The bovine protein α-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress[1–3]

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ABSTRACT

Background: Increased brain serotonin may improve the ability to cope with stress, whereas a decline in serotonin activity is involved in depressive mood. The uptake of the serotonin precursor, tryptophan, into the brain is dependent on nutrients that influence the cerebral availability of tryptophan via a change in the ratio of plasma tryptophan to the sum of the other large neutral amino acids (Trp-LNAA ratio). Therefore, a diet-induced increase in tryptophan availability may increase brain serotonin synthesis and improve coping and mood, particularly in stress-vulnerable subjects.

Objective: We tested whether α-lactalbumin, a whey protein with a high tryptophan content, may increase the plasma Trp-LNAA ratio and reduce depressive mood and cortisol concentrations in stress-vulnerable subjects under acute stress.

Design: Twenty-nine highly stress-vulnerable subjects and 29 relatively stress-invulnerable subjects participated in a double-blind, placebo-controlled study. Subjects were exposed to experimental stress after the intake of a diet enriched with either α-lactalbumin or sodium-caseinate. Diet-induced changes in the plasma Trp-LNAA ratio and prolactin were measured. Changes in mood, pulse rate, skin conductance, and cortisol concentrations were assessed before and after the stressor.

Results: The plasma Trp-LNAA ratio was 48% higher after the α-lactalbumin diet than after the casein diet ($P = 0.0001$). In stress-vulnerable subjects this was accompanied by higher prolactin concentrations ($P = 0.001$), a decrease in cortisol ($P = 0.036$), and reduced depressive feelings ($P = 0.007$) under stress.

Conclusions: Consumption of a dietary protein enriched in tryptophan increased the plasma Trp-LNAA ratio and, in stress-vulnerable subjects, improved coping ability, probably through alterations in brain serotonin. *Am J Clin Nutr* 2000;71:1536–44.

KEY WORDS Tryptophan, α-lactalbumin, serotonin, prolactin, cortisol, stress, mood

INTRODUCTION

Enhanced brain serotonin (5-hydroxytryptamine) activity is an established consequence of stress (1, 2), whereas a deficient central serotonin function has been shown in mood disorders like depression (3, 4). Hence, a rise in serotonin may improve the adaptation to stress (5–8) and prevent a deterioration of mood.

Serotonin is synthesized from the dietary amino acid tryptophan, and brain serotonin concentrations rise with tryptophan administration or with the intake of a carbohydrate-rich, protein-poor (CR-PP) diet. Both raise the ratio of plasma tryptophan to the sum of the other large neutral amino acids (Trp-LNAA ratio) and give tryptophan the advantage in the competition for access to the brain (9–12). An increase in the plasma Trp-LNAA ratio with a CR-PP diet is caused by a carbohydrate-induced rise in glucose, which triggers insulin secretion and facilitates the uptake of the LNAAs except tryptophan into the skeletal muscles. A protein-rich diet decreases the plasma Trp-LNAA ratio, because proteins are poor in tryptophan (1–2%) but rich in the LNAAs valine, tyrosine, leucine, isoleucine, and phenylalanine (25%). These dietary effects on the plasma Trp-LNAA ratio have frequently been shown (13–15).

Several studies have investigated the effect of diet-induced alterations in the plasma Trp-LNAA ratio on mood. Although in clinical populations an elevated plasma Trp-LNAA ratio has been shown to improve mood (15, 16), in healthy subjects such evidence has been weak and often inconsistent (17, 18).

We suggested previously that differences in the vulnerability to stress may explain some of the inconsistent findings in the literature (14, 19). Assuming that vulnerability to stress indicates a frequently elevated level of brain serotonin activity, in stress-vulnerable subjects the serotonergic system may be overloaded during acute stress and, subsequently, serotonin activity may fall below functional needs. In a recent study, we found that a CR-PP diet increased the plasma Trp-LNAA ratio and prevented a

stress-induced rise in depressive mood and cortisol in stress-vulnerable subjects (14). In a subsequent study, a CR-PP diet prevented a cortisol response and depressive mood during controllable and uncontrollable stress in stress-vulnerable subjects (CR Markus, G Panhuysen, A Tuiten, H Koppeschaar, unpublished observations, 1999). The dietary effects on cortisol concentration were surprising because the administration of serotonin precursors is usually found to stimulate cortisol secretion (3). However, under acute stress, increases in brain serotonin activity may facilitate coping and different serotonergic pathways are involved in the initiation as well as the termination of a stress response (7, 8). Stress adaptation is associated with a reduction in cortisol concentration and depression (20, 21); thus, we assume that in stress-vulnerable subjects the CR-PP diet, by increasing brain serotonin, will improve the ability to cope with stress and subsequently reduce a cortisol response.

Serotonin activity might be enhanced more by increases in the Trp-LNAA ratio in stress-vulnerable subjects because of chronic stress-induced sensitization of the serotonergic system. Chronic stress may decrease brain serotonin availability and increase serotonin receptor function by way of a compensatory mechanism (22, 23). Accordingly, changes in serotonin receptor sensitivity are believed to mediate the effect of tryptophan administration or antidepressant treatment on depression (24, 25).

In line with previous findings, the aim of the present study was to test whether increases in tryptophan availability and brain serotonin are responsible for the dietary effect on depressive mood and cortisol responses to stress in stress-vulnerable subjects. If an increased availability of tryptophan and brain serotonin is the main factor, proteins with a high tryptophan content are likely to have the same effect as a CR-PP diet. Because α-lactalbumin has the highest tryptophan concentration of all bovine protein fractions (26), we hypothesized that a diet composed of α-lactalbumin–enriched whey protein may also increase the Trp-LNAA ratio and consequently central serotonin activity and thus prevent depressive mood and cortisol responses in stress-vulnerable subjects under acute stress. To test this hypothesis, subjects with high and low vulnerability to stress participated in a double-blind, placebo-controlled stress experiment in which they received isoenergetic diets containing either α-lactalbumin–enriched whey protein or casein protein. Because the secretion of prolactin is regulated by serotonergic mechanisms in the brain (27, 28), increases in plasma prolactin were measured as indexes of enhanced brain serotonin function (29, 30) and hypersensitivity of the serotonergic system (31, 32).

SUBJECTS AND METHODS

Subjects

Utrecht University students (*n* = 455) filled out the Inadequacy (IN) Scale of the Dutch Personality Inventory, which measures neuroticism (33), and a questionnaire concerning personal details. Neuroticism is closely related to negative affectivity (34), the disposition to see events as alarming, to experience aversive emotional states (35), and to be vulnerable to stress (36). Accordingly, subjects high in neuroticism frequently experience stress (37). On the basis of these findings, 29 subjects (10 men and 19 women) were selected from the students in the highest quartile of IN scores as the highly stress-vulnerable (HS) group (IN score = 26 ± 6; range: 18–38). From the students in the lowest quartile of IN scores, 29 subjects (9 men and 20 women) were selected as the relatively stress-invulnerable (LS) group (IN score = 4.6 ± 1.3; range: 2–6). Subjects' ages ranged from 17 to 34 y (mean age in HS group: 20.5 ± 3.10 y; mean age in LS group: 20.9 ± 3.18 y). Exclusion criteria were chronic and current illness, history of psychiatric illness, taking medication, or consuming irregular diets. All subjects selected for the experiment had a body mass index (BMI; in kg/m²) in the normal range, ie, between 20 and 25 (mean BMI in HS group: 23.15 ± 1.86; mean BMI in LS group: 23.74 ± 2.0). Because oral contraceptive medication may have an effect on cortisol responsiveness (38), female subjects were matched for contraception method. The protocol for the present study was approved by the institutional board of experimental research of University Utrecht.

Experimental procedures

During the 2 experimental days, subjects were placed under experimental stress while following a balanced diet containing either an α-lactalbumin–enriched whey protein (α-lac diet) or casein (casein diet). The diets were isoenergetic and contained equal amounts of protein, carbohydrate, and fat. The order of presentation of the α-lac and casein diets was counterbalanced between subjects. The 2 experimental days were separated by a 4-wk period, allowing for the menstrual phase of the female subjects. The women not taking the contraceptive pill participated during their mid-to-late follicular phase (days 4–10), whereas women taking the contraceptive pill participated during the period in which they actually took the contraceptive pill.

On each experimental day, 2 subjects arrived at the laboratory, one at 0900 and one at 1000. Subjects were sedentary and were allowed only to read in a study room. Subjects had fasted overnight; only water or tea without sugar was permitted. They received breakfast on arrival, a snack at 1015 or 1115, and lunch at 1100 or 1200. One and one-half hour after lunch, a first salivary cortisol sample was taken, followed 3 min later by a blood sample (transferred to a 10-mL tube containing EDTA). Then each subject was brought into a temperature-controlled laboratory room, seated in front of a computer screen, and instructed about the experiment. The electrodes for measuring skin conductance and the finger sensor for measuring pulse rate were attached, and during the next 10 min, baseline physiologic recordings were made. Subsequently, the subject was exposed to a computer-assisted battery of tests and experimental tasks, all integrated into the research software package MINDS (39). The battery consisted of the following: a version of the Profile of Mood States (POMS; 40), a stress-inducing mental arithmetic task (41), and a second version of the POMS. A second salivary sample was taken at the end of the stress-inducing task, ≈25 min after the onset of the stress; a third cortisol sample was taken after completion of the experiment (35 min after the stress). Pulse rate and skin conductance were recorded until the end of the experiment. Subjects spent ≈4 min on the first POMS, 25 min on the stress-inducing task, and 4 min on the second POMS. The diets were administered and the experimental measures were made by a research assistant blind to the purpose of the experiment and dietary conditions.

Diets

On both experimental days, an isoenergetic diet composed of standard products and providing 7995 kJ was used with 10% of energy as protein, 60% of energy as carbohydrate, and 30% of

TABLE 1
Compositions of the α-lac and casein diets[1]

Foods	Nutrient composition			
	Carbohydrate	Protein	Fat	Energy
	g			kJ
α-Lac diet	289 (61.5)[2]	46.8 (9.9)	62 (28.7)	7995
Bread, margarine, fruit jelly, tea, coffee, candy bar, grape juice, chocolate drink				
Casein diet	289 (61.5)	46.6 (9.9)	62 (28.7)	7996
Bread, margarine, fruit jelly, tea, coffee, candy bar, grape juice, chocolate drink				

[1] The casein and α-lactalbumin (α-lac) were added to the chocolate drinks.
[2] $\overline{x}$; percentage of total energy in parentheses.

energy as fat (**Table 1**). The 2 diets were similar with the exception of the composition of a chocolate drink in which the protein sources differed. The chocolate drink of the α-lac diet contained an α-lactalbumin–enriched whey protein (Borculo Domo Ingredients, Borculo, Netherlands) and the chocolate drink of the casein diet contained sodium caseinate (DMV International, Veghel, Netherlands). The chocolate drink was prepared within 20 min of breakfast (first drink) and 20 min before lunch (second drink) by mixing the specially prepared chocolate powders with 200 mL water (under a constant temperature of 75–80 °C). The chocolate drinks were isoenergetic and contained equal amounts of protein, carbohydrate, and fat. Rum flavoring was added to mask any taste differences between the chocolate drinks.

During the experiment, all meals were constantly supervised to make sure that all foods were consumed. The nutrient composition and the amino acid profile of both chocolate drinks were analyzed by HPLC (Ansynth Service BV, Roosendaal, Netherlands) and are given in **Table 2**. As shown, the chocolate drink of the α-lac diet contained 12.32 g/kg tryptophan (Trp-LNAA ratio of 8.7), whereas the drink of the casein diet contained 9.51 g/kg tryptophan (Trp-LNAA ratio of 4.7).

Profile of Mood States

Changes in mood were measured by using 2 scales of the Dutch shortened version of the POMS questionnaire (40) offered on the computer screen with a 5-point interval scale ranging from "strongly disagree" to "strongly agree." The first scale was offered before the start of the stress-inducing task (−5 min) and the second scale was offered after the stress task (25 min). The POMS comprises 5 different subscales for mood. The subscales Anger (range: 7–35), Depression (range: 8–40), Fatigue (range: 6–30), and Tension (range: 6–30) refer to a negative mood state, whereas the subscale Vigor (range: 5–25) concerns a positive mood.

Experimental stress

Subjects were given 18 successive 1-min trials in which they had to do mental arithmetic under time constraints while at the same time receiving different levels of industrial noise (65, 70, or 80 dB) through headphones. During each trial, multiple-choice calculations were presented on a computer screen one at a time. A specified number of calculations (called the criterion) had to be solved correctly. Subjects were told that by their performance they could control the intensity of noise presented to them during the task. If they failed the criterion, they could not choose the level of noise and the computer would then set the

noise level to be presented during the next trial; however, if they met the criterion they could choose the noise level for the next trial. Before the actual test, subjects were given 2 practice trials in which they had to solve a few sums first without noise and then in the presence of 3 successive noise levels. The credibility of the task, as well as the motivation of the subjects, was enhanced by providing the subjects with constant on-screen feedback on the criterion for the particular trial, the number of sums already solved correctly, and the time left for that trial. Experimental stress was induced by manipulating the criterion so that all subjects continued to fail each trial and, thus, could not choose the noise intensity for the next trial. The criterion was always set at one sum above what subjects could manage, as calculated from the average time per sum needed on the previous trial. This task has been shown to be uncontrollable and to induce psychological and physiologic stress (14, 41).

Biochemical analyses

The blood samples are collected in 10-mL evacuated tubes containing EDTA and centrifuged at $2650 \times g_{max}$ for 20 min at 20 °C. The supernate then was stored at −70 °C until analyzed.

For the measurement of amino acids in plasma, a sensitive, reproducible, and fully automated method described previously was used (42). This method is based on reversed-phase HPLC and o-phthaldialdehyde precolumn derivatization, making use of a 5-mm Spherisorb octadecylsilane 2 column (125 × 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 other LNAAs, ie valine, isoleucine, leucine, tyrosine, and phenylalanine.

Prolactin concentrations in plasma were assayed in duplicate by using a standard radioimmunoassay kit (ImmuChem IRMA; ICN Pharmaceuticals, Costa Mesa, CA) with intra- and interassay CVs of 3% and 5%, respectively. Prolactin concentrations are expressed as μg/L.

Measurement of salivary cortisol

One baseline cortisol sample (−15 min), a second poststress sample (25 min), and a third poststress sample (35 min) were obtained with use of the Salivette sampling device (Sarstedt, Etten-Leur, Netherlands). With this device, saliva was collected in small cotton swabs and stored in special tubes until centrifuged. Saliva samples were centrifuged at $2650 \times g_{max}$ for 3 min at 20 °C and then stored at −23 °C until analyzed.

TABLE 2
Compositions and amino acid profiles of the chocolate drinks used in the α-lac and casein diets[1]

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

[1] α-Lac, containing α-lactalbumin–enriched whey protein; Trp:LNAA, the ratio of tryptophan to the sum of the other large neutral amino acids.

Cortisol concentrations were determined without extraction in a licensed laboratory at the University Hospital Utrecht by using an in-house competitive radioimmunoassay with a polyclonal anti-cortisol antibody (K7348). [1,2-^{3}H]N-Hydrocortisone (NET 185; NEN-Dupont, Dreiech, Germany) was used as a tracer after chromatographic verification of its purity. The lower limit of detection was 0.5 nmol/L and interassay variation was 11.0%, 8.2%, and 7.6% at 4.7, 9.7, and 14.0 nmol/L, respectively (n = 20). The reference range for adults is 4–28 nmol/L at 0800–1000.

Measurement of skin conductance

Skin conductance was measured by using Ag-AgCl electrodes (surface area of 0.5 cm^2) filled with solid adhesive gel (ARBO H91; Braunschweig, Germany). These electrodes were placed bipolarly at the thenar palmar and hypothenar palmar sites of the nonpreferred hand of each subject. Skin conductance was measured with a constant voltage of 0.5 V (sampling rate of 2 Hz). Tonic skin conductance levels were recorded starting from a 10-min baseline rest period until the end of the experiment.

Measurement of peripheral pulse frequency

Reflection-photoplethysmography was used to measure changes in peripheral pulse rate at the volar surface of the middle finger of the nonpreferred hand. The signal was transduced by Sat-Trak signal processing (sampling rate of 65 Hz), based on the Quadrature Division Multiplexing (QDM) technique, by the Pulse Oximeter (SensorMedics Corp, Bilthoven, Netherlands). With a sampling rate of 1 Hz, pulse rate samples were collected and stored in the computer.

Experimental design and statistical analysis

The main research questions formulated in the introduction were analyzed by means of repeated-measures multivariate and univariate analyses of variance (MANOVA and ANOVA) by using the general linear model (SPSS version 7.5 for WINDOWS; SPSS Inc, Chicago) with one between-subjects factor, "stress vulnerability" (HS versus LS, as independent variables), and 2 within-subjects factors, "diet" (α-lac diet versus casein diet, as independent variables) and "experimental stress" (measures before versus after the experimental stress task of pulse rate, skin conductance, cortisol, and mood, as dependent variables). Although we counterbalanced for the order of diets (α-lac diet first followed by casein diet, versus the opposite order), the order of diets was preliminarily taken as a between-subjects factor. For the effect of experimental stress on pulse rate, skin conductance, and cortisol concentration, MANOVAs were performed with first- and second-order polynomial contrasts (linear and quadratic effects). Significant multivariate results shown by these procedures were further examined by univariate tests. Huynh-Feldt– or Greenhouse-Geisser–corrected P values, their corresponding epsilons (ϵ), as well as the original (ie, uncorrected) degrees of freedom were reported when the sphericity assumption was not met. Pearson's correlation coefficients were calculated to examine relations of the plasma Trp-LNAA ratio to prolactin. Because order of diets and sex and contraceptive use did not contribute to any of the scores, final analyses were performed with only stress vulnerability as the between-subjects factor. Analyses for variables with expected directions of change (eg, cortisol and depression) were assessed by unidirectional comparisons (43). All statistics were evaluated at a significance level of 5%. Data are reported as means ± SDs.

RESULTS

Plasma Trp-LNAA ratio

Analysis revealed a significant effect of diet ($F_{[1,56]}$ = 327.557, P < 0.0001), ie, a significant 48% increase in the plasma Trp-LNAA ratio after the α-lac dietcomparted with the casein diet. As shown in **Figure 1**, the mean plasma Trp-LNAA ratio was 0.071 ± 0.012 after the casein diet compared with 0.104 ± 0.013 after the α-lac diet. No effects of stress vulnerability or any other effects were found.

Prolactin

ANOVA showed a significant interaction of diet with stress vulnerability ($F_{[1,49]}$ = 5.224, P = 0.027), indicating that the effect of the diet on prolactin concentrations depended on the vulnerability to stress of the subjects. As indicated in Figure 1, in HS subjects there was a significant 40% difference in prolactin between diet conditions (12.60 ± 4.0 μg/L after the casein diet compared with 17.70 ± 8.2 μg/L after the α-lac diet; P = 0.001), whereas in LS subjects, no dietary effects were found (16.21 ± 8.4 and 15.76 ± 9.0 μg/L; P = 0.79). There were no significant between-subjects effects of stress vulnerability. There were also significant positive correlations between changes in the plasma Trp-LNAA ratio and prolactin concentrations during the α-lac diet compared with the casein diet in HS subjects (R = 0.45, P = 0.028) but not in LS subjects (R = 0.24, P = 0.21).

Physiologic measures

MANOVA found a significant effect of experimental stress on pulse rate ($F_{[2,55]}$ = 115.65, P < 0.0001) and skin conductance ($F_{[2,55]}$ = 120, P < 0.0001). As shown in **Figure 2**, experimental stress increased pulse rate from 76.12 ± 10 beats/min before the stress task to 85.20 ± 11.5 beats/min during the stress task ($F_{[1,56]}$ = 21.88, P < 0.0001, ϵ = 0.781); skin conductance increased from 4.0 ± 2.63 to 8.8 ± 4.30 μs ($F_{[1,56]}$ = 201.13, P < 0.0001, ϵ = 0.644). Multivariate analysis also showed a significant 3-way
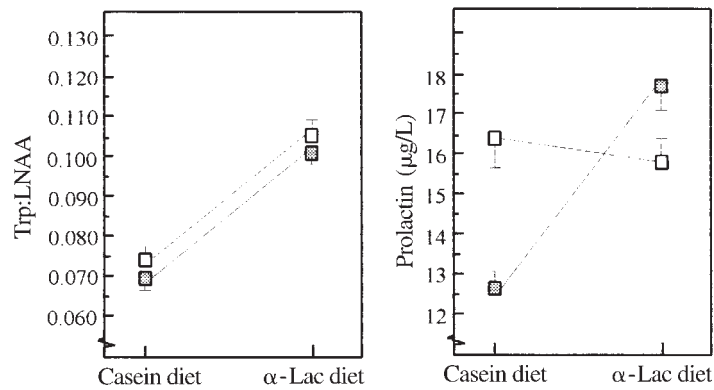
FIGURE 1. The plasma ratio of tryptophan to the other large neutral amino acids (Trp:LNAA) was significantly higher after the α-lactalbumin–enriched whey protein diet (α-lac diet) than after the casein diet in both highly stress-vulnerable (⊠) and relatively stress invulnerable (□) subjects ($P < 0.0001$). Prolactin concentrations were significantly higher after the α-lac diet and only in highly stress-vulnerable subjects ($P = 0.001$). $\bar{x} \pm$ SEM.

interaction of stress vulnerability with diet and experimental stress on pulse rate ($F_{[2,55]} = 6.44$, $P = 0.003$). Further univariate analysis revealed that this interaction effect originated from the first (linear) polynomial contrast ($F_{[1,56]} = 11.71$, $P = 0.001$, $\epsilon = 0.915$), meaning that pulse rate in HS subjects increased more during experimental stress after the casein diet (from 77.90 ± 8.65 to 88.17 ± 10.51 beats/min) than after the α-lac diet (from 80.41 ± 9.10 to 87.93 ± 10.22 beats/min). No effects of stress vulnerability or diet were found on skin conductance.

MANOVA showed a nearly significant interaction of stress vulnerability with diet and experimental stress ($F_{[2,54]} = 2.14$, $P = 0.06$) that originated from a significant linear change in cortisol concentration ($F_{[1,55]} = 3.35$, $P = 0.036$), meaning that a dietary effect on cortisol responses under experimental stress depended on the stress vulnerability of the subject. As shown in Figure 2, in HS subjects cortisol concentrations increased with experimental stress from 9.36 ± 2.41 to 10.65 ± 3.09 nmol/L after the casein diet, whereas after the α-lac diet a cortisol stress response was prevented (concentrations were 10.73 ± 3.64

before and 10.20 ± 3.86 nmol/L after). These effects where not found in LS subjects. There were no other effects of stress vulnerability or diet.

Mood

ANOVA showed a marginally significant interaction effect of stress vulnerability and diet with experimental stress on the mean scores of the depression subscale of the POMS ($F_{[1,56]} = 2.116$, $P = 0.07$). Because the dietary effect was expected only in HS subjects under acute stress, we performed a second analysis among 18 HS and 21 LS subjects who displayed a physiologic stress response during the stress task (measured by increased pulse rate and skin conductance) during the casein diet condition. ANOVA revealed a significant interaction effect of stress vulnerability and diet with experimental stress ($F_{[1,37]} = 6.629$, $P = 0.007$), indicating that the effect of diet on feelings of depression under acute stress depended on the stress vulnerability of the subject. As shown in **Figure 3**, in HS subjects feelings of depression increased slightly with experimental stress from scores of
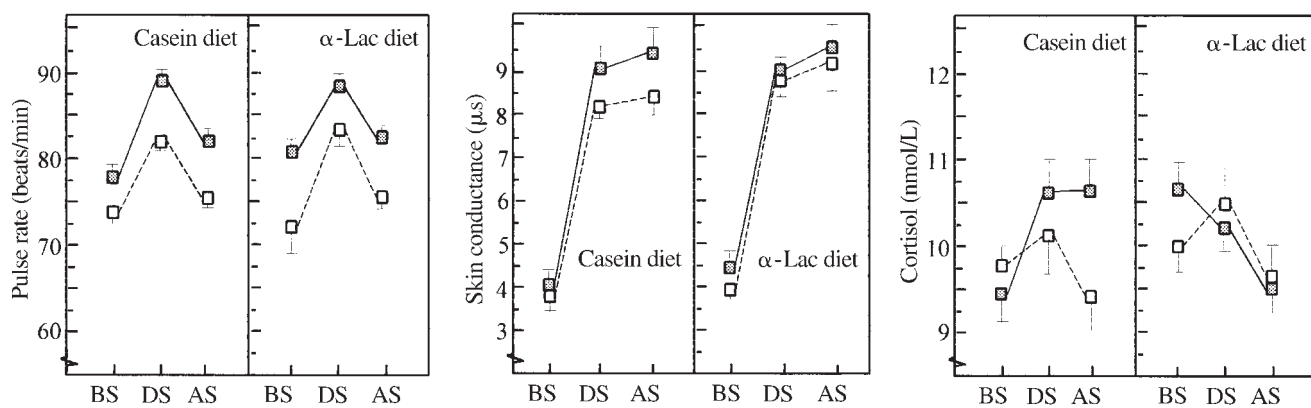
FIGURE 2. Significant increases in pulse rate ($P < 0.0001$) and skin conductance ($P < 0.0001$) were found during experimental stress (DS) compared with before the stress (BS) and after the stress (AS) in highly stress-vulnerable (HS; ⊠) and relatively stress-invulnerable (LS; □) subjects with the α-lactalbumin–enriched whey protein diet (α-lac diet) and the casein diet. A cortisol stress response was prevented in HS subjects but not in LS subjects with the α-lac diet ($P < 0.06$). $\bar{x} \pm$ SEM.
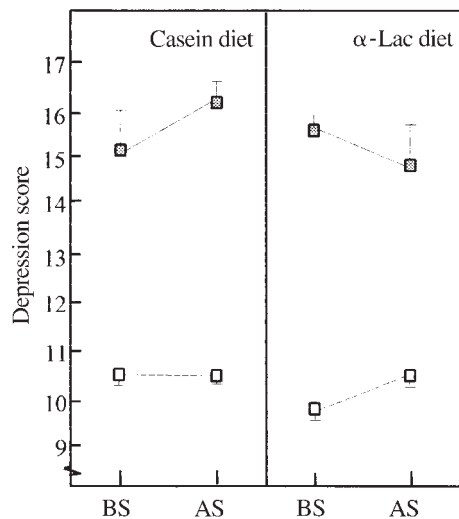
FIGURE 3. In highly stress-vulnerable (▧) subjects but not in relatively stress-invulnerable (□) subjects, the scores on the depression subscale of the Profile of Mood States (40) were lower after experimental stress (AS) during the α-lactalbumin–enriched whey protein diet (α-lac diet) than during the casein diet ($P < 0.007$). BS, before stress. $\bar{x} \pm$ SEM.

15.19 ± 5.7 to 16.19 ± 5.7 after the casein diet, whereas after the α-lac diet the scores declined from 15.62 ± 5.7 to 14.86 ± 5.2. This effect was not found in LS subjects.

Multivariate analysis of the remaining POMS scales of anger, tension, vigor, and fatigue showed a significant effect of experimental stress ($F_{[4,53]} = 16.75$, $P < 0.0001$). Further univariate analysis showed that the effect originated from changes in the anger scores ($F_{[1,56]} = 60.20$, $P < 0.0001$), meaning that the experimental stress significantly increased feelings of anger in both diet conditions (α-lac diet: from 9.59 ± 3.51 to 14.16 ± 6.5; casein diet: from 9.62 ± 3.21 to 13.74 ± 5.5). Results of the analysis of changes in the POMS scores are presented in **Table 3**. Multivariate analysis also showed a significant 2-way interaction effect of stress vulnerability with experimental stress ($F_{[4,53]} = 3.68$, $P = 0.01$), which originated as changes in tension ($F_{[1,56]} = 5.34$, $P = 0.025$) and fatigue ($F_{[1,56]} = 3.90$, $P = 0.05$). The HS group became more tense after the experimental stress (14.4 ± 4.2 after stress compared with 12.2 ± 3.6 before) whereas the rise in tension in LS subjects was negligible (9.91 ± 3.29 after stress compared with 9.38 ± 3.24 before). Furthermore, HS subjects did not experience more fatigue after the experimental stress (16 ± 4.45 after stress compared with 16.73 ± 5.25 before) whereas the LS subjects did (12.70 ± 5.9 after stress compared with 11 ± 5 before).

Multivariate analysis also showed a significant effect of stress vulnerability ($F_{[4,53]} = 8.60$, $P < 00001$) on the baseline values of anger ($F_{[1,56]} = 16.53$, $P < 0.0001$), tension ($F_{[1,56]} = 18.818$, $P < 0.0001$), vigor ($F_{[1,56]} = 15.65$, $P < 0.0001$), and fatigue ($F_{[1,56]} = 18.59$, $P < 0.0001$). Stress-vulnerable subjects reported higher mean scores than did LS subjects on the anger (11.0 ± 3.87 compared with 8.35 ± 2.18), tension (12.12 ± 3.64 compared with 9.38 ± 3.23), and fatigue (16.74 ± 5.26 compared with 11.0 ± 5.0) scales and lower scores on the vigor (13.40 ± 3.37 compared with 16.18 ± 3.91) scale (*see* also Table 3). No other significant effects were found.

DISCUSSION

In this study, we showed that α-lactalbumin–enriched whey protein increased the plasma Trp-LNAA ratio in subjects both highly vulnerable and relatively invulnerable to stress. With the experimental diet, we observed higher prolactin concentrations, an improvement in mood, and a reduced cortisol stress response in subjects who were highly vulnerable to stress.

Internal validity

We believe that the effects of dietary manipulation on blood measures, mood, and cortisol in the present study were not caused by differences in dietary consumption, by expectations about the food or the purpose of the study, or by insufficient statistical power of the experiment. Our study was a completely double-blind, controlled dietary trial in which all subjects consumed, under direct observation, 2 diets that were similar in nutrient composition, appearance, and taste. The research assistant and all subjects were blinded to the purpose of the experiment and the dietary conditions, as was established by a brief interview of each subject at the end of the last experimental day. To detect a small effect of dietary manipulation at a significance level of 5%, ≥ 14 subjects are required; this requirement was greatly exceeded in the present study. Accordingly, multivariate analysis showed a conceivable statistical power of all reported significant effects between 0.70 and 0.81.

Dietary effect on Trp-LNAA ratio and brain serotonin

The plasma Trp-LNAA ratio was 48% higher after the α-lac diet than after the casein diet, indicating that during the α-lac diet, more tryptophan was available for uptake into the brain. This is expected to lead to an increase in central serotonin synthesis (9–12). The higher plasma Trp-LNAA ratio in this experiment exceeded the 42% increase found previously with the CR-PP diet (14). Whereas a protein-rich diet has generally been found to reduce the Trp-LNAA ratio, the present results indicate that a diet composed of α-lactalbumin–enriched whey protein effectively raises the Trp-LNAA ratio; this may be a more practical method to increase brain tryptophan and serotonin concentrations.

Assuming that an increase in the plasma Trp-LNAA ratio with consumption of the α-lac diet raised brain tryptophan and serotonin concentrations, this diet was expected to enhance brain serotonin function most strongly in stress-vulnerable subjects. Because the higher serotonergic activity during stress leads to higher breakdown of serotonin, chronic stress in stress-vulnerable subjects may ultimately lead to a functional shortage of available tryptophan and brain serotonin concentrations. As a consequence, the serotonergic system may become more sensitive because of compensatory receptor sensitization (21, 22). The present findings on prolactin support this assumption: prolactin concentrations were 40% higher after the α-lac diet than after the casein diet in HS subjects only. Because increases in prolactin may reflect increases in central serotonergic neurotransmission and receptor sensitization (30, 31), the present dietary effects on the plasma Trp-LNAA ratio and prolactin concentrations indicate that the serotonergic system is putatively more sensitive in HS subjects than in LS subjects. However, the following annotation should be made here. Although it is likely that fasting prolactin concentrations were comparable on both diet study days within subjects, strictly we do not know whether they were dif-

TABLE 3
Mood score changes in HS and LS subjects under stress after the α-lac and casein diets[1]

Subjects and mood measurement scale	Casein diet		α-Lac diet	
	Before stress	After stress	Before stress	After stress
LS				
Depression	9.8 ± 2.3	10.1 ± 2.6	9.8 ± 2.6	10.2 ± 2.6
Anger	8.5 ± 2.1	11.5 ± 4.4[2]	8.2 ± 2.2	11.8 ± 5.7[2]
Tension	9.2 ± 2.4	9.7 ± 3.3	9.5 ± 4.0	10.1 ± 3.2
Vigor	16.5 ± 3.9	16.4 ± 3.1	15.8 ± 3.9	15.2 ± 3.6
Fatigue	10.5 ± 4.1	12.4 ± 5.4	11.5 ± 5.9	13.0 ± 6.5
HS				
Depression	14.8 ± 5.1	15.8 ± 5.3	15.1 ± 5.1	14.2 ± 5.0
Anger	10.8 ± 3.7	16.0 ± 5.6[2]	11.0 ± 4.0	16.5 ± 6.5[2]
Tension	11.9 ± 3.6	14.1 ± 4.1[3]	12.3 ± 3.7	13.9 ± 4.3[3]
Vigor	13.7 ± 3.4	13.1 ± 2.7	13.1 ± 3.3	13.5 ± 2.6
Fatigue	16.7 ± 4.7	16.9 ± 4.4[3]	16.8 ± 5.8	16.1 ± 4.5[3]

[1] $\bar{x}$ ± SD; n = 29 per group. HS, highly stress vulnerable; LS, relatively stress invulnerable; α-lac, containing α-lactalbumin-enriched whey protein. Mood scores determined by listed subscales of the Profile of Mood States (40).
[2] Significantly different from before stress, $P < 0.0001$.
[3] Significantly different from LS subjects after stress, $P = 0.01$.

ferent between HS and LS subjects. This possibility should be considered in further studies.

The present findings suggest that stress vulnerability constitutes a major factor predisposing the human brain to the beneficial effects of dietary increases in the plasma Trp-LNAA ratio. Enhanced serotonin sensitivity in HS subjects may be a predisposing factor for major depression. Hence, a chronic stress-induced biochemical adaptation may ultimately cause deterioration of the serotonergic system, which then may result in symptoms of a mood disorder (44). Accordingly, stressful life events are linked to the development of depressive disorders (45) that are accompanied by impaired brain serotonin function (3, 4, 24).

Dietary effect on stress: mood and cortisol

In accordance with our previous findings (14), the stress task significantly increased pulse rate and skin conductance and led to a significant deterioration in mood. This indicates successful induction of experimental stress. Assuming that increased serotonergic function in HS subjects constitutes a biological means of coping with stress, it was hypothesized that the α-lac diet would restrain an acute cortisol stress response and depressive mood in HS subjects. Concerning mood, we indeed found that only in HS subjects did feelings of depression tend to be lower after experimental stress with consumption of the α-lac diet compared with the casein diet. In HS subjects who showed a physiologic stress response, this dietary effect on depression was significant ($P = 0.007$). It is important, however, not to draw strong conclusions concerning the clinical implications of this observation because these dietary effects on mood seem to be small. We also found that HS subjects reported more depressive moods at baseline than did LS subjects. This seems to agree with the observation that experiences of chronic stress may lower mood and reflect a vulnerability to depression (45).

In accordance with our previous findings (14), we found that with a diet-increased plasma Trp-LNAA ratio, a cortisol response in HS subjects was prevented during acute experimental stress. At first glance, this decreasing effect on cortisol concentrations seems to contradict findings that the administration of serotonin precursors stimulates cortisol secretion in healthy and clinical subjects (3). Increases in brain serotonin appear to initiate adrenocortical reactivity through alterations in 5-hydroxytryptamine receptor sites 1A and 2, located in the hypothalamus and pituitary (3). However, these facilitating effects of serotonin agents on cortisol concentrations do not necessarily conflict with the notion that, under acute stress, serotonin activity may improve the ability to cope with stress and may contribute to reducing a cortisol response. Serotonergic neurotransmission does not appear to be a unitary mechanism, and different serotonergic pathways are involved in stress adaptation, initiating as well as terminating the activity of the adrenocortical axis (7, 8). Accordingly, an improved ability to cope with stress is often accompanied by a reduced cortisol response and improved mood (20). On the basis of these relations, the present data show that an α-lactalbumin–enriched whey protein diet may reduce in stress-vulnerable subjects the negative consequences of experimental stress on cortisol secretion and mood, probably by enhancing brain serotonin mechanisms that are involved in adaptation to stress.

Effectiveness of dietary manipulation on brain serotonin

Although the significance of changes in the plasma Trp-LNAA ratio for central serotonin function is acknowledged, in humans the extent to which the plasma Trp-LNAA ratio must rise to cause meaningful changes in brain serotonin synthesis has not been clearly delineated. In most studies a diet-induced rise in the plasma Trp-LNAA ratio did not reach >20–25% over baseline values. Some authors have questioned whether this increase would be sufficient to cause a meaningful enhancement of brain serotonin in humans (46, 47). These authors suggested that the plasma Trp-LNAA ratio must rise ≥50% to produce substantial changes in brain serotonin synthesis. However, there is no conclusive evidence to justify the idea that a meaningful change in brain serotonin would occur when the plasma Trp-LNAA ratio increases by ≥50%. One study showed that even an impressive 47% increase in the plasma Trp-LNAA ratio produced by an orange juice drink did not lead to significant increases in 5-hydroxyindolacetic acid in cerebrospinal fluid (47). However, in that study the increase in plasma Trp-LNAA ratio was not significant because of the

small number of subjects ($n = 5$). Furthermore, the subjects under study were neurologic patients (mean age 71 y) diagnosed with normal pressure hydrocephalus, a neuropathologic condition accompanied by a variety of behavioral and biochemical deficits (48). Consequently, the findings of the study do not exclude the notion that increases in the plasma Trp-LNAA ratio <50% could lead to changes in brain serotonin. Accordingly, even smaller increases in the plasma Trp-LNAA ratio might influence brain serotonin synthesis (49). In another study it was found that a 49.5% decline in the plasma Trp-LNAA ratio was accompanied by significantly lower 5-hydroxyindolacetic acid concentrations in cerebrospinal fluid with a balanced diet (50), whereas other groups reported that 20–40% changes in the plasma Trp-LNAA ratio led to neuroendocrine changes mediated by brain serotonin (51, 52).

Conclusion

The present study showed a significantly higher plasma Trp-LNAA ratio with an α-lactalbumin–enriched diet than with a casein diet. Only in HS subjects did the α-lac diet enhance plasma prolactin, decrease cortisol concentrations, and prevent depressive feelings during acute stress. Because increases in cortisol and depressive feelings may reflect low stress adaptation, the present data suggest that a diet composed of tryptophan-enriched whey proteins in healthy but stress-vulnerable subjects could improve the ability to cope with stress by enhancing brain serotonin function. We suggest that a diet either containing α-lactalbumin–enriched whey protein of greater purity than that used in this study or combined with carbohydrates might cause an even greater increase in the plasma Trp-LNAA ratio and may lead to clinically important changes in vulnerable subjects under acute stress. Also, to search for direct evidence of diet-induced changes in serotonin neurotransmission in stress-vulnerable subjects, further research is needed on the effects of postsynaptic serotonin agents.

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