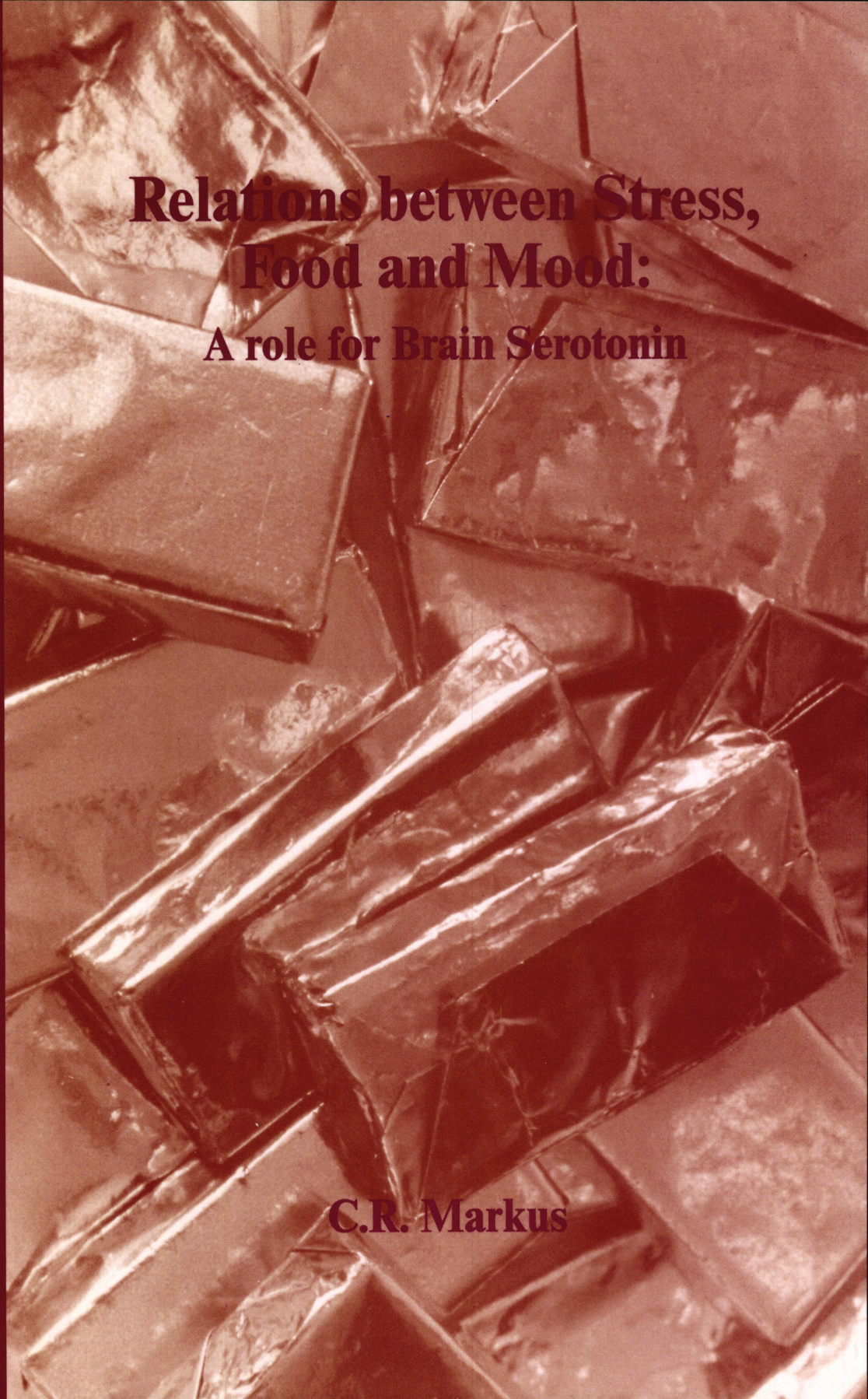
Relations between Stress, Food and Mood:

A role for Brain Serotonin



C.R. Markus

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RELATIONS BETWEEN STRESS, FOOD AND MOOD: A ROLE FOR BRAIN SEROTONIN.

ROB MARKUS

PROMOTIECOMMISSIE: Prof. Dr. L. van Doornen
Prof. Dr. M.N. Verbaten
Prof. Dr. K.R. Westerterp

PARANIMFEN: C. Bouchet
M. van Boven

The Little Sweet Doth
Kill Much Bitterness

John Keats

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RELATIONS BETWEEN STRESS, FOOD AND MOOD: A ROLE FOR BRAIN SEROTONIN.

RELATIES TUSSEN STRESS, VOEDING EN STEMMING:
EN ROL VOOR SEROTONINE IN DE HERSENEN.

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CRISTIAAN ROBERTINO MARKUS

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DRUKKERIJ ELINKWIJK BV, UTRECHT

PROMOTORES: Prof. Dr. E.H.F. de Haan
Prof. Dr. B. Olivier

CO-PROMOTERES: Dr. G.E.M. Panhuysen
Dr. A. Tuiten

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CONTENTS

CHAPTER 1

EFFECTS OF FOOD ON MOOD: A ROLE FOR BRAIN SEROTONIN

INTRODUCTION

Most people have little doubt that certain foods and drinks are able to influence our mood and mental performance. In this context we can think of the psychoactive characteristics of caffeine in the form of coffee, tea or cola drinks, which make us more alert and may raise our concentration and performance. Other examples are the effects of chocolate consumption, including positive feelings and the possible prevention of negative mood states, and alcohol consumption, with its anxiety reducing effects and inducement of euphoric feelings (Rogers, 1995). The apparent effects of food constituents on mood and mental activity have also been noticed by the food industry. During the last decade, food industries have increasingly focused on the effects of different types of food additives on mental activity and emotional behavior. This is evident in the appearance of different esoteric products like "Rise & Shine", "Red Bull", and "Fast Blast", all claiming desirable improvements in mental activity, mood, alertness and physical endurance.

The influence of nutrition on human behavior is also of interest to scientists. For example, the observation that vitamin treatment in certain patients seemed to reduce negative emotional behavior (Clark, 1943) led to the suggestion that vitamin deficiency might be a factor involved in a broad range of psychiatric disorders. The relation between certain food constituents and mental health has also been investigated with respect to schizophrenia, Down's syndrome and Attention Deficit Hyperactivity Disorder. However, much early work on the relation between nutrition and behavior has been criticized on methodological grounds and this has led to growing skepticism about the extent of the relationship between food and human behavior. After the initial work in the 1940's little new work was conducted on the food-mood relationship for several decades.

The 1980's and the 1990's have seen renewed scientific interest in the relationship between food and human behavior. In particular the relationship between carbohydrate intake and human emotion has received much attention. This renewed interest was prompted by Spring et al.'s (1987) integrative overview of the effects of carbohydrates on human behavior, followed by reviews by Christensen (1997) and Bellisle et al. (1998). These authors extensively summarized findings from dietary studies, ranging from the effects of carbohydrate intake on aggression, cognitive performance and pain perception, to the effects of carbohydrate intake on mood and depressive symptoms. In general, the effects of an increased intake of

carbohydrates on feelings of drowsiness, fatigue and depression are reasonably well established (Spring et al., 1987; Christensens, 1997).

The most profound effects of carbohydrate intake on mood are detected among patients who suffer from affective disorders. These subjects show an increased desire for carbohydrate-rich food, particularly during severe episodes of depression (see Wurtman, 1990; Christensen et al., 1993). For instance, patients suffering from bulimia often crave for carbohydrate-rich food during the periods in which they feel most depressed, which has been attributed to the reducing effects of carbohydrates on depressive feelings (e.g. Rosenthal et al., 1986; Christensen et al., 1993). Comparable findings are reported for obese subjects. A majority of subjects suffering from obesity (reflected by a body weight that exceeds the average for a given height by 20%) also endure transient moments of depression. During their depressive moods, these subjects often compulsively consume carbohydrates in order to control or "self-medicate" their negative depressive feelings (e.g. Lieberman et al. 1986a; Wurtman et al., 1986a; see also Christensen et al., 1993). Similar mood improving effects of carbohydrate consumption have been observed in patients with Late Luteal Phase Syndrome (e.g. Wurtman et al., 1989; Wurtman, 1990; see also Christensen et al., 1993) and Seasonal Affective Disorder (e.g. Rosenthal et al., 1989; see also Christensen et al., 1993). Late Luteal Phase Syndrome (or premenstrual syndrome, PMS) refers to a variety of emotional and physical symptoms that show a cyclical relationship to the luteal or premenstrual phase of the menstrual cycle. Premenstrual complaints are, amongst others, characterized by increased feelings of fatigue and depression accompanied by an excessive consumption of carbohydrate-rich foods. Seasonal affective disorder (SAD) is characterized by recurrent episodes of depressive mood that, unlike the Late Luteal Phase Syndrome, occur particularly during fall- and winter time. During their fall-winter depression approximately two-third of these SAD patients reported an increased intake of carbohydrates in order to control their depressive symptoms.

Although positive effects of carbohydrate intake on the mood of patients have been clearly established, in normal controls these dietary effects on mood are less consistent. Often, no effects or just slight and contradictory changes in feelings of sleepiness, calmness, and fatigue are revealed (e.g., Spring et al., 1987).

In the present thesis two important issues are addressed. The first issue relates to the mechanisms underlying the carbohydrate-mood relationship. In particular, it is hypothesized that the intake of an increased proportion of carbohydrates may enhance the activity in those parts of the brain involved in emotional behavior. The second issue refers to inconsistencies in the published results of studies on the effect of carbohydrate intake on the mood of normal subjects. I propose that the inconsistencies can be explained by considering the interrelations between stress, carbohydrate intake and mood; it is hypothesized that an increased proportional

consumption of carbohydrates may improve mood only in chronically stressed (stress-prone) subjects under stress. Continued stress exposure in these subjects may cause biochemical adaptations in the central nervous system that predispose the human brain to benefit more from increased carbohydrate consumption, particularly under acute stress conditions. It is possible that (differences in) stress-proneness may provide not only an important explanation for the inconsistent findings regarding the effects of carbohydrate intake on behavior among normal populations, but also may constitute a commonality among patients suffering from a variety of psychiatric mood disturbances.

The following section starts by describing some mechanisms that have been presented as likely candidates for the formation of the carbohydrate-mood relationship. The most likely explanation for the effects of carbohydrates on mood and behavior is that carbohydrates influence the availability of the precursor of the brain neurotransmitter serotonin (5-hydroxytryptamine). How the activity of serotonin in the brain rises by the intake of increased proportions of carbohydrates is outlined in the following section where empirical evidence supporting the mediating role of the brain serotonergic system in the carbohydrate-mood relationship is also summarized. Finally, a possible reciprocal relationship between stress, food and mood is proposed, and it is argued that the effects of carbohydrates on mood may be mediated by stress-induced adaptations in the brain serotonergic system. In Chapter 2 through 5 four experiments in which the above issues were investigated are described and discussed.

1.1. ATTEMPTS TO EXPLAIN THE CARBOHYDRATE-MOOD RELATIONSHIP.

Of the possible causal explanations that have been proposed to explain the apparent mood-improving effects of carbohydrate intake, only an explanation based on increased availability of tryptophan, a precursor to serotonin, seems viable (e.g. Spring et al., 1987). In the present section, I will nonetheless discuss some other work that has been conducted in the attempt to find alternative explanations. For a more elaborated overview of these alternative explanations, the reader is referred to the work of Christensen (1995) and Bellisle et al. (1998).

1.1.1. *Oro-sensory and cognitive factors.*

One explanation offered for the carbohydrate-mood relationship concerns differential effects of food on the taste and olfactory senses. In contrast to a high protein meal, carbohydrate-rich meals often consist of sweet tasting foods. Because such oro-sensory features may initiate positive emotions, it has been suggested that their presence can explain the effects of carbohydrates on mood. Experimental

findings in neonates have revealed that inborn taste preferences for sweetness are already present at birth. For instance, newborn babies display positive facial expressions (indicating acceptance and a positive emotional state) when tasting a sweet sucrose substance, whereas negative emotional expressions are found in reaction to bitterness (indicative of rejection and negative affect) (Steiner, 1987). Blass et al. (Blass et al., 1991; Blass et al., 1995) further studied the impact of sweet substances on emotional behavior. They demonstrated that the oral administration of a sucrose solution reduced the duration of crying after the taking of a blood sample in 3-years old infants and newborn babies. The authors suggested that carbohydrates may also have analgesic effects on pain perception through alterations in endorphinergic mechanisms in the brain (Blass et al., 1991). In other studies, however, analgesic-like effects of sucrose have not been found on either pain tolerance or intensity (Miller et al., 1994) or direct pain experiences (Barr et al., 1995).

Factors such as the taste, smell, and texture of a meal may also influence emotional behavior through food-related expectancies (Rogers, 1995). Human beings may develop expectancies concerning the effect of food on behavior which, in turn, may cause the onset of that particular behavior when the food is actually consumed. Such food-related expectancies may originate from prior food experiences through associative conditioning (Rogers, 1995). However, it has been demonstrated that people do not develop food-related expectancies concerning nutritional components such as carbohydrates and proteins (Christensen et al., 1985a; Spring et al., 1987; Rosenthal et al., 1989). Even when food-related expectancies are experimentally induced, they do not seem to have meaningful effects on emotional processes (Christensen et al., 1985a). Moreover, most of the studies that do show a carbohydrate-mood relationship have controlled for such cognitive features, making it unlikely that food-related expectancies or learned preferences provide a significant contribution to the carbohydrate-mood relationship (Spring et al., 1987). For example, Spring et al. (1989) compared the effects of a carbohydrate-rich diet, a hedonically similar isocaloric-balanced diet, and a protein-rich diet on mood in normal subjects; only the carbohydrate-rich diet significantly altered mood. This effect could neither be attributed to taste differences nor expectancy effects, since in that case the same effects would have been found with the hedonically similar balanced diet. Further support stems from studies that have controlled for the oro-sensory effects of food. For instance, in a study by Wells et al. (1998) gastric infusion of fat versus carbohydrates had different effects on mood, and these effects could not be attributed to cognitive or oro-sensory influences. In general, neither oro-sensory factors nor food-related expectancies appear to be meaningful determinants of the effects of carbohydrates on mood (Spring et al., 1987).

1.1.2. *Hypoglycemia*

Whether the effects of carbohydrate intake on mood are mediated by a reduction of glucose concentrations in the blood has also been explored. Carbohydrate ingestion is accompanied by a rapid increase of glucose in the bloodstream causing the pancreas to secrete insulin. Since insulin enhances the conversion of glucose into liver glycogen, it is possible that blood glucose levels decline in such a situation until baseline or fasting levels are reached (Marks, 1989). Blood glucose concentrations below a critical level (i.e. under hypoglycemia), may be followed by a rise in the secretion of hormones such as epinephrine and cortisol to restore the glucose level in the blood to normal. The physiological and psychological arousal that are caused by increases in epinephrine (e.g. cardiac responsiveness, trembling, sweating, headaches, poor concentration, and weakness) have been examined as a possible cause of the mood effects of carbohydrate ingestion.

Although insulin-induced hypoglycemia has been found to cause a deterioration of mood in diabetes mellitus patients as well as in normal controls (Hepburn et al., 1995; Merbis et al., 1996), there is no evidence that a carbohydrate-rich diet can do the same. In a series of elegant studies, Spring et al. (1986, 1989) evaluated the relationship between carbohydrate intake, hypoglycemia, and mood. In one study, Spring et al. (1989) demonstrated that a carbohydrate-rich, protein-poor diet alters mood even though the blood glucose level remains above baseline. To most investigators, hypoglycemia does not constitute a sufficient explanation for the effects of carbohydrates on mood (Spring et al., 1987).

In summary, both oro-sensory and cognitive factors as well as hypoglycemia do not appear to be sufficient explanations for the carbohydrate-mood relationship. The explanation with most the support from empirical data is that certain foods provide precursors of neurotransmitters that in turn are known to influence emotional behavior. The most frequently investigated neurotransmitter in this aspect is serotonin (5-hydroxytryptamine or 5-HT). Enhanced serotonin activation in the brain is involved in the regulation of emotional behavior and can be controlled by the intake of increased proportions of carbohydrates. In the following section, the relationships between serotonin, food, and mood is further explored.

1.2. THE INFLUENCE OF CARBOHYDRATE INTAKE ON THE AVAILABILITY OF L-TRYPTOPHAN, THE PRECURSOR OF SEROTONIN (5-HT).

One extensively tested hypothesis for the mood effects of carbohydrates arises from the work of Fernstrom and Wurtman (Fernstrom et al., 1971a, 1971b; Fernstrom et al., 1973; Wurtman, 1987; Wurtman, 1990). This hypothesis is based on the observation that certain food constituents act as neurotransmitter precursors, and thus enhance the activity of the corresponding neurotransmitter systems in the brain.

Meals consisting of high proportions of carbohydrates relative to proteins may influence mood by enhancing the influx of the brain serotonin precursor tryptophan and, subsequently, brain serotonin. Because an increased activity of the brain serotonergic system may lead to an elevation of mood, the emotional effects of carbohydrate intake may be explained by alterations in brain serotonin.

1.2.1. *Relation between serotonin and mood: The serotonin hypothesis of depression*

Half a century ago, serotonin was first isolated from the blood as a serum vasoconstrictor (Rapport et al., 1948). Further investigations revealed that serotonin also naturally occurs in the brain. Accordingly, it seemed that increases in serotonin could counteract the influences of the autonomic nervous system in the onset of negative emotions (Brodie et al., 1957).

Serotonin is involved in a broad range of behavioral and physiological processes, including sleep, locomotor activity, aggression, sexual behavior, pain, and mood disorders (see Wurtman et al., 1981). In particular, behavioral disturbances like mood disorders are attributed to a declined central serotonergic function. Although only 2-5% of the total serotonin content in the human body is located in the brain, its significant role in the pathogenesis of mood disorders is generally accepted (e.g. van Praag, 1980; Maes et al., 1995). The first indications of a role of serotonin in mood disorders were found almost 40 years ago, when the mood improving effects of tricyclic antidepressants with a preferential action at the serotonergic system such as chlomipramine and by monoamine oxidase inhibitors (MAO) like iproniazid were observed. These drugs increased synaptic concentrations of serotonin and were able to improve depressive symptoms. Conversely, serotonin-depleting drugs like reserpine were found to induce depressive mood. Such observations led to the assumption that a reduced level of brain serotonin contributes to the development of clinical mood disturbances (Schildkraut, 1965; Coppen, 1967) and that increased serotonergic transmission plays an important role in the therapeutic effects of antidepressants.
An accumulation of research findings suggest that the brain serotonin level is not the sole factor involved in mood disorders like depression. For example, the absence of reliable cerebrospinal fluid measures of serotonin metabolites in patients, the inconsistent findings on serotonin mediated neuroendocrine and metabolic changes in plasma, and the lack of immediate effects of brain serotonin interventions on mood in normal and depressive subjects suggest that additional factors are present (Maes et al., 1995; Heninger et al., 1996). One reliable method by which the effect of alterations in brain serotonin concentrations on mood has been investigated is serotonin depletion (Delgado et al., 1994; Miller et al., 1996). In this procedure, brain serotonin content is depleted through the manipulation of the level of its precursor, exhausting available tryptophan (Moja et al., 1989). It has been shown that serotonin depletion in untreated depressive subjects does not lead to an increase in depressive mood, whereas in

patients treated successfully with an Selective Serotonin Reuptake Inhibitor (SSRI), serotonin depletion significantly increases the risk of relapse (Delgado et al., 1994; see also Heninger et al., 1996). Moreover, antidepressant treatment with an SSRI does not lead to immediate affective changes, although the SSRI causes an immediate increase in serotonergic activity. Recent findings stress the importance of changes in receptor sensitization in the effectiveness of antidepressant treatment (Charney et al., 1981; see also Heninger et al., 1996). That is, certain antidepressants may desensitize serotonin receptors involved in the inhibition of serotonin release, thereby restoring serotonin concentrations in the brain. However, antidepressant treatment does not always act on serotonin-inhibiting receptor sites, nor does a down-regulation of these sites consistently lead to a simultaneous antidepressant response. Furthermore, changes in serotonin receptor sites induced by electro-convulsive therapy are opposite to those caused by antidepressant drugs. Nowadays, it is assumed that the antidepressant effects on increases in serotonin levels, receptor sensitization, and depression are mediated by changes in intracellular cyclic adenosine monophosphate (cAMP) pathways (Duman et al., 1997).

Although a comprehensive theory of the involvement of serotonin in mood and mood-related psychiatric disorders does not yet exist, it is generally thought that a shortage of serotonin in the brain may contribute to mood deterioration and increase a vulnerability to major depression (e.g. van Praag, 1980; Meas et al., 1995). Evidence for this stems from different research paradigms (see Maes et al., 1995). First, declined serotonergic function appears a common factor in various mood disorders such as depression. Decreased serotonergic function is detected by lower availability of plasma tryptophan, impaired serotonin synthesis- release- reuptake or metabolism, or by disturbances in 5-HT_1 or 5-HT_2 serotonin receptors. Furthermore, a decline in brain serotonin levels has been found to induce depressive mood in patients and, to a lesser extent, in normal subjects (Young et al., 1985; Miller et al., 1992; Delgado et al., 1994; Meas et al., 1995; Smith et al., 1997). For instance, tryptophan depletion was found to lead to a modest increase in depressive feelings in normal men (Young, 1985), to induce depressive symptoms in recovered depressive patients (Delgado et al., 1994) and to alter mood in subjects with a genetic vulnerability to major depression (Benkelfat et al., 1994). Finally, as mentioned earlier, the mood improving effects of antidepressant therapy are partly mediated by increases in brain serotonin synthesis and function (Delgado et al., 1990; Delgado et al., 1993) most likely through changes in 5-HT_{1a} and 5-HT_2 receptor sites (Maes et al., 1995).

1.2.2. *Serotonin in the brain*

Before explaining the relationship between food intake and serotonin functioning in the brain, it is necessary to clarify the sequence of biochemical conversions that leads

to the serotonin synthesis in the brain. Also a brief description of the brain serotonergic system is given.

Since serotonin itself cannot pass the blood-brain barrier, the first step in the synthesis of brain serotonin is the uptake of the amino acid nutrient l-tryptophan from the blood into the brain. As the serotonin precursor l-tryptophan is an essential amino acid, it is only available in the blood after hydrolysis of dietary proteins or tissue proteins. In the central serotonin neuron, l-tryptophan is converted into 5-hydroxytryptophan (5-HTP) by the enzyme *l-tryptophan hydroxylase* and subsequently decarboxylated into serotonin (5-hydroxytryptamine) by the aromatic l-amino acid *decarboxylase*. The most critical or rate limiting step in the formation of serotonin is its hydroxylation to 5-HTP by the enzyme *l-tryptophan hydroxylase*. In vitro as well as in vivo studies have shown that *l-tryptophan hydroxylase* under normal conditions is only half saturated; thus increases in available brain l-tryptophan unconditionally elevate serotonin synthesis and release (e.g. Chaouloff, 1993). After synthesis, serotonin is stored in the nerve endings of the neuron. Serotonin is metabolized by the enzyme *monoamine oxydase* (MAO) to *5-hydroxyindoleacetic acid* (5-HIAA).

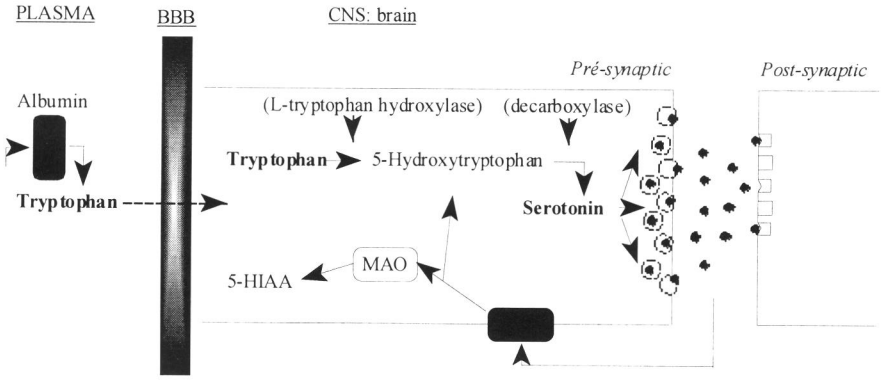

Fig.1: Sequence of biochemical conversions involved in brain serotonin synthesis (from: Markus et al., 1998).

Thanks to the histochemical fluorescent techniques developed by Dahlstrom and Fuxe (1964), the distribution of serotonergic neurons in the brain has been made visible, providing a clear picture of the anatomical architecture of the serotonergic system (reviewed by Soubrié, 1986; Jacobs et al., 1992). Serotonergic cell bodies are mainly concentrated along the midline of the brain stem, constituting the midbrain raphe nuclei. From this area, axonal serotonergic fibers project into different parts of the brainstem, the spinal cord and the forebrain limbic structures. The serotonin system is divided into an ascending and a descending part. Ascending serotonergic neurons

project from structures containing the dorsal and median raphe nuclei (DR and MR) and mainly project to the forebrain, whereas the serotonergic neurons from the descending system are mainly enervating the spinal cord and the cerebellum. It appears that the DR and the MR are common sources of serotonergic input to a variety of forebrain structures, including the hypothalamus, the cerebral cortex, limbic structures, and the basal ganglia.

1.2.3. *Effects of food on brain serotonin*

The assumption that an increased intake of carbohydrates improves mood by increasing brain serotonin function originated from animal studies (Fernstrom et al., 1971a, 1971b; Fernstrom et al., 1973; see also Wurtman, 1987; Wurtman, 1990). A first indication derived from a study of Eccleston et al. (1965) showing that a large amount of l-tryptophan could raise brain serotonin levels. This finding was initially surprising, since it was believed at that time that the human brain was an autonomic system and that the biochemical atmosphere within this system could not be influenced by external artificial manipulations. Fernstrom and Wurtman (1971a) showed that enhanced serotonin level in the brain could also be accomplished within an hour by an oral dose of tryptophan as low as 10 % of the optimum daily intake (12.5 mg/kg). A sequence of impressive studies followed, demonstrating that a meal with a high proportion of carbohydrates also increased brain serotonin synthesis (e.g. Fernstrom et al., 1971b; Fernstrom et al., 1973). This effect of carbohydrates was unexpected and seems to be counterintuitive since the body's supply of tryptophan can only be obtained from dietary protein. However, it appears that a balanced or protein-rich diet leads to a decline in brain tryptophan and serotonin concentrations, whereas a carbohydrate-rich, protein-poor diet causes the opposite result, even though this latter diet does not contain tryptophan. These dietary effects on brain tryptophan availability and serotonin synthesis can be explained by the following mechanism (see Figure 2).

Brain serotonin synthesis is controlled by the influx of plasma tryptophan into the central nervous system. However, plasma tryptophan competes with other large amino acids, valine, tyrosine, isoleucine, leucine and phenylalanine (LNAA's) for transport across the blood-brain barrier (BBB) into the brain. Consequently, increases in brain tryptophan and serotonin concentrations do not depend on the total concentration of plasma tryptophan but, instead, on the plasma ratio tryptophan to the sum of the other LNAA's (Trp/LNAA). An increased plasma Trp/LNAA ratio increases the influx of tryptophan into the brain and causes a subsequent rise in brain serotonin levels, whereas a declined Trp/LNAA ratio has the opposite effect. Even though proteins contain a certain percentage of tryptophan (1-2 %), the intake of proteins could not lead to an increase in brain serotonin because they consist of even larger amounts of LNAA's (25%). Consequently, a balanced or protein-rich diet decreases the plasma Trp/LNAA ratio and declines available tryptophan for transport across the blood-brain barrier into the brain (Fig.2a). Conversely, a carbohydrate-rich, protein-poor diet

increases the plasma Trp/LNAA ratio and gives tryptophan an advantage in the competition for access to the brain (Fernstrom et al., 1971b; Fernstrom et al., 1973; Curzon, 1985; Wurtman, 1987). This increased Trp/LNAA ratio is produced by a carbohydrate-induced elevation of glucose and insulin, that causes the LNAA's, with the exception of tryptophan, to be taken up into the skeletal muscles (Fig.2b). Increases in insulin cause the free fatty acids to be stripped away from the albumin protein circulating in the blood. Since unbound albumin loosely binds with tryptophan, this prevents tryptophan from being taken up in the periphery, whereas the insulin response leads to a 40-60% fall in plasma leucine, isoleucine and valine, and a 30% fall of plasma tyrosine.

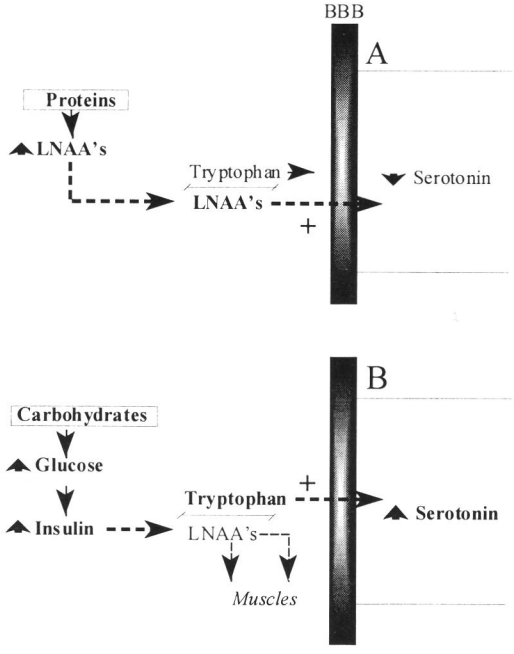

Fig.2: Effects of proteins (A) and carbohydrates (B) on the plasma Trp/LNAA ratio, the influx of brain tryptophan, and serotonin synthesis (from: Markus et al., 1998).

In conclusion, based on the work of Fernstrom and Wurtman (Fernstrom et al., 1971a, 1971b; Fernstrom et al., 1972; Fernstrom et al., 1973; Wurtman, 1987) it appears that the intake of a carbohydrate-rich diet constitutes a practical method to increase brain serotonin concentrations through increases in the plasma Trp/LNAA ratio. An increased Trp/LNAA ratio enhances the influx of the serotonin precursor l-tryptophan into the brain and, subsequently, increases serotonin synthesis. Conversely, a balanced- or protein-rich diet leads to the opposite results: a decline in the plasma Trp/LNAA ratio, brain tryptophan, and serotonin concentrations.

1.2.4. *Serotonergic control of feeding behavior*

As the previous findings show, the increased proportional intake of carbohydrates that is often evidenced by patients may be attributed to the fact that such a diet results in an increased brain serotonin function and subsequent improvement of mood. This suggests that the brain serotonergic system may effectively regulate the proportional intake of carbohydrates and proteins.

Blundell (1984) showed that the central serotonergic system indeed seems to control feeding. Evidence for this originated from pharmacological studies in which brain serotonin levels were manipulated by using direct and indirect-acting drugs that release or block serotonin function. For instance, quipazine (a 5-HT_2 agonist enhancing post-synaptic serotonergic function), fluoxetine and chlorimipramine (increasing synaptic serotonin activation) and fenfluramine (which enhances serotonin release as well as blocks serotonin reuptake) all have been found to reduce food intake. Conversely, a variety of serotonin receptor antagonists and neurotoxins have been found to enhance food ingestion (Fernstrom, 1992). These pharmacological studies proved that an increase in brain serotonin activity controls the selective intake of carbohydrates or proteins, and this has been attributed to serotonergic control at the level of the ventromedial hypothalamus (Leibowitz, 1980; Leibowitz et al., 1986). The activation of serotonergic cells within the paraventricular nucleus of the hypothalamus in particular, leads to a reduced appetite for carbohydrate-rich foods (Leibowitz et al., 1989; Stallone & Nicolaidis, 1989). For instance, Leibowitz et al. (1989) demonstrated that the infusion of serotonin into this region of the rat brain reduced the intake of carbohydrates but had little effect on the intake of fat and protein. In contrast, drugs that destroy serotonergic neurons -- thus inhibiting the synthesis of serotonin -- or block serotoninergic receptors increase food intake especially for carbohydrates (Stallone et al., 1989).

In summary, there seems to be a reciprocal relationship between the selective intake of proteins and carbohydrates and brain serotonergic activity. Accordingly, the intake of a carbohydrate-rich meal may inhibit further carbohydrate consumption by increases in plasma Trp/LNAA and brain serotonin function, whereas protein intake has the opposite effect (Wurtman, 1984).

1.3. REVIEW OF THE EMPIRICAL EVIDENCE

Interests in the relationship between carbohydrates, the plasma Trp/LNAA ratio and brain serotonin levels on the one hand, and between serotonin and mood on the other hand, has led to the completion of a variety of dietary studies carried out during the last two decades. Most of these studies are well documented by Spring et al. (1987), Rogers (1995), Christensen (1997) and Bellisle et al. (1998). An overview of these studies is presented in Tables 1 and 2. In the following section, a few of these studies

will be discussed in more detail. Some attention will also be paid to findings concerning tryptophan administration, because an increased or reduced availability of tryptophan is assumed to be the main factor in producing the carbohydrate-mood effects. Most of the studies also provide information about the direct effects of carbohydrates on cognitive performance. However, in reviewing these studies the main focus will be on the mood effects of dietary manipulation. A brief overview of dietary effects on cognitive performance will be given in a separate section.

1.3.1. *Dietary effects on mood in normal subjects*

Changes in mood have been demonstrated in normal subjects following the ingestion of carbohydrates (Hartman et al., 1977; Leathwood et al., 1982/83; Spring et al., 1982/83; Christensen et al., 1985b; Spring et al., 1989; Lloyd et al., 1996; Wells et al., 1998) and tryptophan (Greenwood et al., 1975; Yuwiler et al., 1981; Lieberman et al., 1983). Concerning the effects of carbohydrates, Hartmann et al. (1977), in a double-blind within-subjects experiment, measured changes in mood in 12 normal subjects after ingestion of a carbohydrate-lipid-drink without proteins, or a protein-drink supplemented with either l-tryptophan, l-leucine or a placebo. Sleepiness was measured at 15 minute intervals after consumption. Two hours after consumption of the carbohydrate drink feelings of sleepiness increased compared with the effects of the protein drink. Comparable mood effects of carbohydrates were found in a study by Leathwood et al., (1982/83). In a double-blind cross-over study, the authors examined changes in mood in 30 males and 30 females after a carbohydrate-rich, protein-poor breakfast (3.3 grams of protein, 2.4 grams of fat and 51.5 grams of carbohydrates) containing either 500 mg tryptophan, 500 mg l-tyrosine, 100 mg caffeine or a placebo. Subjects arrived at 8:00am at the laboratory without having had breakfast. Before breakfasting and at 9:00, 10:00, and 11:00am, after the intake of the breakfast, mood was measured. The first measurement was at the laboratory, whereas the later measurements were taken in the work environment of the subject. The findings of this study revealed that the carbohydrate-rich breakfast supplemented with 500 mg tryptophan significantly shifted the mean scores of mood from alert towards somnolent and lethargic, whereas the breakfast supplemented with caffeine increased wakefulness and energy. The authors concluded that the mood effects of the combined administration of 500 mg of tryptophan with carbohydrates may have been caused by a tripled plasma Trp/LNAA ratio and thus an increase in brain serotonin.

Spring et al. (1982/83) examined whether gender and age would influence the effects of carbohydrates on mood among 129 males and 55 females subjects. In a between-subjects design, subjects were randomly assigned to either a carbohydrate-rich, protein-poor diet (no protein, 4g fat and 57g carbohydrates) or a protein-rich, carbohydrate-poor diet (57g protein, 4g fat, 1g carbohydrates and 30-90mg choline),

offered as breakfast (between 7:15 and 8:30 am) or lunch (between 11:00am and 1:00 p.m.). Both meals were isocaloric and taken after subjects had fasted overnight for approximately 11 hours. Mood was measured once 2 hours after dietary intake, using the Profile of Mood States (POMS), Visual Analogue Scales (VAS) and the Stanford Sleepiness Scale (SSS). Results revealed different effects of dietary manipulation on mood for men and women and for older and younger subjects, depending on the time of day at which the meals were consumed (breakfast vs. lunch). Only women reported higher levels of sleepiness after the carbohydrate meal as compared to the protein meal, whereas males reported higher levels of calmness after carbohydrate intake as compared to protein intake. When the diets were consumed at breakfast, subjects of 40 years or older were calmer and less tense after the carbohydrate compared to the protein meal, whereas in younger subjects this differential effect was not found. These results suggest that carbohydrate foods may have tranquilizing effects, depending on the time of day when they are consumed and on the age and sex of the consumer. The authors attributed the dietary effects to an increased availability of tryptophan in the brain and a subsequent increase in central serotonin activity. Furthermore, the sex difference in dietary effect was explained by differences in qualification of emotional experiences or by a larger body mass in women, whereas the effect of age was attributed to age-related differences in the sensitivity to dietary nutrients. In a consecutive study, Spring and colleagues (1989) tested whether the effects of a carbohydrate-rich lunch on mood were accompanied by changes in blood glucose, insulin or plasma amino acids. Seven women participated in a within-subjects crossover study during four different dietary days separated by 1-week intervals. During the study the women fasted overnight (for 11.5 hours) and ate a standard breakfast in the morning at the laboratory (at 7:30 am). At about noon (12:15) either a lunch or a fasting period followed. When lunch was given, the test meals were isocaloric and were given in a counter balanced order across days, comprising a carbohydrate lunch (105g carbohydrates, 0.7g protein, 42.7g fat), a balanced lunch (76g carbohydrates, 27.7g proteins, 40g fat), and a protein lunch (105g protein, 33.3g fat). The carbohydrate- and balanced lunch were hedonically similar. Blood samples were taken 30 minutes before and 45, 90, 135, and 165 minutes after lunch. Mood changes were measured 45 minutes before- and 30, 75, 120, and 150 minutes after lunch (using the POMS, VAS and SSS). The results revealed that only the carbohydrate lunch significantly altered fatigue as measured two hours after consumption of the meal. In comparison to the other dietary conditions, the carbohydrate lunch caused a profound elevation in blood glucose and led to a significant increase in the plasma Trp/LNAA ratio. These results indicate that the effect of carbohydrates on mood can not be attributed to oro-sensory differences, because the balanced diet was hedonically similar but did not lead to the same results. Furthermore, the effects can not be explained by hypoglycemia since the blood glucose level did not decline and in fact exceeded the glucose concentrations measured during the fasting condition. Accordingly, the

authors attributed the effects of the carbohydrate-rich diet on mood to the significant increase in the plasma Trp/LNAA ratio and brain serotonin function.
Deteriorating effects of carbohydrates on mood have also been demonstrated. In a within-subjects crossover study in 1 male and 3 female subjects, using a single-subjects design, Christensen et al. (1985b) investigated whether dietary change can influence mood. After a dietary baseline period (containing sugar, caffeine and alcohol), subjects were instructed to consume a protein-rich, carbohydrate-poor diet for a period of two weeks and to abstain from caffeine, sugar and alcohol. After two weeks, subjects were exposed to a test consisting of caffeine (100mg), sugar (either 50 mg saccharin, 0.95 mg aspartame, fructose, sucrose) or a placebo. Mood measures were taken before and after the diet condition as well as after the sugar challenge by a self-report of symptoms, and by the MMPI and POMS. Results revealed that the 2-week period of consuming the protein diet without sugar, caffeine and alcohol led to improvement of mood and distress. Moreover, in two subjects, the sucrose challenge after the dietary manipulation period increased feelings of fatigue and decreased feelings of vigor. It remains unclear, however, whether the effects of the dietary change were due to withdrawal of carbohydrates, caffeine or alcohol.

Some studies have also compared the mood effects of dietary carbohydrates to the effects of dietary fat (Lloyd et al., 1994; Lloyd et al., 1996; Wells et al., 1998). For instance in a study by Lloyd et al. (1996) the effect of eating breakfast that differed in carbohydrate and fat content on mood was measured in 2 male and 14 female subjects. Subjects fasted overnight until the start of the experiment the next morning. The four different conditions were: 1) a low-fat, high-carbohydrate breakfast (62% carbohydrates, 10% protein, 27% fat), 2) a medium-fat/medium-carbohydrate breakfast (47% carbohydrates, 9% protein, 44% fat) and 3) a high-fat, low-carbohydrate breakfast (34% carbohydrates, 9% protein, 56% fat), or 4) no breakfast at all. All diets were isocaloric and contained approximately equal amounts of protein. Mood and performance were measured at 30 minutes before and 30, 90, and 150 minutes after consumption of the diet (at 8.30 am) using a questionnaire derived from the Profile of Mood States and the Activation-Deactivation Adjective Checklist. There was a significant reduction in feelings of fatigue after the low-fat/high-carbohydrate breakfast, but no other mood changes or effects of the other dietary conditions were found. It was found that the fat-to-carbohydrate ratio of a breakfast can establish changes in mood independent of energy value or oro-sensory quality. Although these results do not provide conclusive information about the separate contributions of fat and carbohydrates, the mood changes indicate a profound effect of carbohydrates. Further evidence for this was found in a recent experiment of Wells et al. (1998). In this study, the effect of gastric infusion of fat and carbohydrates on mood and several physiological variables was measured in 9 normal subjects. Gastric infusion was introduced to control for oro-sensory and cognitive effects of observable differences in fat and carbohydrate composition. On three separate experimental days, a solution either containing sucrose (100% energy

of carbohydrates), lipid emulsion (100% energy of fat) or a non-nutrient control (0.9% saline) was directly infused in the gastrointestinal tract of the subject. The order in which the subjects received the infusion was counterbalanced using a repeated crossover design, and the subjects were blind to the nature of the dietary condition. Mood and sleepiness were measured approximately 30 minutes before (at 09:45 am) and at regular intervals after (30, 60, 90, 120, 150 and 180 minute intervals) gastric infusion. It was found that hedonic tone and tension improved 3 to 3.5 hours after intake of the sucrose (as well as the saline) solution, whereas subjects reported greater tension and lower hedonic feelings after the fat infusion. These results indicate that sucrose administration has positive effects on mood while fat administration does not.

In normal subjects, positive and negative mood changes are also found after tryptophan administration. As for example, in a within-subjects study (Yuwiler et al., 1981) with 5 males, in which the the effect of single or repeated tryptophan administration (suspended in a 180 ml dairy chocolate drink) on mood, blood tryptophan, and serotonin concentrations was measured. After a baseline period (consumption of chocolate drink without tryptophan, followed by two days of recovery) subjects in the Yuwiler et al. study participated in the following experimental sessions: 1) short-term tryptophan administration at 8:00 am (50 mg/kg) followed by a recovery period of one week, 2) a short-term treatment at a double tryptophan dose (100 mg/kg) followed by a recovery period of one month, and 3) chronic tryptophan consumption (50 mg/kg) daily between 8:00 and 8:45 am for 14 days. Blood samples and measures of mood changes were acquired before and during the baseline period, and several times after the tryptophan loading. It was revealed that the consumption of tryptophan led to dosage-dependent increases in blood tryptophan and serotonin concentrations. Under all load conditions, tryptophan administration led to increased feelings of drowsiness within 30 minutes after ingestion. The authors explained these findings by suggesting that changes in brain serotonin concentration underlie the mood effects of tryptophan administration. Another study showing effects of tryptophan on mood is that of Lieberman et al. (1983) which examined the effect of tryptophan and tyrosine administration on mood, performance and pain perception in 2 experiments with 24 male subjects. In a double-blind placebo controlled study, tryptophan (50 mg/kg), tyrosine (50 mg/kg) or a placebo was administered in a single dose at 7:15 am, followed two hours later by cognitive and affective tests. Mood was measured once, 2 hours after ingestion (VAS and POMS scales). Data revealed a negative mood change only after tryptophan administration; feelings of drowsiness and fatigue were higher than in the placebo condition. The authors assumed that the effects of tryptophan on mood are mediated by changes in serotonergic functioning in the brain.

The studies described above revealed that an increased proportional intake of

carbohydrates or tryptophan in normal subjects may lead to slight and inconsistent changes in mood; increasing feelings of calmness and sleepiness and either improving or deteriorating feelings of fatigue and tension. The inconsistency of the evidence regarding the influence of carbohydrates on mood in normal subjects is further revealed by studies that report no effect of carbohydrates or tryptophan administration on mood (Lieberman et al., 1986b; Smith et al., 1988; Deijen et al., 1989; Reid and Hammersley, 1995). For instance, using a repeated measurement design, Lieberman et al (1986a) studied the effect of carbohydrates on mood in 40 male subjects. These subjects fasted overnight, ate a standard breakfast and consumed a carbohydrate or a protein lunch on two different days separated by a one-week interval. Mood was measured before and several times after the intake of the test diet. Although the rise in sleepiness seemed to be more profound 1-2 hours after the carbohydrate-rich lunch compared with the protein lunch, these differences were not significant. The authors suggested that men may not be very sensitive to carbohydrate induced changes in self-reported mood, either by a failure to experience them or by the unwillingness to report them. In a within-subjects cross-over study, Smith et al. (1988) tested the effect of a protein, starch or sugar lunch on mood in 6 male and 6 female subjects. Subjects were given a standardized breakfast at 08:35 am followed by a test lunch at 13:00 pm. Three different isocaloric lunches were used: a high protein meal (14% starch, 1% sugar, 55% protein), a high starch meal (40% starch, 15% sugar, 15% protein), and a high sugar meal (15% starch, 40% sugar, 15% protein). All lunches contained 30% fat. Mood was measured by VAS scales 45 minutes before and approximately 75 minutes after the onset of the test lunch. No significant effects of diet composition on mood were found. Deijen et al. (1989) investigated the effect of protein and carbohydrate intake among 4 male and 16 female subjects in a between-subjects design. Half of the subjects were assigned to the experimental diet condition. The other half of the subjects were assigned to the control condition and were instructed to consume a standardized balanced diet, approximately containing the same amounts of protein and carbohydrates in the morning and afternoon (160g carbohydrates, 55g proteins). In the experimental condition, subjects were instructed to choose their daily food from a dietary list of different food items for a period of 3 weeks in their natural environment. The list was constructed in such a way that it was certain that breakfast consisted of proteins and dinner was high in carbohydrates. Subjects were required to keep a daily food diary. The protein breakfast was standardized so that approximately 70 grams (63%) of energy were derived from proteins and 25 grams (8%) of energy were derived from carbohydrates. The carbohydrate dinner was set at 10 grams (9%) of energy delivered by protein and 100 grams (31%) of energy by carbohydrates. In all subjects, on the last day of the three-week period, repeated measures of mood were made during the morning (about 10:30 am) and afternoon (about 06:00 pm) at the laboratory. The experimental group reported more profound feelings of anger after the protein breakfast compared with the control condition,

whereas after the carbohydrate lunch no such effects were found. Based on their findings, the authors attributed the higher scores on anger in the morning to the unattractive nature of the protein diet. In a between-subjects, placebo study, Reid et al., (1995) examined mood and food-intake effects of a carbohydrate load among 31 male and 29 female subjects. Subjects fasted overnight and were divided into 3 dietary conditions during which they received either a 110 ml drink containing 40 gram sucrose (160 Kcal), a 56 ml drink containing 4.34 gram saccharine (10 Kcal), or plain water. Before intake, test- and placebo-load subjects took a benzocaine anesthetic and wore a nose clip in order to eliminate sensory cues such as sense, taste and texture. Mood was measured by the POMS before, immediately after and 30 and 60 minutes after the load. After the second mood measure (30 minutes after intake), subjects were allowed to leave the laboratory room and could return the last POMS measure the following day. Results revealed no effect of the sucrose or saccharine load on mood.

Summarizing, the effects of an increased proportional intake of carbohydrates or tryptophan administration on mood in normal subjects yield inconsistent and contradictory findings; either slightly increasing sleepiness and calmness, deteriorating as well as improving feelings of tension and fatigue, or show no effect at all.

EFFECTS OF CARBOHYDRATES AND TRYPTOPHAN ON MOOD IN NORMAL SUBJECTS.

Author	Design	Sample (age in years)	Food/nutrients	Results
Greenwood, Lader, Kantameneni & Curzon, 1975	Within-subjects	6 males and 4 females (21-38)	* Tryptophan * Placebo	Tryptophan increased drowsiness and mental slowing.
Hartmann, Spinweber & Fernstrom, 1977	Within-subjects, double-blind	12 (20-35)	Carbohydrate drink and Protein-drink supplemented with: * Tryptophan * Leucine * Placebo	Carbohydrate drink produced greater sleepiness than protein drink.
Yuwiler, Brammer, Morley, Raleigh, Flannery & Geller, 1981	Within-subjects	5 males (20-50)	Acute and chronic tryptophan (Trp) administration	Acute Trp increases drowsiness. Chronic Trp produced greater lethargy.
Leathwood & Pollet, 1982/83	Within-subjects, double-blind	30 males and 30 females (18-64)	Carbohydrate-rich, protein-poor diet combined with: * Tryptophan (Trp) * Tyrosine * Caffeine * Placebo	Diet with caffeine increased wakefulness. Diet with Trp had a sedating effect.

Spring, Maller, Wurtman, Digman & Cozolino, 1982/83	Between-subjects	129 males and 55 females (18-39 and 40-65)	* Protein-rich, carbohydrate-poor meal * Carbohydrate-rich, protein-poor meal	Women reported greater sleepiness after carbohydrates, whereas men reported greater calmness. Older subjects were more calm and less tense after carbohydrates.
Lieberman, Corkin, Spring, Growdon & Wurtman, 1982/83	Within-subjects	16 males (18-45)	* Tryptophan * Tyrosine * Placebo	Tryptophan increased fatigue, and decreased vigor and alertness.
Christensen, Krietsch, White & Stagner, 1985b	Within-subjects	1 male & 3 females	Protein-rich, carbohydrate-poor diet without sucrose, caffeine and alcohol, for two weeks	Protein-rich, carbohydrate-poor diet improved mood.
Lieberman, Spring & Garfield, 1986a	Repeated measures	40 males (18-28)	* Starch lunch * Protein lunch	No significant dietary effects.
Smith, Leekam, Ralp & McNeill, 1988	Within-subjects	6 males and 6 females	* High protein meal * High starch meal * High sugar meal	No dietary effects on mood. General effect on post-lunch feelings.
Deijen, Heemstra & Orlebeke, 1989	Between-subjects	4 males and 16 females (21-27)	Daily diet for 3 weeks: Protein-rich breakfast in the morning and Carbohydrate-rich diet in the afternoon	Protein diet in the morning increased anger.
Spring, Chiodo, Harden, Bourgeois, Mason, & Lutherer, 1989	Within-subjects	7 females (18-29)	* Protein-lunch * Balanced lunch * Carbohydrate-lunch * No lunch	Carbohydrate and balanced lunches both increased glucose and insulin. Only a carbohydrate lunch increased the Trp/lnaa ratio and increased fatigue.

Lloyd, Green & Rogers, 1994	Within-subjects	3 males and 15 females (27.3)	Low-fat, high-carb (LFHC); Medium-fat, med-carb (MFMC); and high-fat, low-carb (MFMC) lunch.	Subjects were less cheerful after LFHC and HFLC and less tensed after LFHC.
Reid & Hammersley, 1995	Between-subjects	31 males and 29 females	* Sugar * Saccharin * Water (control)	No significant effects on mood and energy intake. Sugar marginally delayed food intake.
Lloyd, Rogers & Hedderley, 1996	Within-subjects	2 males and 14 females (26)	Low-fat, high-carb; medium-fat,medium-carb; high-fat, low-carb breakfast; and no breakfast	Mood improved only after the low-fat, high carbohydrate breakfast.
Wells, Read & MacDonald, 1998	Within-subjects	6 males and 3 females	Gastric infusion: * Sucrose * Lipid * Saline	Sucrose and lipid both increased pulse rate and energy expenditure, although the latter had a more delayed effect. Contrary to sucrose, lipid increased tension and lowered hedonic tone.

1.3.2. *Dietary effects on mood in patients*

I will now turn to the influence of diet manipulations on mood in clinical subjects. Unlike normal subjects, clinical subjects consistently report improvements of mood after the intake of increased proportions of carbohydrates or tryptophan administration (Thomson et al., 1982; Lieberman et al., 1986a; Wurtman et al., 1989; Rosenthal et al., 1989; Steinberg et al., 1994; Sayegh et al., 1995). Only a few studies have shown no effects of diet on mood (Toornvliet et al., 1997). One example is given by Lieberman et al. (1986b), in which the effect of a carbohydrate lunch on mood was measured among 8 male and 23 female obese carbohydrate craving subjects, and 4 male and 10 female non-carbohydrate craving obese subjects. After a standard breakfast (07:30), all subjects received a carbohydrate test lunch at 11:45 (105 g carbohydrates, 0.7 g protein, 42 g fat) that had to be consumed within 15 minutes. Mood was measured 15 minutes before and 2 hours after the carbohydrate lunch using the POMS, VAS and SSS. The non-carbohydrate cravers were shown to become more drowsy, and to grow more fatigued and depressed after the carbohydrate lunch, whereas feelings of depression *improved* among the carbohydrate-craving group after the carbohydrate test lunch. The authors concluded that the positive effects of carbohydrate consumption among the carbohydrate-cravers may have been due to increases in brain tryptophan and serotonin concentrations. Comparable therapeutic effects of carbohydrates have been found in women with Late Luteal Phase Syndrome (Wurtman et al., 1989). Using a within-

subjects design, Wurtman and colleagues measured the occurrence and coincidence of depressive mood and carbohydrate intake among 19 PMS patients and 9 control subjects during two 48-hour periods. In the first 48-hour period, subjects participated while in the early follicular phase of the menstrual cycle (day 4-7) and during the second 48-hour period they were in their late luteal or premenstrual phase (3-5 days before the expected onset of menstruation). During both experimental periods, subjects were allowed to eat as much as they liked from standardized high carbohydrate- or high protein isocaloric meals and between-meal snacks. The high carbohydrate meals contained 13-15 gram carbohydrates and 1-2 gram protein and the protein meals contained 13-15 gram protein. The carbohydrate snacks consisted of 12-13 gram carbohydrates, 1% protein and 5-7 gram fat, whereas the protein snacks contained 11-12 gram protein and 5-7 gram fat. Food intake was measured by differences in weight of the meals before and after consumption. A computer connected to the vending machine which delivered the snacks registered the identity of the subjects (by a code), the type of snack and the time of day the snack was obtained. Mood was measured by the Hamilton Depression Scale and a questionnaire was administered to assess changes in fatigue, appetite, carbohydrate intake and sociability. As expected, depression was found to increase during the luteal phase among patients but not in the control subjects. Patients also increased their intake of carbohydrates during the late luteal phase compared with the early follicular phase, this was not found in the control subjects. Moreover, carbohydrate consumption during the late luteal phase improved depression, tension, anger and fatigue symptoms among patients. No such dietary effect was found during the early follicular phase or among the control subjects during either phase. The authors assigned the therapeutic effects of carbohydrate consumption to increases in brain tryptophan and serotonin function. Sayegh et al. (1995) also examined the effects of carbohydrate loading (known to increase the plasma Trp/LNAA ratio) on mood, appetite and performance in women with Late Luteal Phase Syndrome. In a double-blind, placebo-controlled study, mood was measured before and several times after carbohydrate intake. Results revealed that, compared with the placebo, the carbohydrate load significantly improved feelings of anger and depression 90-180 minutes after intake.

In order to test whether the effects of carbohydrates on mood in clinical populations are accompanied by changes in the plasma Trp/LNAA ratio, Rosenthal et al. (1989) studied the effect of diet on mood and plasma amino acids during the winter months in 16 depressed patients suffering from Seasonal Affective Disorder (SAD) and 16 control subjects. Using a cross-over design, subjects fasted overnight, consumed a standardized breakfast and received two different isocaloric standard meals for lunch: one rich in protein (15g carbohydrates, 105g protein, 33.3g fat) and one rich in carbohydrates (105g carbohydrates, 0.7g protein, 42.7g fat). Mood changes and blood amino acids were measured every hour until 3 hours after the test lunch. Mood was measured by POMS, VAS and SSS. The carbohydrate rich diet

resulted in an approximately 27% increase in the plasma Trp/LNAA and a decrease following the protein diet that was identical in the clinical and control groups. However, the SAD group reported increases in vigor and activation following the carbohydrate diet, whereas the control group reported enhanced feelings of sedation. The authors suggested that the differences in the effects of carbohydrates on mood in patients and controls support the notion that serotonin abnormalities may exist in clinical populations and that a high carbohydrate meal can restore serotonin levels to normal.

Therapeutic effects like those of carbohydrate consumption demonstrated in patients have also been found with tryptophan administration (Steinberg et al., 1994). In study of Steinberg et al. (1994) 13 Late Luteal Phase Syndrome patients were treated with 6 grams of orally administered L-tryptophan during 3 consecutive menstrual cycles. Tryptophan treatment significantly improved dysphoric symptoms and negative mood. This effect was attributed to changes in brain serotonin.

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Author	Design	Sample (age in years)	Food/nutrients	Results
Thomson et al., 1982	Double-blind, within-subjects	115 depressed subjects (18-65)	* Tryptophan * Amytriptyline * Trp & Amytr. * Placebo	All conditions except placebo improved depressive mood.
Lieberman, Wurtman & Chew, 1986b	Between-subjects	31 Carbohydrate-craving and 14 non-carbohydrate craving obese subjects	Carbohydrate-rich, protein-poor lunch	In non-carbohydrate cravers the lunch increased feelings of drowsiness and depression, whereas in carbohydrate-cravers the lunch improved depression.
Rosenthal, Genhart, Caballero, Jacobsen, Skwerer, Coursey, Rogers & Spring, 1989	Within-subjects	16 depressed SAD and 16 control subjects	Carbohydrate-rich, protein-poor lunch, and a Protein-rich, carbohydrate-poor lunch	The carbohydrate-rich lunch increases the plasma Trp/LNAA ratio. Only in SAD the lunch increases vigor, whereas in controls it increases sedation.
Wurtman, Brzezinski, Wurtman & Laferrere, 1989	Within-subjects	19 PMS subjects and 9 controls	Free choice of carbohydrate- and protein-rich meals and between snacks	Only PMS subjects consumed more carbohydrate-rich foods during the luteal phase of the menstrual cycle, which improved mood.
Steinberg, Annable, Young & Belanger, 1994	Open pilot study	13 PMS subjects	Tryptophan administration during 3 luteal cycles	Tryptophan administration improved mood.

Sayegh, Schiff, Wurtman, Spiers, McDermott & Wurtman, 1995	Double-blind within-subjects	Women with PMS	* Carbohydrate load * Placebo	Carbohydrates improved feelings of depression and anger.
Toornvliet, Pijl, Tuinenburg, Elte-de-Wever, Pieters, Frolich, Onkenhout & Meinders, 1997	Double-blind, within-subjects	Carbohydrate-craving and non-carbohydrate craving subjects	High carbohydrate-; high-carb, high-fat-; and high-protein, medium-carb snack	No different effects of dietary manipulation on mood.

1.3.3. *Dietary effects on cognitive performance*

A broad range of studies have been guided by the assumption that carbohydrates may also influence cognitive performance through changes in brain serotonin (Young, 1991). However, a consistent picture of the role of serotonin in the cognitive domain is rather difficult to obtain. In general, serotonin is believed to act as a general inhibitor involved in filtering cognitive computational processes and controlling perceptual-motor responsiveness (Soubrie, 1986; Spoont, 1992). Yet, increases in central serotonin activation may also facilitate motor output (Jacobs et al., 1995) and are believed to cause learning and memory impairments (Altman et al., 1988). Dietary studies have yielded inconsistent findings on the effects of carbohydrate intake on cognitive performance, ranging from improved performance (Kanarek et al., 1990; Benton et al., 1992), declined performance (Spring et al., 1982/83; Connors et al., 1985; Smith et al., 1988) to no effect at all (Lloyd et al., 1996; Deijen et al., 1989; Horton et al., 1987). For a more elaborated overview of the literature on the direct effects of carbohydrates on cognitive performance, the reader is referred to the recent work of Bellisle et al. (1998).

In summary, increasing the proportional intake of carbohydrates compared to proteins and the administration of tryptophan have been found to influence mood in patients and in normal subjects. In patients, carbohydrate or tryptophan administration improves depression (Thompson et al., 1982; Lieberman et al., 1986b; Rosenthal et al., 1989; Wurtman et al., 1989; Steinberg et al., 1994; Sayegh et al., 1995). Conversely, in normal subjects the mood effects of carbohydrates are less consistent, revealing either no effects on mood (Smith et al., 1988; Lieberman et al., 1986a; Deijen et al. 1989; Reid et al., 1995; Wells et al., 1998) or just slight and contradictory alterations in feelings of calmness, tension and fatigue (e.g. Hartmann et al., 1977; Leathwood et al., 1982/83; Spring et al., 1982/83; Spring et al., 1989; Lloyd et al., 1996). A majority of these studies emphasized that the mood effects of carbohydrates and tryptophan administration are caused by alterations in brain serotonin concentrations. Consistent with this hypothesis, it has been shown that carbohydrate or tryptophan induced changes in mood have been preceded by diet

induced alterations in the plasma Trp/LNAA ratio (Yuwiler et al., 1981; Rosenthal et al., 1989; Spring et al., 1989). Since a diet induced change in the plasma Trp/LNAA ratio influences the brain serotonin level (Fernstrom et al., 1971b; Fernstrom et al., 1973; Peréz-Cruet, 1974; Curzon, 1985; Wurtman, 1987; Lyons et al., 1988; Kaye et al., 1988; Anderson et al., 1990), a depletion of the serotonin precursor tryptophan (and thus a reduction in brain serotonin) deteriorates mood (Young et al., 1985; Miller et al., 1992; Delgado et al., 1994, 1995; Smith et al., 1997), a lower serotonin function contributes to depression (van Praag, 1981; Anisman et al., 1992; Maes et al., 1995) and an enhanced brain serotonin function may improve mood and mediates the therapeutic effects of antidepressant drug treatment (Maes et al., 1995; Heninger et al., 1996; Duman, 1997), it seems that the carbohydrate-mood relationship can be best explained by increases in brain tryptophan and serotonin concentrations (Spring et al., 1987).

1.3.4. *Problems and methodological concerns*

A few concerns need to be addressed before assuming that a carbohydrate-rich, protein-poor diet may enhance brain serotonin function and may improve mood.
A first concern refers to the question of the extent to which the plasma Trp/LNAA ratio must increase in order to enhance brain serotonin function in humans. In most studies, a diet-induced rise in the plasma Trp/LNAA ratio did not exceed 20-25% over baseline values. Some authors have questioned whether this increase would also be sufficient to cause a meaningful enhancement of brain serotonin in humans (Ashley et al., 1985; Teff et al., 1989). These authors suggested that the plasma Trp/LNAA ratio must rise at least by 50% to produce substantial changes in brain serotonin synthesis. However, there is no evidence to justify such a general restriction of a meaningful rise in brain serotonin at a 50% increased plasma Trp/LNAA ratio. There is only one study (Teff et al., 1989) that has demonstrated a 47% increased plasma Trp/LNAA ratio, which did not lead to significant increases in 5-HIAA. However, the increased plasma Trp/LNAA ratio in the study may not have reached significance due to small group size (N=5). Moreover, the subjects under study were neurological patients (mean age 71 years) diagnosed with Normal Pressure Hydrocephalus. It should be noted that Pérez-Cruet et al. (1974) found that a significant 49% decline of the plasma Trp/LNAA ratio was accompanied by significantly lower 5-HIAA in CSF following a balanced diet. Furthermore, animal studies have revealed that a less than 35% increase in plasma Trp/LNAA may lead to significant increases in brain serotonin (Fernstrom et al., 1971b, 1972), and in humans, increases in the plasma Trp/LNAA ratio appear to vary with carbohydrate consumption just as they do in rats (Lieberman et al., 1986c). Accordingly, even 20-25% increases in the plasma Trp/LNAA ratio may influence brain serotonin synthesis in men (Lyons et al., 1988). This contention is supported by findings from

Kaye et al. (1988) and Anderson et al. (1990) that a 20-40% change in the plasma Trp/LNAA ratio leads to neuroendocrine alterations that are indicative of alterations in brain serotonin.

A second concern relates to the question of whether increases in tryptophan available for uptake into the brain is controlled by free- or total circulating tryptophan. As was previously mentioned, increases in brain tryptophan and serotonin due to food intake are dependent on the transport of tryptophan from the blood across the blood-brain barrier. 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. Thus, more support is found for the position that both free and total plasma tryptophan can be transported across the blood-brain barrier, thus increasing brain serotonin synthesis (see Chaouloff, 1993).

A third concern relates to the question of why the positive mood effects of carbohydrates are absent or inconsistently reported in normal populations. It may be that the use of various methods and designs has obscured mood effects in some of the data. For example, a recurring and significant methodological error is the absence of a reliable test- or placebo diet. Most studies have failed to use a carbohydrate test diet that significantly differed from the control diet in protein content. This is important because as little as 4% of the energy delivered by dietary proteins can suppress the increasing effects of carbohydrates on the plasma Trp/LNAA ratio (Yokogoshi et al., 1986). Another problem is that dietary manipulations have not always been controlled. For instance, some authors have measured food intake by self-handled food diaries that were filled in in the non-controlled environment of the subject. This method must be criticized since it is likely that such a subjective recall of food intake is easily confounded by, for instance, bad memory, wishful thinking, or unintended environmental influences. Thus, the investigation of the effects of food intake on mood has to take place in a controlled environment and by a controlled dietary manipulation. An additional methodological concern is the absence of a sufficient fasting period before the intake of the test diet. The effect of dietary manipulation on mood can be contaminated by prior food intake. For instance, it has been demonstrated that the effect of carbohydrates on increasing the plasma Trp/LNAA ratio and brain serotonin concentrations declines when the test diet is consumed 2-3 hours after a prior meal containing at least 12% of energy from proteins (Fernstrom et al., 1995). A diet-induced increase in brain tryptophan and serotonin is most likely to occur when the test diet is consumed in the morning after a fasting period of at least 8 hours. A final confounding factor may be the use of inappropriate intervals between the intake of a

test meal and the measurement of mood changes. Because the peak response of diet-induced mood changes most often occurs approximately 2 hours after food intake, earlier or later mood measures may miss meaningful mood effects of dietary manipulation. Furthermore, the investigation of the affective influence of food manipulation also requires pre- and post measures of mood; preferably taken in a constant environment. Some authors either did not measure mood changes by pre- and post-testing or measured mood changes by pre- and post-testing under different environmental conditions.

Even though these methodological problems illustrate the importance of carrying out controlled dietary studies, it is unlikely that they are responsible for the inconsistent and weak mood effects of carbohydrate consumption in normal subjects. In the following section it is argued that the effects of an increased proportional intake of carbohydrates on mood can be mediated by stress.

1.4. RELATION BETWEEN STRESS, FOOD AND MOOD.

Changes in mood and the occurrence of depressive symptoms are determined by biological as well as environmental factors. Among the most potential environmental factors are stressful events. Depressive mood and clinical depression are often preceded by severe stressful experiences (Brown et al., 1987), and it has been frequently demonstrated that stress may lead to poor cognitive performance (Hockey et al., 1983; Mandler, 1984; Smith, 1990; Kramer et al., 1991). In explaining the influence of stress on mood, a prominent role for brain serotonin has been acknowledged. During stress, the activity of the brain serotonergic system rises (Joseph et al., 1983; Stanford, 1993). This increase in brain serotonin function may be a biological condition for coping with stress and for preventing stress-induced mood deterioration (Anisman et al., 1991; Deakin, 1991; Delbende, 1992; Graeff et al., 1996). Because stress is accompanied by increases in brain serotonergic activity, chronic stress exposure will lead to a continuous elevation in serotonin synthesis, resulting in a tryptophan depletion and, hence, reduced brain serotonin concentrations. In animal studies, it has been demonstrated that chronic stress causes an increase in the sensitivity of the brain serotonergic system by a compensatory receptor sensitization (Kennett et al., 1985; Adell et al., 1988). Chronic stress in humans may also cause a compensatory increase in central serotonin sensitization that, in turn, predisposes the human brain for positive mood effects of a diet-induced increase in available brain tryptophan. If brain serotonin function must increase if the organism is to cope with acute stress, then an increase in brain tryptophan may improve stress coping and mood in subjects chronically distressed, especially under conditions of actual stress exposure.

1.4.1. *Biological stress mechanisms: HPA and cortisol.*

Under stress, the activity of the central nervous system rises to prepare the organism for defense and stress coping. The hypothalamic-pituitary-adrenal (HPA) axis is the neural mechanism that integrates various stress inputs into a series of autonomic and neuroendocrinological changes needed for stress adaptation (Ursin et al., 1993; Akil et al., 1995; Maes et al., 1995). In the brain, the serotonergic system plays a prominent role in stress regulation: It controls the activity of the HPA axis and, in turn, mediates the influence of stress perception on the onset and course of stress responsiveness.

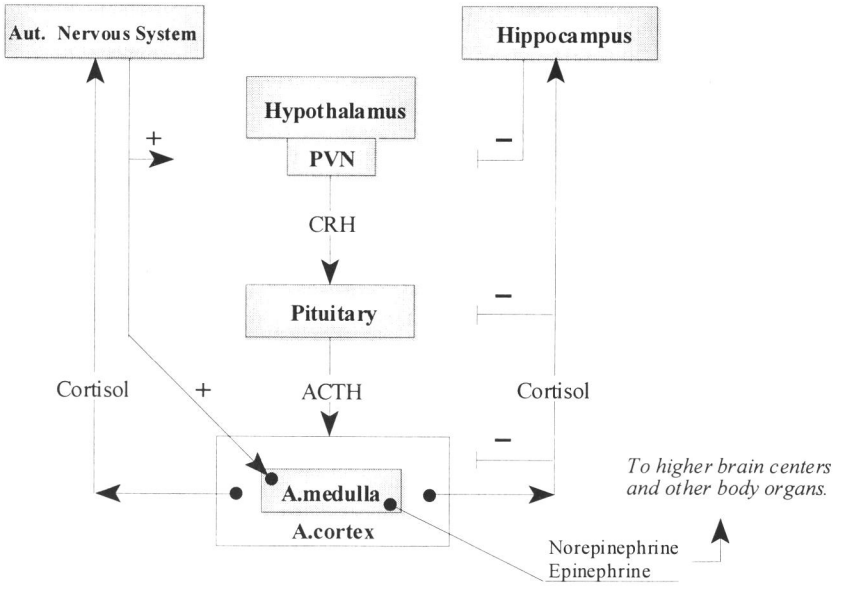

Fig.3: The Hypothalamic-Pituitary-Adrenal axis.

During the perception of threat, different brain areas are stimulated to relay information about the sensory properties of the threatening event through the brain, thereby bringing the nervous system to a higher level of readiness. First, there is a direct sympathetic activation that stimulates the release of norepinephrine from synaptic nerve terminals and plasma epinephrine into the blood stream from the medulla of the adrenal glands. In addition, the liver secretes glucose in the blood, whereas the kidneys are enforced to secrete renin and constrain the excretion of sodium and water. This sympathetic-adreno-medulla activation leads to a general mobilization response that provides the organism with more energy for immediate defense (fight or flight) by speeding up heartbeat, blood pressure and blood glucose levels.

Second, a more delayed system is activated under prolonged stress; intensifying stress adaptation. This system has been described as the pituitary-adrenal-cortex system which, on the one hand, provides extra glucose for sympathetic action and, on the other hand, suppresses the stress response in order to reestablish physiological balance (Munck et al, 1984; Levine et al., 1991). Upon receiving various limbic inputs indicative of stress, cell bodies at the level of the paraventricular nucleus of the hypothalamus (PVN) are stimulated to enhance the release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP). Corticotropin-releasing hormone, in turn, constitutes the main modulator for cell bodies in the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH) and related peptides that originate from the same precursor proopiomelanocortin (POMC). Adrenocorticotropic hormone is transported by the blood to stimulate the chromaphin cells within the adrenal cortex to release glucocorticoid cortisol. Cortisol, in turn, reestablishes the internal balance of the nervous system and the body that was disrupted by the stress perception (Munck et al., 1984) by exerting a variety of actions throughout the brain in order to terminate the stress-response and to prepare the organism for stress coping. These effects of cortisol are regulated by fast and slow negative feedback actions on several levels of the HPA axis. Cortisol binds to mineral- and glucocorticoid receptors that directly or indirectly activate hypothalamic and pituitary structures involved in CRH and ACTH release. A direct cortisol action occurs within minutes by activating both receptor types and stimulating hippocampal and thalamic regions to inhibit CRF and ACTH release. A less immediate action of cortisol is exerted by decreasing gene expression of glucocorticoid receptors, reducing the transcription of those genes that are involved in the ACTH release at the pituitary level.

As an illustration of the involvement of the adrenal-cortical-axis in stress coping, cortisol concentrations measured in saliva or in the blood vary predictably with different psychological demands and with the appearance of depressive symptoms (Mason, 1968; Frankenhaeuser et al., 1980; Frankenaeuser, 1986; Ursin et al., 1993). For instance, Frankenaeuser et al (1980, 1986) tested the differences between sympathetic-adreno-medulla activity (measured by catecholamine release) and pituitary-adrenal-cortex activity (measured by cortisol) under different stress conditions. They showed that increases in cortisol varied as a function of the controllability of the stressor, with more controllability associated with lower cortisol levels. The authors suggest that a catecholamine response is correlated with general arousal and effort, and that cortisol responses are associated with poor stress coping and feelings of helplessness or depression. In accordance with this, Ursin and Olff (1993) concluded that although many stressful events lead to enhanced cortisol levels, cortisol will only return to baseline when stress coping is successful. In general, improved stress coping is accompanied by reduced cortisol levels and by lower feelings of depression, whereas poor stress coping leads to an increase in cortisol and often contributes to depressive mood (Mason, 1968; Ursin, 1980; Henry et al., 1981;

Henry, 1992; Dantzer, 1993; Ursin et al., 1993; Willner, 1993; Dinan, 1994). In accordance with these findings, a hyper-secretion of cortisol has been commonly found among patients with major depression (Maes et al., 1995; Barden et al., 1995).

1.4.2. *Brain serotonin: A biological basis for stress coping*

Previous findings demonstrated that alterations in the pituitary-adrenal-cortical system are necessary for stress adaptation. However, a stress-induced increase of HPA activity is under control of the brain serotonergic system (Fuller, 1992; Maes et al., 1995; Dinan, 1996). Pharmacological drug studies using serotonin precursors such as tryptophan, serotonin releasing drugs such as fenfluramine, or drugs that directly act upon serotonin receptors, have shown that increases in serotonin concentration may alter HPA activation. The administration of serotonin precursors such as tryptophan has been found to alter ACTH and cortisol release by 5-HT_1 and 5-HT_2 receptors at the hypothalamic level (enhancing CRF production), by 5-HT_2 and 5-HT_3 receptors at the level of the anterior pituitary, or by 5-HT_4 receptors directly at the level of the adrenal-cortex. Conversely, the serotonergic system contributes to the prevention or termination of HPA activity and subsequent cortisol responses by directly or indirectly acting on hippocampal structures, thereby enhancing negative feedback control on the HPA axis (Deakin, 1991, Graeff et al., 1996). Accordingly, serotonin depletion may reduce the feedback control of cortisol on the HPA axis, causing increased cortisol concentration in the blood.

In accordance with the findings mentioned above, increases in the brain serotonergic system are believed to constitute a biological condition for adequate stress coping and, in turn, for preventing the negative consequences of stress on mood (Deakin, 1991; Anisman et al., 1992; Graeff et al., 1996). The activity of the brain serotonergic system increases under stress (Joseph et al., 1983; Stanford, 1993) and controls (initiating as well as terminating) HPA activation (Deakin, 1991; Fuller, 1992; Graeff et al., 1996; Dinan, 1996), whereas a lowered serotonin function has been related to either disturbed or hyperactive HPA functioning (Maes et al., 1995; Barden et al. 1995) and to mood disorders such as depression (van Praag, 1980; Maes et al., 1995). It remains unclear, however, through which route an increase in brain serotonin contributes to improved stress coping and, in turn, to the suppression of stress-induced depressive mood. One possibility is that this occurs through an increased activation of the serotonergic pathway connecting the median raphe nucleus (MRN) to the hippocampus. It has been suggested that increased activation of this serotonergic pathway "disconnect" the negative consequences of stress perception on stress experiences and, in turn, prevents the onset of depressive mood (Deakin, 1991; Graeff et al., 1996). Furthermore, brain serotonin terminals at the level of the sensory- and prefrontal cortex are found to be part of the limbic-thalamo-cortical circuit (LeDoux, 1996) and are involved in the planning and execution of emotional actions. On the

basis of findings from Deakin (1991) and Graeff et al. (1996), it can be suggested that enhanced activity of serotonin structures that are part of this circuit may improve emotional planning and override a direct activation of the pituitary-adrenal-cortex (LeDoux, 1996). In accordance with this notion, serotonergic cell bodies in the frontal brain have been shown to be at least partly involved in mediating the therapeutic effects of antidepressant drugs (Drevents et al., 1992), and increases in brain serotonin by these drugs may improve depressive mood by stabilizing the negative feedback control on the HPA axis (Barden et al., 1995).

1.4.3. *Chronic stress effects on brain serotonin: Predisposing conditions for the effects of food on mood*

As we have seen, there is enough evidence to assume that increased functioning of the brain serotonergic system constitutes a biological response to stress and aids in stress coping and the prevention of a deterioration of mood. In the present thesis it is assumed that continuous stress exposure or chronic stress leads to increased responsiveness of the central serotonergic system to increases in available tryptophan and brain serotonin concentrations. This can be explained as follows. Because stress leads to an increased activity of the central serotonergic system (Joseph et al., 1983; Stanford, 1993) chronic stress will be accompanied by continuous elevations in brain serotonin synthesis. When stress is chronic, this continuous serotonergic activation ultimately may lead to a shortage of available tryptophan concentrations: i.e. serotonin expenditure exceeds its synthesis. Such chronic stress-induced depletion of serotonin has been frequently demonstrated in animal studies (Kennett et al., 1985; Adell et al., 1988). In humans, a chronic stress-induced shortage of available brain tryptophan may increase the risk that the serotonergic system will be depleted or overtaxed under conditions that cause a further decline of available brain tryptophan and serotonin. In the studies by Tuiten et al. (1994, 1995) it was assumed that one of these conditions is the premenstrual withdrawal of progesterone, which cause a decline in plasma Trp/LNAA. Such a decline of the plasma Trp/LNAA ratio has only been found in stress-prone women and is associated with a deterioration of mood. However, the most likely condition overtaxing the brain serotonergic system in chronically stressed subjects would be an actual confrontation with a highly stressful situation. In chronically stressed subjects, the required increase in brain serotonin synthesis during an acute stressor can not be fulfilled and will lead to poor stress adaptation and to a subsequent deterioration of mood. Thus, only chronically stressed subjects are able to benefit from an increased supply of brain tryptophan during the occurrence of an acute stress condition. This is expected on the basis of two factors. First, an increased influx of plasma tryptophan into the brain may lead to a meaningful improvement of stress coping and mood by preventing a further critical decline of brain serotonin levels. Second, since the chronic shortage of brain serotonin in chronically stressed subjects

may have caused the system to become more sensitive, only in stress-prone subjects should the brain serotonergic system strongly react to an increased influx of brain tryptophan and serotonin levels. In support of this explanation, a chronic stress-induced depletion of brain serotonin has been found to enhance serotonin sensitization in rats (Adell et al., 1988; Kennett et al., 1985).
Based on the above suggested relationships between stress, food, cerebral serotonin and mood, stress-proneness may constitute an important factor in the consistent mood improving effects of carbohydrates commonly found in several clinical subgroups. In normal subjects, differences in stress-proneness may partly explain the inconsistent dietary effects on mood documented in the literature.

1.5. GENERAL HYPOTHESIS AND RESEARCH QUESTIONS

The aim of the present thesis is to examine whether a diet-induced increase in available plasma tryptophan improves mood under acute stress exposure in stress-prone subjects but not in control (low stress-prone) subjects. It is hypothesized that the chronic stress experienced by stress-prone subjects will have lead to adaptations in the serotonergic system that, in turn, will have made this system more sensitive to dietary induced increases in brain tryptophan and serotonin concentrations. The study of the relationship between stress, food and mood may clarify the inconsistent findings regarding the affective influence of carbohydrate consumption in normal subjects. It may be that among normal subjects, stress-proneness constitutes a mediating factor for the carbohydrate-mood relationship. Additionally, the present thesis aims to provide a theoretical basis for explaining the therapeutic effects of carbohydrate consumption, serotonin precursor administration, or antidepressant treatment in patients suffering from a variety of mood disorders.

The following research questions are of importance:

1) Does a carbohydrate-rich, protein-poor diet significantly increase the plasma Trp/LNAA ratio and improve stress coping and mood in stress-prone subjects under acute stress ?

2) Does a carbohydrate-rich, protein-poor diet prevent a stress-induced deterioration of cognitive performance in stress-prone subjects ?

3) Is an improvement of mood and stress coping in stress-prone subjects under acute stress with a carbohydrate-rich, protein-poor diet attributable to an increased availability of tryptophan and an enhanced cerebral serotonergic function ?

The following chapters (Ch2-Ch5) describe the studies that were performed to investigate the relationship between stress, food and mood and to answer the research questions as previously stated. In the first study (Ch2), it was tested whether a carbohydrate-rich, protein-poor diet is able to increase the plasma Trp/LNAA ratio and, only in stress-prone subjects, improve mood during acute uncontrollable experimental stress. It is suggested that during the actual confrontation with a highly stressful situation, an increased availability of plasma tryptophan for uptake into the brain prevent a further decline in serotonin concentrations in stress-prone subjects but not in controls. In the second study (Ch3), it is tested whether a carbohydrate-rich, protein-poor diet is able to improve mood in stress-prone subjects during controllable- as well as uncontrollable acute stress. It was hypothesized that due to a chronic stress-induced biochemical adaptation of the serotonergic system, the serotonergic system in these subjects will become more sensitive to dietary tryptophan. Subsequently, a diet-induced increase in available brain tryptophan is expected to improve stress coping and mood regardless of the controllability of the acute stressor. In the third study (Ch4) it was investigated whether a carbohydrate-rich, protein-poor diet improves cognitive performance after acute stress. It was expected that the negative consequences of acute stress on mental performance that are frequently described in the literature can be prevented by a diet-induced increase in brain tryptophan. Finally, in the last study (Ch5) it was investigated whether a balanced or protein diet consisting of tryptophan-enriched proteins (compared with tryptophan-low proteins) is able to increase the plasma Trp/LNAA ratio and to improve mood and stress coping in stress-prone subjects by enhancing brain serotonin functioning. It was hypothesized that if a carbohydrate-rich, protein-poor diet improves stress coping and mood in stress-prone subjects by increasing tryptophan and brain serotonin levels, enriching tryptophan in dietary proteins in a balanced diet will have the same effects and should be accompanied by increases in brain serotonergic functioning.

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CHAPTER 2

DOES CARBOHYDRATE-RICH, PROTEIN-POOR FOOD PREVENT A DETERIORATION OF MOOD AND COGNITIVE PERFORMANCE OF STRESS-PRONE SUBJECTS WHEN SUBJECTED TO A STRESSFUL TASK ?

C.R. Markus, G. Panhuysen, A. Tuiten, H. Koppeschaar, D. Fekkes, M. Peters. (1998), *Appetite*, 31, 49-65.

ABSTRACT

This study investigates whether in stress-prone subjects, carbohydrate-rich, protein-poor food (CR/PP) prevents a deterioration of mood and performance under uncontrollable laboratory stress conditions. The assumption was that in stress-prone subjects there is a higher risk of serotonin deficiency in the brain and that carbohydrates may prevent a functional shortage of central serotonin during acute stress, due to their potentiating effect on brain tryptophan. Twenty-four subjects with a high stress proneness (HS) and 24 subjects with a low stress proneness (LS) participated in an uncontrollable stress situation under both a CR/PP and a protein-rich, carbohydrate-poor (PR/CP) diet condition. The plasma ratio of tryptophan to the other large neutral amino acids (ratio Trp/LNAA) was determined as a measure indicating the dietary effect on brain tryptophan and serotonin levels. Significant increases were found in the ratio Trp/LNAA during the CR/PP diet compared with the PR/CP diet. Experimental stress had significant effects on pulse rate, skin conductance, cortisol and mood in all subjects. During the CR/PP diet only the HS subjects did not show the stress-induced rise in depression, decline in vigour, and cortisol elevation that they showed after the PR/CP diet. With respect to cognitive performance, significant dietary effects were found on reaction time. It is suggested that CR/PP food in HS subjects may increase personal control, probably under the influence of higher levels of brain tryptophan and serotonin.

2.1. INTRODUCTION

The present study investigates whether in subjects with a high stress proneness, food rich in carbohydrates but very low in proteins may prevent a lowering of mood and poor performance when they are confronted with an uncontrollable stressful task. The assumption is that a high stress proneness is associated with the risk of a serotonin deficiency in the brain, and that this deficiency is part of the neurophysiological basis of depressive mood and worse performance, as frequently noted under high stress (Mandler, 1984; Smith, 1990; Kramer et al., 1991).

The rise of activity in the serotonergic brain systems is one of the established consequences of stress (Joseph & Kennett, 1983; Stanford, 1993) and a decline of serotonin activity in the brain has been demonstrated in disturbances of mood (van Praag, 1980; Delbende *et al.*, 1992). Hence, it has been suggested that an increased activity of central serotonin is an important biological condition enabling the human brain to cope with stress (Anisman & Zacharko, 1991). Serotonergic activity seems, in particular, to be involved in the maintaining of control over information processing and motor activity (Spoont, 1992) and, it is assumed, in the sense of personal control. As the higher serotonergic activity during stress leads to a higher breakdown of serotonin, continuing stress may lead to a functional shortage of the supply of this neurotransmitter, its expenditure exceeding its synthesis. Consequently, coping, mood and accuracy of performance deteriorate. In accordance with this idea, depletion of the precursor of serotonin synthesis, tryptophan, has been found to increase depressive mood in healthy subjects (Young et al., 1985; Smith et al., 1987) and may even result in depression as a clinical syndrome (Meltzer, 1989, 1990; Anisman & Zacharko, 1991, 1992), particularly in subjects with a prior history of depression (Heninger, Delgado, Charney, et al., 1996). Assuming that stress proneness stands for a frequently elevated level of serotonergic activation, it is probable that, in this condition, particular coincidences will overload the serotonergic system causing serotonin synthesis and activity to fall below functional need.

In previous studies (Tuiten *et al.*, 1994; Tuiten *et al.*, 1995) it was assumed that one of these coincidences is the pre-menstrual withdrawal of progesterone, causing a decline of the plasma ratio of tryptophan to the sum of the other large neutral amino acids (ratio Trp/LNAA). However, a more obvious coincidence overloading the serotonergic system would be an actual confrontation with a highly stressful situation. The risk of overloading the serotonergic system may be lowered by an appropriate diet composition, increasing the ratio Trp/LNAA in the blood and, thus, the influx of tryptophan into the brain (Fernstrom & Wurtman, 1971a, 1972). As insulin sends the LNAAs into the skeletal muscles with the exception of just tryptophan, an adequate way to increase the Trp/LNAA ratio is the elicitation of an insulin response by the intake of food very rich in carbohydrates but very poor in proteins (Curzon, 1985; Fernstrom & Wurtman, 1971b; Fernstrom et al., 1973; Wurtman, 1987). Such a diet will give tryptophan the advantage over the other LNAAs in the competition for access

to the brain and thus may increase central serotonin functioning. In contrast, a protein-rich, carbohydrate-poor diet will not increase but even decrease the plasma ratio Trp/LNAAs, because an insulin-induced fall in LNAAs is more than compensated for by the LNAAs presented in proteins.

Although dietary effects on the plasma ratio Trp/LNAAs are rather impressive, prior research comparing the effect of carbohydrate- and protein meals on mood has yielded contradictory findings (Spring et al., 1994) and opposite results have been found in clinical and non-clinical populations (Lieberman, Wurtman & Chew, 1986; Rosenthal et al., 1989; Spring, et al., 1989). In this respect, stress proneness might be the crux explaining these apparent inconsistencies.

The present study tested the hypothesis that a carbohydrate-rich, protein-poor (CR/PP) diet leads to less deterioration of mood and cognitive performance in stress prone subjects when they are confronted with a stressful task, in comparison with a protein-rich, carbohydrate-poor (PR/CP) diet. In contrast, such a CR/PP diet would have little effect on subjects with a low stress proneness, for in their case there is little risk the serotonin system will be overcharged.

A group with a high stress proneness and a group with a low stress proneness participated in two identical experimental sessions on two different dietary days. On day one they received a CR/PP diet, alternating the other day by a PR/CP diet. During the two experimental sessions they were successively exposed to unfeasible mental arithmetic, inducing uncontrollable stress, and to a memory scanning test (Sternberg, 1975), to measure cognitive performance. Before and after the stress induction task, mood was measured using the profile of mood states questionnaire (POMS). Changes of pulse rate, skin conductance and salivary cortisol from baseline were measured as physiological indices of the stress response, to check the stress manipulation. The dietary effect on the plasma ratio Trp/LNAA (and on the plasma ratio tyrosine/LNAA) was determined on the basis of blood samples taken before the start of the experimental sessions.

2.2. METHODS

Subjects

Three-hundred and thirty-one female and male social science students of the Utrecht University filled in the Inadequacy (IN) Scale of the Dutch Personality Inventory, measuring neuroticism (Luteijn et al., 1975), and also a questionnaire concerning personal details. Neuroticism is closely related to negative affectivity (Luteijn & Bouman, 1988), the disposition to see events as alarming and to experience aversive emotional states (Watson & Clark, 1984), and also to stress proneness (Gallagher,

1990; Wells & Matthews, 1994). In accordance, subjects high in neuroticism frequently experience stress (Bolger & Schilling, 1991; Deary & Matthews, 1993). Based on these connections, from the highest quartile of IN-scores 24 subjects (seven males and 17 females) were selected for the High Stress (HS) group (mean IN=22, range 17-30), and from the lowest quartile of IN-scores 24 subjects (six males and 18 females) for the Low Stress (LS) group (mean IN=5, range 1-6). Subjects' ages ranged from 18 to 25 years (21.2,±0.4 years). Exclusion criteria for participation were chronic and current illness, medication, or irregular diets. All subjects selected for the experiment were of normal weight for height (Body Mass Index [weight(kg)/height(m)^2] between 20-25). As oral contraceptive medication may have an effect on cortisol responsivity (Kirschbaum, 1995), the HS-group as well as the LS-group were matched for contraception. All subjects participating in the experiment signed a letter of (informed) consent and received a financial compensation after completion of the second experimental session.

Procedure

All subjects participated in two experimental sessions. One session was paired with a CR/PP diet, the other with a PR/CP diet. For half of the subjects in each group (both HS and LS) the first experimental session included the CR/PP diet, followed by the PR/CP diet in the second session. The other half of the subjects received the diets in the opposite order.

The two experimental sessions were separated for both male and female subjects by a period of 4 weeks, allowing to account for the menstrual phase of the female subjects with a normal menstrual cycle. These women participated in the experiment during their mid-late follicular phase (day 4-10), while women using oral contraception participated when they actually used the contraception pill.

Both experimental sessions had the following structure. On each experimental day, two subjects arrived at the laboratory at 900 and 1100 hrs, were enabled to read in a study room, and received breakfast at arrival, snack (1015 and 1215 hrs) and lunch (1100 and 1300 hrs), according to their dietary condition. Subjects were instructed not to eat or drink anything during the 11 h before their arrival. One and a half hours after lunch, a salivary cortisol sample was taken. The subject was then brought into a temperature-controlled laboratory room, where a blood sample was taken. After a little rest, the subject was seated in front of a computer screen and instructed about the experiment. The electrodes for skin conductance and the finger sensor for pulse-rate measurements were attached, and during the following 10 min the baseline physiological recordings were made. The subject was then exposed to a computer assisted battery of tests and experimental tasks, all integrated into the research software package MINDS (Brand & Houx, 1992). The battery consisted of (1), a version of the Profile of Mood States (POMS; Wald & Mellenbergh, 1990);

(2), an uncontrollable, high stress inducing, mental arithmetic task (Peters *et al.*, 1998); (3), a second version of the POMS; and (4), Sternberg's memory scanning task. A second salivary sample was taken at the end of the stress inducing task, approximately 30 min after stress onset (see Kirschbaum, 1993), before the second version of the POMS. Pulse-rate and skin conductance were recorded until the end of the memory scanning task. Subjects spent approximately 4 min on the first POMS, 30 min on the stress task, 4 min on the second POMS and 10 min on the memory scanning task.

		Carbohydrates	Proteins	Fats	Caloric value
CR/PP	**Breakfast** Bread, low-fat margarine, jam, tea with sugar, grape-juice. **Snack** Black coffee with sugar, candy bar. **Lunch** Bread, butter, marmelade, grape-juice.	219,1 grams (66,2 %)	11,9 grams (3,6 %)	44,3 grams (30,1 %)	1323 kcal.
PR/CP	**Breakfast** Bread, butter, cheese, black tea, high-fat milk. **Snack** Black coffee, milk, peanuts. **Lunch** Bread, butter, roast beef, tea.	109,5 grams (40,4 %)	71,3 grams (26,3 %)	40,2 grams (33,3 %)	1084 kcal.

Tabel 1: Composition of the carbohydrate-rich, protein-poor (CR/PP) diet and the protein-rich, carbohydrate-poor (PR/CP) diet.

Diets

The diets were composed by an authorised dietician of the University Hospital Utrecht. Both diets complied with the reference daily energy intake for peer groups of both men and women. The total amount of calories and fat in both diets were roughly equal. The ingredients of both diets were all available at the University restaurant and commonly consumed by the students. In order to keep the reducing effect of protein on the increase of the tryptophan ratio small, the total amount of protein in the CR/PP diet did not exceed 5% of the total amount of dietary energy (Yokogoshi & Wurtman, 1986).The composition of both diets is shown in Table 1.

Profile of Mood States (POMS)

Changes in mood were measured using two scales of the Dutch shortened version of

the Profile of Mood States questionnaire (Wald & Mellenbergh, 1990). The POMS comprises five different subscales for mood; four subscales are related to negative mood states (Anger, Depression, Fatigue and Tension) and one subscale concerns a positive mood (Vigour). The scores on the different subscales range from; 7-35, Anger; 8-40, Depression; 6-30, Fatigue; 6-30, Tension; and 5-25, Vigour.

Stress induction task

A mental arithmetic task, performed under noise stimulation, was used as an uncontrollable stress situation. Subjects were given 20 successive 1-min trials during which they had to solve a specific number of multiple choice mental arithmetic (the criterion) under time constraints, while at the same time continuous 80 dB industrial noise could be presented to them through headphones. They were led to believe that the presence or absence of the noise depended on their performance; if they failed the criterion, noise would be present during the next trial; if they met the criterion, noise would be absent. In fact, the criterion was manipulated so that all subjects continued to fail on each trial. The criterion was always set at one sum above what subjects could manage as calculated from the average time per sum needed on previous trials. Accordingly, in only the first trial no noise was presented, whereas on all other trials the noise was present. This task has been demonstrated to induce psychological and physiological stress and to be perceived as highly uncontrollable (Peters *et al.*, in press).

Memory task

A memory-scanning task was used as a cognitive performance (accuracy) test (Sternberg, 1975). This task consisted of four sub-tasks, corresponding to memory sets of 1, 2, 3 and 4 consonants. Each sub-task included a presentation of the particular set to be memorized on the computer screen, as long as each subject needed, and then 60 trials followed. In 30 trials the presented letter belonged to the memorized set, in the other 30 trials this was not the case. Every first 10 trials in each sub-task were used for practice and, therefore, not used for analysis. In each trial the letter was presented in the center of the screen during 1 sec. The order of presentation of letters from within or without the memory set was randomized. Subjects were instructed to decide as fast as possible whether the presented letter was a member of the set of letters kept in memory, by pressing a red button (No) or a green button (Yes) with the preferred hand. Specific button boards were made for both left- and right handed subjects to make sure that the finger-button position for all subjects was equal.

Physiological measurements.

Measurement of electrodermal activity

Skin conductance was measured by the Vitaport system (Jain, 1995). Silver/silver chloride (Ag-AgCI) electrodes (surface 0.5cm^2) were used, filled with adhesive solid gel (ARBO H91, Braunschweig; Germany). These electrodes were bipolarly placed at the thenar palmar and hypothenar palmar sites of the non-preferred hand of the subject. The measurement principle was a constant voltage of 0.5 V with a sampling rate of 3Hz. Tonic skin conductance levels (SCL) were recorded during a 10-min baseline rest period and throughout the stress task as well as the memory task.

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 non-preferred hand. The signal was transduced by Sat-Trak^{TM} signal processing (sampling rate 65Hz), based on the Quadrature Division Multiplexing (QDM) technique. We used the Pulse Oximeter (SensorMedics corporation, Bilthoven; The Netherlands). With a sampling rate of 1Hz, pulse rate samples were collected and stored in the computer.

Measurement of salivary cortisol

Based on findings that peak cortisol levels are obtained 30 min after stress onset (Kirschbaum, 1993), one baseline cortisol sample (-15 min) and one post-stress sample (+30 min) were obtained with the Salivette sampling device (Sarstedt, Etten-Leur; the Netherlands). With this procedure, saliva was collected in small cotton swabs and stored in special salivette tubes until centrifugation. Saliva samples were centrifuged at 3000 rpm for 2 min at 20°C, which resulted in a clear supernatant of low viscosity. After centrifugation the supernatant was frozen at -20°C until analysis.

The analysis proceeded as follows. Cortisol levels were determined without extraction in a licensed laboratory at the University Hospital Utrecht using an in house competitive radio-immunoassay (RIA) employing a polyclonal anticortisol-antibody (K7348). 1,2-^{3}H(N)-Hydrocortisone (NET 185, NEN -Dupont-, Dreiech, Germany) was used as a tracer, following chromatographic verification of its purity. The lower limit of detection was 0.5 nmol/l and inter-assay variation was 11.0; 8.2; and 7.6% at 4.7; 9.7; and 14.0 nmol/l respectively (n=20). Reference values for adults are 4-28 nmol/l.

Determination of the amino acids.

The blood samples were collected in 7 ml EDTA tubes containing lithium heparin and centrifuged at 2650 g_{max} for 20 minutes at 20°C. Then the supernatant was frozen at -30°C until analysis.

For the determination of amino acids in plasma, a sensitive and reproducible fully automated method was used as described in detail by Fekkes *et al.* (1995). This method is based on reversed-phase high-performance liquid chromatography (HPLC) and *o*-phthaldialdehyde precolumn derivatization, making use of a 5-mm Spherisorb ODS 2 column (125 x 3 mm I.D.) for routine determination.

The plasma tryptophan ratio was ultimately calculated by dividing the plasma tryptophan concentration by the sum of the other LNAA's, i.e. valine, isoleucine, leucine, tyrosine and phenylanaline. The plasma tyrosine ratio was calculated in a similar way.

Experimental design and statistical analyses

The experimental design of our study comprised two *"between-subjects"* factors: *"Stress Proneness"* (high vs low stress) and *"Order of Diet"* (CR/PP diet first, or PR/CP first). There were also two *"within-subjects"* factors: *"Diet"* (a CR/PP diet vs a PR/CP diet) and several measures were repeated before and after the *"Stress Induction"* task. The dependent variables were the tryptophan ratio and the tyrosine ratio; the scores on the different scales of the POMS; the reaction time and amount of errors on (the different levels of) the Sternberg task; pulse rate, skin conductance, and salivary cortisol level.

The results on all dependent variables were analysed by means of multivariate analyses of variance with repeated measurements (MANOVA). Only significant results revealed by this procedure were further examined by univariate tests. As Order of Diet only contributed to the score on the Anger subscale of the POMS, mood was analysed by MANCOVA while further MANOVA's of the remaining variables were performed with only Stress Proneness as between-subjects factor. Pearson's correlation procedures did not reveal significant correlation's between alterations in plasma amino acids and changes in mood and physiological responsivity. All tests were based on two-tailed analyses at a significance level of 5%.

2.3. RESULTS

Ratio Trp/LNAA

It was argued that the effect of a CR/PP diet on mood and performance is indirectly

caused by an increase of the ratio of Trp/LNAA, thus, a straight prediction was derived that the tryptophan ratio averaged over all subjects would be higher on the day of the CR/PP diet compared to the day of the PR/CP diet. There were significant Diet on Trp/LNAA effects as expected (see Fig.1). The mean ratio Trp/LNAA significantly increased from 0.074 (±0.01) on the PR/CP day to 0.105 (±0.015) on the CR/PP day [$F(1,45)=171.159$, $p<0.0001$]. (The ratio Tyrosine/LNAA was altered in the opposite direction; the mean ratio Tyrosine/LNAA significantly decreased from 0.132 (±0.025) on the PR/CP day to 0.109 (±0.023) on the CR/PP day [$F(1,45)=44.414$, $p<0.001$]. There were no significant differences in the ratio Trp/LNAA or in the ratio Tyrosine/LNAA between the HS and LS group, in either dietary condition.

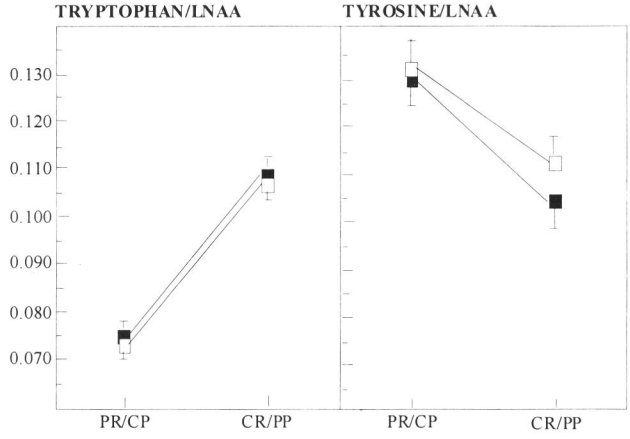

Fig.1: Plasma ratio Trp/LNAA and tyrosine/LNAA in High stress (■) and Low stress subjects (□), under a carbohydrate-rich, protein-poor (CR/PP) diet and a protein-rich carbohydrate-poor (PR/CP) diet.

Mood

Multivariate analysis of variance with repeated measurements revealed a significant overall effect of the stress inducing arithmetic task on changes of the different mood-scales of the POMS [Wilks $F=8.539$, $p< 0.001$] before and after this task. Significant changes in POMS values were found after the stress inducing task on Anger [from 7.64±1.5 to 11.67±5.6; $F(1,45)=35.953$, $p<0.001$], Tension [from 9.22±2.637 to 10.138±3.58; $F(1,45)=4.716$, $p=0.035$], Depression [from 9.76±2.3 to 10.75±3.8; $F(1,45)=7.667$, $p=0.008$], Vigour [from 14.60±3.53 to 13.13±4.0; $F(1,45)=12.503$, $p=0.001$] and Fatigue [from 9.45±3.33 to 11.49±4.0; $F(1,45)=14.773$, $p<0.001$] compared with POMS values before the stress inducing task, with both groups and

dietary conditions combined. These results indicate the success of the stress manipulation we used.

A significant Order of Diet by Diet by Stress Induction effect was found on the Anger subscale of the POMS [$F(1,43)=12.62$, $p=0.001$]. Although the authors had already counterbalanced for the order of administration of the diets, further multivariate analysis of variance was performed with Order of Diet as a covariate (MANCOVA).

It was predicted that the HS group would be less deteriorated in mood by the stress inducing task on the CR/PP day compared to the PR/CP day. This effect would not occur in the LS group. Therefore, a significant interaction effect of Diet by Stress Proneness by Stress Induction was expected on the diverse sub-scales of the POMS. The multivariate analysis of variance revealed a significant interaction effect [Wilks $F(1,45)=2.450$, $p=0.050$]. Figures 2 and 3 present the results of the separate sub-scales of the POMS. The univariate analyses revealed that the significant interaction effect of Diet by Stress Proneness by Stress Induction on mood originated from the scores on only two of the five sub-scales of the POMS: Depression and Vigour. As shown in Fig. 3, in the LS group the stress task led to an increase in feelings of depression (from 9.13±2.05 to 10.33±3.73) and a decrease in feelings of vigour (from 15.70±3.80 to 13.61±3.89) on the CR/PP day as well as on the PR/CP day (from 9.70±3.70 to 10.52±3.8 and from 14.44±3.72 to 13.30±4.90 respectively). In the HS group, on the other hand, there was only an increase of depression (from 9.96±3.29 to 11.83±4.90) and a decrease of vigour (14.58±3.13 to 12.50±3.35) on the PR/CP day after the stress induction, while on the CR/PP day this stress-induced increase of depression (from 10.37 ±2.9 to 10.13 ±2.8) and a decline of vigour (from 13.50 ±3.39 to 13.29 ±4.10) did not occur. These interaction effects were clearly significant for Depression [$F(1,45)=9.466$, $p=0.004$] and Vigour [$F(1,45)=5.653$, $p=0.022$] and were not found for Anger [$F(1,45)=0.002$, $p=0.96$), Tension [$F(1,45)=0.016$, $p=0.90$) or Fatigue [$F(1,45)=0.172$, $p=0.68$). The decline of depression after the stress task in HS subjects on the CR/PP day (10.37 ±2.90) compared to the PR/CP day (12±4.9) showed a trend but was not statistically significant (paired t-test: $t_{23}=11.97$, $p=0.06$).

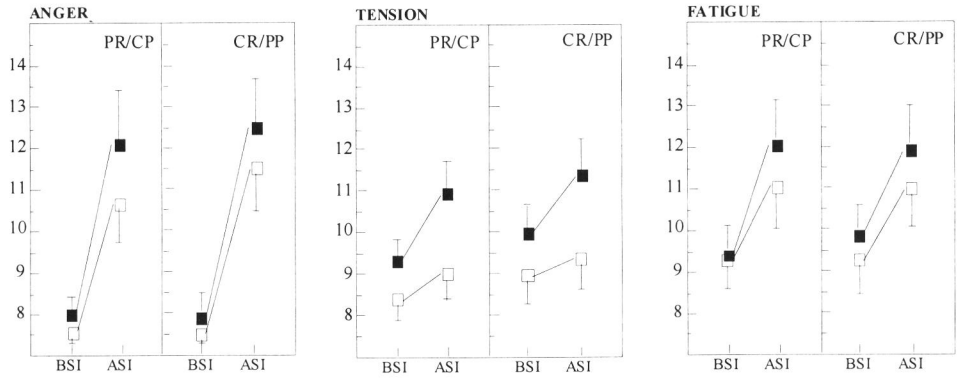

Fig.2: Anger, Tension and Fatigue (subscales of the Profile of Mood States, POMS); ■, High stress; □, Low stress; CR/PP, Carbohydrate-rich, protein-poor diet; PR/CP, Protein-rich, carbohydrate-poor diet; BSI, Before stress induction; ASI, After stress induction.

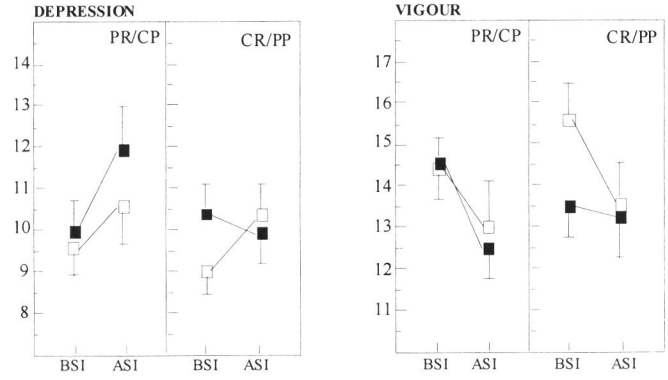

Fig.3: Depression and Vigour (subscales of the Profile of Mood States, POMS); ■, High stress; □, Low stress; CR/PP, Carbohydrate-rich, protein-poor diet; PR/CP, Protein-rich, carbohydrate-poor diet; BSI, Before stress induction; ASI, After stress induction.

Cognitive performance

Accuracy of performance was analysed on the basis of the total number of errors of the subjects over all sub-tasks. It was predicted that HS subjects would make less errors on the CR/PP day than on the PR/CP day, compared with LS subjects. Although the mean number of errors in the HS group seems to be somewhat lower on the CR/PP diet (1.6 ±2) than on the PR/CP diet (2.1±2.8) (Fig. 4), and in the LS group even seems to be higher (1.4±1.44) than in the HS group (1.8±2.6), a significant Diet by Stress Proneness by Stress Induction effect, or any other effect were not found.

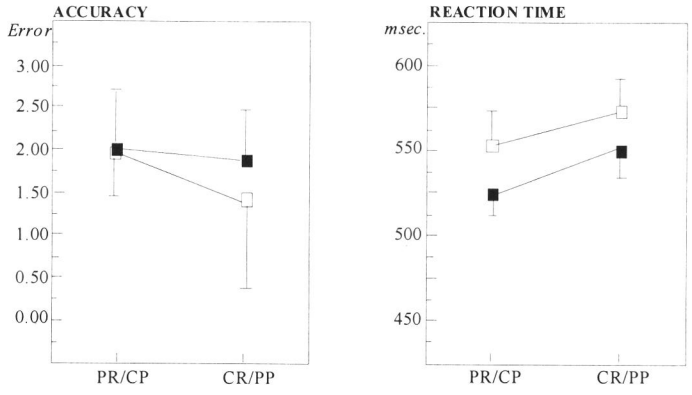

Fig.4: Mean number of errors and reaction time in cognitive performance in High stress (■) and Low stress (□) subjects during a Carbohydrate-rich, protein-poor diet (CR/PP) and a Protein-rich, carbohydrate-poor diet (PR/CP).

The possibility that in the HS group the increase in the number of errors, as a function of memory load, was lower on the CR/PP day than on the PR/CP day (see Fig. 5) was also explored. A linear regression analysis of the number of errors as a function of the number of items to be memorized (performed in SPSS) did not reveal any significant effect, however.

The results concerning reaction time were more informative. As shown in fig. 4, the mean reaction time, with both groups combined, was higher on the CR/PP day (560ms±69) than on the PR/CP day (541ms±68). This effect of diet on reaction time was clearly significant [$F(1,46)=6.780$, $p=0.012$]. Although it seems that HS subjects are somewhat faster (524ms±56) on both dietary days than LS subjects (557ms±76), this difference was not significant [$F(1,46)=0.08$, $p=0.777$].

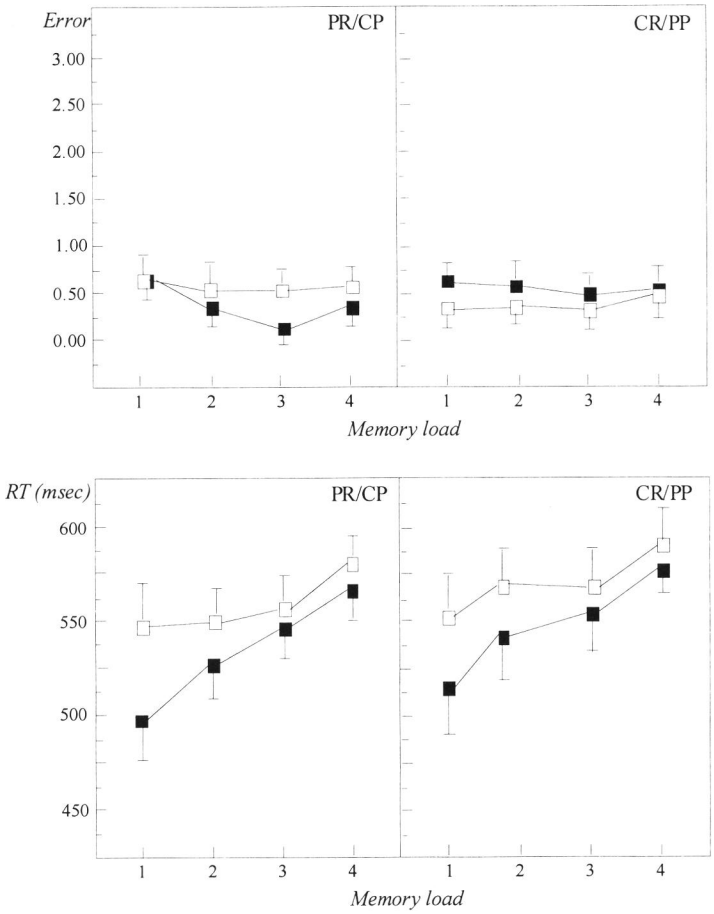

Fig.5: Distribution of errors and reaction time in cognitive performance as a function of memory load in High stress (■) and Low stress (□) subjects during a Carbohydrate-rich, protein-poor diet (CR/PP) and a Protein-rich, carbohydrate-poor diet (PR/CP).

Figure 5 presents reaction time as a function of the size of the memory set. This slope is interpreted by Sternberg (1975) as an indicator of the time needed for scanning an item in memory. As can be seen, in the HS group the mean reaction time decelerated significantly more, on both diets, with the number of items to be scanned in memory than in the LS group [$F(1,42)=4.90$, $p=0.032$]. This means that the HS group in comparison with the LS group, used more time for memory scanning, regardless of the dietary day. There was no interaction effect of Diet by Stress Proneness on the slope data.

Stress prone subjects seem to be faster on the easier part of the memory scanning task on both dietary days than LS subjects. These differences, however, were not significant on the PR/CP day (t_{44}= -1.63; p= 0.11) or on the CR/PP day (t_{44}=-1.27; p= 0.21).

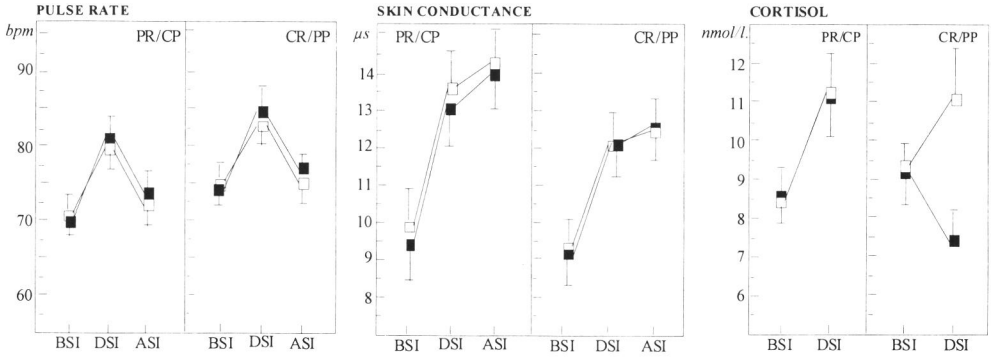

Fig.6: Pulse rate, skin conductance and cortisol in High stress (■) and Low stress (□) subjects during a Carbohydrate-rich, protein-poor (CR/PP) and a Protein-rich, carbohydrate-poor (PR/CP) diet, Before (BSI) and after (ASI) stress induction.

Psycho-physiological measures

To check stress manipulation, it was predicted that the pulse rate, the skin conductance and the salivary cortisol level of all subjects would increase after the Stress Induction task in both dietary conditions. The multivariate analysis of variance revealed a significant effect [Wilks F(1,45)=91.319, p<0.001]. Significant Stress Induction effects were found on pulse rate [from 72.14±9.3 to 82±11; F(1,40)=125.305, p<0.001], skin conductance [from 9.41±4 to 13.2±4.5; F(1,40)=157.994, p<0.001] and cortisol [from 8.77±3.72 to 13.38±4.8; F(1,40)=13.329, p=0.001] (see Fig. 6). Note that after Stress Induction pulse rate rapidly decelerated to baseline, as opposed to skin conductance. Skin conductance at ASI (see fig. 6) was higher on the PR/CP day than on the CR/PP day in HS and LS subjects (paired t-test: t_{47}= 12.49; p= 0.016).

A significant effect of Diet on Pulse rate was also found. Pulse rate was significantly higher on the CR/PP day (83.42bpm±11.20) than on the PR/CP day (70.51bpm±9.79) in both HS and LS groups [F(1,40)=7.030, p=0.011].

As shown in Fig. 6, there was a strong Diet by Stress Proneness by Stress Induction effect on cortisol. In the LS subjects, the cortisol level significantly increased after the stress induction task, regardless of the dietary condition; on the other hand, in HS subjects the cortisol level only increased after stress induction on the PR/CP day, while

it even became lower after stress induction on the CR/PP day. This interaction effect showed a trend but was not statistically significant [$F(1,40)=3.48$, $p=0.069$].

2.4. DISCUSSION

The ratio of Trp/LNAA in blood plasma was significantly higher on the CR/PP day than on the PR/CP day. From this, it may be predicted that during the CR/PP day there were increased levels of brain tryptophan in comparison with the PR/CP day, which in turn is thought to increase serotonin synthesis and activity (Curzon, 1985; Fernstrom & Wurtman, 1971b; Fernstrom et al., 1973; Wurtman, 1987). It was expected that the CR/PP condition would have beneficial effects on mood and cognitive performance in just HS subjects, when subjected to uncontrollable laboratory stress. A significant interaction effect of Diet by Stress Proneness by Stress Induction was found on two of the sub-scales of the POMS. As expected, during a CR/PP diet only in HS subjects there was not the stress-induced rise in depression and decline in vigour that was displayed during the PR/CP condition.

Equivalent interaction effects were not found for cognitive performance. The HS and LS group did not differ in the number of errors made during the memory scanning task. This may be attributed to the simplicity of the task. As demonstrated, the mean number of errors was extremely low (an average of 1.5 over 50 trials) and did not change with the size of the set of consonants to be memorized. Moreover, pulse rate returned nearly to baseline during the memory scanning task, which also may confirm that the task was highly manageable. Based on these results, it is believed that the version of the Sternberg task in the study may have been too easy to reveal the hypothesized differentiation in the dietary effect on performance between the HS and LS group.

The stress induction task led to increases of pulse rate and skin conductance, and to increased feelings of anger and tension, regardless of stress proneness and dietary condition. In the LS group this was also accompanied with an increased salivary cortisol reaction, elevated feelings of depression and reduced feelings of vigour after the stress task on both dietary days. In the HS group, however, this response pattern was only present on the PR/CP day, while on the CR/PP day a stress-induced rise in depression, decline in vigour and cortisol elevation was prevented.

These differentiated response patterns seem to correspond with the different roles of two systems involved in stress adaptation, as indicated by Dantzer (1993), Henry & Meehan (1981), Henry (1992), Mason (1968a, 1968b), Ursin & Olff (1993), and Willner (1993). On the one hand, the sympathetic-adrenomedullary (SAM) system appears to be specifically involved in that part of the stress response that generates a fast and general mobilisation of the physiological resources needed for 'fight or flight', or for the effort of active coping with stress in general. The resulting state of arousal

includes increases in pulse rate and skin conductance, and is paired with feelings of anger and/or anxiety. On the other hand, later on in the stress response, the activation of the pituitary-adrenocortical (HPAC) system increases and slower reacting hormones such as cortisol are released. Henry & Meehan (1981) associated an elevated cortisol excretion with feelings of distress, depression, helplessness and a loss of control, whereas a diminished cortisol reaction should be accompanied with feelings of euphoria, security and control. Frankenhaeuser et al. (1980, 1986) demonstrated that an elevation in cortisol will be lower the more the stressor can be controlled and that after effort without distress even a (slight) decrease in cortisol can be found (Frankenhaeuser et al., 1980; Frankenhaeuser, 1986). According to Ursin & Olff (1993) the HPAC system "may function as a suppressing mechanism to dampen the acute stress response and re-establish physiological balance" (see also Levine & Ursin, 1991; Munck et al., 1984). In their opinion, any challenging or alarming stimulus will raise the cortisol level and only when an individual is successful in coping with the stressor, the cortisol level will return to baseline (Ursin & Olff, 1993). In short, elevations in cortisol excretion will be less if an individual feels able to cope with stress effectively, while when stress is prolonged and paired with a low sense of control, it often leads to a tonic increase of cortisol, with eventually pathological consequences (Ursin, 1980) and depressive symptoms (Dinan, 1994).

The results of this study are in agreement with this differentiated operation of the SAM and HPAC systems. Apart from the results of the HS group on the CR/PP day, just the stress response to an uncontrollable stressor was found that was to be expected from this conception. This may indicate that a CR/PP diet in HS subjects may be beneficial in preventing a decline in personal control and a subsequent increase in cortisol.

The hypothesis tested in this study originated from a rather simple idea that in HS subjects there is an increased risk that the serotonergic system will be overcharged during an acute uncontrollable stressor; the supply of brain serotonin can no longer compensate for a new peak in the already high rate of turnover. Consequently, a CR/PP diet should eliminate this risk by raising the level of tryptophan in the brain. The results of the study seem to support this assumption. However, HS subjects were not found to be more deteriorated in mood by the stress inducing task than the LS subjects during a PR/CP diet, as might have been expected from this assumption. As chronic stress has been found to lead to serotonin sensitisation (Adell et al., 1988), sensitization may be a more helpful notion in this context. Findings consistent with this idea can be found in studies of Delgado (Delgado et al., 1989; Delgado et al., 1994; Heninger et al., 1996). This speculation implicates that the effect of the CR/PP diet on the HS group may also occur in reaction to a controllable stressor, as there is, contrary to the notion of a functional shortage of serotonin supply, no reason to bind the effect to the uncontrollability of an acute stressor.

In summary, the results of the current study support the conclusion that in HS subjects a CR/PP diet prevents a deterioration of feelings of depression and vigour when subjected to uncontrollable stress. Although this stress situation led to a rise of feelings of anger, tension and fatigue, paired with an increase of pulse rate, skin conductance and cortisol, only in HS subjects did a CR/PP diet prevent a stress-induced increase in depression, a decline in vigour and a cortisol elevation. These psycho-physiological response patterns seem to indicate that a CR/PP diet, in HS, may improve coping behaviour, possibly by increasing personal control.

As alterations in the plasma ratio Trp/LNAA has been reported to influence the rate of serotonin synthesis in the brain (Fernstrom & Wurtman, 1971b; Fernstrom et al., 1973), the significant rise of the Trp/LNAA ratio following the CR/PP diet may be seen as indirect evidence for the involvement of the serotonergic system in the effects found. This system seems to be involved in controlling and co-ordinating sensory and motoric processes (Spoont, 1992) and may cause subjects to attend selectively to stimuli important for coping with stress. However, it is doubtful whether the effect found in HS subjects was due to the prevention of a shortage in the serotonin supply during acute stress; instead, serotonin sensitisation may be a more likely mechanism.

The results of the current experiment emphasize the importance of searching for more detailed relationships between acute stress, coping behaviour and serotonin function in stress-prone subjects and for evidence whether the dietary effects found in this study are indeed restricted to uncontrollable stress. In the same way, it needs to be investigated whether different dietary effects can be found on cognitive performance in HS and LS subjects in a more difficult version of the memory scanning task.

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CHAPTER 3

EFFECTS OF FOOD ON CORTISOL AND MOOD IN VULNERABLE SUBJECTS UNDER CONTROLLABLE AND UNCONTROLLABLE STRESS.

C.R. Markus, G. Panhuysen, A. Tuiten, H. Koppeschaar. (under revision)
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ABSTRACT

The aim of this study was to investigate whether in stress-prone subjects, carbohydrate-rich, protein-poor food (CR/PP) diminished depressive mood and a cortisol response under controllable as well as uncontrollable laboratory stress. Twenty-two subjects with a high stress proneness (HS) and 23 subjects with a low stress proneness (LS) participated in a controllable and uncontrollable stress experiment during either a CR/PP or a protein-rich, carbohydrate-poor (PR/CP) diet. Both controllable and uncontrollable laboratory stress significantly increased pulse rate and skin conductance in HS and LS subjects, whereas uncontrollable stress increased feelings of depression, anger, tension, and fatigue and decreased feelings of vigor. Only in HS subjects a CR/PP diet prevented a stress-induced cortisol response and feelings of depression during controllable as well as uncontrollable laboratory stress, suggesting that a CR/PP diet in HS subjects increases the ability to cope with stress. As the CR/PP diet previously has been found to cause a significant 42% increase in plasma Trp/LNAA, seen as an indirect indices of increases in brain tryptophan and serotonin levels, the present results suggest that an enhanced serotonin function in HS subjects may be involved.

3.1. INTRODUCTION

Stressful life events may contribute to the onset and course of mood deterioration and affective disorders such as depression (9,11). Scientists have become increasingly interested in the study of neurochemical imbalances in the brain involved in depression and in the mediating effects of neurotransmitters in stress-related depressive mood. A prominent role for serotonin (5-hydroxytryptamine) in this respect is generally acknowledged (3,15,41). For instance, an increased activity of serotonin in the brain is an established consequence of stress (28,51) and a decline of serotonergic activity has been demonstrated in disturbances of mood and depressive disorders (15,46). Hence, a rise in the activity of central serotonin is regarded as an important biological condition to cope with stress (3,13,14) and to prevent stress-induced depression (13). In contrast, a blunted serotonin function in the brain may contribute to a decreased ability to cope with stress and, thus, may lead to increased depressive feelings.

The synthesis of serotonin in the brain increases by the intake of a carbohydrate-rich, protein-poor (CR/PP) diet. Such a diet raises the plasma ratio tryptophan (Trp: precursor of serotonin) to the sum of the other Large Neutral Amino Acids (Trp/LNAA) and gives Trp the advantage in the competition for access to the brain (12,19,20,21,22). An increased plasma Trp/LNAA by the CR/PP diet is produced by the carbohydrate raising glucose and triggering insulin secretion, causing the LNAA's except Trp to be taken up into the skeletal muscles. Since dietary proteins are scarce in Trp (1-2%) compared with the LNAA's valine, tyrosine, leucine, isoleucine, and phenylalanine (25%), the influx of Trp into the brain declines after consuming a balanced or protein-rich meal. This dietary effect on the plasma ratio Trp/LNAA's has frequently been demonstrated (35,42,47).

Based on the relationships between food intake, the plasma Trp/LNAA and brain serotonin and mood, investigators studied the effect of carbohydrate and protein meals on mood and performance. Yet, a large body of research yielded inconsistent findings and opposite results have been found in clinical and non-clinical populations. For instance, some studies reported positive effects of carbohydrates on mood (37) whereas other studies indicated that carbohydrates have no effect on mood of healthy subjects or has the effect of making them drowsy (49,50). Accordingly, only small mood changes are found in healthy subjects following dietary Trp depletion (59) or no effects at all (5). On the other hand, there is a bulk of literature suggesting that CR/PP meals have anti-depressant effects on clinical populations as in Seasonal Affective Disorder (47), carbohydrate-craving obesity (36) and pre-menstrual distress (57). Correspondingly, declined plasma Trp concentrations are found in clinical depression (41) and Trp depletion appeared to cause depressive symptoms in remitted patients (17,48) and even lowers mood in subjects with a genetic risk for clinical depression (6).

Recently, we have argued that some of the inconsistencies found in literature might be explained by individual differences in stress vulnerability (42). Assuming that an increased serotonin function constitutes a biological condition to improve stress adaptation, in stress prone subjects serotonin activity might be continuously increased following chronic stress experiences. Ultimately this may lead to a functional shortage in serotonin, causing a subsequent deficiency of brain serotonin activity (42,53). Accordingly, it is expected that stress prone subjects should benefit more from a CR/PP meal during acute stress, since such a diet may enhance central serotonin function by rising Trp uptake in the brain.

The present study aims to explore the effects of a CR/PP meal particularly on mood and cortisol responses in stress-prone subjects under acute laboratory stress. Recent research in our laboratory revealed that a CR/PP diet causes a significant 42% increase in plasma Trp/LNAA and prevented a deterioration in depressive mood and vigor and decreased the cortisol concentration in stress prone subjects under high uncontrollable stress (42). This decreasing effect on the cortisol concentration was unexpected and seems to challenge findings that the administration of serotonin precursors like Trp stimulate cortisol secretion in healthy and clinical subjects (41). Increases in brain serotonin appear to modulate adreno-cortical reactivity probably through alterations in 5-HT1a and 5-HT2 receptor sites located at hypothalamic and pituitary brain areas (41). However, these initiating effects of serotonin on cortisol concentrations do not necessarily conflict with the notion that under acute stress serotonin activity also may contribute in terminating a cortisol stress response. Brain serotonin does not appear to be an unitary system. The serotonergic pathways involved in stress adaptation have different functions, initiating as well as terminating the adreno-cortical axis (13,14,41). For instance, increases in serotonergic terminals located at the paraventricular hypothalamus and the pituitary appear to enhance cortisol excretion, whereas a rise in serotonin in hippocampal structures may be involved in the termination of an adreno-cortical response by increasing negative feedback control over the hypothalamic-pituitary axis. Accordingly, contrary to the dorsal raphe nucleus (DRN) the serotonergic route connecting the median raphe nucleus (MRN) to the hippocampus may be involved in disconnecting the negative consequences of stress on the cortisol stress responses and depression (13). In agreement with this idea, stress-induced elevations in cortisol and depressive mood are particular indices of a reduced ability to cope with stress (43,25). Based on these relations, data of our previous study (42) suggest that a CR/PP diet in stress prone subjects more stronger may terminate the negative consequences of acute uncontrollable stress on cortisol secretion and depression, probably by enhancing brain serotonin mechanisms involved in stress adaptation.

It is unclear, however, whether the effect of a CR/PP diet on cortisol and depressive mood in stress prone subjects could be replicated during controllable as well as uncontrollable laboratory stress. Originally we hypothesized that a CR/PP diet would improve stress coping in these subjects particularly under situations of high

uncontrollable stress, since only such stress situations may increase the risk for the already exhausted serotonin concentrations to decline below functional level. However, another possibility is that the dietary effect on mood and cortisol in stress prone subjects is mediated by an increased sensitivity of the serotonergic system, following a chronic stress induced shortage in serotonin concentration (42). In stress prone subjects an increased sensitization of the serotonin system might appear as a biochemical adaptation maintaining appropriate serotonin levels needed for the chronic utilization required. Accordingly, indicative of increases in serotonin sensitization, it has been demonstrated that chronic stress enhances serotonin responsiveness to further acute stress (1) and enlarges behavioral reactivity of the serotonergic system (30). Moreover, changes in sensitization are suggested to mediate the effect of Trp depletion and repletion on depressive mood (18,24), as well as on serotonin induced neuroendocrine alterations (16). If an increased serotonin sensitization mediates the effect of a CR/PP diet on depressive mood and cortisol in stress prone subjects under stress, this would appear during uncontrollable as well as controllable laboratory stress.

The purpose of the present study is to test whether a CR/PP diet prevents depressive mood and a cortisol response in stress prone subjects during controllable, as well as uncontrollable laboratory stress. Both a group with a high stress vulnerability and a group with a low stress vulnerability were randomly assigned to a CR/PP diet or to a protein-rich, carbohydrate-poor (PR/CP) diet. Subjects participated in two experimental sessions, during which stress was manipulated by exposing the subjects to a stress inducing laboratory task. In one session, this task was uncontrollable, alternated the other day by a controllable version. The controllability manipulation was checked by using a self-constructed questionnaire, and mood was measured using the Profile of Mood States (POMS). Changes of pulse rate, skin conductance and salivary cortisol from baseline were measured as physiological indices of the stress response.

Based on our assumptions we predict that in stress vulnerable subjects an increase in depressive mood and cortisol will be prevented during uncontrollable- and controllable laboratory stress, under a CR/PP diet compared with a PR/CP diet. As controllable stress may lead to moderate stress responses, the effect of the CR/PP diet on depressive mood and cortisol in stress prone subjects is predicted to be less pronounced but significantly present after controllable laboratory stress compared with uncontrollable stress.

3.2. METHODS

Subjects

334 female and male social science students of Utrecht University filled in the Inadequacy (IN) Scale of the Dutch Personality Inventory, measuring neuroticism (38), and

also a questionnaire concerning personal details. Neuroticism is closely related to negative affectivity (39), defined as the disposition to see events as alarming and to experience aversive emotional states (55), and also to stress proneness (23). In accordance, subjects high in neuroticism frequently experience stress (7). Based on these connections, from the highest quartile of IN-scores 22 subjects (5 males and 17 females) were selected for the High Stress (HS) group (25±4.73; range 20-37), and from the lowest quartile of IN-scores 23 subjects (6 males and 17 females) for the Low Stress (LS) group (3±1.18; range 1-5). Subjects' ages ranged from 19 to 26 years (22 ± 2.3 years). Exclusion criteria for participation were chronic and current illness, medication, or irregular diets. All subjects selected for the experiment were of normal weight for height (Body Mass Index [weight(kg)/height(m)^2] between 20-25). Because oral contraceptive medication may have an effect on cortisol responsiveness (32), the HS-group as well as the LS-group were matched for contraception (in each group 7 women used oral contraception). All subjects participating in the experiment signed a letter of (informed) consent and received a financial compensation after completion of the experiment.

Procedure

All subjects were randomly assigned to either the CR/PP diet or the PR/CP diet and participated in two experimental sessions. On both experimental sessions, the subjects were confronted with a stress inducing mental arithmetic task following their diet condition. For half of the subjects in each group (both HS and LS) the first experimental session included a version of this task supposed to induce controllable stress (CS), followed by a version inducing uncontrollable stress (US) in the second session. The other half of the subjects received the conditions in the opposite order. Both experimental sessions were separated by a four-week period interval, allowing to account for the menstrual phase of the female subjects with a normal menstrual cycle. These women participated in the experiment during their mid-late follicular phase (day 4-10), while women using contraception (N=14) participated when they actually used the contraception pill.

On each experimental day, two subjects arrived at the laboratory at respectively 0900 hours and 1000 hours. They were enabled to read in a study room, and received breakfast at arrival, snack (1015 hours and 1115 hours) and lunch (1100 hours and 1200 hours), according to their dietary condition. Dietary intake was constantly supervised to make sure that all foods were consumed. Subjects fasted overnight for eleven hours, except for water and tea without sugar. One and a half hour after lunch, a first salivary cortisol sample was taken. Then the subject was brought into a temperature-controlled laboratory room, where he or she was seated in front of a computer screen and instructed about the experiment. The electrodes for skin conductance and the finger sensor for pulse-rate measurements were attached, and during the following 10 min the baseline physiological 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 (8). The battery consisted of (A) a version of the POMS (54), (B) the CS or US mental arithmetic task (45), (C) a self-constructed Controllability Questionnaire (CQ) and (D) a second version of the POMS. A second salivary sample was taken directly at the end of the stress inducing task, approximately 22 min after stress onset, and a third cortisol sample was taken at the end of the experiment (30 min after stress onset). Pulse-rate and skin conductance were recorded until the end of the experiment. Subjects spent approximately four min on the first POMS, 22 min on the stress inducing task and eight min on the PCQ and the second POMS.

TABLE 1

Composition of the CR/PP diet and the PR/CP diet.

		CARBOHYDRATE	PROTEIN	FAT	CALORIC VALUE
CR/PP	**Breakfast, snack and lunch**: Bread, margarine, jam tea & sugar, grape-juice. Black coffee & sugar candy bar.	219,1 grams (66,2 %)	11,9 grams (3,6 %)	44,3 grams (30,1 %)	1323 kcal.
PR/CP	**Breakfast, snack and lunch**: Bread, butter, cheese, meat, fat-milk, tea, black coffee, peanuts.	123,1 grams (41 %)	81,2 grams (27 %)	42,2 grams (32 %)	1200 kcal.

Diets.

Two different diets were used; a carbohydrate-rich, protein-poor diet (CR/PP) and a protein-rich, carbohydrate-poor diet (PR/CP). The diets were composed by an authorized dietitian of the Academic Hospital Utrecht (AZU, The Netherlands) and complied with the reference daily energy intake for similar groups of both men and women. As shown in Table 1, the total amount of calories and fat in both diets were approximately equal. Moreover, in order to keep the reducing effect of protein on the increase of the Trp ratio small, the total amount of protein in the CR/PP diet did not exceed 5% of the total amount of dietary energy (58). Both diets are identical to the meals that have been used in a prior study with comparable groups (42). In this earlier study, the CR/PP diet causes a significant 42% increase in plasma Trp/LNAA compared with the PR/CP diet [$P<0.0001$] (see figure 1).

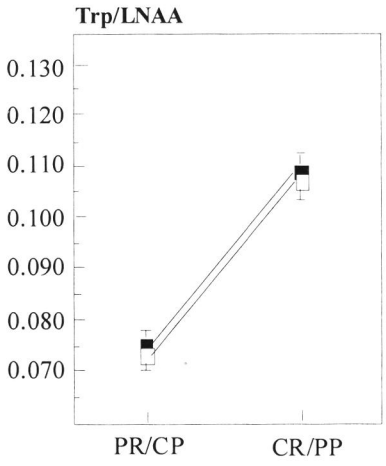

Fig.1: The plasma Trp/LNAA ratio significantly increased after the carbohydrate-rich, protein-poor (CR/PP) diet compared with the protein-rich, carbohydrate-poor (PR/CP) diet in HS (■) and LS (□) subjects (from: Markus et al., 1998).

Perceived controllability.

In order to check the effect of manipulation of controllability across the controllable and uncontrollable stress task (CS versus US), we designed a computerized Controllability Questionnaire (CQ) for the constructs 'perceived control' and 'effort'. The CQ comprises seven questions indicating subject's experience of control and seven questions indicating the amount of effort required by the task. With respect to the former, questions like 'I could control the amount of successes during the task', 'I feel incompetent to perform well' and 'I knew what kind of sums I could expect' were used, whereas questions like 'Performing this task took me a lot of energy' and 'The task required much effort' were used for the effort construct. Subjects were asked to rate their level of agreement on an 8-point interval scale ranging from 'strongly agree' to 'strongly disagree'.
Factor analysis was performed on the total sample scores, for which the results of factor extraction (by Principal Component Analysis with Varimax, rotation; SPSS 7.5.2 for Windows) revealed an optimal factor structure of two factors corresponding to the constructs as intended. Both scales revealed a strong reliability in the range of 0.85 (Cronbach's α).

Profile of Mood States (POMS).

Changes in mood were measured using two scales of the Dutch shortened version of

the Profile of Mood States questionnaire (54), offered at the computer-screen on a 5-point interval scale ranging from `strongly disagree' to `strongly agree'. The first scale was offered before the start of the stress inducing mental arithmetic task (-5 min) and the second scale was offered after the stress inducing task and the PCQ (+26 min). The POMS comprises five different subscales for mood. The subscale 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. Analysis were performed on the raw POMS scores.

Stress Induction task.

A mental arithmetic task, performed under noise stimulation, was used as a stress inducing situation and was offered in a controllable version (CS) and an uncontrollable version (US) (45). Subjects were given 18 successive one-min trials in which they had to do mental arithmetic under time constraints, while at the same time different levels of industrial noise (either 65, 70 ore 80 dB) were presented to them through a headphone. During each trial, multiple choice sums were presented on a computer screen one at a time in a self-paced manner for which a specified number of sums (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 select the noise level to be present during the next trial; if they met the criterion they could choose the noise level for the following trial. Before the actual test, subjects were given two practice trials in which they had to solve a few sums first without noise and then in the presence of three successive levels of noise (4 min). The credibility of the task, as well as motivation, was enhanced by providing the subjects with constant on-screen feedback of the criterion in a particular trial, the number of sums already solved correctly, and the time left for that trial.

Controllability was manipulated by altering the total number of sums to be solved in each trial (the criterion). For each subject as well as each trial a new criterion was established based on the average calculation time needed for previous trials (for the first trial, the average time per sum during practice was used). In the CS version, controllability was accomplished by manipulating the criterion so that all subjects had success in most of the trials, and could thus choose the noise intensity for the following trial. One restriction was that two of the louder intensities had to be selected three times each, but the order of appearance was determined by the subjects themselves. In the US version all subjects continued to fail on each trial and, thus, could not choose the 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. Successful task performance was

excluded by giving only incorrect multiple choice alternatives in case a subject threatened to meet the criterion after all (i.e. when only one sum was left to solve).

Physiological measurements.

Measurement of electrodermal activity.

Skin conductance was measured using silver/silver chloride (Ag-AgCI) electrodes (surface 0.5cm^2), filled with adhesive solid gel (ARBO H91, Braunschweig; Germany). These electrodes were bipolarly placed at the thenar palmar and hypothenar palmar sites of the non-preferred hand of the subject. The measurement principle was a constant voltage of 0.5 V with a sampling rate of 1Hz. Tonic skin conductance levels (SCL) 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 non-preferred hand. The signal was transduced by Sat-Trak^{TM} signal processing (sampling rate 65Hz), based on the Quadrature Division Multiplexing (QDM) technique, by the Pulse Oximeter (SensorMedics corporation, Bilthoven; The Netherlands). With a sampling rate of 1Hz, pulse rate samples were collected and stored in the computer.

Measurement of salivary cortisol.

One baseline cortisol sample (-15 min), a second post-stress sample (+22 min) and a third sample (+30 min) were obtained with the Salivette sampling device (Sarstedt, Etten-Leur; the Netherlands). With this procedure, saliva was collected in small cotton swabs and stored in special salivette tubes until centrifugation. Saliva samples were centrifuged at 3000 rpm for three min at 20°C, and frozen afterwards at -20°C until analysis.

Cortisol levels were determined without extraction in a licensed laboratory at the University Hospital Utrecht using an in house competitive radio-immunoassay (RIA) employing a polyclonal anticortisol-antibody (K7348). 1,2-^{3}H(N)-Hydrocortisone (NET 185, NEN -Dupont-, Dreiech, Germany) was used as a tracer, following chromatographic verification of its purity. The lower limit of detection was 0.5 nmol/l and inter-assay variation was 11.0; 8.2; and 7.6% at 4.7; 9.7; and 14.0 nmol/l, respectively (n=20). Reference values for adults are 4-28 nmol/l.

Experimental design and statistical analysis

The experimental design of our study comprised three *between-subjects* factors: *Stress Proneness* (high versus low stress), *Diet* (CR/PP versus PR/CP) and *Order of Controllability Condition* (CS/US versus US/CS). There were also two *within-subjects* factors: *Controllability* (CS versus US), and *Laboratory Stress* (repeated measures of mood, pulse rate, skin conductance, and salivary cortisol concentrations before, during and after the laboratory stress task).

The main research questions formulated in the introduction were analyzed by means of repeated measures multivariate analysis of variance (MANOVA; General Linear Model; SPSS 7.5 for Windows) with Controllability and Laboratory Stress as within-subjects factors. As pulse rate, skin conductance and cortisol were measured throughout the whole experiment, multivariate analyses of variance for these variables were performed with first- and second order polynomial contrasts. Only significant multivariate results revealed by these procedures were further examined by univariate tests. As Order of Controllability Condition contributed to the scores on the Controllability Questionnaire (CQ), these scores were analyzed by MANCOVA with Order of Controllability Condition as a covariant. Since oral contraceptive and gender did not contribute to any effect, further MANOVA's of the remaining variables were performed with only Diet and Stress Proneness as between-subjects factors. Analyses for variables whose direction of changes were expected (depression and cortisol) were assessed by unidirectional comparisons (26). All tests were evaluated at a significance level of 5%. Data are reported as means ± SDs.

3.3. RESULTS

Perceived controllability.

As multivariate analysis of variance detected a significant main effect of the Order of Controllability Condition on the scores of the CQ [$F(2, 37) = 4.665$, $p = 0.04$], further analysis was performed with Order of Controllability Condition as a covariant. Analysis only revealed a significant main effect of Controllability [$F(3. 36) = 10.264$, $p < 0.001$], meaning that the amount of control experienced during task performance depended on the controllability condition of the concerning stress inducing task (US vs. CS). As shown in figure 2, there was a significant decline in experiences of control (from 42.57±7.29 to 22.0±6.81: $F(1, 38) = 9.301$, $p = 0.004$) and an increase in effort (from 14.79±3.71 to 20.95±2.39: $F(1, 38) = 17.481$, $p < 0.001$) in all subjects after the US condition compared with the CS condition. No effects were found for Diet or Stress Proneness. These results indicate a successful manipulation of controllability in both versions of the US and CS stress inducing task.

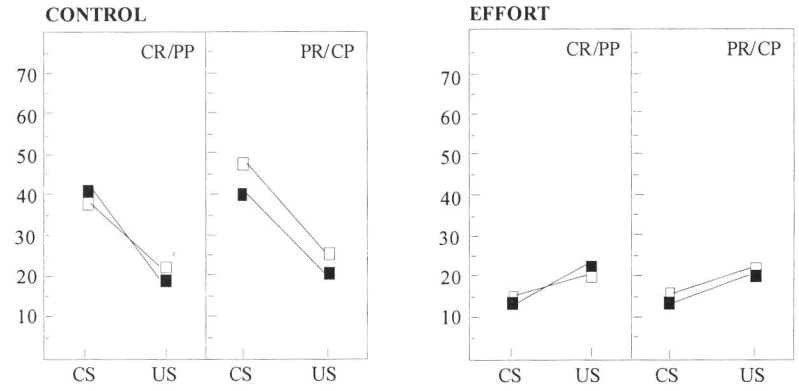

Fig.2: Effect of controllable laboratory stress (CS) and uncontrollable laboratory stress (US) on experiences of control and effort during the CR/PP diet and PR/CP diet in HS (■) and LS (□) subjects.

Mood (POMS).

It was hypothesized that the CR/PP diet only in HS subjects prevents a deterioration of depressive mood regardless of Controllability. Multivariate analysis with repeated measures revealed a significant three way interaction effect of Stress Proneness by Diet by Laboratory Stress on the different mood scales of the POMS [$F(5,35) = 2.176$, $p = 0.04$]. Univariate analysis revealed that this three-way interaction originated from changes in the scores on depression [$F(1, 39) = 3.876$, $p = 0.025$], meaning that a dietary effect on alterations in depressive feelings under laboratory stress depended on stress proneness. As shown in Figure 3, in HS subjects increases in feelings of depression were absent under a CR/PP diet after the US task (from 13.67±4.4 to 13.5±1.6) and after the CS task (from 12.75±4.63 to 11.58 ±3.1), whereas during the PR/CP diet feelings of depression increased after US (from 14.30±3.27 to 17.4±4.86) but there was hardly any increases after CS (from 12.30±3.19 to 12.50±4.79).

Multivariate analysis of variance also revealed a significant interaction effect of Controllability by Laboratory Stress [$F(5, 35) = 9.270$, $p < 0.0001$], meaning that the effect of laboratory stress on mood changes depended on the controllability of the laboratory stress inducing task. Hence, the US version more pronounced deteriorate feelings of Anger [$F(1, 39) = 34.771$, $p < 0.0001$], Tension [$F(1, 39) = 8.058$, $p = 0.007$], Depression [$F(1, 39) = 6.143$, $p = 0.018$], Vigor [$F(1, 39) = 28.301$, $p < 0.0001$] and Fatigue [$F(1, 39) = 15.941$, $p < 0.0001$] compared with the CS version. All statistics for these mood scales are presented in table 2. Although it seems that the interaction between Controllability by Laboratory Stress was more pronounced in HS subjects, there was no significant effect of Stress Proneness by Controllability by

Laboratory Stress [F(5, 35) = 0.717, p = 0.62], nor was there any other interaction effect. However, we found a main effect of Stress Proneness [F(5, 35) = 12.387, p<0.0001], indicating that HS subjects compared with LS subjects had higher baseline scores of Anger [F(1,39)=11.956; p=0.001], Tension [F(1,39)=42.105; p<0.0001], Depression [F(1, 39) = 44.484, P < 0.0001], and Fatigue [F(1,39)=15.286; p<0.0001], and lower scores on Vigor [F(1,39)=6.723; p=0.013] (see table 2).

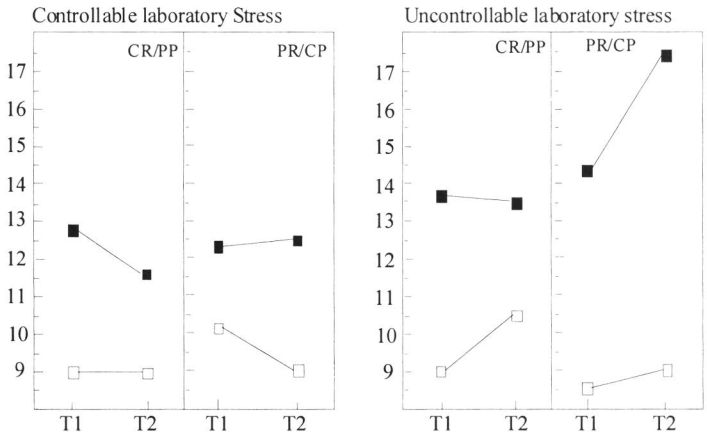

Fig.3: Depression before (T1) and after (T2) controllable- and uncontrollable laboratory stress in HS (■) and LS (□) subjects, during a CR/PP diet and a PR/CP diet.

TABLE 2

Mood before (t1) and after (t2) controllable laboratory stress (CS) and uncontrollable laboratory stress (CS) following a CR/PP diet and a PR/CP diet.

	CR/PP				PR/CP			
	CS		US		CS		US	
	T1	T2	T1	T2	T1	T2	T1	T2
LS								
POMS Depression	8.9±1.20	8.9±1.34	9.18±1.25	10.30±2.90	10.18±4.68	9.0±1.26	8.55±1.21	9.0±1.61
POMS Anger	8.0±1.05	8.30±1.64	8.09±1.70	11.90±6.40*	8.55±3.17	7.82±1.47	7.73±1.62	11.36±6.34*
POMS Tension	8.50±2.07	6.40±2.59	8.36±2.29	8.91±3.08	8.73±2.69	7.91±1.51	9.36±5.59	8.55±2.21
POMS Vigor	17.40±3.20	18.60±1.65	16.18±4.60	16.27±3.60	15.55±4.39	17.0±4.43	16.55±4.18	14.73±4.86
POMS Fatique	9.10±2.73	9.10±3.75	10.64±6.44	10.00±5.22	11.64±5.84	9.73±3.66	9.73±3.82	10.64±3.20
HS								
POMS Depression	12.75±4.63	11.58±3.1	13.67±4.4	13.5±1.6	12.00±3.11	12.18±4.60	14.3±3.27	17.4±4.86*
POMS Anger	9.92±3.29	10.75±5.12	9.58±2.87	17.50±6.30***	10.00±2.20	9.36±2.54	10.70±3.50	17.40±5.68**
POMS Tension	10.67±3.94	12.17±4.49	11.17±3.46	14.58±3.40**	14.27±4.00	13.91±5.70	15.40±4.09	17.90±5.04
POMS Vigor	15.0±4.45	15.25±4.52	16.0±3.64	12.08±4.38*	13.18±2.75	14.27±3.17	14.90±2.60	14.20±3.29
POMS Fatique	13.17±6.60	14.42±7.01	12.83±4.75	16.58±5.28*	16.45±4.50	15.00±5.90	13.20±4.32	15.80±5.71

* P = 0.05 ** P = 0.01 *** P = 0.001

Psycho-physiological measures

The multivariate analysis of variance revealed a significant main effect of Laboratory Stress on pulse rate and skin conductance [$F(2,37)=40.226$, $p<0.0001$]. As shown in figure 4, the laboratory stress inducing task significantly increased pulse rate from 74.72±10.44 to 84.0±12.83 [$F(1, 38) = 78.345$, $p < 0.0001$] and skin conductance from 7.0±3.13 to 11.27±4.75 [$F(1, 38) = 83,157$, $p < 0.0001$] with the scores of Stress Proneness, Controllability and Diet combined. No effects are found for Diet or Controllability, nor were there significant differences in this respect between HS and LS subjects.

We predicted that a CR/PP diet only in HS subjects would prevent or lower a stress-induced cortisol response during the US and the CS condition. Multivariate analysis of variance revealed a significant three-way interaction effect of Stress Proneness by Diet by Laboratory Stress [$F(2, 37) =3.451$; $p = 0.02$], which originated from the linear polynomial contrast [$F(1, 38) = 5.135$, $p = 0.015$]. As shown in figure 4, in HS subjects within a CR/PP condition there was a decline in cortisol after US (from 8.65±2.85 to 6.97±2.62) and after CS (from 8.78±2.1 to 7.74±2.22), whereas during a PR/CP condition cortisol increased after US (8.6±2.72 to 9.72±3.0) and remained stable after CS (8.44±1.9 to 8.31±2.34). This effect was not found in LS subjects. Notice, that in LS subjects the cortisol level remained increased 8 min after the end of the stress task (30 min after stress onset) only during a CR/PP diet, whereas during the PR/CP diet cortisol returned to baseline after completion of the task. However, this latter effect as not significant on the quadratic polynomial contrast.

We predicted that increases in cortisol after the laboratory stress task were more pronounced after US than after CS. As mentioned, in HS subjects within a PR/CP condition, cortisol concentrations were increased during US (from 8.6±2.7 to 9.72±3.0) but not during CS (from 8.44±1.91 to 8.3±2.34). In LS subjects cortisol only increased marginally during US (from 9.0±3.0 to 9.82±3.2) compared with the CS task (from 9.26±2.9 to 9.6±2.9), with the scores of both diet conditions combined. However, analysis of variance did not reveal significance towards an interaction between Controllability by Laboratory Stress.

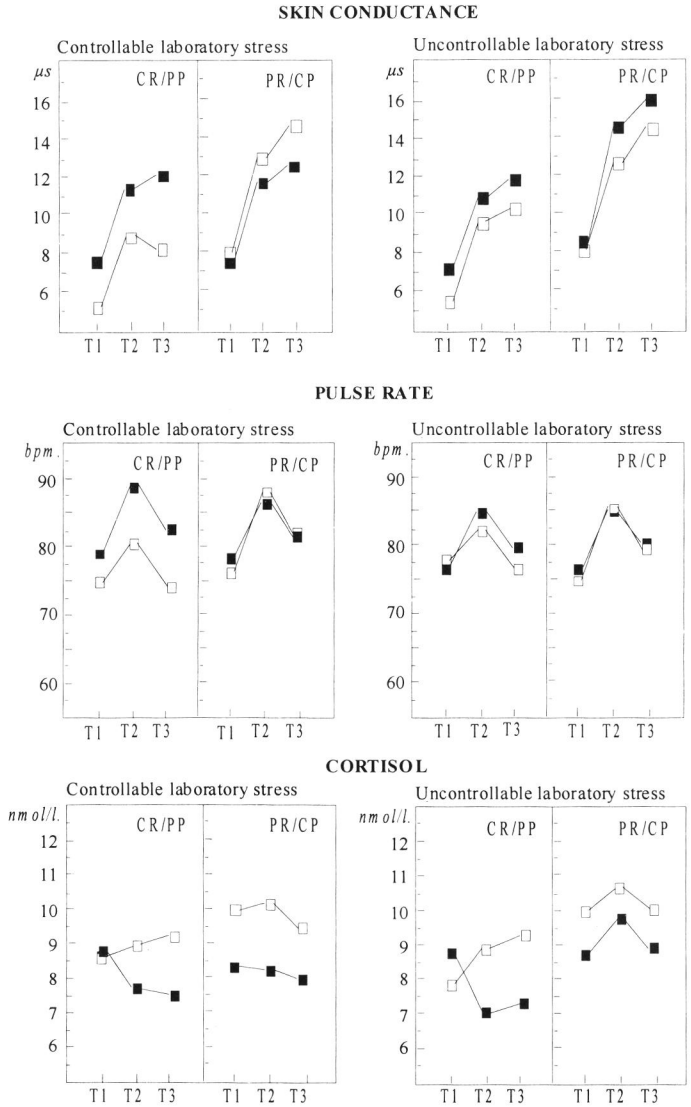

Fig.4: Pulse rate, skin conductance and cortisol before (T1), during (T2), and after (T3) controllable and uncontrollable laboratory stress in HS (■) and LS (□) subjects, during a CR/PP diet and a PR/CP diet.

3.4. DISCUSSION

The purpose of the present study was to examine whether a CR/PP diet in HS subjects prevented depressive mood and a cortisol response during acute laboratory stress, regardless of the controllability of the stressor. This research question was based on the premise that the central serotonergic system in HS subjects is hypersensitive, due to a functional shortage of available serotonin following chronic stress exposure. Since the CR/PP diet has been found to increase the plasma ratio Trp/LNAA (42), which is an indirect indication of an increased brain Trp and serotonin level (20,22), we hypothesized that this may prevent depressive mood and a cortisol response in HS subjects regardless of stress controllability. Accordingly, the present findings reveal that only in HS subjects the CR/PP diet prevented depressive feelings and a cortisol response after controllable as well as uncontrollable laboratory stress induction.

Changes in the scores on the Controllability Questionnaire (CQ) between the US and CS task revealed a successful manipulation of Controllability. There was a significant decline in feelings of control and an increase in effort after the US task compared with the CS task. Pulse rate and skin conductance significantly increased during both the CS and US conditions, whereas cortisol showed a tendency to increase more after the US than after the CS task. Moreover, after the US task all subjects reported a significant deterioration of feelings of anger, tension, depression, fatigue and vigor, compared with the CS task. Comparable results were found in a recent study (45) in which a deterioration of mood, control experience and an increase in cortisol exclusively were found after the US condition.

The different response patterns found in the US and the CS versions of the stress inducing task seem to reflect the two distinctive arousal responses involved in stress adaptation (25,43,56). On the one hand, increases in pulse rate and skin conductance are linked to an increased activation of the sympatho-adreno-medullary system (SAM), which is associated with effortful coping. On the other hand, increases in cortisol and feelings of depression refer to an increased and prolonged activation of the pituitary-adreno-cortical system (HPAC), indicating unsuccessful coping. Based on these findings, it may be concluded that the US version of the stress inducing task in the present study was less controllable and more distressing than the CS version.

We found remarkable between-subjects differences in mood. As demonstrated, the HS group showed lower moods at baseline measured on the POMS scales of anger, tension, depression, vigor and fatigue, compared with the LS subjects. These results seem to correspond to findings that chronic experiences of daily stress lead to mood deterioration and, subsequently, might reflect a trait of liability to clinical depression (9,10). Next, we predicted that the CR/PP diet in HS subjects would prevent or diminish depressive mood regardless of the controllability of the stressor. Multivariate analysis of variance indeed showed an exclusive three-way significant interaction effect of Stress Proneness by Diet by Laboratory Stress; indicating that during the

CR/PP diet HS subjects had lower scores on depression after US, and to a less extent after CS, compared with the PR/CP diet.

Replicating our previous findings (42), we found a significant three-way interaction effect of Stress Proneness by Diet by Laboratory Stress on cortisol concentrations. Like depression, only in HS subjects the CR/PP diet condition prevented a cortisol response after US and diminished cortisol after CS. As mentioned before (see introduction), these results on cortisol do not necessarily contradict with findings that the administration of serotonin precursors promote cortisol secretion (41). Under conditions of acute stress, increases in brain serotonin may improve stress coping (3) and thus may contribute to the initiation as well as termination of a cortisol stress response by an enhanced activation of different serotonergic pathways in the brain (3,13,14). Although we did not find between-subjects differences in baseline cortisol concentrations, the present data suggest that under conditions of acute stress the pathway involved in terminating an adreno-cortical stress response may be more enhanced by a CR/PP diet in stress prone subjects.

Although not significant, in LS subjects laboratory stress induced increases in cortisol during the CR/PP diet extended till the end of the experiment (+30 min after stress onset). This extended cortisol response was not found during the PR/CP diet. These results seem to be consistent with findings that peak cortisol levels occur approximately 30 min after stress onset (31), though it remains unclear why the course of a cortisol response in the present study tend to be influenced by dietary intake after completion of the stress inducing task. It is suggested that low glucose levels inhibit adreno-cortical responsiveness during stress in healthy subjects (33), as the access to energy (glucose) might be a prerequisite to an adreno-cortical stress response. However, we did not find such an effect during the laboratory stress task, even though the glucose level is expected to rise after a CR/PP diet compared with the PR/CP diet. Perhaps this might be a factor attenuating the reversion of cortisol to its original level; in particular, when the stress response is completed and is not required anymore. However, this does not seem to be a factor involved in the effect of the CR/PP diet on the prevention of a cortisol response in HS subjects during acute stress as has been demonstrated previously (42) and was replicated in the present study.

The present study was based on the assumption that a CR/PP diet increases available Trp and serotonin in the brain, and particularly in HS subjects enhances serotonin function due to a chronic stress mediated sensitization of the serotonin system. Following on from the assumption that increases in serotonin might be a biological basis to cope with stress, the CR/PP diet particularly was expected to lower stress-induced cortisol responses and depressive mood in HS subjects regardless of the controllability of the acute stressor. As the current CR/PP diet previously caused a significant 42% increase in plasma Trp/LNAA (42), the present results on depression and cortisol direct to the involvement of the serotonin system as suggested.

In human studies, dietary induced increases in plasma Trp/LNAA commonly do not exceed 15-20% from baseline values. Accordingly, it has been questioned whether diet-induced changes in plasma Trp/LNAA in human may cause meaningful changes in brain serotonin (4,52). Teff et al. (52) reported that even an impressive 47% increased plasma Trp/LNAA by an orange juice drink could not lead to significant increases in the serotonin metabolite 5-HIAA in cerebrospinal fluid. However, the increased plasma Trp/LNAA in this latter study was not significant due to a small group of subjects (N=5). Moreover, their subjects were neurological patients (mean age 71 years) diagnosed with Normal Pressure Hydrocephalus; a neuropathology accompanied by several behavioral, biological and biochemical deficits. Pérez-Cruet et al. (44) found that a significant 49.5% decline of the plasma ratio Trp/LNAA was accompanied with significant lower 5-HIAA in CSF following a balanced diet, whereas in other studies even a 20-40% change in plasma Trp/LNAA let to brain serotonin alterations (2,29). Accordingly, it is suggested that a 34% increase in plasma Trp/LNAA might have an effect on brain serotonin synthesis depending on the glycemic index of carbohydrates (40). In the light of the present findings, a 42% increased plasma Trp/LNAA as previously found by the CR/PP diet used here too (42) may have caused at least modest changes in brain serotonin.

Assuming that the serotonin system is involved, findings of the present study emphasizes the need to investigate the pathway through which dietary induced increases in brain Trp and serotonin may, directly or indirectly, terminate a cortisol response and depressive mood in HS subjects. Although the precise pathway involved in the serotonergic control of the adreno-cortical axis still remains unclear, possible directions have been emphasized. It may be suggested that increases in brain Trp in HS subjects particularly act upon the serotonin pathway connecting the median raphe nucleus to the hippocampus, which inhibits HPA activation (27,41) and contribute to stress coping and (13,14,25). A chronic stress induced serotonin shortage in these subjects particularly may lead to moderate increases in serotonin sensitization in hippocampal structures, resulting in a more pronounced termination of the adreno-cortical route after increasing brain Trp availability. Dietary induced increases in brain serotonin also may enhance higher cortical brain structures involved in planning and cognitive appraisal, which then may override an immediate activation of the limbic adreno-cortical system; serotonergic cell bodies within the prefrontal cortex are part of the limbic-thalamo-cortical circuit and are involved in the planning and execution of emotional actions (34). However, these suggestions remains highly speculative and further research should first focus upon the more direct neuroendocrine and metabolic reflections of brain serotonin involvement. In this respect, testing the effect of dietary Trp alterations on behavior as well as on serotonin function within a double-blind placebo design, might offer stronger support for the involvement of the serotonergic system.

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CHAPTER 4

CARBOHYDRATE INTAKE IMPROVES COGNITIVE PERFORMANCE OF STRESS-PRONE INDIVIDUALS UNDER CONTROLLABLE LABORATORY STRESS.

C.R. Markus, G. Panhuysen, L.M. Jonkman, M. Bachman. (Under revision).
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ABSTRACT

Cognitive performance has been found to decline after exposure to stress, particularly in stress-prone subjects. This study investigated whether a carbohydrate-rich, protein-poor (CR/PP) diet, which may enhance cerebral serotonin function in stress-prone subjects due to increases in the available tryptophan, improves the performance of stress-prone subjects after exposure to stress. Twenty-two high stress-prone (HS) subjects and 21 low stress-prone (LS) subjects aged between 19 and 26 years performed a memory scanning task after controllable and uncontrollable stress, following either a CR/PP or a protein-rich, carbohydrate-poor (PR/CP) isocaloric diet. Uncontrollable stress reduced feelings of control [$F(1,38)=9.30$; $P=0{\cdot}004$), whereas pulse rate and skin conductance increased after both stress tasks [$F(1,38)=78.34$; $P=0{\cdot}0005$ and $F(1,37)=83.16$; $P=0{\cdot}0004$). Diet, stress-proneness and stress controllability interacted [$F(1,36)=9.46$; $P=0{\cdot}004$) such that performance in HS subjects was better with the CR/PP diet than the PR/CP diet, but only after controllable stress. As the CR/PP diet has been found to increase the plasma Trp/LNAA ratio, indicating an increased availability of cerebral tryptophan and serotonin levels, it appears that an increased availability of brain serotonin in HS subjects after controllable laboratory stress.

4.1. INTRODUCTION

It is common knowledge that stress can have a detrimental effect on cognitive performance (Mandler, 1984; Smith, 1990). Marked examples are found following traumatic stress experiences (Bremner *et al.* 1992; Marmar *et al.* 1994), chronic life events (Cohen *et al.* 1980) and short-lived acute stressors (Hockey & Hamilton, 1983; Loeb, 1986; Smith, 1990; Kramer *et al.* 1991).

The onset of cognitive disturbances following stress may depend on the interaction between the intensity of the stressor and an emotional disposition to become aroused (Eysenck, 1982). For instance, Sorg and Whitney (1992) found that highly anxious subjects performed worse than non-anxious subjects in a reading span task under stress, whereas they performed better than control subjects during a nonstressful condition. Comparable effects were demonstrated by Stelmack *et al.* (1984) in a study in which anxious subjects showed improved performance relative to controls in a moderate stress situation, but not in an intense stress situation. As anxiety reflects an emotional susceptibility for stress, these findings suggest that in subjects with chronic stress experiences (stress-prone subjects), acute stress may lead to poor cognitive information processing. However, this relationship has not been thoroughly investigated. Moreover, the neurophysiological mechanisms mediating the effects of stress on cognitive performance are not well understood.

A possible factor mediating the negative effect of stress on cognitive performance is biochemical imbalances in the brain. In particular, increased serotonin (5-hydroxytryptamine) activity in the brain is a well established consequence of stress (Joseph & Kennett, 1983; Stanford, 1993) and declined serotonin function has been demonstrated in mood disturbance (Delbende *et al.* 1992) and cognitive disorder (Altman & Normile, 1988) patients. Hence, an increased serotonin function may be a prerequisite to cope with stress (Anisman & Zacharko, 1991; Deakin, 1991; Deakin & Graeff, 1991) and to maintain control over cognitive information processing (Spoont, 1992). Accordingly, differences in stress-related cognitive disturbances between stress-prone subjects and controls may partly be attributed to differences in serotonin function. If chronic stress in stress-prone subjects results in frequently elevated levels of cerebral serotonin activity, serotonin release may be exhausted resulting in the depletion of available tryptophan and brain serotonin concentrations (Markus *et al.* 1998; Tuiten *et al.*, 1995). As a consequence, the serotonergic system in stress-prone subjects may be overloaded during confrontations with actual stressors, bringing already low concentrations below functional requirements and resulting in poor performance.

Serotonin levels in the brain can be increased by the intake of a carbohydrate-rich, protein-poor (CR/PP) diet. In contrast to a protein-rich diet, a carbohydrate-rich, protein-poor diet raises the plasma ratio tryptophan (the precursor of serotonin) to the sum of the other large neutral amino acids (Trp/LNAA), giving tryptophan the advantage in the competition for access to the brain thus increasing brain tryptophan

and serotonin (Fernstrom & Wurtman, 1971*a*, *b*; Fernstrom & Wurtman, 1972; Fernstrom *et al.* 1973; Curzon, 1985; Wurtman, 1987). This dietary effect on the plasma Trp/LNAA ratio has been well documented (e.g. Rosenthal *et al.* 1989; Christensen & Redig, 1993; Pijl *et al.* 1993; Markus *et al.* 1998).

The relationship between brain serotonin function and cognitive performance, and between a CR/PP diet and serotonin availability, has inspired a broad range of dietary studies based on the assumption that carbohydrates may improve cognitive performance through changes in brain serotonin (Young, 1991). However, dietary studies yield inconsistent findings, revealing that carbohydrates may either improve performance (Kanarek *et al.*, 1990), decline performance (Spring *et al.*, 1982/83) or show no effect at all (Lieberman *et al.*, 1986; Lloyd *et al.*, 1996; for a review see Bellisle *et al.* 1998).

A crucial factor in explaining the inconsistent findings in literature may be that stress-proneness interacts with the serotonin level. A carbohydrate diet may improve performance in stress-prone subjects, particularly under conditions of actual stress, by preventing a critical decline in serotonin functioning. A recent study (Markus *et al.* 1998) investigated whether a carbohydrate-rich, protein-poor diet improved cognitive performance in stress-prone subjects after uncontrollable laboratory stress. Although the diet significantly increased the plasma Trp/LNAA ratio, and in stress-prone subjects prevented a stress-induced increase in depression and a cortisol response, no effects were found on performance in a Sternberg memory scanning task (Sternberg, 1969, 1975). Although the memory load conditions (1, 2, 3 and 4 different digits) may have been too low to reveal an effect, the absence of a dietary effect on performance *after* laboratory stress might also be caused by the use of a highly uncontrollable stressor. The uncontrolled stress may have depleted the extra brain serotonin concentrations supplied by the carbohydrate-rich diet.

The present study was designed to test whether a carbohydrate-rich, protein-poor diet improves performance on the memory scanning task (Sternberg, 1969; 1975) after laboratory stress in stress-prone subjects, and whether this depends on the controllability of the stressor. High and low stress-prone subjects were randomly assigned to a CR/PP diet or a protein-rich, carbohydrate-poor (PR/CP) diet condition. During two experimental days subjects successively consumed the diet, were exposed to laboratory stress, and performed the Sternberg memory scanning task. During one day, laboratory stress was uncontrollable, alternated the other day by a controllable version. Changes of pulse rate and skin conductance from baseline were measured as physiological indices of the stress response. It was expected that the CR/PP diet only in stress-prone subjects improves memory scanning, depending on the controllability of the stressor.

4.2. METHODS

Subjects

Three-hundred and thirty-one Utrecht University social science students filled in the Inadequacy (IN) Scale of the Dutch Personality Inventory (measuring neuroticism, Luteijn *et al.* 1975), and a questionnaire concerning personal details. Neuroticism is closely related to negative affectivity (Luteijn & Bouman, 1988), the disposition to see events as alarming and to experience aversive emotional states (Watson & Clark, 1984), and also to stress-proneness (Gallagher, 1990; Wells & Matthews, 1994). In accordance, subjects high in neuroticism frequently experience stress (Bolger & Schilling, 1991; Deary & Matthews, 1993). Twenty-two subjects (6 males and 16 females) were selected from the highest quartile of IN-scores for the High Stress (HS) group (mean score 25, range 20-37), and 21 subjects (5 males and 16 females) were selected from the lowest quartile of IN-scores for the Low Stress (LS) group (mean score 3, range 1-5). Subjects' ages ranged from 19 to 26 years (mean age 22·5 years). Exclusion criteria for participation were chronic and current illness, medication, or irregular diets. All subjects selected for the experiment were of normal weight for height (BMI between 20-25 kg/m^2). All subjects participating in the experiment signed a letter of informed consent. The protocol was approved by the ethics committee of the clinical health department of Utrecht University.

Procedure

Equal numbers of both HS and LS subjects were randomly assigned to a carbohydrate-rich, protein-poor (CR/PP) diet or a protein-rich, carbohydrate-poor (PR/CP) diet condition; containing breakfast, lunch and one between-meal snack. On each of two experimental days, subjects completed a demanding stress-inducing mental arithmetic task. During one day, a controllable version of the stress task (Controllable Stress = CS) was presented, alternated with an uncontrollable version (Uncontrollable Stress = US) the other day. The order of presentation of CS and US was counterbalanced between subjects. The experimental days were separated by a 4-week interval in order to account for the menstrual phase of the female subjects. Women participated during their mid-late follicular phase (day 4-10).

On each experimental day, two subjects arrived at the laboratory at 9.00 am (first subject) and at 10.00 am (second subject), were allowed to read in a study room, and received breakfast (9.00-9.30 or 10.00-10.30 hours), a snack (10.15-10.30 or 11.15-11.30 hours) and lunch (11.00-11.30 or 12.00-12.30 hours), according to their dietary condition. Subjects did not eat or drink anything during the 11 hours before their arrival. One and a half hours after lunch the subject was brought into a laboratory room, seated in front of a computer screen, and instructed about the

experiment. The electrodes for skin conductance and the finger sensor for pulse-rate measurements were attached, and during the following 10 min baseline physiological recordings were made. The subject then was exposed to a computer assisted battery of experimental tasks (MINDS; Brand & Houx, 1992). The battery consisted of (1) a CS or US version of the laboratory stress task (Peters, Godaert, Ballieux, van Vliet, Willemsen, Sweep, & Heijnen, 1998), (2) a self-constructed questionnaire measuring experiences of control (PCQ), and (3) a Sternberg memory scanning task. Pulse-rate and skin conductance were recorded continuously until the end of the experiment. Subjects spent approximately 22 min on the stress task, 4 min on the PCQ and 10 min on the memory scanning task.

TABLE 1:

Composition of the carbohydrate-rich, protein-poor (CR/PP) diet and the protein-rich, carbohydrate-poor (PR/CP) diet.

	NUTRIENT COMPOSITION (g)			
DIET	Carbohydrates	Protein	Fat	Caloric value (kJ)
CR/PP				
Bread, margarine, jam, marmelade, tea, black coffee, sugar, candy bar and grape-juice.	219,1 (66,2 %)	11,9 (3,6 %)	44,3 (30,1 %)	5555,3 (1323 Kcal)
PR/CP				
Bread, margarine, cheese, roast beef, tea, black coffee, high-fat milk, and peanutes.	123,1 (41 %)	81,2 (27 %)	42,2 (32 %)	5039 (1200 kcal)

Diets

Two different diets were used; a carbohydrate-rich, protein-poor diet (CR/PP) and a protein-rich, carbohydrate-poor diet (PR/CP). The diets were composed by an authorised dietician of the Academic Hospital Utrecht (AZU, The Netherlands) and complied with the reference daily energy intake for peer groups of both men and women. As shown in Table 1, the total amount of calories and fat in both diets were approximately equal (the difference of 123 Kcal is negligible in regard to behavioural consequences). In order to keep the reducing effect of protein on the increase of the plasma Trp/LNAA ratio small, the amount of protein in the CR/PP diet did not exceed 5% of the total amount of dietary energy (Yokogoshi & Wurtman, 1986).

Both diets were identical to meals used in a prior study with similar groups (Markus *et al.* 1998). In the former study, the plasma Trp/LNAA ratio was significantly increased by 42% during the CR/PP diet compared with the PR/CP diet [P<0.0001].

Perceived Controllability Questionnaire (PCQ).

Experience of control refers to a subjective sensation of the ability to influence the outcome of self-directed actions and is commonly accompanied by increased feelings of mastery and control. Control experiences can be influenced by, among other factors, manipulating the difficulty of a task, the chance of successful performance, and the expectations about the outcome. In order to measure the effectiveness of manipulating the controllability of the stress task (CS vs. US), a computerised questionnaire was designed for the constructs "experiences of control" and "effort". This questionnaire comprised seven questions examining subject's experiences of control (e.g. "I could control the number of successes during the task"; "I feel incompetent to perform well") and seven questions measuring the amount of effort required by the task (e.g. "performing this task took me a lot of energy" and "the task required much effort"). Subjects were asked to rate their level of agreement on an 8-point interval scale ranging from "strongly agree" to "strongly disagree".

Factor analysis was performed on the total sample scores, for which the results of factor extraction (by Principal Component Analysis with Varimax, rotation; SPSS 7.5.2 for Windows) revealed an optimal factor structure of two factors. Both scales revealed strong reliability in the range of 0.85 (Cronbach's α).

Two additional questions (e.g. "I have confidence in performing well during the following task") tested the effect of stress controllability on expectations of competence (mastery). Subjects were asked to rate their level of agreement on an interval scale ranging from 1 (strongly agree) to 8 (strongly disagree).

Laboratory stress task

A mental arithmetic task performed under noise stimulation, was used as a stress inducing task and was offered in a controllable version (CS) and an uncontrollable version (US) (Peters *et al.* 1998). Subjects were given 18 successive one-minute trials in which they had to solve mental arithmetic problems under time constraint, while at the same time different levels of noise (either 65, 70 or 80 dB) were presented through a headphone. During each trial, multiple choice arithmetic questions were presented on a computer screen one at a time for which a specified number of sums (called the criterion) had to be solved correctly. Subjects were told that their performance would control the intensity of noise presented to them during

the task. If they failed the criterion, the computer would set the noise level to be present during the next trial; however, if they met the criterion they could choose the noise level for the following trial. Before the actual test, subjects were given two practice trials in which they became familiar with the task and the noise levels. The credibility of the task, as well as motivation, was enhanced by providing the subjects with constant on-screen feedback of the criterion in a particular trial, the number of sums already solved correctly, and the time left for that trial.

Controllability was manipulated by altering the total number of sums to be solved in each trial (the criterion). For each subject as well as each trial a new criterion was established based on the average calculation time needed for previous trials. In the controllable version (CS), controllability was accomplished by manipulating the criterion so that all subjects had success in most of the trials, and could thus choose the noise intensity for the following trial. One restriction was that two of the louder intensities had to be selected three times each, but the order of appearance was determined by the subjects themselves. In the uncontrollable version (US) subjects continued to fail on each trial and, thus, could not choose the intensity of the noise. The criterion was always set at one sum above what subjects could manage.

Memory task

Cognitive performance refers to the observable outcome of mental computations involved in the perception, recognition and encoding of a stimuli (input level) as well as the preparation and organisation of a response (output level). A Sternberg memory scanning task (Sternberg, 1969, 1975) was used as a cognitive performance test since this task allows for subtracting different levels of mental processing. This task consisted of four sub-tasks, corresponding to memory sets of 3, 4, 5 and 6 different consonants. Each sub-task started with a presentation on the computer screen of the particular set to be memorised, as long as each subject needed, after which 60 trials followed. In 30 trials the presented letter belonged to the memorised set, in the other 30 trials this was not the case. Every first 10 trials in each sub-task were used for practice and, therefore, not used in the analysis. In each trial the probe letter was presented at the center of the screen for 1 sec (machine-paced; with an interval range of 1 sec). The order of presentation of the 60 letters was randomised. 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 button (No) or a green button (Yes) with the preferred hand. Specific button boards were made for both left- and right handed subjects.

Measure of electrodermal activity

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

Measure of pulse rate.

Reflection-photoplethysmography was used to measure changes in peripheral pulse rate at the volar surface of the middle finger of the non-preferred hand. The signal was transduced by Sat-Trak^{TM} signal processing (sampling rate 65Hz), based on the Quadrature Division Multiplexing (QDM) technique, using the Pulse Oximeter (SensorMedics corporation, Bilthoven; The Netherlands). With a sampling rate of 1-Hz, pulse rate samples were collected and stored in the computer. Pulse rate recording started from a 10-min baseline rest period until the end of the experiment.

Experimental design and statistical analysis

The design of our study included *Diet* (the CR/PP vs PR/CP diet) and *Stress-Proneness* (HS vs LS) as between-subjects factors. To test the effect of the *Order of Stress-Controllability* (CS first followed by US, versus the opposite order) and *Gender*, these variables were also taken as between-subjects factors in a preliminary analysis. The within-subjects factors were *Stress-Controllability* (CS vs. US) and *Memory Load* (the four memory sets with 3, 4, 5, and 6 different consonants that had to be memorised during the memory scanning task). The dependent variables were *Laboratory-Stress* (increases in pulse rate and skin conductance measures before and after the laboratory stress task) and the results for the PCQ and memory scanning.

The results for pulse rate, skin conductance, the PCQ and memory scanning were analysed by means of repeated measures multivariate analyses of variance (MANOVA: General Linear Model; SPSS 7.5 for Windows). For pulse rate and skin conductance (measured before, during and after the stress-task) as well as for the scores on the four different consecutive memory sets of the memory scanning task, multivariate analyses of variance were performed with first- and second order polynomial contrasts (testing linear and quadratic effects). Only significant multivariate results were further examined by univariate tests. Huynh-Feldt and Greenhouse-Geisser corrected p values, their corresponding epsilons (ϵ) as well as the

original uncorrected, degrees of freedom are reported when the sphericity assumption was not met. The study was designed to detect a large effect size ($\mu^2 = 0.20$) for a power of 0.80 at alpha = 0.05. All significant effects on memory scanning are represented with their corresponding power estimations. As order of Stress-Controllability only contributed to the scores on the PCQ questionnaire, these scores were analysed with *Order of Stress-Controllability* as a covariate. Since there were no effects of gender (P = 0.84), *Gender* was not included in the final analysis. All statistics were evaluated at a significance level of 5% (two-tailed).

4.3. RESULTS

Perceived Controllability Questionnaire (PCQ).

As expected, there were significant effects of *Stress-Controllability* on the scores of the PCQ [F(2,37) = 10.26; P = 0.001]. As shown in Figure 1, in all subjects the mean scores on the subscale of Experience of Control significantly increased after CS (42; SD = 7.3) compared with US (22; SD = 7) [F(1,38) = 9.3, P = 0.004], and there was a significant reduction in the mean scores on Effort after CS (14.8; SD = 3.7) compared with US (21; SD = 2.4) [F(1,38) = 17.50, P < 0.001]. Multivariate analysis of variance did not reveal any differences in the effect of *Stress-Controllability* between HS and LS subjects, nor was there an effect of *Diet*.

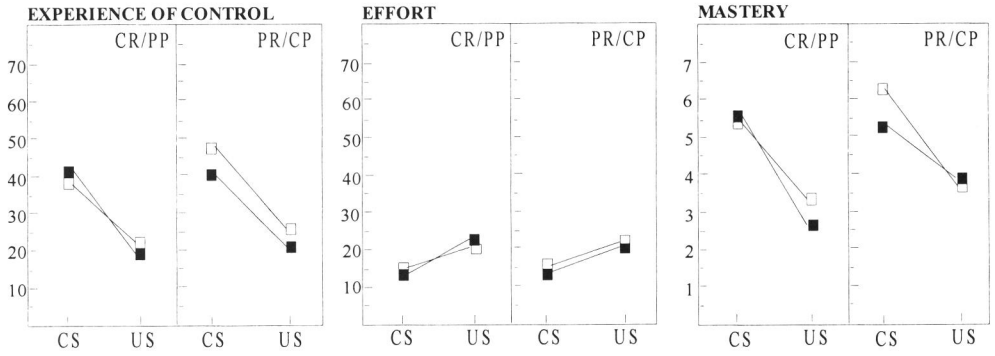

Fig.1: Significant effects of *Stress-Controllability* (P=0.001) on the scores of the Perceived Controllability Questionnaire (PCQ), indicating increases in experience of control (P=0.004) and mastery (P<0.001), and a reduction in effort (P<0.001), after controllable stress (CS) compared with uncontrollable stress (US), during a carbohydrate-rich, protein-poor (CR/PP) diet as well as during a protein-rich, carbohydrate-poor (PR/CP) diet. (■ = HS, □ = LS).

Analysis of variance also revealed a significant effect of *Stress-Controllability* on feelings of mastery [$\underline{F}(1,37) = 42.88$, $\underline{P} < 0.001$]. As shown in fig.1, experienced competence was lower after the US stress task ($\underline{M} = 3$, SD = 1.47) than after the CS task ($\underline{M} = 6$, SD = 1.40) (with the scores of *Diet* and *Stress-Proneness* combined). There were no differences between HS and LS subjects, nor was there an effect of *Diet*. These results indicate a successful manipulation of the controllability of the stress task.

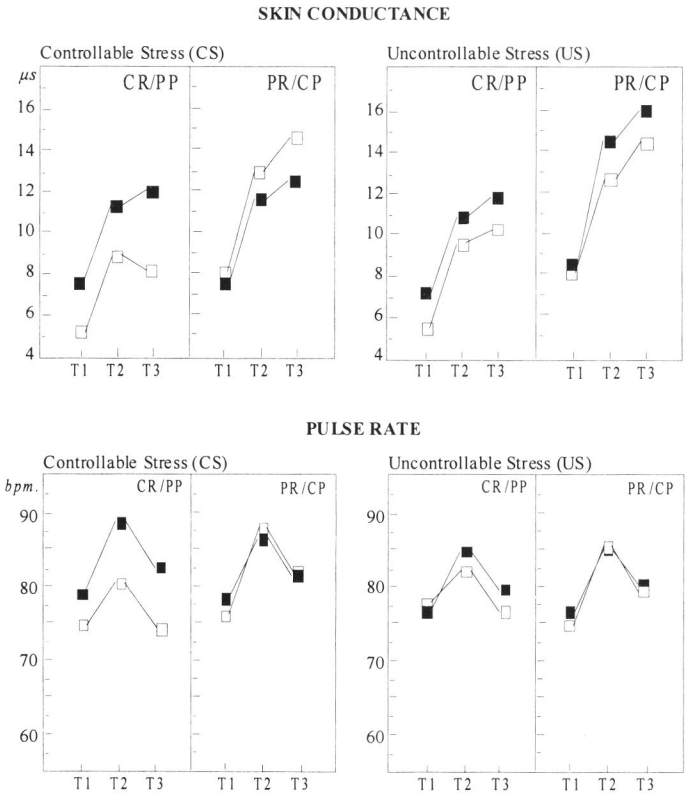

Fig.2: Significant effects of *Laboratory-Stress* on changes in skin conductance ($P<0.001$) and pulse rate ($P<0.001$) before (T1), during (T2) and after (T3) controllable and uncontrollable stress, during a carbohydrate-rich, protein-poor (CR/PP) diet and during a protein-rich, carbohydrate-poor (PR/CP) diet. (■ = HS, □ = LS).

Psycho-physiological measures

Multivariate analysis of variance revealed a significant effect *of Laboratory Stress* on changes in pulse rate and skin conductance [Wilks's $F(2,37) = 40.23$, $P < 0.001$]. No significant effects were found for *Diet*, *Stress-Proneness* or *Stress-Controllability*. As shown in figure 2, the laboratory stress task increased mean pulse rate from 74.72 (SD = 10.44) to 84.0 (SD = 12.83) [$F(1,38) = 78.34$, $P < 0.001$] and skin conductance from 7.0 (SD = 3.13) to 11.27 (SD = 4.75) [$F(1,37) = 83.16$, $P < 0.001$] with the scores of *Diet*, *Stress-Proneness* and *Stress-Controllability* combined.

Cognitive performance: memory scanning

It was predicted that the HS group within the CR/PP diet condition would perform better after laboratory stress, compared to the PR/CP condition, depending on the controllability of the stress task (CS vs US). In the LS group this effect was not expected. To test this, the mean RT across the four subtasks was analysed as an overall measure of performance efficiency. Multivariate analysis of variance revealed an effect of *Stress-Controllability* [Wilks' $F(1,36) = 31.32$, $P < 0.001$; Power = 1.0] and a significant three-way interaction effect of *Diet x Stress-proneness x Stress-Controllability* [Wilks' $F(1,36) = 9.46$, $P = 0.004$; Power = 0.85]; indicating that in the HS group only the mean RT was faster in the CR/PP diet condition ($M = 649$ ms, SD = 106 ms) than in the PR/CP diet condition (M = 780 ms, SD = 170 ms) after CS, whereas after US these differences were not found (see fig. 3). Further testing revealed that RT in HS subjects significantly improved after CS compared with US during the CR/PP diet condition [t -2.36; df=11, P=0.038], whereas the apparent decline in RT in HS subjects after CS compared with US during the PR/CP diet condition was not significant [t 1.54, df=11, P=0.22]. Furthermore, the apparent difference in RT in HS subjects between CS and US during the PR/CP diet condition was not significant (P>0.60). In LS subjects no effects of *Diet* or *Stress-Controllability* were found.

It was further explored whether in the HS group the increase in RT as a function of memory load was less during a CR/PP day than during a PR/CP day, depending on the controllability of the stress task (CS vs US). This slope -- the rate at which RT increases with memory load -- is interpreted by Sternberg (1969, 1975) as an indicator of the time needed for scanning an item in short-term memory. As a near significant effect of *Order of Stress-Controllability* [Wilks' $F(3,34) = 2.76$, $P = 0.06$] was found on this slope, a multivariate analysis of variance was performed with *Order of Stress-Controllability* as a covariate. However, analysis only revealed a significant effect of *Memory Load* on RT [Wilks' $F(3,34) = 7.91$, $P < 0.001$; Power = 0.98]. Further univariate analysis showed that this effect mainly originated from the second polynomial contrast [$F(1,36) = 8.69$, $P < 0.001$; $\epsilon = 0.588$; Power = 0.91], reflecting a quadratic effect, even though the linear contrast was also significance [$F(1,36) = 4.09$;

P = 0.05; Power = 0.51]. As can be seen in figure 3, this quadratic effect seems to be caused by a learning effect during the first memory set and may obscure the linear increase in RT during the following three memory sets. To test this, a second multivariate analysis was performed without the first memory set. Again there was a significant effect of *Memory Load* [Wilks' $\underline{F}(2,35) = 6.62$, $\underline{P} = 0.004$; Power = 0.89], without an *Order of Stress-Controllability* effect [Wilks' $\underline{F}(2,35) = 0.52$, $\underline{P} = 0.60$]. This time, the effect of *Memory Load* only originated from a linear increase in RT [$\underline{F}(1,36) = 9.31$, $\underline{P} < 0.001$; $\epsilon = 0.692$; Power = 0.92].

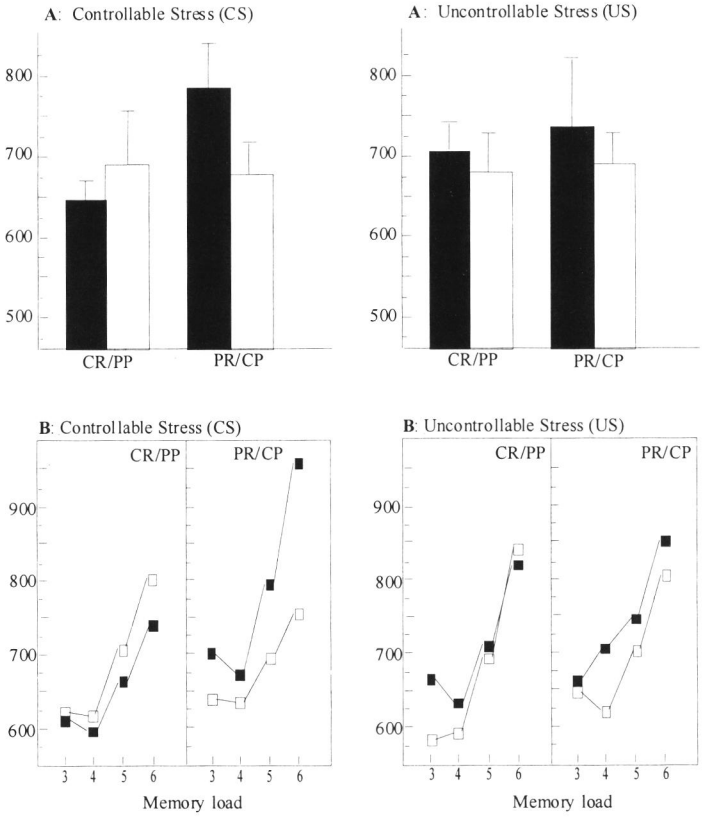

Fig.3: Mean reaction time (**A**) in HS subjects (■) but not in LS subjects (□) improved after controllable but not uncontrollable stress, under a carbohydrate-rich, protein-poor (CR/PP) diet condition compared with a protein-rich, carbohydrate-poor (PR/CP) diet condition (*Diet* x *Stress-Proneness* x *Stress-Controllablity*; P = 0.004). Reaction time as a function of Memory Load (**B**) increased particularly during the last three memory sets of 4, 5, and 6 items (P<0.001).

It was expected that subjects in the HS group would make fewer errors on memory scanning after laboratory stress in the CR/PP diet condition than in the PR/CP condition. Figure 4 shows the mean number of errors across the four sub-tasks. Only in HS subjects did the mean amount of errors appear to be lower during a CR/PP diet than during the PR/CP diet after CS, and --to a lesser extent-- after US. Note that HS subjects within a CR/PP diet condition also made fewer errors than LS subjects after CS, which is reversed within a PR/CP diet condition. However, these latter observations were not significant [P > 0.25].

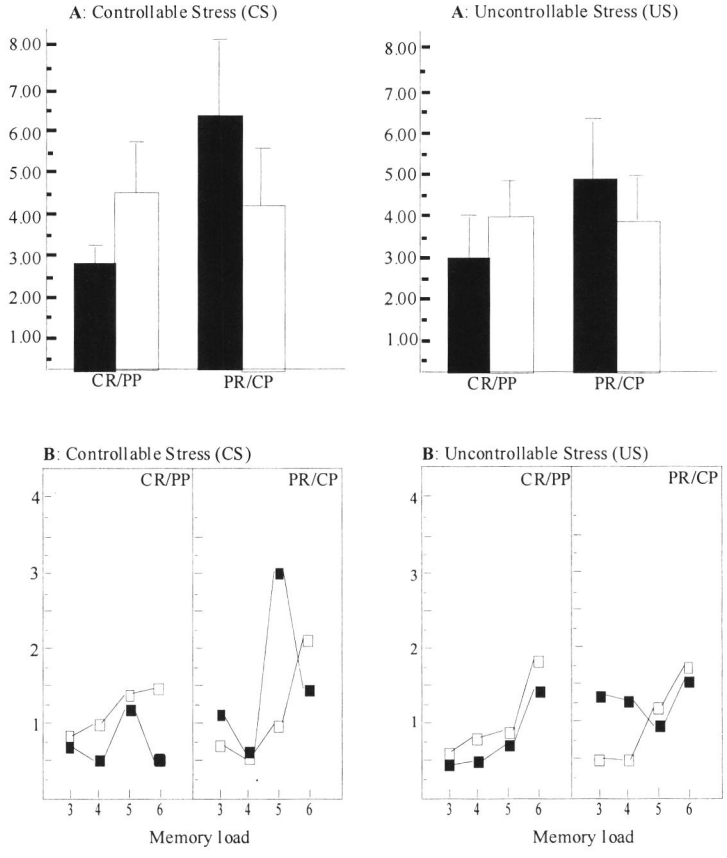

Fig.4: There were no significant effects on the mean amount of errors (**A**) of the memory scanning task after controllable and uncontrollable stress, neither during a carbohydrate-rich, protein-poor (CR/PP) diet condition or a protein-rich, carbohydrate-poor (PR/CP) diet condition. The amount of errors significantly increased as a function of Memory Load (**B**) ($P<0.001$). (■ = HS, □ = LS)

Figure 4 presents the number of errors as a function of memory load. Multivariate analysis of variance revealed an effect of *Memory Load* [Wilks' $\underline{F}(3,35) = 4.09$, $\underline{P} < 0.01$; Power = 0.80]. Further polynomialanalysis revealed that this effect originated from a linear increase in the amount of errors as a function of memory load [$\underline{F}(1,37) = 9.59$, $\underline{P} < 0.001$; $\epsilon = 0.75$; Power = 0.85], indicating that all subjects made more errors when more items had to be memorised (see fig.4). In HS subjects, there appeared to be a marked increase in errors when five items had to be memorised, followed by a recovery during the memory set of six items, particularly after CS. However, this latter interaction was not significant ($P > 0.85$).

4.4. DISCUSSION

In the present study it was investigated whether a CR/PP diet in HS subjects improves memory scanning following acute stress, depending on the controllability of the stressor. This research question was based on the assumption that in HS subjects there is a chronic stress induced serotonin deficiency in the brain. As the CR/PP diet previously has been found to increase the plasma Trp/LNAA ratio (Markus *et al.* 1998), a measure indicative of increases in brain tryptophan and serotonin (Fernstrom *et al.* 1973; Fernstrom & Wurtman, 1971*b*), it was assumed that this diet would enhance serotonin function in HS subjects, thereby reducing the negative effects of stress on memory scanning. Accordingly, the present data reveal that after controllable but not uncontrollable stress, memory scanning in HS subjects improves with a CR/PP diet compared with a PR/CP diet. No such differences were found for LS subjects.

Internal validity

We believe that the effects of diet manipulation on performance in the present study are not caused by differences in food intake, expectations about the food, or insufficient statistical power. First, dietary intake was under continuous observation in the laboratory to ensure that all subjects consumed the whole diet and that no other foods were taken. Second, it is unlikely that one does hold personal expectancies about the behavioural effects of nutrients like carbohydrates (Christensen *et al.*, 1985; Spring *et al.*, 1987; Rosenthal *et al.*, 1989). Moreover, food-related expectancies do not represent a meaningful factor involved in the effect of dietary manipulation on human behaviour (Spring *et al.*, 1987). In support of this notion, non of the subjects seemed to consider the dietary condition as a factor involved, as was established by a brief interview of each subject at the end of the last experimental day. Third, in order to detect a large effect of dietary manipulation on performance for a power of 0.82 at an alpha of 0.05, a group size of 17 subjects is required. This was exceeded in the present study. Accordingly, all significant

multivariate effects were reflected by conceivable power values above 0.80. Moreover, all null findings were accompanied by probabilities between 0.22-0.89.

Controllability manipulation

Scores on the PCQ questionnaire (measuring experience of control) revealed a successful manipulation of the controllability of the stress task. Although pulse rate and skin conductance significantly increased during both the CS and US condition, indicating an enhancement of general arousal and effort, there was only a significant reduction in experienced control and mastery during the US task. Similarly, in a study by Peters *et al.* (1998) lower control experiences as well as increases in cortisol were found exclusively after a US condition. Based on these findings, the US version in the present study may be considered as less controllable than the CS version.

Dietary effects on performance

Concerning memory scanning, multivariate analysis showed a significant three-way interaction effect of *Diet* by *Stress-Proneness* by *Stress-Controllability* on the mean RT across all four memory sets [P=0.004]. Further testing indicated that this interaction effect originated from faster memory scanning in HS subjects during the CR/PP diet condition after CS compared with US (P0.038), instead of slower performance during the PR/CP diet condition after CS compared with US [P=0.22]. This means that the CR/PP diet in HS subjects improved memory scanning after controllable laboratory stress, rather than that the PR/CP diet impaired performance.

There was a significant linear increase in RT as a function of memory load particularly across the last three memory sets. The first memory set was thought to be subjected to a learning effect, as it caused a quadratic change at the start of the memory task particularly during the first experimental day. The slope of the RT's 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 input and output stages of information processing regardless of memory load (Sternberg, 1996, 1975). As there were no dietary effects on the slope of RT, the three-way interaction effect on the mean RT across all subtasks may indicate that HS subjects benefit from a CR/PP diet regarding both input and output stages of information processing after laboratory stress, but only when the stressor is experienced as controllable.

In a previous study, a dietary effect on cognitive performance in HS subjects was not found after high uncontrollable laboratory stress (Markus *et al.* 1998). It was assumed by the authors that due to low memory load conditions, this task might have been too easy to reveal a dietary effect. However, the present data do not confirm such an explanation as we still did not find a dietary effect on memory scanning after US

with a memory load of 3, 4, 5, and 6 items. Rather, the effect of a CR/PP diet on performance *after* acute stress in HS subjects seems to depend on the controllability of the stressor.

Studies that investigated the *direct* effects of carbohydrate consumption on performance yield inconsistent findings; either showing an improvement (Kanarek *et al.*, 1990), a reduction (Spring *et al.*, 1982/83) or no effect at all (Lieberman *et al.*, 1986; Lloyd *et al.*, 1996). Results of the present study are the first in showing that the effect of carbohydrate consumption on performance may *indirectly* be mediated by stress-proneness during an actual confrontation with stressors that differ in controllability.

Conclusion: explaining the dietary effects on performance

The question remains why memory scanning after stress in HS subjects improves during a CR/PP diet only when the preceding stressor is controllable. First, since the CR/PP diet used in the present study previously has been found to increase the plasma Trp/LNAA ratio (Markus *et al.*, 1998), we assume that after the CR/PP diet condition there is an increased supply of available tryptophan and brain serotonin concentrations. Second, because a mental stress induced increase in cerebral serotonin activity is accompanied by an enhanced breakdown of this neurotransmitter, there might be more serotonin available after CS than after US. Chronic stress in HS subjects ultimately may induce a shortage of brain serotonin concentrations. Because the serotonergic system becomes more sensitive under chronic stress because of compensatory receptor sensitisation (Kennett *et al.* 1985; Adell *et al.* 1988; Cancela & Molina, 1990), an increased availability of serotonin after CS during the CR/PP diet condition particularly in HS subjects may have profound beneficial effects on performance. In support of this line of reasoning, the CR/PP diet previously has been found to improve stress coping during high uncontrollable laboratory stress only in HS subjects (Markus *et al.*, 1998); suggesting a more enhanced serotonergic functioning in HS subjects than in LS subjects. However, to find evidence for the involvement of the brain serotonergic system as suggested, it is necessary to use a more direct measure of cerebral tryptophan and serotonin alterations.

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CHAPTER 5

THE BOVINE PROTEIN α-LACTALBUMIN INCREASES THE PLASMA TRP/LNAA RATIO, AND IN VULNERABLE SUBJECTS RAISES BRAIN SEROTONIN ACTIVITY, REDUCES CORTISOL AND IMPROVES MOOD UNDER STRESS.

C.R. Markus, B. Olivier, G. Panhuysen, J. Van der Gugten, M. Alles, H. Westenberg, A. Tuiten, D. Fekkes, H. Koppeschaar, E. de Haan. (Submitted).

ABSTRACT

Increased serotonin function in the brain may be important to cope with stress, whereas a decline in serotonin activity is involved in mood disturbance and depression. 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 consequently improve stress coping and mood, particularly in stress-vulnerable subjects. In the present study, we tested whether an α-lactalbumin enriched whey-protein fraction, with a high tryptophan content, may cause an increase in the plasma Trp/LNAA ratio and thereby reducing depressive mood and cortisol concentrations in stress-vulnerable subjects under experimental stress by enhancing brain serotonergic function. Twenty-nine high stress-vulnerable subjects and 29 low stress-vulnerable subjects participated in a double-blind placebo-controlled cross-over study. They were exposed to experimental stress after the intake of a balanced diet with either an α-Lactalbumin enriched whey-protein fraction (α-Diet) or sodium-caseinate (c-Diet). Dietary-induced changes in the plasma Trp/LNAA ratio, prolactin, and 5-hydroxy-indoleacetic acid (5-HIAA) were measured. Changes in mood, pulse rate, skin conductance and cortisol were assessed before and after the stress task. The α-Diet caused a significant increase of 48% in the plasma Trp/LNAA ratio [$\underline{P}$ = 0.0001]. In stress-vulnerable subjects, this increase in the plasma Trp/LNAA ratio was accompanied by a significant 40% rise in prolactin [$\underline{P}$ = 0.001], prevented an increase in cortisol [P = 0.036] and reduced depressive feelings [$\underline{P}$ = 0.007] under acute stress. This study showed that the enrichment of tryptophan in dietary protein, by an α-Lactalbumin enriched whey-protein fraction, increases the plasma Trp/LNAA ratio and, only in stress-vulnerable subjects, improves stress coping probably by alterations in brain serotonin.

5.1. INTRODUCTION

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

Serotonin is synthesized from the dietary amino acid tryptophan, and brain serotonin concentrations rise by tryptophan administration or by the intake of a carbohydrate-rich, protein-poor 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 into the brain (9-12). An increase in the plasma Trp/LNAA ratio by a carbohydrate-rich, protein-poor diet is caused by a carbohydrate-induced rise in glucose; which triggers insulin secretion and facilitates the uptake of the LNAA's except tryptophan into the skeletal muscles. In general, a balanced protein-rich diet decreases the plasma Trp/LNAA ratio, as dietary proteins are poor in tryptophan (1-2%) but rich in the LNAA's valine, tyrosine, leucine, isoleucine, and phenylalanine (25%). These dietary effects on the plasma Trp/LNAA ratio have been demonstrated frequently (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 increased plasma Trp/LNAA ratio have been shown to reduce depressive mood (15,16), in normal subjects such evidence is weak and yields contradictory findings (17, 18).

Recently, we suggested that differences in the vulnerability to stress may explain some of the inconsistent findings in literature (14,19). Assuming that stress-vulnerability or chronic stress stands for a frequently elevated level of brain serotonin activity, in stress-vulnerable subjects the serotonergic system may be overloaded during an acute stressor and, subsequently, serotonin activity may fall below functional need. In a recent study we found that a carbohydrate-rich, protein-poor diet compared with a balanced protein-rich diet increased the plasma Trp/LNAA ratio by 42% and prevented a stress-induced increase in depressive mood and cortisol in stress-vulnerable subjects (14). In a subsequent study a carbohydrate-rich, protein-poor diet prevented a cortisol response and depressive mood during controllable as well as uncontrollable experimental stress in stress-vulnerable subjects (Markus et al., 1998; unpublished manuscript). The dietary effects on cortisol were surprising, because the administration of serotonin precursors have been found to stimulate cortisol secretion (3). However, under an actual stressor increases in brain serotonin activity may facilitate stress 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 reduce in cortisol and depression (20,21), and thus we assume that in stress-vulnerable subjects the carbohydrate-rich diet, by increasing brain serotonin function, will improve the ability to cope with stress and subsequently reduce or

preclude a cortisol stress response. In these subjects serotonin activity might be more enhanced by increases in the Trp/LNAA ratio than in low stress-vulnerable subjects, due to a chronic stress induced sensitization of the serotonergic system. Chronic stress in stress-vulnerable subjects may decrease brain tryptophan and serotonin availability and increases 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 the previous findings, the aim of the present study is to test whether increases in tryptophan availability and brain serotonin concentrations 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 carbohydrate-rich, protein-poor diet. Since α-Lactalbumin protein has the highest tryptophan concentration of all bovine protein fractions (26), we hypothesized that a balanced diet composed of α-Lactalbumin enriched whey-protein may also increase the Trp/LNAA ratio and consequently central serotonin activity, and ultimately will prevent depressive mood and cortisol responses in stress-vulnerable subjects who are placed in a stressful situation. To test this hypothesis, subjects with a high and low stress-vulnerability participated in a double-blind placebo controlled stress experiment; receiving an isoenergetic balanced diet either containing α-Lactalbumin enriched whey-protein or casein protein. Since the secretion of prolactin is regulated by serotonergic mechanisms in the brain (27,28), increases in plasma prolactin were measured as indices of an enhanced brain serotonin function (29,30) and hypersensitivity of the serotonergic system (31,32). We also investigated a possible relationship between these indices of central serotonin function and changes in the plasma serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), since this metabolite is assigned as a peripheral indicator of changes in brain serotonin system (33). To our knowledge, the present study was the first to tested this relationship between α-Lactalbumin enriched proteins, brain serotonin function, and stress.

5.2. SUBJECTS AND METHODS

Subjects

455 Students of Utrecht University filled in the Inadequacy (IN) Scale of the Dutch Personality Inventory, measuring neuroticism (34), and a questionnaire concerning personal details. Neuroticism is closely related to negative affectivity (35), the disposition to see events as alarming and to experience aversive emotional states (36), and also to stress-vulnerability (37). In accordance, subjects high in neuroticism frequently experience stress (38). Based on these findings, from the highest quartile

of IN-scores 29 subjects (10 males and 19 females) were selected for the High Stress (HS) or experimental group (IN= 26±6; range 18-38), and from the lowest quartile of IN-scores 29 subjects (9 males and 20 females) for the Low Stress (LS) or control group (IN= 4.6±1.3; range 2-6). Subjects' ages ranged from 17 to 34 years (20.7±3.14). Exclusion criteria for participation were chronic and current illness, psychiatric history, medication, or irregular diets. All subjects selected for the experiment were in the normal range for the Body-Mass Index (BMI in kg/m^2 between 20-25). Because oral contraceptive medication may have an effect on cortisol responsiveness (39), female subjects were matched for contraception. The protocol for the present study was approved by the institutional board of experimental research of the University Utrecht.

Experimental Procedure

During two experimental days, subjects were placed under experimental stress following a balanced diet containing either an α-Lactalbumin enriched whey-protein fraction (α-Diet) or casein (c-Diet). Both diets were isoenergetic and contained equal amounts of protein, carbohydrate and fat. The order of presentation of the α-Diet and the c-Diet was counterbalanced between subjects. Both days were separated by a four-week period, allowing to account for the menstrual phase of the female subjects. These women participated during their mid-late follicular phase (day 4-10), while women using contraception participated when they actually used the contraception pill.

On each experimental day, two subjects arrived at the laboratory at respectively 0900 and 1000 hours. Subjects were sedentary and only allowed to read in a study room. Subjects fasted overnight; only water or tea without sugar were permitted. They received breakfast at arrival, snack at 1015 or 1115 hours, and lunch at 1100 or 1200 hours. One and a half hour after lunch, a first salivary cortisol sample was taken, followed three minutes later by a blood-sample (transferred to a 10 ml tube containing EDTA). Then the subject was brought into a temperature-controlled laboratory room, seated in front of a computer screen, and instructed about the experiment. The electrodes for skin conductance and the finger sensor for pulse-rate measurements were attached, and during the following 10 minutes baseline physiological 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 (40). The battery consisted of, (A) a version of the POMS (41), (B) a stress inducing mental arithmetic task (42), and (C) a second version of the POMS. A second salivary sample was taken directly at the end of the stress inducing task, approximately 25 minutes after stress onset, and a third cortisol sample was taken after completion of the experiment (35 minutes after stress onset). Pulse-rate and skin conductance were recorded until the end of the experiment.

Subjects spent approximately four minutes on the first POMS, 25 minutes on the stress inducing task and 4 minutes on the second POMS. The administration of the diets as well as the acquisition of the experimental measures was conducted by a research assistant blind to the purpose of the experiment and dietary conditions.

TABLE 1
Composition of the α-Diet and the c-Diet.

	Nutrient composition (*g*)			
	Carbohydrate	Protein	Fat	Caloric value (kJ)
α-Diet				
Bread, margarine, fruit-sprinkles, tea, coffee, candy bar, grape-juice, chocolate drinks	289 (61.5 %)	46.8 (9.9 %)	62 (28.7 %)	7995 kJ
c-Diet				
Bread, margarine, fruit-sprinkles, tea, coffee, candy bar, grape-juice, chocolate drinks	289 (61.5 %)	46.6 (9.9 %)	62 (28.7 %)	7996 kJ

Diets

On both experimental days, an isoenergetic diet containing 7995 kJ was used with 10% of energy as protein, 60% of energy as carbohydrate, and 30% of energy as fat, composed of standard products (see Table 1). The diet complied with the recommended average daily energy intake for both men and women (Dutch Board of Nutrition, 1989). The two 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-Diet contained an α-Lactalbumin enriched whey-protein fraction (Borculo Domo Ingredients, Borculo; The Netherlands) and the chocolate drink of the c-Diet contained sodium-caseinate (DMV International, Veghel; The Netherlands). The chocolate drink was prepared within twenty minutes before breakfast (first drink) and twenty minutes before lunch (second drink) by mixing the manipulated chocolate powder with 200ml of water (under constant temperature of 75-80°C). Both chocolate drinks were isoenergetic and contained equal amounts of protein, carbohydrates and fats. Rum-aroma 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 B.V., Roosendaal; The Netherlands) and are given in Table 2. As shown, the chocolate drink of the α-Diet contained 12.32 g/kg tryptophan (Trp/LNAA= 8.7), whereas the drink of the c-Diet contained 9.51 g/kg tryptophan (Trp/LNAA= 4.7).

TABLE 2

Composition and amino acid profile of the chocolate drink used in the α-Diet and the c-Diet.

	α-Diet	c-Diet
Composition (*g*)		
α-lactalbumin enriched whey-protein	20	0
sodiumcaseinate	0	15.5
Cacao	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/ΣLNAA	**8.7**	**4.7**

Profile of Mood States (POMS)

Changes in mood were measured using two scales of the Dutch shortened version of the Profile of Mood States questionnaire (41), offered at the computer-screen on 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 minutes) and the second scale was offered after the stress task (+25 minutes). The POMS comprises five different subscales for mood. The subscale 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

A mental arithmetic task, performed under noise stimulation, was used as an experimental stress situation. Subjects were given 18 successive one-minutes trials in which they had to do mental arithmetic under time constraints, while at the same time different levels of industrial noise (either 65, 70 ore 80 dB) were presented to them through a headphone. 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 then would set the noise level to be present during the next trial; however, if they met the criterion they could choose the noise level for the following trial. Before the actual test, subjects were given two practice trials in which they had to solve a few sums first without noise and then in the presence of the three noise levels successively. The credibility of the task, as well as motivation, was enhanced by providing the subjects with constant on-screen feedback of the criterion in a 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 on each trial and, thus, could not choose the 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 demonstrated to be highly uncontrollable and induced psychological and physiological stress (14,42).

Biochemical Analyses

The blood samples are collected in 10 ml vacutainer tubes containing EDTA and centrifuged at 2650 g_{max} for 20 minutes at 20°C. Then the supernatant was stored at -70°C until analysis.

For the determination of amino acids in plasma, a sensitive and reproducible fully automated method was used as previous described (43). This method is based on reversed-phase HPLC and *o*-phthaldialdehyde precolumn derivatization, making use of a 5-mm Spherisorb ODS 2 column (125 x 3 mm I.D.) for routine determination. The plasma tryptophan ratio was ultimately calculated by dividing the plasma tryptophan concentration by the sum of the other large neutral amino acids, i.e. valine, isoleucine, leucine, tyrosine and phenylalanine.

Prolactin concentrations in plasma were assayed in duplicate by using a standard radioimmunoassay kit (ImmuChem™ IRMA, ICN Pharmaceuticals) with intra- and interassay coefficients of variation of 3% and 5% respectively. Prolactin concentrations are expressed in ng/ml.

5-Hydroxy-indoleacetic acid (5-HIAA) was analyzed in duplicate by HPLC, using a Hewlett Packard Model 1090 liquid chromatograph equipped with a Metrohm model 641 VA electrochemical detector. The detector was equipped with a glassy carbon working electrode and operated at a potential of 800 mV vs. an Ag/AgCI reference electrode and a temperature of 40°C. The components were separated on a reversed-phase column (CP-Sphere C8, 250 x 4.6 mm ID, particle size 7 μm, Chrompack, the Netherlands) at a temperature of 40°C, using a mobile phase consisting of a 0.1 M acetic acid/sodium acetate buffer (pH = 5.0), containing 0.15 mM sodium heptanesulfonate, 0.25 mM EDTA and 30 ml methanol per liter.

The flow rate was 1 ml/min. The sensitivity of the method is 2.5 fmol/l and the coefficient of variation is less than 6%. The concentrations are expressed in ng/ml.

Measurement of salivary cortisol

One baseline cortisol sample (-15 minutes), a second post-stress sample (+25 minutes) and a third post-stress sample (+35 minutes) were obtained with the Salivette sampling device (Sarstedt, Etten-Leur; the Netherlands). With this procedure, saliva was collected in small cotton swabs and stored in special salivette tubes until centrifugation. Saliva samples were centrifuged at $2650g_{max}$ for three minutes at 20°C and then were stored at -23°C until analysis.

Cortisol concentrations were determined without extraction in a licensed laboratory at the University Hospital Utrecht (AZU, the Netherlands) using an in house competitive radio-immunoassay (RIA) employing a polyclonal anticortisol-antibody (K7348). 1,2-^{3}H(N)-Hydrocortisone (NET 185, NEN -Dupont-, Dreiech, Germany) was used as a tracer, following chromatographic verification of its purity. The lower limit of detection was 0.5 nmol/l and inter-assay variation was 11.0; 8.2; and 7.6% at 4.7; 9.7; and 14.0 nmol/l, respectively (n=20). Reference values for adults are 4-28 nmol/l (0800-1000 hour).

Measurement of skin conductance

Skin conductance was measured using silver/silver chloride (Ag-AgCI) electrodes (surface 0.5cm^2), filled with adhesive solid gel (ARBO H91, Braunschweig; Germany). These electrodes were bipolarly placed at the thenar palmar and hypothenar palmar sites of the non-preferred hand of the subject. Skin conductance was measured with a constant voltage of 0.5 V (sampling rate 2Hz). Tonic skin conductance levels were recorded starting from a 10-minutes 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 non-preferred hand. The signal was transduced by Sat-Trak^{TM} signal processing (sampling rate 65Hz), based on the Quadrature Division Multiplexing (QDM) technique, by the Pulse Oximeter (SensorMedics corporation, Bilthoven; The Netherlands). With a sampling rate of 1Hz, 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 (GLM: SPSS 7.5 for Windows) with one between-subjects factor "*Stress-Vulnerability*" (HS vs LS; as independent variables) and two within-subjects factors "*Diet*" (α-Diet vs c-Diet; as independent variables) and "*Experimental Stress*" (measures before vs after the experimental stress task of pulse rate, skin conductance, cortisol, and mood; as dependent variables). Although we counterbalanced for *Order of Diet* (α-Diet first followed by c-Diet, versus the opposite order) *Order of Diet* was preliminary taken as a between-subjects factor. For the effect of Experimental Stress on pulse rate, skin conductance and cortisol, multivariate analyses of variance were performed with first- and second order polynomial contrasts (linear and quadratic effects). Significant multivariate results revealed by these procedures were further examined by univariate tests. Huynh-Feldt or Greenhouse-Geisser corrected *P* values, their corresponding epsilons (∈) as well as the original, i.e. 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 and 5-HIAA. Because *Order of Diet*, as well as *gender* and *pill-use*, did not contribute to any of the scores, final analyses were performed with only *Stress-Vulnerability* as between-subjects factor. Analyses for variables with expected directions of change (cortisol, depression) were assessed by unidirectional comparisons (44). All statistics were evaluated at a significance level of 5%. Data are reported as means ± SD.

5.3. RESULTS

Plasma Trp/LNAA ratio

Analysis revealed a significant effect of *Diet* [$F(1,56)=327.557$, $P<0.0001$], indicating that there was a significant increase in the plasma Trp/LNAA ratio (48%) following the α-Diet. As shown in figure 1, the mean plasma Trp/LNAA ratio increased from 0.071±0.012 after the c-Diet condition to 0.104±0.013 after the α-Diet condition. No effects of *Stress-Vulnerability* or any other effects were found.

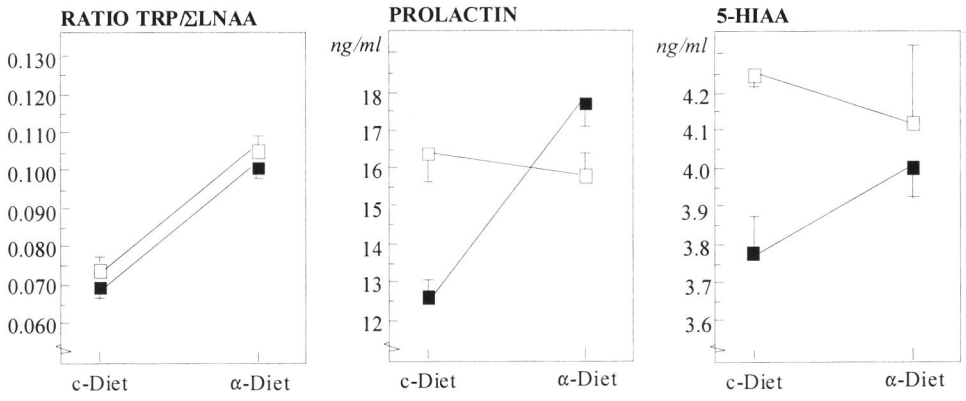

Fig.1: The α-lactalbumin enriched whey-protein diet (α-Diet) significantly increases the plasma Trp/LNAA ratio in HS (■) and LS (□) subjects compared with the casein diet (c-Diet) [P<0.0001], and only in HS subjects increases prolactin [P=0.001] and led to modest but non-significant increases in 5-HIAA. (Error-bars are expressed in SEM)

Prolactin

Analysis of variance revealed a significant interaction effect of *Diet* by *Stress-Vulnerability* [F(1,49)=5.224; P=0.027], indicating that the effect of the diet on prolactin concentrations depended on the stress-vulnerability of the subjects. As figure 1 indicates, in HS subjects there was a significant 40% increase in prolactin from 12.60±4.0 ng/ml after the c-Diet to 17.70±8.2 ng/ml after the α-Diet (P=0.001), whereas in LS subjects no dietary effects were found (from 16.21±8.4 ng/ml to 15.76±9.0 ng/ml; P=0.79). There were no significant between-subjects effects of *Stress-Vulnerability*.

There were also significant positive correlation's between changes in the plasma Trp/LNAA ratio and prolactin concentrations during the α-Diet as opposite to c-Diet in HS subjects [R = 0.45, P=0.028] but not in LS subjects [R = 0.24; P=0.21].

5-HIAA

As shown in figure 1, only in HS subjects 5-HIAA slightly increased from 3.77±1.1 after the c-Diet condition to 4.03±1.0 after the α-Diet condition, whereas in LS subjects 5-HIAA remained approximately equal (from 4.25±1.0 ng/ml to 4.17±1.1 ng/ml respectively). However, analysis of variance did not reach significance for the interaction between *Diet* by *Stress-Vulnerability* [F(1,55)=2.047; P=0.16]. There were no significant correlation's between changes in 5-HIAA and changes in the plasma Trp/LNAA ratio, nor between 5-HIAA and prolactin.

Physiological measures

Multivariate analysis of variance revealed 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 before the stress task to 85.20±11.5 during the stress task [$F(1,56)=21.88$, $P<0.0001$; $\epsilon=0.781$], and skin conductance from 4.0±2.63 to 8.8±4.30 [$F(1,56)=201.13$, $P<0.0001$; $\epsilon=0.644$]. Multivariate analysis also revealed a significant three-way interaction effect of *Stress-Vulnerability* by *Diet* by *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 c-Diet (from 77.90±8.65 to 88.17±10.51) than after the α-Diet (from 80.41±9.10 to 87.93±10.22). No *Stress-Vulnerability* or *Diet* effects were found on skin conductance.

Multivariate analysis of variance revealed a near significant interaction effect of *Stress-Vulnerability* by *Diet* by *Experimental-Stress* [$F(2,54)= 2,14$; $P=0.06$], originated from a significant linear change in cortisol [$F(1,55)= 3.35$; $P=0.036$], meaning that a dietary effect on cortisol responses under experimental stress depended on the stress-vulnerability of the subject. As demonstrated in figure 2, in HS subjects cortisol increased by experimental stress from 9.36±2.41 to 10.65±3.09 following the c-Diet, whereas after the α-Diet a cortisol stress response was prevented (from 10.73±3.64 to 10.20±3.86). These effects where not found in LS subjects. There were no other effects of *Stress-Vulnerability* or *Diet*.

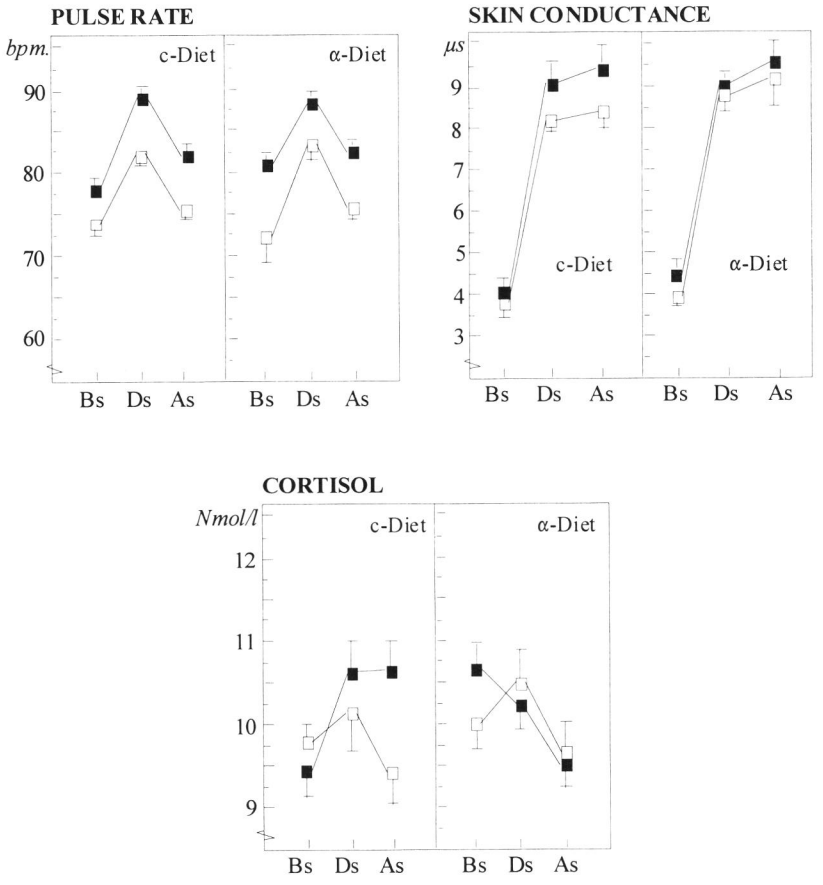

Fig.2: Significant increases in pulse rate [P<0.0001] and skin conductance [P<0.0001] were found during experimental stress (Ds) in HS (■) and LS (□) subjects, during the α-lactalbumin enriched whey-protein diet (α-Diet) and the casein diet (c-Diet). In HS subjects but not in LS subjects, a cortisol stress-response was prevented during experimental stress (Ds) following the α-Diet compared with the c-Diet [P=0.036]. (Error-bars are expressed in SEM)

Mood (POMS)

Analysis of variance revealed a marginal significant interaction of *Stress-Vulnerability* by *Diet* by *Experimental-Stress* on the mean scores of the depression subscale of the POMS [F(1,56)=2.116; P=0.07]. Since the dietary effect is only expected in HS subjects under acute stress, we performed a second analysis among 18 HS and 21 LS subjects who displayed a physiological stress response during the stress task (measured by increased pulse rate and skin conductance) during the control (c-Diet) condition.

Analysis of variance revealed a significant interaction effect of *Stress-Vulnerability* by *Diet* by *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 slightly increased by experimental stress from 15.19±5.7 to 16.19±5.7 after the c-Diet, whereas after the α-Diet they declined from 15.62±5.7 to 14.86 ±5.2. In LS subjects this effect was not found.

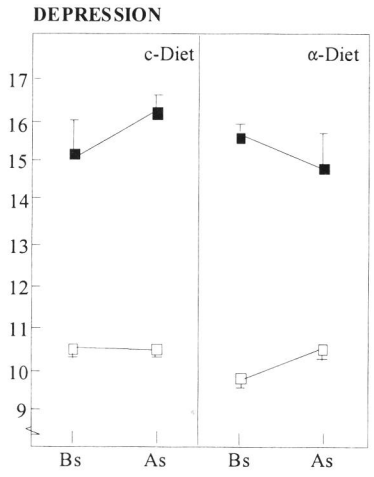

Fig.3: In HS (■) subjects but not in LS (□) subjects, the scores on the depression subscale of the POMS decreased after experimental stress (As) during the α-lactalbumin enriched whey-protein diet (α-Diet) compared with the casein diet (c-Diet) [P=0.007]. (Error-bars are expressed in SEM)

Multivariate analysis of the remaining POMS scales of anger, tension, vigor and fatigue revealed a significant effect of *Experimental-Stress* [F(4,53)=16.75; p<0.0001]. Further univariate analysis revealed that this originated from changes in the scores of anger [F(1,56)=60.20; p<0.0001], meaning that experimental stress significantly raises feelings of anger in both diet conditions (α-Diet: from 9.59±3.51 to 14.16±6.5, in the c-Diet: from 9.62±3.21 to 13.74±5.5). Results of the analysis on changes in the POMS-scores are presented in table 3. Multivariate analysis also revealed a significant two way interaction effect of *Stress-Vulnerability* by *Experimental-Stress* [F(4,53)=3.68, p=0.01], which was originated from 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 tensed after experimental stress (from 12.2±3.6 to 14.4±4.2) whereas the rise in tension in LS subjects was negligible (from 9.38±3.24 to 9.91±3.29). Furthermore, HS subjects did not experience more fatigue after experimental stress (from 16.73±5.25 to 16±4.45) as opposite to LS subjects (from 11±5 to 12.70±5.9).

Multivariate analysis also revealed a significant effect of *Stress-Vulnerability* [F(4,53)=8.60; P< 0,0001] 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 on anger (11.0±3.87 vs 8.35±2.18), tension (12.12±3.64 vs 9.38±3.23), fatigue (16.74±5.26 vs 11.0±5.0) and lower scores on vigor (13.40±3.37 vs 16.18±3.91) than LS subjects did (see also Table 3). No significant effects were found for *Stress-Vulnerability* or *Diet*, nor were there baseline differences in mood between physiological stress-responders and non-responders.

TABLE 3

Mood changes in HS and LS subjects under stress, after the α-Diet and c-Diet.

Moodscale	c-Diet		α-Diet	
	Bs	As	Bs	As
LS				
POMS Depression	9.79±2.3	10.14±2.61	9.83±2.55	10.24±2.63
POMS Anger	8.48±2.13	11.45±4.38[a]	8.21±2.23	11.83±5.73[a]
POMS Tension	9.24±2.43	9.72±3.28	9.52±4.04	10.10±3.23
POMS Vigor	16.52±3.90	16.38±3.11	15.83±3.92	15.24±3.55
POMS Fatigue	10.48±4.07	12.41±5.38	11.45±5.91	13.00±6.49
HS				
POMS Depression	14.83±5.13	15.80±5.3	15.10±5.14	14.20±5.04
POMS Anger	10.76±3.70	16.03±5.61[a]	10.97±4.03	16.48±6.54[a]
POMS Tension	11.90±3.56	14.14±4.10[b]	12.34±3.72	13.93±4.29[b]
POMS Vigor	13.69±3.42	13.14±2.67	13.10±3.31	13.45±2.59
POMS Fatigue	16.72±4.70	16.90±4.42[b]	16.76±5.81	16.10±4.47[b]

a: significant main effect of *Experimental-Stress*, meaning that stress-vulnerable subjects (HS) and control subjects (LS) had higher scores on anger after experimental stress (As) as compared to before experimental stress (Bs) (p<0.0001).

b: significant interaction effect of *Experimental-Stress* by *Stress-Vulnerability*, meaning that HS subjects became more tensed but less fatigue after experimental stress than LS subjects (p=0.01).

5.4. DISCUSSION

In this study, we showed that an α-Lactalbumin enriched whey-protein fraction increased the plasma Trp/LNAA ratio in both high (HS) and low (LS) stress-vulnerable subjects. We observed an increase in prolactin, an improvement of depressive mood, and a reduced cortisol stress response in those subjects that were highly vulnerable to stress (HS).

Internal validity

We believe that the effects of diet manipulation in the present study on blood measures, mood and cortisol are not caused by differences in dietary consumption, expectations about the food or purpose of the study, inappropriate biochemical analysis, or an insufficient statistical power of the experiment. Our study was a complete double-blind controlled dietary trial in which all subjects consumed the diet under direct observation that was similar in nutrient composition, appearance and taste. The research assistant and all subjects were blind 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. All blood samples were taken by an authorized assistant, directly stored at the appropriate temperature, and analyzed in duplicate within two months by standard procedures. In order to detect a medium effect ($\eta^2 = 0.2$) of dietary manipulation on mood at a significant level of 5% a group sample is required of at least 17 subjects, which was largely exceeded in the present study. Accordingly, analysis revealed a conceivable statistical power of all reported significant effects between 0.70 and 0.81.

Dietary effect on Trp/LNAA and brain serotonin.

The α-Diet caused a significant 48% increase in the plasma Trp/LNAA ratio compared with the c-Diet, indicating that during the α-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 increase of the plasma Trp/LNAA ratio in this experiment exceeds the 42% increase previously found with the carbohydrate-rich, protein-poor diet (14). Has a balanced or protein-rich diet generally been found to decline the Trp/LNAA ratio, the present results indicate that a balanced diet composed of an α-Lactalbumin enriched whey-protein fraction effectively raises the Trp/LNAA ratio; constituting a more conclusive and practical method to increase brain tryptophan and serotonin concentration.

Assuming that an increased plasma Trp/LNAA ratio by the α-Diet raises brain tryptophan and consequently serotonin concentrations, this diet was expected to enhance brain serotonin function most strongly in stress-vulnerable subjects. Stress-vulnerable subjects may experience chronic stress. As the higher serotonergic activity during stress leads to a higher breakdown of serotonin, chronic stress ultimately may lead to a functional shortage of available tryptophan and brain serotonin concentrations. As a consequence of chronic stress, the serotonergic system may become more sensitive because of compensatory receptor sensitization (22,23). The present findings on prolactin tended to support this assumption, since the α-Diet caused a significant 40% rise in prolactin only in HS subjects. Because increases in prolactin may reflect increases in central serotonergic neurotransmission

and receptor sensitization (31,32), the present dietary effects on both the plasma Trp/LNAA ratio and prolactin concentrations indicate that the serotonergic system putatively is more sensitive in HS subjects than in LS subjects. This suggests that stress-vulnerability constitutes a major factor predisposing the human brain for the beneficial effects of dietary induced increases in the plasma Trp/LNAA ratio. It may also be suggested that an enhanced serotonin sensitivity in normal HS subjects is a predisposing factor for major depression. Hence, a chronic stress induced biochemical adaptation may ultimately deteriorate the serotonergic system; which then may onset the symptoms of a mood disorder (45). Accordingly, stressful life events are linked to the development of depressive disorders (46) which, as previously mentioned, are accompanied by impaired brain serotonin function (3,4,25).

Since the α-Diet increased the plasma Trp/LNAA ratio and in HS subjects increased prolactin, and thus brain serotonin concentration, it was expected that the α-Diet would also cause a significant increase in plasma 5-HIAA. In concordance with changes in prolactin, we found an exclusive increase in plasma 5-HIAA during the α-Diet but not during the c-Diet in HS subjects. However the interaction between *Stress-Vulnerability* and *Diet* for 5-HIAA was not significant. It may be possible that the non-significance of the effects on plasma 5-HIAA are due to peripheral influences. Although changes in plasma 5-HIAA are partly related to alterations in brain serotonin, a majority of 5-HIAA in plasma is derived from peripheral sources (47).

Dietary effect on stress; mood and cortisol

In accordance with our previous findings (14), the current experimental stress task significantly increased pulse rate and skin conductance and led to a significant mood deterioration. This indicates a successful induction of experimental stress. Assuming that an increased serotonergic function in HS subjects constitutes a biological condition to cope with stress (see introduction), it was hypothesized that the α-Diet restrains an acute stress-induced cortisol response and depressive mood in HS subjects. Concerning depressive mood, we indeed found that only in HS subjects feelings of depression tended to be lower after experimental stress on the α-Diet compared with the c-Diet. In those HS subjects who showed a physiological stress response, this dietary effect on depression was highly significant ($P = 0.007$). It is important, however, not to draw too strong conclusions concerning the clinical implications from this observation, as the current dietary effects on depressive mood seem to be small. We also found that HS subjects reported higher mood deterioration at baseline compared with LS subjects. This seems to agree with the observation that experiences of chronic stress may lower mood and reflect a vulnerability to depression (46).

In accordance with our previous findings (14), we found that under a dietary induced increase in the plasma Trp/LNAA ratio an increase of cortisol concentration in HS subjects was prevented during acute experimental stress. At first sight, this decreasing effect on cortisol concentrations seems to contradict findings that the administration of serotonin precursors stimulate cortisol secretion in normal and clinical subjects (3). Increases in brain serotonin appear to initiate adreno-cortical reactivity through alterations in 5-HT_{1A} and 5-HT_2 receptor sites located in the hypothalamus and the 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 stress coping and may contribute in decreasing a cortisol response. Serotonergic neurotransmission does not appear to be an unitary mechanism; and different serotonergic pathways are involved in stress adaptation, initiating as well as terminating the activity of the adreno-cortical axis (7,8). Accordingly, an increased ability to cope with stress is often accompanied by a declined cortisol response and improved depressive mood (20,21). Based on these relations, the present data shows that an α-Lactalbumin enriched whey-protein diet (α-Diet) in stress-vulnerable subjects may terminate the negative consequences of experimental stress on cortisol secretion and mood; probably by enhancing brain serotonin mechanisms that are involved in stress adaptation.

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 it has not been clearly delineated to what extent the plasma Trp/LNAA ratio must rise to cause meaningful changes in brain serotonin synthesis. In most studies a diet-induced rise in the plasma Trp/LNAA ratio did not exceed 20-25% over baseline values. It is questioned by some authors whether this increase would also be sufficient to cause a meaningful enhancement of brain serotonin in human (48,49). These authors suggested that the plasma Trp/LNAA ratio must rise at least by 50% to produce substantial changes in brain serotonin synthesis. However, there is no conclusive evidence to justify such a general constriction. Only in one study it was demonstrated that even an impressive 47% increase in the plasma Trp/LNAA ratio by an orange juice drink could not lead to significant increases in 5-HIAA in cerebrospinal fluid (49). However, in this study (49) the increased plasma Trp/LNAA ratio did not reach significance due to a small subjects group (N=5). Furthermore, the subjects under study were neurological patients (mean age 71 years) diagnosed with Normal Pressure Hydrocephalus; a neuropathological condition accompanied by a variety of behavioral, biological and biochemical deficits (50). Consequently, the findings of this study (49) do not exclude the notion that increases in the plasma Trp/LNAA ratio of less than 50% could lead to changes in brain serotonin. Accordingly, it was suggested that even

smaller increases in the plasma Trp/LNAA ratio might influence brain serotonin synthesis (51). Moreover, in another study (52) it was found that a 49.5% decline of the plasma Trp/LNAA ratio was accompanied by significant lower 5-HIAA concentrations in CSF following a balanced diet, while other groups reported that 20-40% changes in the plasma Trp/LNAA ratio led to brain serotonin mediated neuroendocrine alterations in bulimic (53) and dieting subjects (54).

Conclusion

The present study demonstrated a highly significant (48%) increase in the plasma Trp/LNAA ratio by an α-Lactalbumin enriched whey-protein diet (α-Diet) as opposite to the control or casein diet (c-Diet). Only in HS subjects this α-Diet enhanced plasma prolactin, decreased cortisol concentrations and prevented depressive feelings during acute experimental stress. Since increases in cortisol and depressive feelings may reflect a decline in mastery and coping, the present data suggest that a balanced protein diet composed of tryptophan-enriched whey-proteins in healthy but stress-vulnerable subjects improves the ability to cope with stress by enhancing brain serotonin function. We suggest that a balanced protein diet either comprising α-Lactalbumin enriched whey-proteins with increased purity or combined with carbohydrates, might be a way to induce even stronger increases in the plasma Trp/LNAA ratio. More pronounced increases in brain serotonin activity (48) may also lead to clinically important changes in vulnerable subjects under acute stress. Also, to search for direct evidence on diet-induced changes in serotonin neurotransmission in HS subjects, more research is needed on the effect of post-synaptic serotonin agents.

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CHAPTER 6

SUMMARY AND CONCLUDING REMARKS.

During the past two decades there has been a renewed scientific interest in the relationship between food and human behavior. In particular the effects of an increased proportional intake of carbohydrates compared to protein on mood has received much attention. However, a large body of research has yielded inconsistent findings and opposite results have been found in patients and normal subjects. In normal subjects, carbohydrates either have no effects on mood or have contradictory influences on feelings of sleepiness, tension or fatigue (Spring et al., 1987; Spring et al., 1994). Conversely, there is a bulk of literature suggesting that an increased intake of carbohydrates has mood improving effects in patients suffering from depressive symptoms (Rosenthal et al., 1989; Spring et al., 1989; Wurtman et al., 1989). The main purpose of the present thesis was to investigate whether carbohydrate intake improves mood and performance under acute experimental stress in normal subjects suffering from chronic stress, by increasing the plasma Trp/LNAA ratio and, consequently, brain serotonin function. An increased cerebral serotonergic activity is a well established consequence of stress and is believed to constitute a biological condition to improve stress coping and to prevent the negative consequences of stress on mood and cognitive performance. As an increased serotonin activity during stress will lead to a higher breakdown of this neurotransmitter, it is assumed that chronic stress exposure in chronically stressed or stress-prone subjects may ultimately cause a functional shortage of available tryptophan and brain serotonin concentrations. As a consequence, compensatory biochemical changes in the brain serotonergic system may occur predisposing the serotonergic system to respond strongly to a diet-induced increase in available brain tryptophan. Accordingly, particularly under conditions of acute stress exposure, stress-prone subjects may benefit more from a diet-induced increase in the plasma Trp/LNAA ratio. Based on the interrelationship between stress, food, serotonin and mood, stress-proneness may provide a crucial factor explaining the inconsistent findings in normal populations. Furthermore, among patients suffering from a clinical disorder stress-proneness may constitute a common factor contributing to the pathogenesis of a broad range of apparently disparate mood disturbances. In the present chapter first the main findings that have been reported in the empirical chapters (Ch2-5) will be summarized. Then, the internal validity of these studies will be briefly discussed. Next, the main research questions formulated in the first chapter are restated and addressed, and some general conclusions will be provided. Finally, some recommendations for further investigations will be given.

SUMMARIZING THE MAIN EMPIRICAL FINDINGS

In the first study (Ch2), the main purpose was to test whether a carbohydrate-rich, protein-poor diet is able to increase the plasma Trp/LNAA ratio and will only in stress-prone subjects improve mood and cognitive performance under acute uncontrollable experimental stress. It was hypothesized that in stress-prone subjects there is a higher risk of a serotonin deficiency in the brain, due to a chronic stress induced shortage of brain serotonin concentrations. Accordingly, a carbohydrate-rich, protein-poor diet was expected to improve stress coping, mood and cognitive performance by preventing the brain serotonergic system from being overcharged during the confrontation with an actual uncontrollable experimental stressor, presumably by an increased supply of cerebral tryptophan and serotonin concentrations. 24 subjects with a high stress proneness and 24 control subjects with a low stress proneness participated in an uncontrollable stress experiment under both a carbohydrate-rich, protein-poor diet and a protein-rich, carbohydrate-poor diet. Increases in the plasma Trp/LNAA ratio were determined as a peripheral measure for increases in available brain tryptophan and serotonin concentrations. Results of this study revealed a significant 42% increase in the plasma Trp/LNAA ratio during the carbohydrate-rich, protein-poor diet compared with the protein-rich, carbohydrate-poor diet. During the carbohydrate-rich, protein-poor diet, only stress-prone subjects were prevented from a stress-induced rise in depressive mood, a decline in vigor, and cortisol stress responses. Contrary to our expectations, there was no improvement in performance during the Sternberg memory scanning task after completion of the experimental stress task in stress-prone subjects under the carbohydrate-rich, protein-poor diet condition.

Findings from the first experiment raised two important questions. The first question related to the nature of the mechanism underlying the beneficial effect of carbohydrate consumption on mood and cortisol in HS subjects during an acute uncontrollable stress task. Assuming that in stress-prone subjects a high uncontrollable stressor may overcharge the serotonergic system, it was expected that a carbohydrate-rich, protein-poor diet will only in these subjects improve stress coping due to an increased plasma Trp/LNAA ratio and, thus, a rise in available tryptophan and serotonin concentrations. In contrast to what would be expected, stress-prone subjects were not found to be more deteriorated in mood by the experimental stressor than control subjects after consuming the protein-rich, carbohydrate-poor diet. As the serotonergic system becomes more sensitive under chronic stress exposure due to compensatory receptor sensitization (Adell et al., 1988), this led to the suggestion that an increased sensitization of the serotonergic system is a more helpful explanation for the beneficial effects of carbohydrates found in stress-prone subjects. This speculation implicates that the effect of a carbohydrate-rich, protein-poor diet on mood (and cortisol) in stress-prone subjects may also occur in reaction to a controllable experimental stressor. A second question concerns the absence of a dietary effect on cognitive performance in stress-prone subjects. The carbohydrate-rich diet was found

to slow down reaction time in all subjects during the memory scanning task, whereas the diet did not appear to have an exclusive beneficial effect on performance in stress-prone subjects. One possibility that has been offered was that the version of the memory scanning task used in this study was too easy to reveal the hypothesized differentiation in the dietary effect on performance between the stress-prone and control subjects.

In the second study (Ch3), the focus was on the first research question whether the carbohydrate-rich, protein-poor diet is capable of preventing a deterioration of mood and a cortisol response in stress-prone subjects during controllable- as well as uncontrollable experimental stress. Assuming that stress-prone subjects experience chronic stress, it was hypothesized that the serotonergic system in these subjects might have become more sensitive due to compensatory receptor sensitization. Accordingly, a dietary induced increase in the plasma Trp/LNAA ratio, assumed to increase available cerebral tryptophan and serotonin concentrations, was expected to improve stress coping and mood in stress-prone subjects regardless of the controllability of the experimental stressor. Twenty-two subjects with high stress proneness and 23 subjects with low stress proneness participated in a controllable and uncontrollable stress experiment during either a carbohydrate-rich, protein-poor diet or a protein-rich, carbohydrate-poor diet. Both diets were similar to those used in the first study (Ch2). As was expected, only in stress-prone subjects a carbohydrate-rich, protein-poor diet prevented a stress-induced cortisol response [$P=0.02$] and feelings of depression [$P=0.025$] during controllable as well as uncontrollable experimental stress.

In the third experiment (Ch4), it was tested whether a carbohydrate-rich, protein-poor diet improves cognitive performance in stress-prone subjects after acute experimental stress during the Sternberg memory scanning task (that was made more difficult than in the first study). In the first experiment (Ch2) it was found that performance during the Sternberg memory scanning task in stress-prone subjects did not improve by a carbohydrate-rich diet after a high uncontrollable experimental stress task. In the third study, it was tested whether this absence of an effect might have been caused by the low level of difficulty of the memory scanning task or by the highly uncontrollable nature of the experimental stressor. Following either a carbohydrate-rich, protein-poor diet or a protein-rich, carbohydrate-poor diet, 22 high stress-prone subjects and 21 low stress-prone subjects performed a Sternberg memory scanning task after controllable and uncontrollable experimental stress. Only in stress-prone subjects performance on the memory scanning task improved with the carbohydrate-rich, protein-poor diet compared to the protein-rich, carbohydrate-poor diet, exclusively after the controllable experimental stressor. After the uncontrollable experimental stressor, no dietary effects were found on performance. These findings suggest that after completion of an experimental stress task performance in stress-prone subjects may only improve by a carbohydrate-rich, protein-poor diet when the preceding stressor is not too strong.

Data from the previous studies strongly suggest that the effects of a carbohydrate-rich, protein-poor diet on depressive mood and cortisol in stress-prone subjects are caused by an increased plasma Trp/LNAA ratio and thus by an increased availability of brain tryptophan and serotonin concentrations. In the last study (Ch5), it was investigated whether the dietary effects on mood and cortisol in stress-prone subjects are attributable to an increased availability of tryptophan for uptake into the brain and, consequently, to an enhanced brain serotonin function. It was hypothesized that if an increased availability of cerebral tryptophan and serotonin constitutes a crucial factor in the mediation of the dietary effects on mood and cortisol in stress-prone subjects, a balanced protein-rich diet containing proteins with an enriched tryptophan content should have the same effects as those that were previously found with the carbohydrate-rich, protein-poor diet. Since α-Lactalbumin whey-proteins have the highest tryptophan concentration of all bovine protein fractions (Heine, 1996), it was hypothesized that a balanced protein-rich diet containing proteins with an α-Lactalbumin enriched whey-protein fraction should also increase the Trp/LNAA ratio, enhance brain serotonin functioning, and should prevent depressive mood and cortisol responses in stress-prone subjects under acute experimental stress. Twenty-nine high stress-prone subjects and 29 low stress-prone subjects participated in a double-blind placebo-controlled study. They were placed under experimental stress after the intake of a balanced protein-rich diet comprising an α-Lactalbumin enriched whey-protein fraction or the same diet containing placebo proteins (sodium-caseinate). Dietary-induced changes in the plasma Trp/LNAA ratio, prolactin, and 5-hydroxy-indoleacetic acid (5-HIAA) were measured as indices of brain serotonin function. The α-Lactalbumin enriched protein diet caused a significant increase (48%) in the plasma Trp/LNAA ratio. Only in stress-prone subjects this increase in the plasma Trp/LNAA ratio was accompanied by a significant 40% rise in prolactin [P=0.001], and was able to prevent a cortisol stress response [P=0.036] and depressive mood [P=0.007] under acute experimental stress.

INTERNAL VALIDITY

The effects of diet manipulation on mood, cortisol and performance in stress-prone subjects that were presented in the previous empirical chapters do not appear to be caused by differences in dietary intake, oro-sensory quality, or food-related expectancies. Dietary intake during each experimental session was under continuous observation to ensure that all the diets were consumed and that no other foods were taken. Furthermore, subjects were instructed to fast 11 hours before the onset of each experimental day and were excluded from further participation and payment when they did not obey this requirement (they were led to believe that fasting was checked by taking a salivary sample after each dietary condition).

In the first three experiments a diet was used differing in carbohydrates and protein. Yet, it is unlikely that differences in oro-sensory quality between the diets may have

contributed to the main effects reported. Oro-sensory differences do not appear to have a meaningful contribution to the mood effects of carbohydrate consumption (Spring et al., 1989; Wells et al., 1998; see Introduction). Accordingly, findings reported in the last double-blind placebo controlled study (Ch5) revealed similar effects of a diet-induced increase in the plasma Trp/LNAA ratio on mood and stress coping in stress-prone subjects.

Finally, the effects of a dietary manipulation on mood and stress coping do not seem to be caused by differences in food-related expectancies. First, the influence of food-related expectancies on human behavior has been investigated and it is shown that one does not hold personal expectancies about the behavioral effects of nutrients like carbohydrates (Christensen et al., 1985; Spring et al., 1987; Rosenthal et al., 1989). Therefore, it is unlikely that food-related expectancies play an important role in the effects of dietary manipulation on human behavior (Spring et al., 1987) and measurement of such expectations are commonly omitted in dietary studies. Second, subjects in the present studies were always ignorant to the purpose of the experiment and were scheduled within the same dietary condition when participating in the experimental session. Furthermore, as part of the informed consent that was signed before participation in the experiment, subjects were led to believe that they all received identical diets during the morning to compensate for the fasting period and to ensure similar satiating conditions at the onset of the "real" experiment. Accordingly, it appeared that they did not consider the dietary condition as a meaningful factor involved in the experiment, as was established by a brief interview at the end of the last experimental day.

The strongest support for the assumption that the dietary effects on mood and cortisol reported in the previous studies are not mediated by factors other than changes in the plasma Trp/LNAA ratio, originated from the last experiment (Ch5). This study revealed similar effects on mood and cortisol in stress-prone subjects in double-blind controlled dietary trials.

ANSWERING THE MAIN RESEARCH QUESTIONS

1) Does a carbohydrate-rich, protein-poor diet significantly increase the plasma Trp/LNAA ratio and improve stress coping and mood in stress-prone subjects under acute stress ?

In the present thesis, it was demonstrated that a carbohydrate-rich, protein-poor diet comprising 66% of energy from carbohydrates and 3.6% of energy from proteins causes a significant increase in the plasma Trp/LNAA ratio [$P<0.0001$]. This diet-induced increase in the plasma Trp/LNAA ratio confirms previous findings in the literature (Lieberman et al., 1986; Spring et al., 1989; Rosenthal et al., 1989). The carbohydrate-rich, protein-poor diet used in the present studies increased the plasma

Trp/LNAA ratio by 42%, which largely exceeds the 20-25% that is mostly reported in the literature. This marked increase in the plasma Trp/LNAA ratio by the carbohydrate-rich, protein-poor diet used in the present study may be attributed to the low percentage (3.6%) of proteins, which should keep the reducing effect of proteins on the plasma Trp/LNAA ratio small (Yokogoshi & Wurtman, 1986). Most studies have failed to use a test diet that differed from the control diet in this respect.

Findings reported in the present thesis further revealed that the carbohydrate-rich, protein-poor diet slightly but significantly improved feelings of depression and reduced a cortisol response in stress-prone subjects during acute experimental stress. Although the dietary effect on cortisol in the first study (Ch2) was nearly significant, it appeared to be significant in the second and fourth study. A possible explanation for these differences might be the small difference in stress-proneness; in the first study stress-proneness was lower (range 17-30; mean 22) than in the remaining studies (range 20-37; mean 26).

The assumption that a carbohydrate-rich, protein-poor diet in stress-prone subjects improves stress coping is supported here. First, the dietary effect on mood and cortisol in stress-prone subjects was always found during the actual confrontation with the experimental stress task. Also, no direct dietary effects on mood or cortisol were found approximately 1,5-2 hours after diet consumption. Second, the dietary effects on mood appeared to be stronger in stress-prone subjects who actually displayed a physiological stress response (see Ch5). Third, a diet induced improvement of mood under experimental stress was exclusively found for feelings of depression, and was always accompanied by a reduction in cortisol. Since reduced feelings of depression and the lack of a cortisol response are indications of improved stress coping (Mason, 1968; Ursin, 1980; Henry & Meehan, 1981; Henry, 1992; Dantzer, 1993; Ursin & Olff, 1993; Willner, 1993; Dinan, 1994), the dietary influence on depression and cortisol reported in the present thesis suggests that a carbohydrate-rich, protein-poor diet in stress-prone subjects may improve stress coping. Assuming that a carbohydrate-rich, protein-poor diet improves stress adaptation in stress-prone subjects, the question why the scores on the PCQ (a self-constructed questionnaire intended to measure experiences of control; Ch3 & Ch4) in stress-prone subjects did not reveal a stronger sense of control under a carbohydrate-rich, protein-poor diet. Perhaps this self-constructed questionnaire was not sensitive enough to detect small changes in personal experiences of control, whereas it appeared to be suitable to detect the large differences in the experience of control given the controllability or uncontrollability of the stress task.

2) Does a carbohydrate-rich, protein-poor diet prevent a stress-induced deterioration of cognitive performance in stress-prone subjects ?

In the present thesis, the influence of a carbohydrate-rich, protein-poor diet on cognitive performance was studied in two experiments (Ch2 & Ch4). Only in one study (Ch4) it was found that after acute stress the carbohydrate-rich, protein-poor diet improved performance during the Sternberg memory scanning task in stress-prone subjects. In the first study (Ch2), the carbohydrate-rich, protein-poor diet did not improve memory scanning in stress-prone subjects after uncontrollable experimental stress. The absence of a dietary effect on performance in this study was attributed to the simplicity of the memory scanning task; comprising a memory set of 1 to 4 items. However, results form the third study (Ch4) revealed that effects of a carbohydrate-rich, protein-poor diet on performance are dependent on the controllability of the stress task. Hence, the carbohydrate-rich, protein-poor diet in this study only in stress-prone subjects improved memory scanning (reaction time) after a controllable experimental stressor but not after an uncontrollable stressor. Assuming that the carbohydrate-rich, protein-poor diet enhances the availability of cerebral tryptophan and thus serotonin concentrations, it might be that after the uncontrollable stress task the performance of stress-prone subjects was not improved because there was not enough serotonin left for efficient task processing. However, this is a rather hypothetical explanation and it certainly needs further exploration.

3) Is the improvement of mood and stress coping in stress-prone subjects under acute stress with a carbohydrate-rich, protein-poor diet attributable to an increased availability of tryptophan, and an enhanced cerebral serotonergic function ?

Different findings from the present studies suggest that the beneficial effects of the carbohydrate-rich diet on mood and cortisol in stress-prone subjects are mediated by elevations in the plasma Trp/LNAA ratio and by subsequent increases in brain serotonergic function. First, compared to the protein-rich, carbohydrate-poor diet the carbohydrate-rich, protein-poor diet led to a significant increase in the plasma Trp/LNAA ratio. Increases in the plasma Trp/LNAA ratio have been found to enhance central serotonin synthesis (Curzon, 1985; Fernstrom et al., 1971a, 1971b; Fernstrom et al., 1972; see also Ch1). Second, when the effects of a carbohydrate-rich, protein-poor diet on stress coping and mood are due to an increased availability of tryptophan and cerebral serotonin, tryptophan enriched proteins in a double-blind placebo controlled dietary trial will lead to the same effects. In the fourth study (Ch5) this is exactly what was found. Although a protein-rich diet generally has been found to decrease the plasma Trp/LNAA, in this study the protein-rich diet enriched with proteins with high tryptophan content significantly increased the plasma Trp/LNAA ratio in a double-blind placebo controlled dietary trial. This increase in the plasma Trp/LNAA ratio again reduced depressive feelings and prevented a cortisol stress response only in stress-prone subjects. Furthermore, only in stress-prone subjects the diet induced increase in the plasma Trp/LNAA ratio was accompanied by a significant

increase in plasma prolactin. These findings suggest that the effects of a carbohydrate-rich, protein-poor diet on mood and cortisol in stress-prone subjects are mediated by an increased plasma Trp/LNAA ratio and, subsequently, by an enhanced availability of cerebral tryptophan and serotonin. Since increases in prolactin are indices of increases in central serotonergic neurotransmission (Clemens et al., 1980; Delgado et al., 1989), findings of the last study support the hypothesis that a diet-induced increase in the plasma Trp/LNAA ratio in stress-prone subjects improves stress coping and mood by an enhanced brain serotonin function.

This refers to the last question, whether a diet-induced increase in the plasma Trp/LNAA ratio is able to improve stress coping, mood and performance in stress-prone subjects by preventing the negative effects of stress on behavior. Based on the findings reported in the present thesis, it was concluded that the beneficial effects of a diet-induced increase in the plasma Trp/LNAA ratio is more likely to be attributed to an enhanced sensitization of the brain serotonergic system. First, a carbohydrate-rich, protein-poor diet increased the plasma Trp/LNAA and in stress-prone subjects improved mood and stress coping regardless of the controllability of the stressor (Ch2). If the dietary improvement of mood and stress coping was due to the prevention of an acute stress-induced depletion of the brain serotonin level, different behavioral effects should have been found during the controllable and uncontrollable stress task. Second, during the protein-rich, carbohydrate-poor diet mood nor performance deteriorated more in stress-prone subjects compared with controls. Although hypothetical, this may indicate an enhanced sensitization of the serotonergic system, compensating the shortage in brain serotonin concentrations. Third, a diet-induced increase in the plasma Trp/LNAA ratio was accompanied by increases in prolactin *only* in stress-prone subjects. Since increases in prolactin are thought to be indicative of increased receptor sensitization (Clemens et al., 1980; Delgado et al., 1989), these latter findings also suggest that in stress-prone subjects the serotonergic system might have become more sensitive due to chronic stress exposure.

Summarizing, it appeared that a diet-induced increase in the plasma Trp/LNAA ratio causes improved mood and stress coping particularly in stress-prone subjects. This might be caused by compensatory increases in receptor sensitization of the serotonergic system. This sensitization
may contribute to an enhanced serotonergic function under conditions of an increased supply of cerebral tryptophan concentrations.

MAIN CONCLUSION

Findings reported in the present thesis support the hypothesis that an increased proportional intake of carbohydrates relative to proteins, as well as the consumption of tryptophan-enriched proteins, significantly increases the plasma Trp/LNAA ratio, and in stress-prone subjects improves stress coping and mood during actual stress.

Furthermore, a diet induced increase in the plasma Trp/LNAA ratio also improves performance (reaction time) in stress-prone subjects after acute stress exposure, particularly when the preceding stressor is not too strong. Although serotonin activation could not be directly measured in the human brain, the present findings regarding changes in the plasma Trp/LNAA ratio and prolactin suggest that the dietary effects on mood and stress coping in stress-prone are mediated by an enhanced brain serotonin function. Although hypothetical, these dietary effects in stress-prone subjects may be due to a chronic stress induced compensatory receptor sensitization of the serotonergic system.

Findings reported in the present thesis have meaningful consequences for further research. First, it stresses the importance to control for differences in stress vulnerability, and for differences in acute stress experiences, when exploring possible influences of food on mood and performance in normal subjects. Accordingly, differences in stress-proneness may explain some of the inconsistent findings reported in the literature concerning mood improving effects of carbohydrate intake in normal subjects. Second, chronic stress exposure may constitute a common factor involved in the pathogenesis of several apparently disparate clinical disorders like depression, bulimia, or Late Luteal Phase Syndrome. Accordingly, the consistent positive mood effects of carbohydrate consumption reported in these patients may partly be mediated by a chronic stress induced compensatory receptor sensitization of the serotonergic system.

Further investigations are necessary to uphold the hypothesis that the brain serotonergic system may be profoundly enhanced in stress-prone subjects under stress, during a diet induced increase in the plasma Trp/LNAA ratio. One interesting option is to investigate behavioral (mood, coping, performance) and biochemical (amino acids, prolactin, serotonin metabolites) changes under a tryptophan-enriched diet while using drugs that selectively act on the serotonin system. For instance, assuming that the effects of a dietary induced increase in the plasma Trp/LNAA ratio are caused by alterations in brain serotonin function, these effects will disappear by using drugs that antagonise serotonin function. Conversely, drugs that enhance serotonin function (like fenfluramine) may cause a profound effect of a diet induced increase in the plasma Trp/LNAA. Accordingly, a protein diet may be used either comprising alpha-lactalbumin enriched whey-proteins with an increased purity or combined with more carbohydrates, which may lead to a stronger increase of the plasma ratio Trp/LNAA and, consequently, to more profound changes in brain serotonin.

Further investigations may also focus on the pathways through which increases in the plasma Trp/LNAA ratio, and thus available brain tryptophan, improve mood and stress coping in stress-prone subjects. Deakin and colleagues (Deakin, 1991; Graeff et al., 1996) have suggested that an increased brain serotonin function improves stress coping by enhancing the activity of the serotonergic pathway connecting the median raphe nucleus (MRN) to the hippocampus. An enhanced activation of this neuronal

route may "disconnect" the negative consequences of stress perception on the onset of depressive mood and cortisol responses (Deakin, 1991; Graeff et al., 1996). The results reported in the present thesis are in accordance with this idea. However, further research may explore whether the beneficial effects of increases in brain tryptophan and serotonin concentrations are established primarily at higher levels of cortical processing (improving planning, evaluation, and mental control) or lower levels of autonomic activation (directly acting upon limbic structures inhibiting the release of cortisol). LeDoux (1996) mentions that the perception of threat appears to initiate an adrenal-cortical response by parallel transmission to the amygdala; via a direct subcortical route (short autonomic pathway) and via an indirect cortical route (long cortical pathway). Through the direct subcortical route, stressful events may directly initiate a conditioned adreno-cortical stress response without interference of higher cortical or mental processes. Conversely, through the indirect cortical route, which is involved in the planning, evaluation and execution of emotional actions, the brain is provided with a cortical representation of the stressful event and may prevent or override an autonomic adreno-cortical stress response (LeDoux, 1996). Assuming that a diet induced increase in the plasma Trp/LNAA ratio raises brain tryptophan and serotonin concentrations, the question remains whether this improves mood either by enhancing serotonergic activation at the autonomic level (preventing an adreno-cortical response and, consequently, improving mood) or at the level of cortical processes (improving planning, mental control and mood). When a dietary induced increase in the plasma Trp/LNAA ratio enhances serotonin function primarily at the cortical level, alterations in cortisol are not expected to contribute to the mood improvement. Conversely, when a diet induced increase in the plasma Trp/LNAA ratio activates serotonergic cell bodies directly in limbic structures like the hippocampus (inhibiting cortisol), the improvement of mood in stress-prone subjects under stress may also be influenced by the reduced level of cortisol. In order to investigate whether the reported effects are primarily caused by an enhanced serotonergic function at higher cortical levels, one might investigate whether a diet-induced increase in the plasma Trp/LNAA ratio will still improves mood in stress-prone subjects when cortisol is kept constant (for instance by using synthetical glucocorticoids or by drugs that block cortisol release).

Another field of interest is whether a diet induced increase in the plasma Trp/LNAA ratio may additionally increase the therapeutic effects of serotonin releasing drugs used in the treatment of clinical disorders. Assuming that a carbohydrate- or tryptophan-rich diet increases the availability of cerebral tryptophan, such a diet may improve therapeutic effects of serotonin releasing drugs by increasing the supplying of cerebral tryptophan concentrations necessary for the drug to remain effective. For instance, several serotonin releasing drugs are used in the treatment of obese subjects who are dangerously overweight. These serotonin releasing drugs are applied because they are supposed to reduce appetite and food intake by acting on serotonergic cell bodies at the level of the ventromedial hypothalamus (Leibowitz,

1980; Leibowitz & Shor-Postner, 1986). Yet, a long-term weight control by the use of these serotonin releasing drugs is seldom successful, and it appear that only 10-30% of these treated obese subjects are successful in establishing long-term change in body weight. This may be explained partly by the fact that most of these drugs are acting on the paraventricular nucleus of the hypothalamus, thereby particularly reducing the intake of carbohydrate-rich foods and enhancing protein intake (Leibowitz et al., 1989; Stallone & Nicolaidis, 1989). Although an increased supply of brain tryptophan is a necessary basis for these serotonin releasing drugs to enhance brain serotonin synthesis successfully, the reducing effect of these drugs on carbohydrate intake might paradoxically decline the supply of brain tryptophan thereby reducing the plasma Trp/LNAA ratio. Accordingly, when serotonin releasing drugs are not combined with a tryptophan-enriched diet, these drugs ultimately may lead to a shortage of serotonin concentrations and, thus, hamper further improving action of pharmacological treatment (further weight loss). Conversely, when serotonin releasing drugs are accompanied by a tryptophan-enriched diet this may provide the brain with a sufficient supply of available tryptophan, providing a prolonged action of the drug treatment (Markus, Tuiten, Panhuysen et al., unpublished manuscript).

In conclusion, data of the empirical studies presented in the present thesis revealed support for the hypothesis that a large 42-48 % increase in the plasma Trp/LNAA ratio, either by the intake of carbohydrates or tryptophan-enriched proteins, improves mood and stress coping in stress-prone subjects by enhancing brain serotonin function. Nevertheless, it is necessary to collect additional evidence to proof that a rise in cerebral serotonin is indeed involved. The investigation of pharmacological drug effects on mood and stress coping in double-blind dietary trials proofs to be reliable method to uphold the stress-food-mood hypothesis emphasized in the present thesis. Only then we may further explore practical features of carbohydrates or tryptophan-enriched proteins as sweet medicines for depressive feelings under stress.

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SAMENVATTING (SUMMARY IN DUTCH)

Wetenschappers hebben gedurende de laatste twee decennia een omvangrijk aantal studies uitgevoerd naar de relatie tussen voeding en gedrag. Daarbij heeft men effecten gevonden van koolhydraten op de stemming. Onderzoeksresultaten in de literatuur veronderstellen dat een verhoogde consumptie van koolhydraten ten opzichte van proteïne een gunstig effect kan hebben op de stemming; met name bij patiënten met stemmingsstoornissen en psychosomatische aandoeningen (Rosenthal et al., 1989; Spring et al., 1989; Wurtman et al., 1989). Daarentegen worden bij gezonde proefpersonen nauwelijks positieve voedingseffecten gevonden en blijken koolhydraten inconsistente en zelfs tegenstrijdige effecten te hebben op de stemming (Spring et al., 1987; Spring et al., 1994). In dit proefschrift wordt een mogelijke verklaring gegeven voor de tegenstrijdige effecten van voeding op de stemming bij gezonde proefpersonen in vergelijking met patiënten. In vier laboratorium experimenten is onderzocht of een verhoogde consumptie van koolhydraten ten opzichte van proteïne de stressweerbaarheid en dus de stemming kan verbeteren bij gezonde maar stressgevoelige proefpersonen wanneer zij onder acute stress worden geplaatst, en of dit kan worden toegeschreven aan een verhoogde serotonerge activiteit in de hersenen. De neurotransmitter serotonine (5-HT) in de hersenen speelt een belangrijke rol bij stress, stemming en depressie; onder stress wordt de serotonerge activiteit in het brein verhoogd, hetgeen wordt gezien als een biologische voorwaarde voor stresshantering en dus ter voorkoming van de nadelige gevolgen van stress op de stemming en cognitieve prestatie. Koolhydraten kunnen de concentratie van serotonine in de hersenen verhogen via een toename van tryptofaan (precursor van serotonine) in het bloedplsama ten opzichte van de andere grote neutrale aminozuren (de plasma Trp/LNAA ratio). Omdat de toegenomen serotonerge activiteit in het brein onder stress gepaard gaat met een grotere afbraak van deze neurotransmitter, wordt in dit proefschrift aangenomen dat bij mensen die continu onder stress staan (stress-gevoelige personen) er een functioneel tekort kan ontstaan in de hoeveelheid tryptofaan en serotonine beschikbaar in de hersenen. Juist hierdoor zouden stress-gevoelige proefpersonen baat kunnen hebben bij een dieet-geinduceerde verhoging in de plasma ratio Trp/LNAA, met name in situaties van acute stress waarin een verhoogde serotonerge activiteit een vereiste is om met de stressor te kunnen omgaan. Mogelijkerwijze is het serotonerge systeem bij stress-gevoelige mensen vatbaarder geworden voor een dieet-geïnduceerde toename in beschikbare tryptofaan; het serotoninesysteem lijkt gesensitiseerd als biochemische compensatie voor het serotonine tekort. Deze veronderstelde relatie tussen stress, serotonine, voeding en stemming zou de tegenstrijdige resultaten van de effecten van dieetmanipulaties op de stemming bij gezonde proefpersonen kunnen verklaren.

In het eerste hoofdstuk wordt de relatie belicht tussen serotonine en stemming en wordt beschreven op welke wijze dieetmanipulaties de activiteit van serotonine in de

hersenen kunnen beïnvloeden. Vervolgens wordt een samenvatting gegeven van gepubliceerde studies naar de relatie tussen voeding en stemming bij gezonde proefpersonen en bij patiënten. Resultaten uit deze studies laten zien dat koolhydraten met name bij patiënten met affectieve aandoeningen positieve effecten sorteren op stemming, terwijl dergelijke effecten niet of nauwelijks worden gevonden bij gezonde proefpersonen. Vervolgens wordt in het eerste hoofdstuk een beschrijving gegeven van een mogelijke interaktie tussen stress, voeding en stemming die resulteert in de hypothese dat een verrijkte inname van koolhydraten alleen bij stress-gevoelige personen de stemming zal verbeteren onder acute stress.

In hoofdstuk twee wordt een studie beschreven waarin is nagegaan of een koolhydraat-rijk, proteïne-arm dieet de plasma Trp/LNAA ratio kan verhogen en of dit met name bij stress-gevoelige proefpersonen de stemming en cognitieve prestatie kan verbeteren onder acute laboratoriumstress. Resultaten uit deze studie tonen aan dat een koolhydraat-rijk, proteïne-arm dieet de plasma Trp/LNAA ratio met 42% verhoogt en dat dit alleen bij stress-gevoelige proefpersonen resulteert in een verbeterde stemming en een verlaagde cortisol-stressrespons onder acute laboratoriumstress. Er werden geen verschillende dieeteffecten gevonden op de cognitieve prestatie tussen stressgevoelige proefpersonen en proefpersonen uit de controlegroep.

In hoofdstuk 3 wordt een studie gepresenteerd waarin is gekeken of de hiervoor beschreven dieeteffecten op de stemming en cortisol afhankelijk zijn van de controleerbaarheid van de acute stressor. In deze studie wordt uitgegaan van de hypothese dat bij stressgevoelige proefpersonen het serotonine systeem in de hersenen gevoeliger is geworden voor verhogingen in tryptofaan- en serotonine-concentraties in het brein. Als gevolg hiervan zou een verhoogde Trp/LNAA ratio onder een koolhydraat-rijk, proteïne-arm dieet evenredige gevolgen moeten hebben op de stemming en op cortisol tijdens controleerbare en oncontroleerbare laboratoriumstress. Alleen stressgevoelige proefpersonen rapporteerden een verbeterde stemming en vertoonde een verlaagde cortisol response onder zowel controleerbare als oncontroleerbare laboratorium stress.

In hoofdstuk 4 wordt een derde experiment beschreven waarin is onderzocht of het koolhydraat-rijke, proteïne-arme dieet bij stressgevoelige proefpersonen de cognitieve prestatie tijdens een geheugentaak kan verbeteren na controleerbare en oncontroleerbare laboratoriumstress. Alleen stressgevoelige proefpersonen vertoonden een verbeterde prestatie (sneller in memory searching) onder de koolhydraat-rijke dieetconditie uitsluitend na controleerbare laboratorium stress.

De resultaten beschreven in de drie voorafgaande studies veronderstellen dat de effecten van het koolhydraat-rijke, proteïne-arme dieet kunnen worden toegeschreven aan een verhoogde plasma Trp/LNAA ratio en, dus, een verhoogde serotonerge activiteit in de hersenen. In de laatste studie (H5) is onderzocht of de effecten van een dergelijk dieet op stemming en cortisol in stress-gevoelige proefpersonen werkelijk kunnen worden toegeschreven aan een verhoogde

beschikbaarheid van tryptofaan en serotonine in het brein. Indien het werkelijk om een verhoogde beschikbaarheid van tryptofaan gaat dan zouden vergelijkbare effecten op de plasma Trp/LNAA ratio, stemming en cortisol in stress-gevoelige proefpersonen moeten worden gevonden onder een proteïne-dieet waarin gebruik is gemaakt van proteïne verrijkt met tryptofaan. Bovendien zouden eventuele veranderingen in de stemming en cortisol vergezeld moeten gaan met hormonale veranderingen in plasma die onder controle staan van serotonine in het brein. Resultaten laten zien dat een proteïne-dieet opgebouwd uit tryptofaan-verrijkte proteïne (alpha-lactalbumine) de plasma Trp/LNAA met 48% verhoogt en alleen bij stressgevoelige proefpersonen resulteert in een verbeterde stemming, een afname in de cortisolrespons en tevens gepaard gaat met een verhoging in plasma prolactine.

Resultaten van deze studie ondersteunen de hypothese dat een koolhydraat-rijk, proteïne-arm dieet, evenals een proteïne dieet verrijkt met tryptofaan, de plasma Trp/LNAA ratio kan verhogen en met name in stress-gevoelige proefpersonen de stresshantering (gemeten middels stemming en cortisol) kan verbeteren onder acute stress. Ondanks de serotonerge activiteit in het brein niet direct is gemeten, hetgeen een algemene beperking is bij humaan onderzoek, zijn de verhogingen in de plasma Trp/LNAA ratio en prolactine aannemelijke indicatoren voor de betrokkenheid van serotonine in het brein. Uitkomst van deze studie toont tevens het belang om eventuele individuele verschillen in stress-gevoeligheid in verdergaand onderzoek onder controle te houden.

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Utrecht, RU, 6199
(Hendrikus)

CURRICULUM VITAE

Cristiaan Robertino (Rob) Markus was born on October 14th 1963 in Barneveld, The Netherlands. After completing his secondary school education he attended the College of Social Studies (Academy of Social Work) in 1986 where he in 1989 obtained his Bachelor's degree (*cum laude*). From 1988 until 1991 he worked as a social therapist in several clinical institutions. In 1989 he attended to study Psychology at the Utrecht University and in 1993 he obtained his Master's degree from the Department of Psychonomics, majoring in Experimental and Theoretical Psychology. From January 1993 until January 1994 he worked as a Research Associate at the Department of Clinical and Health Psychology at the Utrecht University. Subsequently, from January 1994 through December 1998 he worked as a Research Trainee on the project which culminated in the present thesis at the Department of Psychonomics, Sub-Department of Experimental Psychology, Utrecht University. Rob Markus will continue his research on the relationship between food, the central nervous system and behavior as a research fellow at the TNO Nutrition and Food Research Institute in Zeist, The Netherlands.