not appear to result from differences in dietary consumption, expectations about the food or purpose of the study, or inappropriate biochemical analyses. Our study was double-blind and controlled and all subjects consumed the diet (which was similar in nutrient composition, appearance, and taste) under direct observation. In addition, all blood samples were handled in accordance with standardized procedures. Because increases in the plasma Trp-LNAA ratio are considered to be indirect indexes of elevated brain tryptophan and serotonin concentrations, these findings emphasize the involvement of brain serotonin. However, further research is needed to find conclusive evidence on diet-induced changes in cerebral serotonin to justify this assumption.

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