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Performance functional foods

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Contents

Preface

This book is about rapid innovation and market change. Functional foods are still new to many of us. There have been other books about them that tend to assume that there is a clear, common definition of functional foods. I consider them to be foods that are designed to perform a function in us other than the avoidance of starvation. There has been much written about the marketing of functional foods and little about the science behind them. This book should help to fill that gap.

In editing this book it has become clear to me that a key issue is whether science is reassuring or worrying consumers. There has been a surprising amount of fundamental science done on traditional products such as ginseng, caffeine and the other substances reviewed by experts in this book. The sheer quantity and at times complexity of this research might help to overcome some people's natural reticence about consuming biologically active chemicals in staple items of their diet. But from a regulator's point of view, it boils down to how well science is answering the following questions:

- What evidence underpins the claims that might be made about a functional food?
- Are such foods safe? (Surely one cannot rely solely on largely historical surmise that a product new to our diet must be safe because it has been used in another context for centuries?)

It is of growing importance that these questions are answered. For some time there have been indications that some rather unusual herbal and other ingredients were starting to appear in a range of products such as soft

drinks. To me, at least, it was odd to see claims on some of these products' labels that my health would improve in a very specific way if I drank particular products. This seemed something more than the usual marketing ploys. Admittedly, I brought with me a background in natural toxicants in food that aroused particular interest in such claims. But what got me really interested in this topic was the extent to which new products containing 'traditional' plant medicines were being launched, particularly in the USA. What happens in the USA in food manufacturing and marketing tends to happen soon after on the other side of the 'pond'. I wanted to know more to be able to make an effective judgement as a consumer.

I first read about functional foods in one of the 'heavy' Sunday newspapers in the USA and saw them being bought in everyday life from shops in New England. However, I lacked the objective scientific information to assess the health claims associated with these functional products that, as a chemist, I have been trained to review. Since then there has been widespread debate in the media about foods that help fight disease. This debate even seems to be extending to the functional benefits of a traditional fungal toxin – alcohol! There are moves, as I write, to regulate further on label claims in the European Union and there are already EU controls on novel foods. It is essential that science does not get left behind in assessing and regulating performance and other functional foods. Airing the scientific work is a key part of keeping science linked to the debate. This book contributes objective information and commentary to a growing discussion that might otherwise lead to consumer rejection, as it has already in some parts of Europe, of irradiated food.

Given the potentially controversial nature of this subject, it is especially important for me to make my usual statement: the views above and those of the chapter authors in this book are our own and not necessarily those of our employers.

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Introduction

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The global functional foods market was estimated to be worth $47.6 billion in 2001, having grown by nearly 60% in six years (Sloan, 2002). The regional breakdown of the market is shown in Table 1.1. Perhaps the most mature market for products linked specifically to improvements in mood and cognitive performance is Germany, with overall functional food sales estimated at $5.6 million in 2001. Herbal supplements such as *Ginkgo biloba* and St John's wort have been on the market since the 1960s.

Other markets have developed more recently, notably the USA during the 1990s. The share of the health-care market in the USA represented by products claiming to improve mood and cognitive function is shown in Table 1.2. Consumer research suggests that depression is one of the fastest-growing health issues in the USA. Over 80% of US consumers report the need to reduce stress (Roper/CHPA, 2001). As a result of these concerns, 60% of consumers have expressed an interest in foods that enhance mood (MSI, 2001; NMI, 2002). Just under 70% of US consumers also expressed an interest in trying foods which improve cognitive function (MSI, 2001). Indeed, 14% of US consumers say they already choose healthy foods and beverages because they improve mental performance (Roche, 2001).

The plants most closely associated with improvements in mood and cognitive function are: *Ginkgo biloba*, ginseng, St John's wort and kava kava. Although they represent a modest percentage of the total health care product and herbal supplements market in the United States (Table 1.3), sales exposure of these showed significant growth during the 1990s, as

Table 1.1 The functional food market in 2001

	$ billion	% share (rounded)
Total	47.6	100
USA	18.3	38
Europe	15.4	32
Japan	11.8	25
Rest of world	2.1	4

Source: adapted from *Nutrition Business Journal*

Table 1.2 Top 10 categories in the US health-care products market in 2001

Category	% (rounded)
Sports/energy/weight loss	26
General health	24
Joint health	5
Cold/flu/immune system	4
Heart health	4
Bone health	4
Cancer prevention	3
Diabetes	2
Mood	2
Cognitive function	2

Source: adapted from *Nutrition Business Journal*

Table 1.3 US sales of herbal supplements influencing mood and cognition ($ millions)

	2000	% total
Total market for all herbal supplements	4000	100
Ginkgo biloba	250	6.25
Ginseng	173	4.3
St John's wort	170	4.25
Kava kava	53	1.3

Source: adapted from SPINS/A C Nielsen

measured by the number of new product launches, with growth slowing in the period 2000–2001 (Table 1.4). New product launches for *Ginkgo biloba* and St John's wort, in particular, slowed significantly, suggesting a maturing market.

Table 1.4 Top 10 herbal supplements in the United States, measured by new product launches*

Herb	1993	1995	1997	1999	2001	% of total product launches in 2001
Ginseng	97	202	205	258	223	14
Echinacea	61	165	131	196	226	14
Saw palmetto	21	135	87	177	189	12
Kava kava	–	52	122	142	171	11
Valerian	42	114	67	181	160	10
Ginger	22	121	45	140	146	9
St John's wort	–	76	104	240	141	9
Milk thistle	17	71	57	148	139	8
Garlic	42	147	23	184	126	8
Ginkgo biloba	33	36	144	227	92	6

* Totals measure the number of times a herb is mentioned as an ingredient.
Source: adapted from Marketing Intelligence Service

A key issue in ensuring continued stable, long term growth for this sector is to consolidate the research on the complex links between food chemical safety, the use of herbal and other supplements, mood and cognitive performance. This collection seeks to review the range of research in this important new area. Chapter 2 discusses recent research on the interactions between stress, food and mood, including the role of carbohydrates. The following chapter surveys the evidence for the impact of a number of nutritional and other supplements on both mood and cognitive performance, ranging from vitamins to herbs such as St John's wort and kava kava. A final introductory chapter looks at the range of medicinal plants which have an effect on mental and physical performance. Following these introductory reviews, a number of chapters consider specific nutrients and herbs. There are chapters on phyto-oestrogens and polyphenols, ginseng and *Ginkgo biloba*, functional ingredients in sports drinks and the recent research on the impact of caffeine on mood and mental performance.

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2

Interactions between stress, food and mood

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2.1 Introduction

There is no longer any doubt that what we eat can influence mood and mental performance. In the context of mood, the intake of carbohydrates and protein has received the most attention. Profound effects of an increased intake of carbohydrates on mood have been particularly detected among depressive patients, whereas in non-clinical subjects often no effects or just slight and contradictory changes in mood are revealed (e.g. Spring et al., 1987; Christensen, 1997; Bellisle et al., 1998). This chapter reviews the most commonly mentioned neurochemical basis underlying the carbohydrate–mood relationship and provides an explanation for some of the inconsistent mood-improving effects of carbohydrate intake. Firstly, biochemical mechanisms involved in mood and the effect of food on mood will be described. Then some of the inconsistent findings in non-clinical populations as opposed to clinical populations are reviewed. Finally, the importance of stress and stress-induced biochemical adaptations that may predispose the human brain to the behavioral effects of carbohydrate consumption is discussed. In addition, individual differences in susceptibility to stress may partly explain the inconsistent findings among normal populations and possibly constitute a commonality among patients suffering from a variety of mood and behavioral disorders.

2.2 Brain mechanisms involved in mood

Since the early 1990s, behavioral scientists have gained more insight about the general outline of brain mechanisms involved in emotional activity and

mood changes. Based on evidence from animal research and observation of trauma patients, it is known that a change in emotional state involves the activation of different integrated neuronal circuits in the brain rather than individual brain regions. These brain circuits are mainly organized across three hierarchical levels including the hindbrain, the midbrain and the neocortex and the most frequently described are those involving the prefrontal cortex, basal ganglia, thalamus, hippocampus and the amygdala (e.g. Thompson, 1990; Davidson et al., 2002). For instance, within the hindbrain several circuits are interrelated and involve the elicitation of somatic and visceral activity during physical arousal and forward all kinds of sensory information to higher brain centers. Most of the structures involved are located at the base of the brain. These serve the elemental needs for food, protection and defense. At the level of the midbrain, circuits particularly within the limbic system and prefrontal cortex contribute to the inhibition and excitation of emotional activity by stimulating cortical arousal through the reticular activating tract and providing hedonic tone of our mood via activation of the pain tract or periventricular system and the pleasure tract or medial forebrain bundle (e.g. Thompson, 1990). The main structures involved are the hypothalamus, the amygdala and the hippocampus. At the level of the forebrain, the frontal lobes in particular integrate sensory information from the internal and external environment with plans and memory and decide whether to increase or inhibit emotional activity. It is believed that the frontal lobes involve evaluating and planning action and expression and are the site of our conscious emotional experiences.

Within the different brain circuits and structures involved in mood, particular neurotransmitters are involved in controlling and connecting different neuronal circuits in a hierarchical fashion so that each contributes to a more complex organized level of mood. These neurotransmitters function at the level of the synapse, selectively altering neural communication and activation. Among the neurotransmitters involved, the monoamine serotonin (5-HT) system has received the most attention. The serotonin system projects from the midline raphe nuclei of the brainstem to different parts of the brainstem, the spinal cord, the forebrain limbic structures and the cerebral cortex.

A significant role of 5-HT in mood and the pathogenesis of mood disorders has been generally accepted (e.g. Praag, 1980; Maes and Meltzer, 1995). First indications were found almost 40 years ago, when the mood-improving effects of tricyclic antidepressants with a preferential action on the serotonergic system were observed. These drugs increased synaptic concentrations of serotonin and were able to improve mood and reduce depressive symptoms. Conversely, serotonin-depleting drugs were found to induce depressive mood. Such observations led to the assumption that a reduced level of brain serotonin contributes to the development of mood disturbances, and that increased serotonergic transmission plays an important

role in the therapeutic effects of antidepressants. Although this monoamine hypothesis of mood endured several revisions (e.g. Heninger et al., 1996; Duman et al., 1997), the significance of a decline in serotonin function in the onset and course of mood deterioration has been generally accepted. Evidence for this stems from different research paradigms. First, reduced serotonergic function appears a common factor in various mood disorders. This is indicated by lower availability of the plasma 5-HT precursor 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. For instance, tryptophan depletion was found to lead to a modest increase in depressive feelings in normal men (Young et al., 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 increasing brain serotonin synthesis and function (Delgado et al., 1990; Delgado et al., 1993; Maes and Meltzer, 1995).

2.3 Food, the brain and mood

2.3.1 The biochemical mechanism

There is no longer any doubt that certain foods are able to modify brain chemistry and function involved in mood and emotional behavior. In the context of mood, the macronutrient carbohydrate and its effect on brain 5-HT synthesis is the most relevant. The connection between carbohydrate intake and brain 5-HT occurs because the production of this neurotransmitter is limited by the availability of its precursor, dietary amino acid tryptophan. Brain concentrations of this precursor were found to be controlled by the intake of carbohydrates as compared to protein.

Since tryptophan cannot be enzymatically synthesized in mammals, this precursor for the synthesis of 5-HT must come from protein sources. However, in a sequence of impressive studies Fernstrom and Wurtman demonstrated that a balanced or protein-rich diet causes a decline in brain tryptophan and serotonin concentrations. Contrarily, a carbohydrate-rich, protein-poor diet caused the opposite effects and was found to increase brain tryptophan and serotonin levels (e.g. Fernstrom and Wurtman, 1971; Fernstrom et al., 1973). This apparently anomalous observation is explained by the following biochemical mechanism.

Brain serotonin synthesis is limited by the influx of available plasma tryptophan into the central nervous system. However, plasma tryptophan competes with the other large neutral amino acids, valine, tyrosine,

isoleucine, leucine and phenylalanine (the LNAAs), for transport across the blood-brain barrier into the brain since they share the same transport carrier. Consequently, increased transport of tryptophan into the brain does not depend on the total concentration of plasma tryptophan but is proportional to the ratio of its concentration to that of the sum of the other competing LNAAs in plasma (plasma Trp/LNAA ratio). 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 declining Trp/LNAA ratio has the opposite effect. Even though proteins contain a certain percentage of tryptophan, the intake of proteins causes a decline in the plasma Trp/LNAA ratio because they contribute proportionately more competing LNAAs. Consequently, a balanced or protein-rich diet reduces the available tryptophan for transport across the blood-brain barrier into the brain and reduces brain 5-HT synthesis. 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 and Wurtman, 1971; Fernstrom et al., 1973; Curzon, 1985; Wurtman, 1987).

This increase is produced by a carbohydrate-induced elevation of glucose and insulin that causes the LNAAs, with the exception of tryptophan, to be taken up into the skeletal muscles. Increases in insulin cause the free fatty acids to be stripped away from albumin circulating in the blood by promoting their uptake by adipocytes. Since unbound albumin loosely binds with tryptophan, tryptophan is prevented from being taken up in the periphery, whereas the insulin response leads to a meaningful fall in free circulating plasma amino acids. So, an increase in bound tryptophan compensates for the slight fall in circulating free tryptophan caused by an insulin-induced increase in periphery uptake. In addition, the total amount of plasma tryptophan (free plus bound) determines the rate of influx of tryptophan into the brain because the affinity of the transport carrier at the blood-brain barrier for tryptophan is much greater than that of albumin for tryptophan.

2.3.2 Inconsistent dietary effects in the literature

Inasmuch as the brain 5-HT system was known to play an important role in the control of mood, and the ingestion of carbohydrates had been reported to have a mood-improving effect, it was soon suggested that carbohydrate ingestion improves mood by increasing brain 5-HT. However, results reveal that the beneficial effect of carbohydrates particularly appears in clinical subjects, whereas in non-clinical subjects dietary effects on mood are rather inconsistent.

2.4 Carbohydrates and mood

2.4.1 Effects in clinical subjects

Increased intake of carbohydrates as compared with protein is frequently found to improve mood and depressive symptoms in clinical subjects who suffer from, for instance, carbohydrate craving obesity, late luteal phase syndrome or seasonal affective disorders (e.g. Wurtman and Wurtman, 1984; 1986; Lieberman et al., 1986b; Rosenthal et al., 1989; Wurtman et al., 1989; Steinberg et al., 1994; Sayegh et al., 1995). Most of these subjects crave carbohydrate-rich food particularly when they feel most depressed, probably as a way of self-medication by increasing brain 5-HT. For instance, in a study by Lieberman et al. (1986b), the effect of a carbohydrate lunch on mood was measured among carbohydrate craving and non-carbohydrate craving, obese subjects. After a standard breakfast, mood was measured before and after all subjects received a carbohydrate test lunch. 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 test lunch.

Therapeutic effects of carbohydrates have also been found in women with late luteal phase syndrome (Wurtman et al., 1989; Sayegh et al., 1995). For instance, Wurtman and colleagues measured the occurrence and coincidence of depressive mood and carbohydrate intake among PMS patients and 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 and during the second 48-hour period they were in their late luteal or premenstrual phase. During both periods, subjects were allowed to eat as much as they liked from standardized high carbohydrate- or high protein-meals and between-meal snacks. 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. Sayegh et al. (1995) also examined the effects of carbohydrate loading 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 reduced feelings of anger and depression.

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 depressed patients suffering from seasonal affective disorder and control subjects. Using a crossover design, subjects consumed a standardized breakfast and received two different isocaloric standard meals for lunch: one rich in protein and one rich in carbohydrates. Mood changes and blood amino acids were measured every hour until 3 hours after the test lunch. The carbohydrate-rich diet resulted in an approximately 27% increase in the plasma Trp/LNAA and a decrease following the protein diet in all subjects. However, only patients reported improved mood following the carbohydrate diet, whereas the control group reported enhanced feelings of sedation.

2.4.2 General effects in healthy subjects

Although beneficial effects of carbohydrates on mood and depression have been clearly demonstrated among clinical populations, in non-patients the mood effects of carbohydrates are less consistent; revealing either no effects on mood (Lieberman et al., 1986a; Smith et al., 1988; Deijen et al. 1989; Reid and Hammersley, 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 and Pollet, 1982/83; Spring et al., 1982/83; Christensen et al., 1985; Spring et al., 1989; Lloyd et al., 1996). For instance, in a double-blind, within-subjects experiment of Leathwood and Pollet (1982/83), changes in mood were measured after a carbohydrate-rich, protein-poor breakfast containing either tryptophan, L-tyrosine, caffeine or a placebo. Before and several times after breakfasting, mood was measured. It was shown that the carbohydrate-rich breakfast supplemented with tryptophan significantly caused a decline in vigor, whereas the breakfast supplemented with caffeine increased wakefulness and energy. In a study of Spring et al. (1982/83), contradictory mood effects of carbohydrates were found depending on gender and age. In a between-subjects design, subjects were randomly assigned to either a carbohydrate-rich, protein-poor diet or a protein-rich, carbohydrate-poor diet, offered as breakfast or lunch. Mood was measured once 2 hours after dietary intake. 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 that were 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.

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 one week intervals. During the study the women ate a standard breakfast in the morning and at about noon either a lunch or a fasting period followed. During lunch, the test meals were given in a counterbalanced order across days comprising a carbohydrate lunch, a balanced lunch and a protein lunch. Blood samples and mood measures were taken before and several times after lunch. 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.

2.4.3 Negative effects in healthy subjects

Negative effects of carbohydrates on mood in healthy subjects have also been demonstrated by Christensen et al. (1985). 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, sugar or a placebo. Mood measures were taken before and after the dietary challenge as well as after the sugar challenge. Results revealed that the two week period of consuming the protein diet without sugar, caffeine and alcohol led to improvement of mood and distress. Moreover, in two subjects sucrose challenge, after the dietary manipulation period, increased feelings of fatigue and decreased feelings of vigor.

Besides inconsistent effects found regarding the influence of carbohydrates on mood in normal subjects, studies also revealed no effect of carbohydrates on mood (Lieberman et al., 1986b; Smith et al., 1988; Deijen et al., 1989; Reid and Hammersley, 1995). For instance, in a study by Lieberman et al. (1986a) male subjects 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. No differences were found between dietary conditions. In a study by Smith et al. (1988), the mood effect of a protein, starch or sugar lunch was tested in male and female subjects. After a standardized breakfast, subjects consumed a test lunch comprising a high protein meal, a high starch meal and a high sugar meal. Mood was measured before and after the test lunch. However, no significant effects of diet composition on mood were found. Reid and Hammersley (1995) examined mood and food-intake effects of a carbohydrate load among male and female subjects. Subjects fasted overnight and were divided into 3 dietary conditions during which they received plain water or a drink containing either sucrose or saccharin. Before intake, test- and placebo-load subjects took a benzocaine anesthetic and wore a nose clip in order to eliminate sensory cues such as

taste and texture. Mood was measured before and several times after the load. Results revealed no effect of the sucrose or saccharin load on mood.

2.4.4 Conclusion: effect of carbohydrate on mood

In summary, the consumption of a carbohydrate-rich, protein-poor meal is found to enhance brain tryptophan and 5-HT. In clinical subjects, such a diet has frequently been found to improve mood and depressive symptoms, whereas in non-clinical populations the effects of carbohydrates on mood are rather inconsistent; they reveal modest positive as well as negative effects of carbohydrate consumption or no effects at all. The following section discusses the possibility that these inconsistent findings on the beneficial effects of an increased proportional intake of carbohydrates may be partly explained by individual differences in proneness to stress.

2.5 Interactions 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 potent environmental factors are stressful events (Brown et al., 1987). 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 and Kennett, 1983; Stanford, 1993) and it is suggested that an increased serotonin function in the brain constitutes a biological condition for coping with stress and for preventing stress-induced mood deterioration (Anisman and Zacharko, 1991; Deakin, 1991; Delbende et al., 1992; Graeff et al., 1996). The involvement of brain 5-HT in stress and stress-induced alterations in mood offers a likely explanation for the inconsistent behavioral effects of food in normal subjects as compared with clinical subjects. It is hypothesized that continuous stress exposure predisposes the brain for positive effects of a diet-induced increase in available brain tryptophan, due to compensatory biochemical 5-HT alterations (Markus et al., 1998, Markus et al., 2000).

2.5.1 Biological stress mechanisms

It is hardly possible to see mood and emotional reactivity without some sort of physiological arousal and bodily responses. These responses are based on the activity of different branches of the central nervous system that integrates various stress inputs into a series of autonomic changes needed for stress adaptation. The brain serotonergic system plays a prominent role in stress regulation: it controls the activity of the central mechanisms and, in turn, mediates the influence of stress perception on the onset and course of stress responsiveness and mood changes.

During the perception of threat, different areas in the brain are stimulated to relay information about the sensory properties of the stressful event through the brain, thereby bringing the central nervous system and the body to a higher level of readiness. First, there is a direct sympathetic activation that stimulates the release of both 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 forced 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 re-establish physiological balance. 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, among other things, the release of corticotropin-releasing hormone (CRH). This hormone, in turn, is 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 pro-opiomelanocortin. Adrenocorticotropic hormone is transported by the blood to stimulate chromaphin cells within the adrenal cortex to release glucocorticoid cortisol. Cortisol, in turn, re-establishes the internal balance of the nervous system and the body that was disrupted by the stress perception by exerting a variety of actions throughout the brain in order to terminate the stress response and to prepare the organism for coping with stress. These effects of cortisol are regulated by fast and slow negative feedback actions on several levels of the hypothalamic-pituitary-adrenal axis (HPA). 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 CRH 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 coping with stress, cortisol concentrations measured in saliva or in the blood vary predictably with different psychological demands and with the appearance of depressive symptoms (Mason, 1968a, 1968b; Frankenhaeuser et al., 1980; Frankenhaeuser, 1986; Ursin and Olff, 1993). For instance, Frankenaeuser and colleagues (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 reduced levels of cortisol. In general, catecholamine responses appear to be correlated with general arousal and effort, whereas cortisol responses are associated with poor stress coping and feelings of helplessness or depression (Mason, 1968a; Ursin, 1980; Henry and Meehan, 1981; Henry, 1992; Dantzer, 1993; Ursin and Olff, 1993; Willner, 1993; Dinan, 1994).

2.5.2 Brain serotonin and stress adaptation

Previous findings demonstrated that biological alterations at the level of the HPA are necessarily involved in stress adaptation. In addition, a stress-induced change in activity of this system is under the control of the brain serotonergic system (Fuller, 1992; Maes and Meltzer, 1995). Pharmacological studies using the serotonin precursor tryptophan, serotonin-releasing drugs such as fenfluramine or drugs that act directly upon serotonin receptors have shown that increases in serotonin concentration may alter HPA activation. For instance, administration of tryptophan has been found to alter ACTH and cortisol release by 5-HT_1 and 5-HT_2 receptors at the hypothalamic level (enhancing CRH 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 concentrations in the blood.

Based on these findings, it is suggested that increased functioning of the brain serotonergic system constitutes a biological condition to cope with stress and to prevent a stress-induced deterioration of mood (Deakin, 1991; Anisman and Zacharko, 1992; Graeff et al., 1996). Hence, brain serotonin activity increases under stress (Joseph and Kennett, 1983; Stanford, 1993) and controls HPA activation (Deakin, 1991; Fuller, 1992; Dinan, 1994; Graeff et al., 1996), whereas lowered serotonin function has been related to disturbed or hyperactive HPA functioning (Barden and Holsboer, 1995; Maes and Meltzer, 1995) and to stress-related mood disorders such as depression (Praag, 1980; Maes and Meltzer, 1995).

2.5.3 Stress alterations in 5-HT and the effects of food on mood

Since brain serotonin activity increases as a way to cope with stress and to prevent mood deterioration, the inconsistent dietary effects on mood may

partly be explained by individual differences in stress proneness and subsequent stress-mediated biochemical alterations within the brain serotonin system (Markus et al., 1998, 2000). Because stress leads to an enhanced activity of the central serotonergic system (Joseph and Kennett, 1983; Stanford, 1993), continued stress exposure requires continuous elevations in brain serotonin function. In addition, frequent experiences of distress as found in stress-prone or emotionally-vulnerable subjects may ultimately increase the risk of a shortage of available brain tryptophan and serotonin concentrations. In support of this notion, chronic stress-induced depletion of brain serotonin has clearly been demonstrated in animal research (Kennett et al., 1985; Adell et al., 1988). Stress-induced depletion of brain serotonin may result in the following two mechanisms. Brain serotonin activity might be enhanced more by temporal increases in available tryptophan for uptake into the brain, due to chronic, stress-induced, compensatory receptor sensitization. In support of this, a chronic stress-induced depletion of brain serotonin has been found to enhance serotonin sensitization in rats (Kennett et al., 1985; Adell et al., 1988). Alternatively, a chronic stress-induced reduction in brain serotonin levels may increase the risk that the serotonergic system will be overtaxed under severe, acute stress conditions that require sufficient increases in brain serotonin activity; this causes a critical decline of available brain tryptophan and serotonin levels. Based on these assumptions, it is likely that stress-prone subjects with an emotional vulnerability to frequently experienced, daily stress may benefit from dietary-induced increases in brain tryptophan and serotonin levels. In addition, the beneficial effects of dietary-induced alterations in brain tryptophan and serotonin levels in these subjects may be more pronounced during acute stress.

Indirect support for the assertion that stress may mediate the beneficial behavioral effects of dietary-induced alteration in brain tryptophan and serotonin levels comes from studies demonstrating increased preferences for carbohydrate-rich food particularly during stressful events (Oliver et al., 2000; Wardle and Gibson, 2002). For instance, in a study by Oliver and colleagues (2000) food choice was measured during the stress caused by public speaking in 68 healthy subjects. Stressed, emotionally-susceptible subjects ate more high-carbohydrate fatty foods than unstressed, non-susceptible subjects.

More direct support for the hypothesis that stress mediates the beneficial mood and behavioral effects of food-induced alterations in available tryptophan for uptake into the brain has recently been found in our laboratory (Markus et al., 1998, 2000, 2002). For instance, in one study (Markus et al., 1998), 24 healthy subjects with a high proneness to stress and 24 healthy control subjects with a low stress proneness participated in an acute stress experiment using both a carbohydrate-rich, protein-poor diet and a protein-rich, carbohydrate-poor diet. Results 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 and a cortisol stress response. Beneficial effects of this dietary condition on cognitive performance have also been demonstrated exclusively in stress-prone subjects (Markus et al., 1999).

A recent study investigated whether increasing tryptophan in dietary protein in a double-blind, placebo-controlled design could also increase plasma Trp/LNAA and mood depending on stress-proneness (Markus et al., 2000). It was suggested by the authors that if an increased availability of cerebral tryptophan and serotonin constitutes a crucial factor in the mediation of the dietary effects on mood 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 alpha-lactalbumin whey-proteins have the highest tryptophan concentration of all bovine protein fractions, it was hypothesized that a balanced protein-rich diet containing proteins with an alpha-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 healthy but stress-prone subjects under acute experimental stress. After the intake of a balanced protein-rich diet comprising an alpha-lactalbumin enriched whey-protein fraction or the same diet containing placebo proteins (sodium caseinate), 29 stress-prone subjects and 29 low stress prone subjects were placed under experimental stress. The alpha-lactalbumin enriched protein diet caused a significant increase (47%) in the plasma Trp/LNAA ratio. Only in stress-prone subjects was this increase in the plasma Trp/LNAA ratio able to prevent a cortisol stress response and depressive mood under acute experimental stress.

2.6 Conclusions and future trends

For the relationship between food and mental performance, the effects of an increased proportional intake of carbohydrates compared to protein on brain 5-HT and mood have received the most attention. Opposite dietary effects have been found on mood in patients and normal subjects. In normal subjects, carbohydrates either have no effects on mood or have contradictory influences, whereas in clinical subjects there is a bulk of literature suggesting that an increased intake of carbohydrates has mood-improving effects particularly in those who suffer from severe depressive symptoms. Recent findings reveal that the beneficial mood and behavioral effects of dietary-induced alterations in brain tryptophan and 5-HT in healthy subjects are mediated by stress. In addition, stress-prone subjects in particular may benefit from dietary-induced alterations in brain tryptophan

and 5-HT, probably due to chronic stress-induced alterations in 5-HT sensitization.

Recent findings on the relation between stress, food, brain 5-HT and mood may have meaningful consequences for further research. First, it stresses the importance to control for individual differences in stress-proneness when exploring possible influences of food on mood in normal subjects. Accordingly, differences in stress-proneness may (partly) explain some of the inconsistent findings reported in the literature concerning the mood-improving effects of carbohydrate intake in normal subjects. Second, an emotional vulnerability to experiencing mental stress frequently may constitute a common factor involved in the pathogenesis of several apparently disparate clinical disorders like depression, late luteal phase syndrome and eating disorders like bulimia, and other disorders that seem to share the brain 5-HT mechanism as a likely mechanism involved. However, additional research is necessary to test this hypothesis. In the near future, we have to explore the influence of continued mental stress, and individual differences in stress-proneness, on the onset and course of brain 5-HT dysfunction by more direct cerebral assessments. In addition, we have to investigate whether an emotional vulnerability to experiencing stress frequently is a common factor involved in mood and behavioral disorders, and whether they share a 5-HT vulnerability for dietary induced alterations in brain tryptophan and 5-HT levels either or not in combination with pharmacological treatment.

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3

Mood, cognitive function and nutritional and other supplements

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3.1 Introduction

This survey reviews a number of nutrients, herbal and other supplements that have been linked to improvements in mood and cognitive function. It begins with a range of nutrients: selected B vitamins, the antioxidant vitamins C and E, omega-3 fatty acids, and phospholipids such as lecithin. It then reviews a range of herbs including St John's wort, kava, *Ginkgo biloba* and ginseng, as well as acetyl-L-carnitine.

3.2 B vitamins

There have been a number of studies suggesting that taking B vitamins may produce improvements in mood and cognitive function (Benton et al., 1995). Studies indicate that increased intake of thiamine (vitamin B_1) provides cognitive benefits, including increased reaction times (Benton et al., 1997; Wilkinson et al., 1997). Work has also been done on the coenzyme form of niacin (vitamin B_3) known as nicotinamide adenine dinucleotide (NADH). A small number of short-term studies done with NADH have shown that it has slight to moderate benefits for depression, Parkinson's disease, Alzheimer's disease and chronic fatigue syndrome (Birkmayer et al., 1991, 1993, and 1996; Forsyth et al., 1998).

Homocysteine, a derivative of the amino acid methionine, has been identified as an important risk factor in both heart disease and age-related cognitive decline (Parnetti et al., 1997; Refsum et al., 1998). It has been shown that adequate intake of folic acid, vitamins B_6 and B_{12} helps to ensure that

homocysteine levels are kept low (Woodside et al., 1998). One study investigated the relationship between blood concentrations of homocysteine, vitamins B_{12}, B_6 and folate, and the cognitive performance of 70 male subjects aged between 54 and 81 (Riggs et al., 1996). Lower concentrations of vitamin B_{12} and folate, and higher concentrations of homocysteine, were associated with poorer memory.

S-adenosyl-methionine (SAMe), a compound made from the amino acid methionine, is a methyl donor involved in the syntheses of dozens of important compounds in the body. SAMe is required in numerous methylation reactions involving nucleic acids, proteins, phospholipids, amines and other neurotransmitters. It has been suggested that SAMe supplementation may also help folate and vitamin B_{12} metabolism and thus alleviate such conditions as depression, dementia and peripheral neuropathy (Bottiglieri et al., 1994; Cestaro, 1994). A survey of European research on SAMe concluded that the efficacy of using SAMe in treating depressive syndromes and disorders was superior to that of placebo and comparable to that of standard tri-cyclic antidepressants (Bressa, 1994). Other studies have confirmed its potential value in treating depression (Salmaggi et al., 1993; Bell et al., 1994). Another methyl donor, dimethyl-amino-ethanol (DMAE), has been shown to help improve mood and motivation in older patients with dementia though studies show limited impact on memory (Ferris et al., 1977; Caffarra, 1980; Fisman et al., 1981).

3.3 Antioxidants

The cell membranes of neurons are made mostly of phospholipids which contain fatty acids. Nerve fibres travelling from the brain to the spinal cord, and from the spinal cord to the rest of the body, are also insulated with a white-coloured fatty substance called myelin. With time, these fats can become oxidized, interfering with proper nerve activity. This process of lipid peroxidation contributes to brain ageing and can accelerate degenerative disorders such as Alzheimer's disease. A number of studies suggest that patients with Alzheimer's disease may have impaired antioxidant defence mechanisms (De Deyn et al., 1998; Marcus et al., 1998). Long term studies also suggest that the use of antioxidants may help to counteract lipid peroxidation over time and thus alleviate conditions such as Alzheimer's. One study over a 20 year period at the University of Bern in Switzerland, for example, found that higher levels of antioxidants, particularly vitamin C and beta-carotene, were associated with better performance in memory testing (Seitz et al., 1998). Epidemiological studies also suggest that older individuals with high levels of antioxidants in their bloodstream, including vitamin E, maintain a sharper memory (Perkins et al., 1999), a finding also supported by animal studies (Joseph et al., 1998). A number of studies have shown that the use of vitamins E and C supplements may help reduce the

risk of developing Alzheimer's disease and in slowing down the progression of the disease (Sano et al., 1997; Morris et al., 1998). Other studies have examined the potentially therapeutic role of thyme oil and other natural antioxidants which enter the brain such as the soy isoflavones (Deans et al., 1993).

Lipoic acid (LA), also known as alpha-lipoic acid, is a natural coenzyme important in the regulation of carbohydrate metabolism and has important antioxidant properties. It can regenerate other antioxidants such as vitamin C and vitamin E, and raises intracellular glutathione levels, making it potentially useful in the treatment of oxidative brain and neural disorders involving free-radical processes (Packer et al., 1997). Animal studies suggest some improvement in memory function with the use of LA (Stoll et al., 1993).

Some studies have concentrated on the problem of plaque, a characteristic microlesion found in the brains of subjects with age-related cognitive decline and Alzheimer's. The main component of plaque is a peptide called Beta-amyloid. In Down's Syndrome, for example, an excess of Beta-amyloid is produced and the incidence of early onset Alzheimer's is very high (Mann, 1989). Beta amyloid has also been shown to be toxic to nerve cells, producing free radicals which oxidize polyunsaturated fatty acids (PUFAs) in the nerve cell membranes (Davis, 1995; Bradbury, 1996; Kalaria and Hedeira, 1996). The neurotoxic effects of beta-amyloid can be blocked by antioxidants such as vitamin E (Behl et al., 1992).

3.4 Polyunsaturated fatty acids

Around 60% of the brain consists of lipids that make up the cell membrane of every brain cell. The types of fats present in the brain influence the fluidity of the cell membrane and thus how well brain cells interact and communicate. The fats that make up brain-cell membranes are much more resistant to changes in diet than the fats forming the cell membranes of other tissues in the body. The brain is, for example, able to preserve its fatty composition despite shortages of essential fats in the diet. However, animal studies show it is possible to alter the fat content of the brain through diet (Yehuda et al., 1998). Building on this foundation, epidemiological studies suggest that differing types of fat in the diet may be linked to mental state. Hibbeln (1998) compared fish consumption and rates of depression in a number of countries and concluded that there was a link between increasing rates of depression in the twentieth century, the consumption of increased amounts of saturated fatty acids and omega-6 fatty acids, and the decreased consumption of omega-3 fatty acids found in fish. Studies also indicate that levels of docosahexanoic acid (DHA), a particularly important omega-3 fatty acid, are low in red-blood-cell membranes in depressed subjects (Peet et al., 1998).

A double-blind placebo-controlled study by Stoll et al. (1996) showed that patients suffering from manic-depression responded positively to concentrated fish oils that are rich in omega-3 fatty acids. Dietary supplementation with fish oil has also been found to alleviate the symptoms of schizophrenia (Laugharne et al., 1996; Peet et al., 1998). The latter study suggested that eicosapentaenoic acid (EPA), another very important omega-3 fatty acid, may be more effective in some circumstances than is DHA. Alpha-linolenic acid (ALA), an omega-3 fatty acid found predominantly in flaxseed and hemp-seed oil, was also found to have antidepressant effects (Stoll et al., 1993; Sahelian, 2000). However, studies suggest that not all individuals are able to metabolize ALA effectively (Drevon, 1992; Gerster, 1998). There have been fewer studies on the effects of fish oils on cognitive function, although animal studies suggest some enhancing effect (Suzuki et al., 1998).

It is known that the intake of PUFAs from such sources as fish oil is crucial in the neonatal development of the retina and the brain, which contains very high levels of PUFAs in the membranes of brain cells (Feldman et al., 1992). If the mother eats a grossly deficient diet in the three to six months before or during pregnancy, the lack of PUFAs may lead to premature, low birth-weight babies with brain damage such as cerebral palsy (Clandidin et al., 1992; Recsan et al., 1995). In lesser cases, babies may develop visual problems and learning deficits (Neuringer and Connor, 1989). Conversely, PUFAs from such sources as fish oil have been found to improve infants' brain development and learning ability (Makrides et al., 1995).

3.5 Phospholipids

3.5.1 Definition and role

Phospholipids are found in high concentrations in the lining of almost every cell of the body, including brain cells. They are compounds made of two fatty acids attached to glycerol, the mineral phosphorus, and an amine. The two most common fatty acids attached to phospholipids in the brain are DHA, an omega-3 fatty acid, and arachidonic acid (AA), an omega-6 fatty acid. Phospholipids play several roles in the brain. They not only determine the flow of nutrients in and out of the cell, but also influence communication between brain cells by influencing the shape of receptors and promoting the growth of dendrites. The fatty-acid composition of phospholipids can deteriorate with ageing and disease. With ageing, many of the long-chained polyunsaturated fatty acids, such as DHA, can become shortened and more saturated. This can interfere with the optimal functioning of neurons. Animal studies have indicated that omega-3 fatty acids added to

the diet of rats are able to travel to the brain-cell membranes and become part of the phospholipids present in the cell (Jumpsen et al., 1997).

3.5.2 Levels in the human body

Dietary intake of phospholipids is at a historic low due to trends in food consumption, such as a reduced intake of offal meats and increased use of refined oils (Cairella et al., 1994). Levels of phospholipids may reach 3% in virgin oils, but in refined oils they are virtually undetectable. Production of phospholipids in the liver may be also sub-optimal because it is complex, slow and energy intensive. It is slowed even further by multiple micronutrient depletion, which is common in the elderly. Antioxidant depletion also increases the rate of phospholipid oxidation. As a result, phospholipid profiles in cell membranes (and in the brain) deteriorate in brain with age, with particularly significant reductions in phospatidyl serine. This membrane shift is a major component in the ageing process in many tissues (Deans et al., 1993 and 1994). Increased oxidation of phospholipids, and the resulting accumulation of the oxidation end-product, lipofucsin, leads to a progressive loss of membrane and of other cellular functions (Nandy et al., 1988; Deans et al., 1993 and 1994).

3.5.3 Phosphatidylcholine and phosphatidylserine

Phosphatidylcholine (PC) is the most abundant phospholipid in brain-cell membranes, comprising about 30% of the total phospholipid content, while phosphatidylserine (PS) makes up less than 10%. Several studies have been done with PC to investigate its effects on memory. The results of the studies have not been consistent. Some have shown positive responses (Sorgatz, 1987; Ladd et al., 1993), while others showed no difference in memory or learning after administration of lecithin, a mix of phospholipids derived from sources such as egg, soy and meats in which PC predominates (Gillin et al., 1981).

There have been a number of studies evaluating the role of bovine cortex phosphatidylserine (BC-PS) in cognitive function, particularly in age-associated memory impairment and Alzheimer's disease. Most of these studies have indicated that BC-PS improves memory and cognition in those with age-related cognitive decline (Crook et al., 1991; Cenacchi et al., 1993), and helps improve memory and recall in patients with Alzheimer's disease (Crook et al., 1992; Engel et al., 1992).

Phosphatidylserine supplementation has been used to improve membrane and particularly nerve cell membrane function. In elderly animals, many of the symptoms of an ageing brain (including failing circadian and oestral rhythms, memory loss, and the loss of nerve cell connections in the brain) can be prevented or reversed by increased PS intake (Toffano, 1987;

Nunzi et al., 1990). There are over 40 clinical studies of PS used to treat Alzheimer's, and its pre-clinical precursor Age-Related Cognitive Decline (ARCD). These trials show improved learning and memory, enhanced recall, and maintenance of concentration while reading, conversing and performing various tasks (Palmieri et al., 1987; Crook et al., 1991; Crook et al., 1992). Due to methodological weaknesses, these studies, although encouraging, need to be confirmed.

3.5.4 Choline

Choline, widely available in foods such as eggs, fish, legumes, nuts, meats and vegetables, helps form both PC and acetylcholine, which also influences memory. According to the results of several animal studies, providing choline during pregnancy enhances memory and learning capacity in the foetus (Zeisel, 1997; Williams et al., 1998). The results of adult studies have been mixed, with some showing positive results (Sitaran et al., 1978) but others showing no improvement (Mohs et al., 1980). Choline has been used in the treatment of manic-depression and in improving mental performance in patients with Alzheimer's disease (Stoll et al., 1996; Cacabelos et al., 1996). Cytidine 5-diphosphocholine (CDP-choline) is a potent form of choline. Studies suggest that CDP-choline helps make phosphatidylcholine (PC) in human brain-cell membranes in older individuals (Babb et al., 1996); improves mental performance in patients with Alzheimer's disease (Cacabelos et al., 1996); and improves memory in elderly patients with memory deficits (Alvarez et al., 1997; Porciatti et al., 1998).

3.6 Aluminium

The course of Alzheimer's may be exacerbated in some cases by depletion of the trace element manganese. Electrochemical activity involved in the transmission of information between neurons creates wear and tear in the synapses. Repairing this damage involves transporting material between the synapses and the cell body, in axonal microtubules comprised of Tau proteins. In a healthy cell, the Tau proteins remain separate. Under certain circumstances, Tau proteins stick together and build up into the helical filaments found in the brain 'tangles' that are a characteristic feature of Alzheimer's.

Some studies have shown that in Alzheimer's, Tau proteins in the filaments have too many phosphate groups attached to them – known as hyperphosphorylated Tau, or Hyper-T (Iqbal et al., 1989; Lee et al., 1991). Hyper-T has been found to disrupt axonal flow (Alonso et al., 1994). In Alzheimer's disease it has been suggested that the nerves die back because of disruption in axonal flow degeneration (Braak et al., 1994). A reduction in the phosphorylation of Tau proteins might restore axonal flow and help to slow the course of Alzheimer's disease. One group of enzymes, the Tau

phosphatases, does this. These enzymes have been shown to be underactive in Alzheimer's brain tissue (Gong et al., 1993). They can be activated by the trace element manganese, which has been suggested as a nutritional factor in the management of Alzheimer's (Gong et al., 1994). This hypothesis is still being debated. Some studies suggest that most Tau proteins in the paired helical filaments are not over-phosphorylated (Lai et al., 1995; Wischik et al., 1995). Some suggest that hyper-phosphorylated Tau might discourage, rather than encourage, filament formation (Wischik et al., 1995).

Some studies also suggest that chronic accumulation of aluminium in the brain may contribute to, or accelerate, damage in the brain such as that found in Alzheimer's (Harrington et al., 1994). Aluminium in the body causes cell damage, dysfunction and brain damage. A number of epidemiological studies have suggested that areas where the water had the highest content of soluble aluminium also had the highest incidence of Alzheimer's disease (Flaten, 1990; Martyn, 1992). Further investigation indicated that it was in areas where water was high in aluminium and low in silicon (which has a neutralizing effect on aluminium) that the incidence of Alzheimer's was highest. In areas where the water was silicon-rich, and aluminium-poor, Alzheimer's was relatively uncommon (Birchall and Chappell, 1989). Where silicon is in excess of aluminium, aluminium salts are precipitated out as biologically inert aluminium silicate, or sand.

3.7 Nerve growth and cerebrovascular factors

Cell suicide in the brain can be triggered by hormonal changes that occur with age. Normally, nerve cells are sustained by a range of nerve growth factors. If the growth factors are not present, cells die. Oestrogen in hormone replacement therapy (HRT) may function as a nerve growth factor (Gould et al., 1990). This has been suggested as an explanation of why women who take HRT have a lower risk of developing Alzheimer's (Henderson et al., 1994; Tang et al., 1996). If true, a high intake of soy products (which contain oestrogen-like compounds), for example, might have a similar protective effect.

Another factor which can trigger apoptotic cell death is a sustained reduction in the oxygen supply. About 50% of all dementias, including Alzheimer's, have a cerebrovascular component. Sustained oxygen depletion, as in cerebrovascular atheroma, causes neuronal apoptosis; sudden oxygen deprivation, as after a stroke, causes cell necrosis. Circulatory problems are therefore risk factors for dementia, and studies have indeed linked hypertension with an increased risk of Alzheimer's (Skoog et al., 1996). A course of anti-atheroma nutrition reduces the likelihood of cerebral infarcts, or mini-strokes, which can exacerbate the underlying Alzheimer's and reduce the loss of brain tissue if a stroke does occur (Van der Worp et al., 1998).

3.8 Herbal and other supplements

3.8.1 St John's wort

St John's wort (*Hypericum perforatum* L.) is a shrubby, aromatic perennial herb belonging to the family of Hypericaceae. It is native to Europe, western Asia and North Africa, and is naturalized in North America and Australia (Hobbs, 1989). A variety of St John's wort preparations is commercially available, including tablets, extracts and juice, produced under a variety of conditions such as air drying, grinding and extraction with water, sunflower or olive oil, ethanol, methanol, glycerol or supercritical carbon dioxide.

Hyperforin is one of the main components (2–4%) of the dried herb and has been shown to inhibit the reuptake of serotonin, dopamine and norepinephrine. It has therefore been identified as the most likely antidepressant compound in St John's wort (Bhattacharya et al., 1998; Chatterjee et al., 1998a, 1998b; Muller et al., 1998; Singer et al., 1999). Hyperforin has been shown to be a dose-related marker for antidepressant efficacy in humans (Dimpfel et al., 1998; Laakmann et al., 1998; Shellenberg et al., 1998). Another component of St John's wort believed to have antidepressant effects is the flavonoid amentoflavone (Baureithel et al., 1997; Nahrstedt and Butterweck, 1997). Baureithel et al. (1977) suggested that amentoflavone exerts its antidepressant action by binding benzodiazepine receptors in the brain. Clinical studies have indicated that St John's wort has antidepressant effects, with successful treatment of patients with mild and moderate depression (Linde et al., 1996; Hippius, 1998; Lenoir et al., 1999).

In an analysis of 23 randomized trials involving over 1700 patients, Linde et al. (1996) found St John's wort to be significantly superior to a placebo and as effective as standard antidepressants with fewer side effects. Although it is successful in treating mild to moderate depression, it is not effective in treating severe depression (Shelton et al., 2001). A number of recent studies have confirmed its role in treating moderate depression (Philipp et al., 1999; Brenner et al., 2000; Schrader, 2000; Stevinson and Ernst, 2000; Woelk, 2000).

3.8.2 Kava

The root of the kava plant (*Piper methysticum*) contains a variety of chemicals known as kavalactones. Specific names for some of these kavalactones include kawain, dihydrokawain, methysticin, and yangonin. Kavalactones influence a number of the brain receptors involved in relaxation and mental clarity. Its anti-anxiety effects have been noted in a number of human studies. One of the most comprehensive human trials, involving giving a course of kavalactones to over 100 patients with a range of anxiety disorders, found improvements in tension and mood (Volz and Kieser, 1997). The German Commission E Monograph on herbal medicines, issued by the

Federal Institute for Drugs, has summarized the effects of kava on anxiety as well as on mental alertness and concentration (Blumenthal, 2000). These include enhanced reaction times in dealing with a range of mental and verbal tasks as well as improvements in mood. Kava is licensed in both Germany and France as a drug for conditions such as mild anxiety and sleep disorders. Other studies discussing its use include those by Sahelian (1998); Muller and Komorek (1999); Nemecz and Lee (1999); and Pepping (1999). The liver toxicity issue is now largely discounted (Waller, 2002).

3.8.3 Adaptogens and other herbal extracts

Adaptogens

Ashwagandha (*Withania somnifera*), a shrub cultivated in India and North America, contains flavonoids, together with several active ingredients of the withanolide class, and is a powerful antioxidant (Elsakka et al., 1990; Bhattacharya et al., 1997; Dhuley, 1998).

Huperzine A is an extract from a club moss (*Huperzia serrata*) that has been used for centuries in Chinese folk medicine. Its action has been attributed to its ability to inhibit strongly acetylcholinesterase, the enzyme that breaks down acetylcholine in the synaptic cleft between neurons. Acetylcholine is involved in memory and learning. By inhibiting the enzyme that breaks it down, more acetylcholine becomes available to stimulate neurons (Cheng et al., 1996). Huperzine A has been found to improve cognitive function and memory in older individuals suffering from Alzheimer's disease and dementia (Zhang et al., 1991; Xu et al., 1995; Skolnick, 1997).

Vinpocetine is chemically related to, and derived from, vincamine, an alkaloid found in the periwinkle plant. It has been found to dilate blood vessels, enhance circulation in the brain, improve oxygen utilization, make red blood cells more pliable, and inhibit aggregation of platelets (Kiss and Karpati, 1996). Vinpocetine may even have antioxidant properties (Orvisky et al., 1997). Studies have found that vinpocetine improved cognitive performance and memory in both subjects suffering from dementia and chronic cerebral dysfunction (Balesteri et al., 1987; Hindmarch et al., 1991) though not in patients suffering from Alzheimer's disease (Thal et al., 1989). It has also been found to improve cognitive performance in healthy subjects (Subhan, 1985).

Other herbals

Ginkgo biloba contains flavonoids, particularly kaempferol, quercetin and isorhamnetin, and terpene lactones, particularly ginkgolides and bilobides. The active ingredients in ginkgo are believed to produce their beneficial effects by acting as antioxidants, preventing red blood cells and platelets from aggregating to form clots, allowing more oxygen to reach neurons, and by inducing relaxation of the muscles surrounding blood vessels (Chung et al., 1999). Since it improves communication between

nerve cells and enhances blood flow to the brain, it has been used in the treatment of Parkinson's disease and Alzheimer's, helping to improve cognitive performance (Le Bars et al., 1997). *Ginkgo biloba* is discussed in more detail in Chapter 7.

There are several genera of ginseng: Asian ginseng (*Panax ginseng*), American ginseng (*Panax quinquefolius*) and Siberian ginseng (*Eleutherococcus chinensis*). The roots of Chinese and American ginseng contain several saponins named ginsenosides. They inhibit the formation of lipid peroxides (fat oxidation) in cardiac muscle or in the liver, and decrease blood coagulation, cholesterol, and sugar levels in blood (Purmova and Opletal, 1995). They stimulate the immune system and may have anti-tumour properties (Wakabayashi et al., 1998). Studies of ginseng use in humans have shown both improvements in cognitive function (D'Angelo et al., 1986), including its use in combination with *Ginkgo biloba* (Wesnes et al., 1997), as well as reduced anxiety and greater sense of well-being (Sotaniemi et al., 1995; Caso Marasco et al., 1996). Ginseng is discussed in more detail in Chapters 4 and 6.

3.8.4 Acetyl-L-carnitine

Acetyl-L-carnitine (ALC) has been identified as helpful to those with Alzheimer's disease, age-related cognitive decline and depression. It helps form the important brain chemical acetylcholine, a neurotransmitter which is depleted in patients with Alzheimer's, and may also, by improving mitochondrial stability and energy throughput, boost neuronal ergonomics and repair mechanisms. Animal studies have also found that administration of ALC can induce the production of nerve-growth factor, a type of protein that helps regenerate neurons (Piovesan et al., 1994). Some patients with Alzheimer's are deficient in the enzyme that converts carnitine to acetyl-L-carnitine, and may benefit from ALC supplement (Kalaria and Harik, 1992). There have been a number of studies on the use of ALC in the treatment of Alzheimer's disease suggesting a slower rate of deterioration for some groups taking ALC supplements (Rai et al., 1990; Spagnoli et al., 1991; Pettegrew et al., 1995; Thal et al., 1996). Acetyl-L-carnitine has also been found to improve cognitive functioning and mood in those suffering from age-related cognitive decline (Bella et al., 1990; Cipolli and Chiari, 1990; Passeri et al., 1990).

3.9 References

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4

The range of medicinal plants influencing mental and physical performance

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4.1 Introduction

The use of plants for medicinal reasons started thousands of years ago and is still a major part of medical practice in most parts of the world despite significant progress in modern medical and pharmaceutical developments. The World Health Organization has estimated that as much as 80% of the world's population relies mainly on traditional medicine in the form of plants and plant extracts (WHO/IUCN/WWF, 1993). In recent years, due to the desire of the general public for alternative medicines and nutraceuticals, medicinal plants have rapidly regained the greater position that they possessed in past centuries in Western medicine. This has resulted in a tremendous surge of interest in medicinal plants and their products have become a multibillion dollar industry in Europe, North America and, recently, Asia. In the USA alone, the consumption of medicinal herbs is rising at approximately 15% annually. The current value of the functional food market in Europe, at $14 billion, is second only to that of the USA at $17 billion (Gruenwald and Pearl, 2000). This market trend has attracted considerable attention from industry and the research community leading to increasing volumes of research and development in medicinal plants and nutraceuticals.

In the past, almost all of the uses of medicinal plants for the treatment of human ailments were based on tradition, folklore or accidental discovery. Recent research has generated considerable information such as identifying biologically active components that are responsible for the claimed therapeutic effects. These scientific findings lead to a better understanding of the principles of medicinal plant uses. However, numerous complicated

mechanisms are likely to be involved in the various actions of a single plant, and more in-depth research is needed for medicinal plants to be accepted into the mainstream medical world.

4.2 Medicinal plants, mental and physical performance

The herbal medicine industry has expanded rapidly in the last decade and herbal products are readily available in drugstores and supermarkets. It has been estimated that one of every three Americans has used herbal remedies (Brevoort, 1998) either to maintain general health, to improve mental and physical performance, or to search for a cure for specific ailments. On the other hand, any toxicity or side effects (see Section 4.5) that occur during the consumption of herbs may have reverse effects on the level of performance. To understand how medicinal plants may influence or improve the mental and physical performance of human beings, it is necessary to realize that there are many factors playing major roles.

Healthy body: Regular physical activity throughout life is important for maintaining a healthy body and is required for the maintenance of certain levels of physical performance. Exercise, keeping to a proper body weight, ingesting a balanced diet and eliminating bad habits, such as smoking, drinking, drug dependency and substance abuse, are key factors for the maintenance of a healthy body (Last, 1997).

Mental health: Mental health is a state of successful mental functioning or performance resulting in productive activities and the ability to adapt, change and cope with adversity. Depression is one of the most common disorders affecting the level of mental performance. It is often associated with other medical conditions, such as heart disease, cancer and diabetes, as well as anxiety and eating disorders (Anonymous, 2000), and these need to be addressed before depression can be treated successfully.

Ageing: Every single human being faces an ageing process, which starts from birth and becomes a major concern after reaching middle age. One theory is that ageing is caused by the oxidants produced by mitochondria as by-products of normal metabolism (Liu et al., 2002). Reduced capacity for physical activity and a lowering of mental agility are two common complaints among mature or older adults. However, age-related changes may be slowed by regular exercise, a hazard-less environment, proper nutrition with herbal supplements (Riedel and Jorissen, 1998) and dietary supplements such as acetyl-L-carnitine and alpha-lipoic acid (Liu et al., 2002).

Stress: A stress-free environment has never existed in the world. Everybody will, sooner or later, face some kind of stress in everyday life. Stress presents a complex assault on the psychological, physiological, and biochemical processes in the body (Kapoor et al., 2001). Any kind of stress, be

it psychological (Takemura et al., 1999), or related to job, family, finances, or the environment, will influence physical and mental performance levels (Flynn, 1996).

Disease: A healthy body is capable of achieving the highest level of mental and physical performance within each age group and this can be impaired by disease. If the disease is cured the person should be able to return to the desired performance level.

Nutrition and fluid intake: Normal nutritional practices have a major impact on the health of human beings. The nutrient requirements depend on the activity level of the individual. A calorie-rich carbohydrate diet with low levels of fat and protein is beneficial; those who exercise regularly require more calories and an increase of most nutrients (Bruce et al., 1985; Caso et al., 1996). Water is another important yet often forgotten item for a healthy body. Inadequate fluid in the body may cause discomfort in the form of muscle cramps, reduced strength, headaches, dizziness and nausea.

Environment: The quality of the environment is a global concern, because it plays a major role in the health of individuals and thus directly affects mental and physical performance. Environmental concerns centre on air and water quality, soil, transportation, land use, industry and agriculture (Anonymous, 2000).

4.3 Functions of medicinal plants on mental and physical performance

Recently, researchers from botany, chemistry, medicine, pharmacognosy, and pharmacology have joined forces with traditional herbalists and manufacturers to promote research. The claimed therapeutic value of medicinal plants has gradually switched from traditional folklore to scientifically-based fact. During the last decade, people have started to realize that certain medicinal plants can not only serve as useful surrogates for prescription drugs to treat certain diseases but also may contribute to the maintenance of general health with proper mental and physical performance. It was reported that mental health can be improved by reducing stress with essential fatty acid supplements such as linoleic acid and alpha-linolenic acid (Kapoor et al., 2001) and these can be found in many medicinal herbs.

Some of the natural compounds in medicinal plants may prove to be relevant to human health and so directly affect physical performance. The major functions of the phytochemicals in medicinal plants are preventative. They act by stimulating the immune system or by suppressing human ailments. Medicinal plants with preventative functions include herbs used for their adaptogenic and tonic capacities. Medicinal plants with adaptogenic

Table 4.1 Medicinal plants with adaptogenic effects on human health

Scientific name	Common name
Aralia racemosa L.	Spikenard
Codonopsis pilosula (Franch) Nannfeldt.	Codonopsis
Eleutherococcus senticosus (Rupr. ex Maxim) Maxim	Siberian ginseng
Ganoderma lucidam (Leyss. Fr.) Karst.	Reishi
Panax ginseng C. A. Mayer	Asian ginseng
P. notoginseng (Burk.) F. H. Chen	Tian Qi
P. quinquefolium L.	American ginseng
Rhodiola rosea (L.) Scop.	Roseroot or golden root
Schisandra chinensis (Turcz.) Baill	Schizandra
Syringa vulgaris L.	Lilac
Withania somnifera Dunal	Ashwagandha (Indian ginseng)

effects (Table 4.1) assist the body to adapt to stress and support normal functions. An adaptogen is a substance that is safe, increases resistance to stress, and has a balancing effect on body function. Plants with tonic functions may exert a restorative or nourishing action on the body by increasing or restoring physical or mental tone. A tonic is a substance that exerts a gentle strengthening effect on the body. Besides this general function, a tonic may have a more specific effect on digestive, cardiac, liver or uterine functions (Table 4.2). Natural compounds that suppress the progression of particular ailments include the herbs *Eleutherococcus senticosus*, *Glycyrrhiza glabra*, *Panax ginseng* or *P. quinquefolium*, and *Rhodiola rosea* to treat stress (Flynn, 1996; Darbinyan et al., 2000). Natural compounds in medicinal plants used to treat specific ailments can also improve physical and mental performance indirectly, such as taxol for cancer (Whiterup et al., 1990; Wickremesinhe and Arteca, 1998), stevioside for diabetes (Duke and duCellier, 1993; Brown et al., 1998), hypericin for depression (Jakovljevic et al., 2000) and ginkgolides for Alzheimer's disease (Perry et al., 1999).

It has been proved that some medicinal plants are valuable alternatives to or supplements for essential nutrient requirements. It is well known that plants have a highly advanced chemical potential, especially with respect to the synthesis of different and often complex molecules. Some of these compounds are already being utilized, while others are being developed for medicinal and dietary applications. In the course of daily living, human beings are inevitably exposed to the by-products of normal oxidative metabolism which could eventually cause the collapse of the health defence system in the body and directly affect physical performance. It has been

Table 4.2 Medicinal plants with tonic effects on human health

Function	Scientific name	Common name
General	*Acer rubrum* L.	Maple
	Achillea millefolium L.	Yarrow
	Acorus calamus L.	Sweet flag
	Agathosma betulina (Berg.) Pillans	Buchu
	Agrimonia eupatoria L.	Agrimony
	Agropyrum repens (L.) Beav.	Couch grass
	Aletris farinosa L.	Aletris
	Allium cepa L.	Onion
	Aloe socotrina (L.) Burm. f.	Aloe
	Anethum graveolens L.	Dill
	Apium graveolens L.	Celery
	Arctium lappa L.	Burdock
	Arctostaphylos uva-ursi (L.) Spreng	Bearberry
	Arnica latifolia Bong	Arnica
	Artemisia absinthium L.	Wormwood
	Asarum canadense L.	Ginger
	Astragalus americana Bunge	Astragalus
	Avena sativa L.	Oats
	Berberis vulgaris L.	Barberry
	Capsicum frutescens L.	Capsicum
	Castanea dentata (Marsh.) Borkh	Chestnut
	Chelone glabra L.	Balmony
	Chenopodium album L.	Lamb's quarters
	Chimaphila umbellata Nutt.	Pipsissewa
	Cichorium intybus L.	Chicory
	Cimicifuga racemosa (L.) Nutt.	Black cohosh
	Cinnamomum verum J. S. Presl.	Cinnamon
	Citrus aurantium L.	Bitter orange
	Citrus aurantium L. var. sinensis	Sweet orange
	Codonopsis pilosula (Franch) Nannfeldt.	Codonopsis
	Coix lachryma-jobi L.	Job's tears
	Coptis groenlandica Salisb.	Gold thread
	Cornus florida L.	American dogwood
	Crocus sativus L.	Saffron
	Cypripedium calceolus L.	Lady's slipper
	Cytisus scoparius (L.) Link.	Broom tops
	Eleutherococcus senticosus (Rupr. ex Maxim) Maxim.	Siberian ginseng
	Ephedra nevadensis Wats	Mormon tea
	Equisetum arvense L.	Horse tail
	Eriodictyon californicum (Hook. et Arn.) Torr.	Mountain balm
	Eucalyptus globulus Labill.	Eucalyptus
	Eupatorium perfoliatum L.	Boneset
	Euphrasia officinalis L.	Eyebright
	Fagus sylvatica L.	Beech tree
	Fraxinus americana L.	White ash
	Ganoderma lucidum (Leyss. ex. Fr.) Karst.	Ganoderma
	Glecoma hederacea L.	Ground ivy
	Hamamelis virginica L.	Witch hazel

Table 4.2 *Continued*

Function	Scientific name	Common name
	Humulus lupulus L.	Hops
	Hydrangea arborescens L.	Hydrangea
	Hydrastis canadensis L.	Goldenseal
	Hyssopus officinalis L.	Hyssop
	Iris versicolor L.	Blue flag
	Juglans nigra L.	Black walnut
	Larrea tridentata (DC) Cov.	Chaparral
	Laurus sassafras (Nutt.) Nees.	Lavender
	Lonicera caerulea L.	Honeysuckle
	Lycium barbarum L.	Wolfberry
	Magnolia virginiana L.	Magnolia
	Marrubium vulgare L.	Horsehound
	Matricaria chamomilla L.	Chamomile
	Matricaria recutita L.	German chamomile
	Medicago sativa L.	Alfalfa
	Myrica cerifera L.	Bayberry
	Myristica fragrans Houtt.	Nutmeg
	Myrtus communis L.	Myrtle
	Nasturtium officinale R. Br.	Water cress
	Nepeta cataria L.	Catnip
	Origanum vulgare L.	Marjoram
	Paeonia lactiflora Pall.	White peony
	Paeonia suffruiticosa Andr.	Tree peony
	Panax ginseng C. A. Meyer	Asian ginseng
	Panax quinquefolium L.	American ginseng
	Petraselinum crispum Nym. ex A. W. Hill	Parsley
	Piper methysticum Forst.	Kava
	Piper nigrum L.	Pipper
	Piscidia piscipula (L.) Sarg.	Dogwood
	Plantago major L.	Plantain
	Podophyllum peltatum L.	May apple
	Polygenum bistorta L.	Bistort root
	Populus tremuloides Michx.	Poplar
	Prunus virginiana L.	Choke cherry
	Pyrethrum parthenium Sm.	Feverfew
	Quercus robur L.	Oak
	Quillaja saponaria Mol.	Quillaia
	Rosa canina L.	Dog rose
	Rosa laevigata Michx	Cherokee rosehip
	Rosmarinus officinalis L.	Rosemary
	Rubus chamaemorus L.	Cloudberry
	Rubus fruiticosus L.	Blackberry
	Rubus idaeus L.	Raspberry
	Salix nigra Marsh.	Willow
	Salvia officinalis L.	Sage
	Sanguinaria canadensis L.	Bloodroot
	Sassafras albidum (Nutt.) Nees	Sassafras
	Satureja hortensis L.	Savory
	Saussurea lappa Clarke	Costus

Table 4.2 *Continued*

Function	Scientific name	Common name
	Schisandra chinesis (Turcz.) Ball.	Schizandra
	Scutellaria baicalensis Georgi	Baical skullcap
	Serenoa repens (Bart.) Small.	Saw palmetto
	Simmondsia chinensis (Link) C. Schneid.	Jojoba
	Smilax medica Schlecht.	Sarsaparilla
	Solanum nigrum L.	Garden nightshade
	Solidago canadensis L.	Golden rod
	Stachys officinalis (L.) Trev.	Betony
	Stellaria media (L.) Vill.	Chickweed
	Sterculia urens Roxb.	Karaya gum
	Syringa vulgaris L.	Lilac
	Tanacetum vulgare L.	Tansy
	Taraxacum officinale G.H. Weber ex Wagg.	Dandelion
	Thymus vulgaris L.	Thyme
	Tilia cordata Mill.	Linden
	Turnera diffusa Willd.	Damiana
	Tussilago farfara L.	Coltsfoot
	Urtica dioca L.	Nettle
	Vaccinium myrtillus L.	Huckleberry
	Valerian officinalis L.	Valerian
	Verbascum blattaria L.	Mullein
	Verbena hastata L.	Wild hyssop
	Vinca minor L.	Periwinkle
	Viola tricolor L.	Pansy
	Vitis quinquefolia Lam.	American ivy
	Withania somnifera Dunal	Indian ginseng
	Zanthaxylum americanum Mill.	Prickly ash
Diarrhoea	*Geranium macrorrhizum* L.	Geranium
Digestion	*Agrimonia eupatoria* L.	Agrimony
	Collinsonia canadensis L.	Pilewort
	Coriandrum sativum L.	Coriander
	Mentha pulegium L.	Pennyroyal
Hair	*Arctium lappa* L.	Burdock
	Humulus lupulus L.	Hops
	Polygonum multiflorum Thunb.	Fo-Ti (Polygonum)
	Polymnia uvedalia L.	Bear's foot, leafcup
	Carthamus tinctorius L.	Safflower
Liver	*Peltiger canina* L.	Ground liverwort
Uterine functions	*Ananas comosus* (L.) Merr.	Pineapple
	Lamium album L.	Nettle

demonstrated that some of the medicinal plants have an antioxidant function with a very high content of compounds such as ascorbate, tocopherols, and carotenoids (Shahidi, 1997; Yeh and Hu, 2001), as are contained in many vegetables and fruits.

4.4 Effects of medicinal plants on mental and physical performance

Some phytochemicals in medicinal plants are essential and they are useful for treating specific ailments. Other plants with non-essential compounds may be relevant to human health and directly affect physical performance such as adaptogens and tonics. However, the recent in-depth research has shown that a few of the medicinal plants with adaptogenic and tonic functions may also have therapeutic value for specific ailments. There are several medicinal plants with adaptogenic (Table 4.1) and tonic (Table 4.2) functions which may maintain or improve human health. Humans consume herbs such as ginseng to enhance their endurance performance, induce muscular hypertrophy and strength, and enhance performance in sport events (Bucci, 2000). The following discussion indicates the most common and widely utilized herbs which have, direct or indirectly, an effect on mental and physical performance.

4.4.1 Asian and American ginseng

Asian and American ginseng (*Panax ginseng* C. A. Meyer; *P. quinquefolium* L.) are perennial aromatic herbs, 60–80 cm tall, originating in China and Canada respectively (Li, 1995). Ginseng has a bifurcated root with compound, verticilated, oval to oblong leaves. It is used routinely by millions of people to stimulate and maintain energy levels and achieve a sense of well-being (Li, 1995). It is one of the most popular medicinal plants with adaptogenic and tonic effects that have been associated with mental and physical performance. The effects of ginseng on stress and fatigue have long been expounded by Chinese herbalists (Xiao et al., 1987). Ginseng is ineffective in the absence of stress, but can return body processes to normal when there is stress or disorder, irrespective of the source (Fulder, 1977). Various compounds in ginseng have been shown to increase nonspecific resistance to physical, chemical and biological stresses. Some of the manifestations include a decrease in body temperature, relaxing of muscle tone and analgesia (Muwalla and Auirmeileh, 1990; Hikino, 1991). This implies a more efficient use of body fuel reserves during physical activity, possibly helping to overcome the strain of exercise in humans. It has been found that ginseng can reduce heart rate during strenuous exercise, reduce blood lactate, increase the efficiency of oxygen utilization, decrease reaction times and increase pulmonary function (Tsung et al., 1964); it can also maintain metabolic balance by eliciting a haemostatic effect (Lewis, 1988). Ginseng can act as immune stimulant or supportive agent (Boik, 2001), although the mechanisms by which these compounds, saponins, exert their immunostimulant effects are not well understood. The major active constituents of ginseng are now generally accepted as dammarane saponins, commonly referred to as ginsenosides (Schulten and Soldati, 1980; Shibata et al., 1985;

Tanaka, 1994). Crude root extracts have effects on cognitive performance, mood, and energy (Lieberman, 2001) and can decrease reactions to various noxious and stressful stimuli such as general hypoxia and cardiac ischaemia and exert a stimulating effect on the metabolism by significantly altering lipid and carbohydrate mobilization and utilization (Hu, 1977; Liu and Xiao, 1992; Wang and Lee, 1998).

Studies of patients with senile dementia of the Alzheimer type indicate that this disease is associated with degeneration of the cholinergic nerve tracts projecting from the medial forebrain complex to cortical and hippocampal regions (Bartus et al., 1982). In a laboratory study, Benishin et al. (1991) found that the ginsenoside Rb_1 could improve memory deficits induced by anticholinergic drug treatment and facilitate acetylcholine release from rat brain hippocampal slices. The increase in acetylcholine release was associated with an increase in the uptake of the precursor choline and the continuous administration of Rb_1 increased the maximum velocity of choline uptake in the hippocampus regions (Benishin, 1992). The specific effect of Rb_1 on cholinergic functions may warrant its further study for enhancing short-term memory acquisition and retention in senile dementia (Wang, 1996).

You et al. (1995) reported that ginseng can act prophylactically as an anti-inflammatory agent in humans. It can also increase antibody levels, stimulate natural killer cells, and stimulate the release of the chemical messenger interferon (Wang et al., 1982); interferon in turn can activate the immune system. Ginseng also possesses anti-complementary activity. The complement system is a complex set of proteins that acts as a defence against invading organisms or particles. A chemical from the leaves of *P. ginseng* that is considered anti-complementary to heteroglycans can alter the speed at which the complement system can recognize, attack and destroy pathogens (Gao et al., 1991). Ginseng can lower cholesterol (Court, 2000) or adjust the ratio between high-density lipoprotein (HDL) and low-density lipoprotein (LDL) (Court, 2000) by either stimulating cholesterol transport or stimulating an enzyme involved in cholesterol metabolism (Kao, 1983; Court, 2000). The decrease of cholesterol may also be due to an increased conversion of cholesterol into bile acids and/or the direct excretion of cholesterol (Yamamoto et al., 1983). Because LDL is linked to such conditions as atherosclerosis and heart disease a reduction of LDL and a decrease in the HDL/LDL ratio favours the maintenance of normal cardiovascular health. Ginseng has also been studied for its effects on metabolism of nucleic acids, proteins and lipids, but the results from these studies are highly contradictory (Hikino, 1991).

In addition to adaptogenic and tonic effects on human health, recent research results have indicated that ginseng has therapeutic effects on immune function (You et al., 1995), on cancer (Hikino, 1991; Mochizuki et al., 1995; Yun and Choi, 1995), on cardiovascular diseases (Hikino, 1991; Muwalla and Auirmeileh, 1990) and on sexual function (Vogler et al., 1999).

It may help reduce the healing period required in patients with chronic bronchitis who are taking antibiotic drugs (Scaglione et al., 2001), maintain blood sugar level in diabetic patients (Vuksan et al., 2000) and synergistically inhibit cancer cell growth *in vitro* (Duda et al., 1999).

4.4.2 Siberian ginseng

Siberian ginseng (*Eleutherococcus senticosus* (Rupr.ex Maxim) Maxim) is a winter hardy perennial deciduous shrub with erect, scarcely branched and slender prickly stems 2.4–4.5 m in height. Its dark green leaves have hairy veins and three or five oval to oblong fine toothed leaflets (Li, 2001). It is a popular medicinal plant in Eurasia and North America. It has been used by the Chinese for over 2000 years. Recently, imported products of this plant have become available in North America, with a market share of 3.1% of the medicinal herbal industry (Li, 2001). Traditionally, Siberian ginseng has been used as an adaptogen (Farnsworth et al., 1985; Davydov and Krikorian, 2000), to restore vigour, improve general health and increase longevity (Duke, 1985), to improve auditory disturbances, mental alertness and work output, the quality of work under stressful conditions, and athletic performance (Farnsworth et al., 1985). Although the mechanisms by which compounds within ginseng exert their immunostimulant effects are not well understood, Siberian ginseng can act as an immune stimulant or supportive agent (Boik, 2001). Siberian ginseng has been used to improve human physical fitness (McNaughton, 1989) and working capacity in Japan (Asano et al., 1986), and in the USA, it is used to improve exercise performance (Dowling et al., 1996) and athletic performance including more rapid recovery from exercise (Azizov, 1997). In Mongolia, Siberian ginseng has been used to accelerate adaptation of newly arrived people to the harsh environment in mountain and desert areas (Zhekalov, 1995). It was reported that Siberian ginseng increases resistance to stress and has a balancing effect on body function (Singh et al., 1991; Rege et al., 1999), and it is used as a folk remedy for rheumatic complaints, weak liver and kidney, bronchitis heart ailments, and hypertension (Bown, 1995). In the last few decades, laboratory and clinical research have confirmed that Eleutherosides A–G (or eleutherans), isofraxidin, and polysaccharides are the major ingredients (Brekhman and Dardymov, 1969; Farnsworth et al., 1985). The medicinal benefits of Siberian ginseng to humans include alleviating upper respiratory tract infections (Thom and Wollan, 1997), impotence (McLeod, 1993), carcinostatic problems (Baranov, 1982; Bespalov et al., 1992), antitoxic effects (Gol'dberg et al., 1971), antiviral effects (Tong et al., 1994), preventing or alleviating artheriosclerosis (Shi et al., 1990), immunoprotective effects against breast, stomach, oral cavity, skin and ovarian carcinoma (Hikino et al., 1986). It was reported that the glycosides contained in Siberian ginseng have the function of lowering blood pressure and tranquillizing the central nervous system (Huang, 1999). Szolomicki et al. (2000)

reported that root extracts of *Eleutherococcus senticosus* affect cellular defence and physical fitness, as well as lipid metabolism. This herb is prescribed in Russia for anaemia, depression, asthenia, any chronic and debilitating disease particularly of the heart and blood vessels, surgery, convalescence, chronic infections such as tuberculosis and simply old age (Fulder, 1980).

4.4.3 Indian ginseng

Indian ginseng (*Withania somnifera* Dunal) is an erect, branching perennial shrub up to 1.5 m high. The roots are used for medicinal purposes. They are light brown, 10–18 cm long and 6–12 cm in diameter. The main ingredients are an essential oil (ipuranol), a crystalline alcohol (whitaniol), hentriacontane, phytosterols and fatty oils. It also contains the alkaloids withanine and somniferene (Chauhan, 1999). Indian ginseng is valued as a potent tonic that delays ageing, greying of hair and provides physical as well as mental strength.

4.4.4 Echinacea

Echinacea (*Echinacea purpurea*, *E. angustifolia*) is a winter hardy herb with stout, hairy and either single or branched stems 30–100 cm in height. Its rough and hairy ovate to lanceolate 3–5 veined leaves are 15–30 cm long; the upper leaves are sessile while the lower leaves have long petioles (Li, 1998). It has been used as a health remedy since the beginning of the twentieth century more than any other herb in the West (Gilmore, 1911). In the past, crude extracts from *E. angustifolia* have been used for treating insect bites (Hill et al., 1996), snake bites (Busing, 1952) and wounds (Seidel and Knobloch, 1957). Recently, it has been demonstrated that the *Echinacea*-derived phytochemicals, such as cichoric acid, alkamides (dodecatetraenoic acid isobutylamides) (Bauer, 1999), polysaccharides, flavonoids, and polyacetylenes (Tubaro and Tragni, 1987; Bauer and Wagner, 1991) act as stimulants of those cells responsible for nonspecific immunity as the first line of defence against virus-infected or transformed cells (Sun et al., 1999). It has also been used for the common cold, coughs, bronchitis, upper respiratory infections and some inflammatory conditions (Percival, 2000).

4.4.5 Sea buckthorn

Sea buckthorn (*Hippophae rhamnoides* L.) is a hardy, deciduous shrub belonging to the family Elaeagnaceae. It bears yellow or orange berries and has been used for centuries in both Europe and Asia for food and pharmaceutical purposes (Li and Schroeder, 1996). The nutritional value is often based on its berry, which is one of the most nutritious and vitamin-rich fruits known (Magherini, 1986). Sea buckthorn is one of the leading medicinal

plants with very high antioxidant activity. The berry, including seeds, contains large amounts of essential oils and vitamin C (Novruzov and Aslanov, 1983). The vitamin C concentration in the berries varies depending on species, geographical location and physiological maturity from 360 mg/100 g of berries for the European subspecies *rhamnoides* (Bernath and Foldesi, 1992) to 2500 mg/100 g of berries for the Chinese subspecies *sinensis* (Yao and Tigerstedt, 1994; Yang and Kallio, 2001), which is higher than that for strawberry (64 mg/100 g), kiwi fruit (100–470 mg/100 g), orange (50 mg/100 g) or tomatoes (12 mg/100 g) (Lu, 1992). Sea buckthorn is also high in carotene, fatty acids, and vitamin E (Beveridge et al., 1999). The leaves of sea buckthorn contain many nutrients and bioactive substances and are suitable for animal feed (Morar et al., 1990). Sea buckthorn oil is approved for clinical use in hospitals in Russia and in China where it was formally listed in the *Pharmacopoeia* in 1977 (Xu, 1994). The most important pharmacological functions of sea buckthorn oil can be summarized as diminishing inflammation, treatment of bacterial infection, relief of pain and promoting regeneration of tissues. It also can be used for skin grafting, cosmetology and treatment of corneal wounds. More recently, studies on the anti-tumour effects of sea buckthorn oil have shown positive results in China (Zhong et al., 1989).

4.4.6 Roseroot

Roseroot (or Golden Root) (*Rhodiola rosea*, syn. *Sedum roseum*) is a perennial herb about 40 cm high, belonging to the Crassulaceae family. It has alternate succulent leaves on a leafy stem with over a hundred pink to purple flowers on the tips of branches. Roseroot is considered a valuable herbal medicine because of its stimulating and adaptogenic qualities; these help the body maintain homeostasis by assisting the adaptation to environmental stressors at a cellular level, promoting mental and physical vitality and increasing alertness and physical endurance (Small and Catling, 1999). They also delay ageing, and improve memory (Petkov, 1986). The active constituents responsible for phytomedicinal properties are cinnamyl-D-glycosides such as rosavin, rosin, and rosarin, *o*-mono- and *o*-dihydroxyphenols (Furmanowa et al., 1999), salidroside and thyrosol (Linh, 2000). Experimentation has also suggested that they have anti-cancer properties (Salikhova, 1997), stimulate the central nervous system and protect the liver (Small and Catling, 1999).

4.4.7 Schizandra

Schizandra (*Schisandra chinensis*) is an aromatic woody vine with alternate, petiolate and oblong leaves and pink or white odoriferous flowers. This Chinese herb has excellent tonic and adaptogenic functions, it is antitussive and is used as a stimulant. The dried berries are used medicinally. It con-

tains several hydrocarbon derivatives, including sesquicarene, -bisabolene, -chamigrene, and -ylangene (Huang, 1999). The pericarp of the fruit contains lignans such as schizandrin, schizandrol, and deoxyschizandrin. The active ingredient, schizandrin, has a protective effect on the liver and can lower glutamyl transferase activity (Huang, 1999). It has been claimed that schizandra can improve mental function and antagonize the central convulsive effect of caffeine. In combination with *Panax ginseng* it has been found to be beneficial in memory consolidation (Nishiyama, 1995). Mucilage from a decoction of stems and branches is used to treat cough, dysentery, and gonorrhoea (Duke and Ayensu, 1985). It was also reported that the berries are aphrodisiac.

4.4.8 Angelica

Angelica sinensis (Chinese angelica or Dong Quai), *A. acutiloba* (Japanese angelica), *A. archangelica* (European angelica) and *A. tropurpurea* (American angelica) are all biennial or perennial plants with grooved stems 1–2 m in height. The plants thrive in damp mountain ravines and on river banks, but can also be cultivated. The roots and rhizomes are the parts most extensively used for medicinal purposes. The chemical compounds of Chinese and Japanese angelica that are responsible for their medicinal actions are coumarins, essential oils (mainly phellandrene, alpha-pinene and limonene in the roots and osthol, angelicin, umbelliferone, bergapten and psoralene in the seed) and flavonoids (Duke, 1985; Foster, 1993; Murray, 1995). It was also reported that angelica contains a significant quantity of polysaccharides and the vitamins B_{12}, and E (Huang, 1999), which may contribute to the immune-modulating activity by enhancing the activity of white blood cells, increasing interferon production and stimulating nonspecific defence mechanisms (Kumazawa et al., 1982; Yamada et al., 1984). Many formulas containing Chinese angelica (*A. sinensis*) are recommended by Chinese pharmacopoeias for the treatment of female ailments (Huang, 1999).

4.4.9 Garlic

Garlic (*Allium sativum*) is a perennial plant of the Liliaceae. It is a very popular and pungent plant and is cultivated worldwide. Fresh or dehydrated, the bulb has been used for medicinal purposes since the beginning of recorded history (Rivlin, 2001). Over the last decade, hundreds of articles have been published on all aspects of its chemistry, pharmacology and clinical trials. Garlic contains a volatile oil composed of sulphur-containing compounds including allicin, diallyl disulphide and diallyl trisulphide. It also contains high concentrations of trace minerals, vitamins, glucosinolates and enzymes such as allinase, peroxidase and myrosinase (Murray, 1995). The modern use of garlic has focussed on its ability to lower cholesterol (Stevinson et al., 2000; Yeh and Liu, 2001), reducing risk factors for car-

diovascular disease (Nagourney, 1998), lowering blood pressure (Murray, 1995), and its anti-cancer properties (Milner, 2001). Garlic has antitumour and immunotherapeutic effects, and prevents immune suppression in malignancies, particularly cancer of the bladder (Lamm and Riggs, 2001), colon or stomach (Fleischauer et al., 2000).

4.4.10 Reishi mushroom

Reishi mushroom (*Ganoderma lucidum* (Fr.) Lloyd) is the dried fructification of a Chinese mushroom. The fruiting bodies and mycelium are used for therapeutic purposes and it has a long history in Chinese folk medicine for adaptogenic and tonic functions. Recently, researchers started to isolate and identify the principal substances from the reishi mushroom that have antitumour, cholesterol-lowering, hypoglycemic, hepatoprotective, antiviral and antibacterial effects (Kim et al., 1997; Wang et al., 1997; El-Mekkawy et al., 1998; Wasser and Weis, 1999). The mushroom contains ergosterol, ganoderiols A and B, ganodermatriol, ganolactone, fungal lysozyme, proteinases, amino and other organic acids and polysaccharides (Huang, 1999). In China, reishi is currently used in the treatment of hyperlipemia, angina pectoris, chronic bronchitis, hepatitis and leucopoenia with a 60–90% effective rate in clinical trials (Huang, 1999).

4.4.11 Goldenseal

Goldenseal (*Hydrastis canadensis* L.) is a herbaceous perennial belonging to the Ranunculaceae family with high medicinal value. It has an erect hairy stem about 50 cm high with 3–4 yellowish scales at the base of each stem and possesses a distinct odour and a bitter taste. The knotted, tortuous, subcylindrical rhizomes are used for medicinal purposes (Foster, 1991). Goldenseal is used to treat a wide variety of disorders including urinary tract infections and internal haemorrhage (Tyler, 1994), inflammation of vaginal and urethral mucous membranes, malaria, nasal congestion, sore gums and cancer (Leung and Foster, 1996), gastritis, anorexia, peptic ulcers, conjunctivitis, tinnitus and catarrhal deafness (Newall et al., 1996). Its pharmacological properties are attributed to hydrastine, beberine (Newall et al., 1996) and benzylisoquinoline alkaloids (Scassocchio et al., 1998). Other biochemical components of goldenseal include albumin, biotin, calcium, chlorine, choline, collagenic acid, inositol, iron, lignin, manganese, volatile and essential oils, phosphorus, potassium, resin, and vitamins A, B complex, C, and E (Duke, 1985).

4.4.12 Tea

Tea is the fragrant beverage made from leaves of the tea tree [*Camellia sinensis* (L.) Kuntze]. It has been used by humans for thousands of years.

Its potential health properties have attracted attention from the research community and recent scientific reports are gradually validating its claimed therapeutic values. Tea has become a source of dietary antioxidants with a potential role in the prevention of chronic diseases (Balentine and Paetau-Robinson, 2000). Tea leaves contain alkaloids including caffeine, theophylline, theobromine, xanthine, tannic acid and polyphenols such as (–) epigallocatechin gallate (EGCG) (Huang, 1999), flavonoids and methylxanthines (Balentine and Paetau-Robinson, 2000) which have cancer-prevention properties. Tea leaves also contain important trace elements such as aluminium, boron, calcium, copper, fluorine, iodine, manganese, phosphorus, potassium, selenium and zinc.

According to recent epidemiological studies, tea plays a major role in protection against cardiovascular disease and acts as a dietary antioxidant (Imai and Nakachi, 1995; Li et al., 1999; Kaegi, 1998). It was reported that green tea consumption in smokers reduces the frequency of sister chromatid exchange in peripheral lymphocytes to a level similar to that found in nonsmokers (Shim et al., 1995). Tea has potent antioxidant flavonoids that act as free radical scavengers and as inhibitors of LDL oxidation (McAnlis et al., 1998).

4.5 Concerns about the safety and quality of medicinal plants

The two major consumer concerns about medicinal plants, quality and safety, are the most important factors to address for gaining the confidence and acceptance of herbal medicines. Strong regulations and precise quality control, from farm production via product manufacture to retail stores are the best measures for monitoring the industry. Similar regulations should apply to imported materials or products to avoid adulteration, substitution, heavy metal contamination, pesticide residues, and illegally added prescription drugs.

Research results indicate that not all medicinal plants are safe to use. For example, the major component of goldenseal, hydrastine, is toxic. Eating fresh plant material may ulcerate and inflame the mucous membrane of the mouth and large quantities of hydrastine will overstimulate the nervous system; they may produce convulsions, respiratory failure or overproduction of white blood cells (Huang, 1999). Public education is needed to raise awareness that some herbs are potent and dangerous to use. Great care should be taken to prevent consumption by children, pregnant women, the sick and the old. Furthermore, additional information is needed on the optimal usage of medicinal plants, such as dosage, frequency and usage period, physical condition and sensitivity of the user and possible interactions with prescribed drugs. This will help to build up public confidence and make the marketing of medicinal plants sustainable.

Toxicity refers not only to lethal effects but also to body reactions such as allergies, irritation, and sensitivity. For example, angelica is generally considered to be safe, but it contains substances that can react with sunlight to cause severe sunburn. Other adverse effects include excessive bleeding and fever due to hypersensitivity (Huang, 1999). An increasing number of herbal products available on the market are mixtures of two or more herbs; this causes great concern because there is very limited research on the combined effect of two or more medicinal plants on humans. Another urgent need is to have reliable information on the interactions between herbal supplements and prescription drugs to provide physicians with better guidance in this area.

4.6 Future trends

Members of the general public are increasingly concerned about their health. Even with available and affordable modern medical care, people like to have traditional practices as an alternative. In the past decade, there has been a considerable surge of interest in medicinal plants because of their low cost, the side effects of prescription drugs and loss of confidence in modern medicine. In Europe, phytomedicine is much more popular, due to powerful regulatory control, than in North America. The causes are a lack of proper scientific evaluation, limited regulation, absence of quality control and limited education of herbal practitioners in the latter region (Small and Catling, 1999), although the market demand for herbal supplements is increasing rapidly. To meet the surging demand, more research and development by professionals (including agronomists, botanists, biochemists, food researchers, in processing and analysis, pharmacologists, and physicians) are needed to make the industry both credible and sustainable.

4.7 Sources of further information and advice

There are many sources of information on medicinal plants. Information on the Internet may or may not be reliable, and counsel with a professional is strongly recommended:

1. *Government agencies*
   US Dept. Health (http://www.health.gov/healthypeople)
   Health Canada (http://www.he-sc.gc.ca)
   Agriculture & Agri-Food Canada (http://agrisource.ncr.agr.ca)
   Alberta Agriculture, Food and Rural Development
   (http://www.agric.gov.ab.ca)
   US National Toxicology Program (http://ntp-server.niehs.nih.gov)
   Developmental therapeutics program online database
   (http://dtp.nci nih.gov)

2. *Universities*
   The University of Georgia Gerontology Centre (http://geron.uga.edu/ageing)
   Alternative Medical Resources, University of Pittsburgh (http://www.pitt.edu/~cbw/alm.html)
   Bastyr University Natural Health Sciences (http://www.bastyr.edu)
   Purdue University (http://www.purdue.edu)
   UC Davis (http://ucdavis.edu)
   University of Guelph (http://uoguelph.ca)
   Harvard University (http://www.hsph.harvard.edu/hai/home.html)

3. *Organizations*
   American Botanical Council and the Herb Research Foundation (http://www.herbalgram.org)
   American College Advancement in Medicine (http://www.acam.org)
   J. Am. Diet Assoc. (http://www.eatright.org),

4. *Other Internet sources*
   Sport nutrition (http://www.discount-vitamins-herbs.net)
   Central Soya Co. (http://www.centralsoy.2/)
   Sports medicine (http://www.thorne.com/almedrev/fulltext/sports2-4.html)
   Health Web (http://healthweb.org)
   Alternative medicine (http://www.alternativemedicine.com)

5. *Books and scientific journals – see references.*

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5

Phyto-oestrogens and cognitive function

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5.1 Introduction

A large body of observational and experimental data points in the direction of a beneficial effect of exogenous oestrogen on cognitive function and prevention of Alzheimer's disease (Stampfer and Colditz, 1991). Conventional hormone replacement therapy (HRT) is associated with considerable adverse effects, such as increased risks of venous thromboembolism (Daly et al., 1996; Grodstein et al., 1996; Hulley et al., 1998; Jick et al., 1996; Vandenbroucke and Helmerhorst, 1996) breast (Beral, 1997; Colditz et al., 1995; Steinberg et al., 1994) and endometrial cancer (Beresford et al., 1997; Jain et al., 2000). To diminish the increased endometrial cancer risk, progesterone is added to the oestrogen in HRT treatment (Beresford et al., 1997; Jain et al., 2000), which in turn leads to renewal of vaginal bleeding, with associated problems of therapy compliance and increase in cardiovascular diseases and breast cancer risk (Writing group WHI, 2002). Conventional HRT is an unattractive therapy to which many women are unable to adhere for long time spans (Barentsen, 1996). Phyto-oestrogens, plant chemicals that are capable of exhibiting oestrogen-like activity due to their capacity to bind to the oestrogen receptor, have been suggested as alternatives.

Phyto-oestrogens occur in three main classes: isoflavones, coumestans, and lignans. Phyto-oestrogens are found in various plants including grain, beans, green vegetables, fruits, nuts, and grasses. Isoflavones are primarily found in soya beans and soya foods. In this chapter we will first explain the main aspects involved in cognitive function and briefly review the available literature on HRT and cognitive function. Then the chemical structures and

main food sources of phyto-oestrogens will be discussed and finally the observational and experimental data regarding phyto-oestrogens and cognitive function are analysed.

5.2 Cognitive function

Cognitive function is the conjunct of intellectual abilities including memory, learning, perception, abstract reasoning, attention and judgement. In cognitive testing several domains are recognized, of which verbal memory is found to be the most sensitive to oestrogens. In verbal memory tests, subjects are shown words or short stories, which they have to recall or recognize. Immediate recall is used for short-term memory while recall after 30 minutes refers to long-term memory. In visual memory tests the subjects are shown pictures, objects or drawings, which they have to recognize or copy.

A separate part of memory function is working memory: the ability to hold information in mind and manipulate it. This function is typically tested using digit span reversed: the subject has to repeat a number of digits backwards. Attention and concentration are a crucial part of all cognitive tests and therefore are also tested as one separate entity. An example of an attention test is the digit symbol substitution test where numbers and symbols have to be paired. Motor speed is assessed using reaction time to a stimulus. Abstract reasoning and concept formation refer to the quality or process of thinking. This aspect of cognition is tested using, for example, abstract patterns in which the subject has to tell what pattern does not belong in a list. There are also many tests related to such qualities as verbal skills, word fluency and naming skills.

This overview is far from complete – many more cognitive sub-domains, with their respective tests, could be recognized. But this is beyond the scope of this chapter. The results of cognitive tests can be influenced by several factors. In the normal process of ageing cognition is affected. Short-term memory and learning are especially impaired while long-term memory remains intact (Petersen et al., 1992). Depression causes an overall worsening of cognitive function, but most pronounced effects can be seen in the frontal lobe function of selective attention and working memory (Landro et al., 2001). Several types of medication, especially psychotropic drugs and tranquillizers, but also nicotine and caffeine, influence cognitive function (Park et al., 2000; Rees et al., 1999).

5.3 Conventional hormone replacement therapy (HRT) and cognition

5.3.1 HRT and cognitive function

Substantial evidence exists for positive effects of HRT on several domains of cognitive function. Menopause is associated with a dramatic decline in endogenous oestrogen levels. Oestrogen, a steroid sex hormone produced mainly by the ovaries, plays a major role in reproductive function. Oestrogens have important stimulating effects on the reproductive tissues, uterus, ovaries, breast and Fallopian tubes. Besides, oestrogen has clear-cut effects on bone, vascular endothelium skin and other tissues. Oestrogen is a small lipophilic molecule that easily passes into the central nervous system. Oestrogen receptors are located throughout the brain, especially in regions related to cognitive function such as the hippocampus and the amygdala. Research has suggested a relationship between lifelong oestrogen exposure and cognitive function (Yaffe et al., 2000).

Several trials and observational studies have aimed to investigate the relation between HRT and cognitive function in peri- and postmenopausal women. The available evidence was reviewed recently (LeBlanc et al., 2001). The Jadad score was used for grading the quality of randomized controlled trials and the criteria of the US Preventive Services Task Force (USPSTF) were used for observational research. Nine randomized trials and eight observational studies were identified. Two trials and four observational studies focused on verbal memory. Both trials and one of the observational studies found improvement of immediate verbal recall in HRT-users, but in delayed verbal memory only one out of four studies found improvement. Visual memory does not seem to be affected by HRT. Six studies looked at this of which only one small cohort study found a positive effect. The studies on attention and working memory have rather mixed results. Three trials looked at attention and found no effect on this test, but with neuro-imaging used in one of these studies an increased activation of the relevant brain areas was visible. The digit span for working memory was used in six studies of which one found improvement in HRT-users. Abstract reasoning was found to improve in one trial and in one cohort study while one other large cohort study did not find this effect. An overall trend in all the studies was that the effect of HRT was more prominent in symptomatic postmenopausal women than in asymptomatic women. In overview of the evidence one may conclude that HRT can affect cognitive function beneficially, especially in symptomatic postmenopausal women, with the most consistent effects being those on verbal memory and abstract reasoning.

5.3.2 HRT, dementia and Alzheimer's disease

Research has also suggested a positive influence of HRT on the incidence of dementia, both vascular dementia and Alzheimer's disease (AD). The underlying mechanisms causing the reduced incidence of vascular dementia are thought to be the positive effect of oestrogen on the lipid spectrum in the blood and the improvement of vascular endothelial function (Applebaun-Bowden et al., 1989; Gangar et al., 1991; Janowsky et al., 2000; Paganini-Hill et al., 1988; Sullivan and Fowlkes, 1996). For AD the mechanisms are quite different. Oestrogen might stimulate the dendrites' spine density in the brain and protect the neurons against harm caused by oxidative stress (Brinton et al., 2000; McEwen and Alves, 1999). Another theory is that oestrogens are able to promote the breakdown of β-amyloid, thereby preventing the formation of neurofibrillary tangles.

So far only data from observational studies are available, but several large scale, long-term clinical trials are currently looking further into this matter. A meta-analysis comprising two cohort studies and ten case-control studies found a 34% decreased risk of AD (95% confidence interval, 18–47%) for current HRT-users compared to non-users (LeBlanc et al., 2001). The major limitation of these studies is a healthy user effect; intervention was not randomly allocated and HRT-users tend to be healthier, smoke less and exercise more. Although the subjects were matched for the known confounders, residual confounding may have caused an overoptimistic estimate. Furthermore, several studies used in the meta-analysis relied on interviewing next of kin with regard to the HRT status of the demented subjects. This may lead to underreporting of HRT use, especially in the demented subjects, leading to an overoptimistic effect estimate. Although the data are very promising, the results from large trials have to be awaited.

Altogether it is reasonable to assume that oestrogens can positively influence cognitive function both short term in healthy postmenopausal women and in the long run by reducing the incidence of dementia. Unfortunately, long-term use of HRT involves considerable adverse effects, with associated problems of therapy compliance. Conventional HRT is an unattractive therapy to which many women are unable to adhere for long time spans.

5.4 Phyto-oestrogens

5.4.1 Chemistry of phyto-oestrogens

Phyto-oestrogens are chemicals occurring in plant foods that can exhibit oestrogen-like activity due to their capacity to bind to the oestrogen receptor (Miksicek, 1995; Shutt and Cox, 1972).

There are three main classes of phyto-oestrogens: isoflavones, coumestans and lignans, all occurring either in plants or in their seeds (Fig. 5.1).

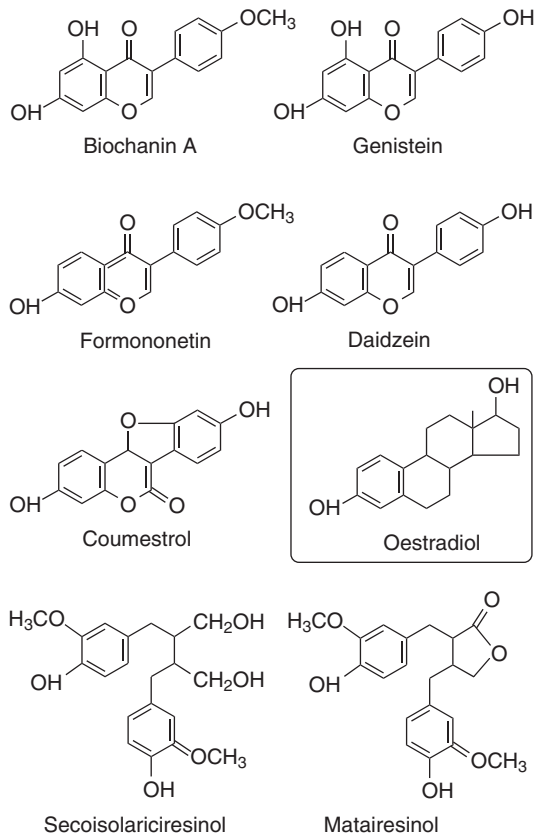

Fig. 5.1 Classes of phyto-oestrogens.

The major isoflavones genistein and daidzein commonly exist bound to glucosidases. They may also be derived from their precursors biochanin A and formononetin. Coumestrol and 4′-methoxycoumestrol are the most important coumestans with oestrogenic activity in human food. The oestrogenically active lignans enterodiol and enterolactone are derived from the compounds secoisolariciresinol (SECO) and matairesinol (MAT).

After consumption of isoflavones or lignans by humans, heterocyclic phenols with a structure similar to that of oestrogens are formed by complex enzymatic metabolic conversions in the gastrointestinal tract (Setchell et al., 1984). Daidzein is eventually metabolized to both equol and O-desmethylangolensin (O-DMA). Genistein is metabolised to 6′-hydroxy-O-DMA (Joannou et al., 1995; Setchell and Adlercreutz, 1988). The plant lignans SECO and MAT are converted by human gut bacteria to the human lignans enterolactone and enterodiol (Borriello et al., 1985).

Isoflavone and lignan absorption and utilization require a series of deconjugation and conjugation steps. After utilization, conjugated isoflavones and lignans are excreted into urine as well as into bile. After excretion into the latter, deconjugation by gut bacteria and re-absorption may take place, resulting in further metabolism and degradation in the intestine (Setchell and Adlercreutz, 1988; Xu et al., 1995). Concentrations of the various phyto-oestrogen metabolites vary widely between individuals, although excretion of metabolites is generally highly correlated with dietary intake (Adlercreutz et al., 1991; Chen et al., 1999; Karr et al., 1997; Kirkman et al., 1995).

5.4.2 Sources of phyto-oestrogens

Phyto-oestrogens are found in various plants including grains, beans, green vegetables, fruits, nuts, and grasses. Isoflavones are primarily found in soya beans and soya foods. These contain approximately 0.2–1.6 mg of isoflavones/g dry weight (Coward et al., 1997; Murphy, 1982; Wang and Murphy, 1994). Chickpeas and other legumes, such as mung beans and clover, are other isoflavone sources. From published information on urinary phyto-oestrogen excretion in humans, it is clear that soya bean consumption is only significant in populations in the Far East (Bingham et al., 1998). The mean daily isoflavone intake in Asian populations has been estimated to be approximately 30 mg/day (Messina, 1995). In Western populations, beans and peas (45%), tea and coffee (25%), nuts (10%) and grains, rice and cereals (5%) are the main sources of isoflavone intake (de Kleijn et al., 2001).

Lignans are found in seeds (e.g., flaxseed, linseed, sunflower seeds and pumpkin seeds), grains (e.g., oats, wheat, barley and rye) and vegetables such as carrots, garlic and broccoli. They can also be found in peanuts, tea and coffee. Lignan consumption is more widespread in Western populations, due to the more widespread occurrence of lignans in common foods, but studies on intake levels are still scarce (Boker et al., 2002; de Kleijn et al., 2001; Horn-Ross, 2001; Pillow et al., 1999). Fruits (25%), vegetables (20%), berries (15%), grains, rice and cereals (10%), tea and coffee (10%) and nuts (10%) are the main sources of isoflavone intake (de Kleijn et al., 2001). Coumestans, mainly coumestrol, are found in alfalfa, clover and the sprouts of soya beans. The richest coumestrol source is mung bean shoots (Adlercreutz and Mazur, 1997).

5.5 Effects on cognitive function: animal studies

So far two oestrogen receptors have been identified: oestrogen receptor α (ERα) and oestrogen receptor β (ERβ). Phyto-oestrogens are able to bind to both receptors, but some phyto-oestrogens, especially genistein, have a

higher affinity for ERβ than for ERα. It is therefore hypothesized that the receptor-mediated effects of phyto-oestrogens are mediated through this receptor. The distribution of ERα and ERβ is tissue-specific, which may explain the observed tissue-specific variability of phyto-oestrogen actions (Kuiper et al., 1996; Kuiper et al., 1997; Kuiper et al., 1998). In the brain ERβ is more abundant. High concentrations of ERβ have been found in the hippocampus, basal ganglia and frontal cortex (McEwen et al., 1997; McEwen and Alves, 1999), all regions that play a major role in cognitive function in general and, more specifically, in memory function.

Aged, ovariectomized cynomolgus monkeys were randomized into three groups receiving whole soya protein, extracted soya protein containing no phyto-oestrogens or extracted soya protein combined with conjugated equine oestrogens (CEE). Immunoblot analysis was used to measure expression of the Tau protein of neurofibrillary tangles that plays a major role in AD. There was a suppression of this Tau protein in animals receiving whole soya, but not in animals receiving extracted soya protein (Kim et al., 2000). In another study (Pan et al., 1999a; Pan et al., 1999b) both young and retired ovariectomized breeder rats were divided into three groups: non-treated controls and those receiving 17β-estradiol (E_2) and those receiving soya phyto-oestrogens. Treatment was given for eight weeks. Effects on the regulation of biomarkers important for cognitive function, namely choline acetyltransferase (Chat), nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) were studied. Both soy and E_2 increased the production of Chat and BDNF in the frontal cortex of the ovariectomized, retired breeder rats. The NGF in the hippocampus in the soy group was intermediate between the control and E_2-treated groups. In a separate study (Pan et al., 2000) the same research group investigated whether soya phyto-oestrogens could improve working memory in rats and whether the phyto-oestrogens would attenuate the effects of E_2 on working memory. Ovariectomized, retired breeder rats were divided into 12 groups combining four levels of E_2 with three levels of phyto-oestrogens. In the groups given only E_2 or only phyto-oestrogens there was a dose-dependent improvement in test performance on a radial maze. Furthermore, the results of E_2 were not attenuated by the addition of phyto-oestrogens.

Visual spatial memory in rats is sex-dependent with male rats normally outperforming female rats. In another experiment (Lund et al., 2001) male and female rats were on a lifelong high phyto-oestrogen diet or on a phyto-oestrogen-free diet. In a radial maze test requiring visual spatial memory the female rats on the high phyto-oestrogen performed significantly better than female rats on the low phyto-oestrogen diet. In male rats the opposite effect was seen. After 80 days half of the rats on the high phyto-oestrogen diet were switched to the low diet. The female rats that stayed on the high phyto-oestrogen diet performed better than those on the low phyto-oestrogen diet. Again, in male rats the effects were opposite. A follow-up study (Lund and Lephart, 2001a) showed that male rats that were treated

with an androgen receptor blocker had the same improvement in visual spatial memory when treated with phyto-oestrogens. An analysis of the neuro-protective proteins in the brains of these treated rats showed an increase in CALB, a neuro-protective calcium binding protein. A rat study aiming to investigate the effects of phyto-oestrogens on body weight, consuming behaviour and anxiety in both male and female rats found that phyto-oestrogens were able to decrease anxiety in both male and female rats as measured with an elevated maze (Lund and Lephart, 2001b).

In conclusion, there is ample evidence for the beneficial effect of phyto-oestrogens on cognitive function in both monkey and rat experiments. On the one hand, there seems to be a direct receptor-mediated effect in the frontal cortex and hippocampus to enhance visual spatial memory in females that is counteracted by circulating androgens in male rats. On the other hand, a positive effect is seen on different proteins related to AD and the normal ageing processes in the brain. However, results in animal research cannot directly be extrapolated to the situation in humans. Factors to bear in mind when trying to translate these results to the human situation are the differences in metabolism, especially in the production of equol, an active compound with strong oestrogenic activity that is synthesized from isoflavones by the gut microflora in virtually 100% of the rats, but in only one third of humans (Lampe et al., 1998). In addition to this, the doses of isoflavones used may exceed the doses that can be applied in human research.

5.6 Effects on cognitive function: human studies

5.6.1 The KAME study

Research done on humans to investigate the effect of phyto-oestrogens on cognitive function and the incidence of AD is still scarce. In humans, two large cohort studies have been performed in which the association between tofu consumption and cognitive function could be assessed (White et al., 2000). In the KAME study population, a longitudinal cohort study of Japanese Americans aged over 65 years living in King County, Washington, USA, 1836 non-demented persons were screened with the Cognitive Abilities Screening Instrument (CASI) at baseline, and 1604 were re-screened two years later. Tofu consumption was categorized as low (<1 serving/week), intermediate (1–2/week) or high (>3/week). Cognitive decline was defined as a two-year loss of >5.15 points/100 on CASI. High tofu consumers had significantly lower CASI scores than low or intermediate consumers, although this was not significant in men or in women who never used HRT. Longitudinally, no associations were observed between tofu consumption and two-year change in CASI-score. An important limitation of this study, as noted by the authors, is that the total isoflavone or phyto-oestrogen consumption is not known in this population. Data for a sample

of female KAME participants showed that tofu accounts for about half of the isoflavone intake of this population.

5.6.2 The Honolulu Asian Ageing Study

In the Honolulu Asian Ageing Study (HAAS), a large cohort of Japanese American men in Hawaii has been followed since 1965 as a part of a project on heart disease, stroke and cancer (Rice et al., 2000). Standardized interviews on the use of certain foods in the week prior to interview were made during the periods 1965–1967 and 1971–1974. According to their answers, participants were divided into categories. If in the first interview they recorded fewer than 2 servings of tofu per week and in the second interview no serving of tofu, participants were classified as low-low consumers. If they reported in both interviews more than two servings of tofu they were classified as high-high consumers. Cognitive function was tested during the 1991–1993 investigations when the participants were between 71 and 93 years old (N = 3734). In a sub-group brain atrophy was measured using neuro-imaging (N = 574) or autopsy (N = 290). For 502 wives of these men the husband's intake was taken as a proxy for their wives' tofu intake and cognitive testing was performed. The participants who had a high-high tofu consumption scored worse on the cognitive tests, had more brain atrophy and more pronounced dilation of the cerebral ventricles. For the wives there was also a significant relationship between cognitive impairment and the tofu intake of their husbands. The odds ratios for cognitive impairment comparing the high-high group with the low-low group, depending on the test used, were 1.6–2.0.

An editorial accompanying the paper of the HAAS study stresses that the provocative data must be regarded as preliminary (Grodstein et al., 2000). At present we do not know whether tofu itself was the cause of the numerous indications of accelerated brain ageing or merely a marker for some other unfavourable exposure. In this population, the men with the higher tofu consumption came from poorer immigrant families and perhaps experienced more childhood privation, which may be related to their brain development and subsequent cognitive functioning. In addition, since tofu is part of a traditional diet, it may simply mark a dietary pattern that might be harmful for the brain. Finally, the men with high tofu consumption also experienced more strokes than men with lower intake and it is therefore possible that vascular causes of cognitive impairment contributed to the observed results. Unfortunately, no experimental data on the incidence of dementia and, more specifically, Alzheimer's disease and the relation with phyto-oestrogen intake are available.

5.6.3 Three other studies: 'Soy and health'

Three studies looked in detail at the direct effects of phyto-oestrogen supplementation on cognitive function (File et al., 2001) (Preliminary results

of the other two studies were presented at 'Soy and health 2002' in London). The first human trial on cognitive function and phyto-oestrogens was conducted in 2001 (File et al., 2001). In this study 27 students (15 men and 12 women), with an average age of 25 years, were randomly allocated to receive a high (100 mg/day) or low (0.5 mg/day) phyto-oestrogen diet for ten weeks. At baseline and after ten weeks cognitive function was assessed. An improvement was found on immediate verbal recall, long term visual memory and a mental flexibility task in both males and females. In a verbal fluency task and a planning task an improvement was seen only in the females. On two complex tasks including the digit symbol substitution task, a sustained concentration task and a test for short-term non-verbal memory, no effect was seen. However, this study had serious limitations. The numbers of participants were rather small and results of both sexes were combined, whilst as noted above rat experiments show specific effects in male and female animals of phyto-oestrogens. The most important limitation was that the participants knew which diets they were given.

The same research group performed a second trial (preliminary results presented at 'Soy and health 2002' in London) in which 33 postmenopausal women aged 50–65 years not using HRT were randomized to receive a soya supplement (solgen containing 60 mg/day isoflavones) or placebo. Cognitive testing took place at baseline and after 12 weeks of treatment. The researchers found significantly greater improvements in episodic memory, mental flexibility and planning in the soya group. Based on both experiments they concluded that soya phyto-oestrogens are able to improve cognitive function in both males and females in younger and older categories.

We performed a large double-blind trial on the relation of phyto-oestrogens and cognitive function ourselves (preliminary results presented at 'Soy and health 2002' in London). In this trial, 202 postmenopausal women aged 60–75 years were randomized to receive a food supplement containing 99 mg of isoflavones or a casein food supplement (placebo), daily for one year. 75% of the subjects completed the trial, the most important reason for early withdrawal being gastrointestinal complaints. The participants underwent cognitive testing at baseline and after 12 months. A digit symbol substitution test and trail making were used to test attention and concentration; a word list, immediate recall, delayed recall and recognition were used for verbal memory. Furthermore, the Doors test was used for visual memory, digit span for working memory, verbal fluency and the Boston naming task to test verbal skills. In this trial no differences in test results were seen between the soya group and the placebo group after the intervention and not even a trend was observed. We concluded therefore that phyto-oestrogens, when supplemented long after menopause, are not able to exert a positive influence on cognitive function.

5.6.4 Raloxifene and its evaluation

Raloxifene, a selective oestrogen receptor modulator (SERM), is in certain aspects comparable to phyto-oestrogens. This SERM specifically activates the ERβ, as do phyto-oestrogens, while blocking ERα and so the effects on the brain of these compounds are expected to be similar. For this reason the results of the only large clinical trial on the effects of raloxifene on cognitive function are presented here.

In the Multiple Outcomes of Raloxifene Evaluation (MORE) trial 7478 postmenopausal women with osteoporosis with a mean age of 66 years were enrolled to receive 60 mg of raloxifene, 120 mg of raloxifene or placebo for three years (Yaffe et al., 2001). Cognitive function was assessed using the short blessed test for orientation, concentration and memory; the trail-making test for attention; the word list memory and recall for verbal memory and a verbal fluency test at baseline; six months, one, two and three years. In this trial no significant differences were found between the groups but a trend towards less decline was detected for the combined raloxifene group in the verbal memory tests (relative risk 0.77 (95% CI: 0.59–1.00)) and the concentration task (relative risk 0.87 (95% CI: 0.74–1.02)). The researchers concluded that treatment with raloxifene might lower the risk of a decline in verbal memory and concentration but deemed further studies necessary to confirm this relationship.

5.7 Summary and conclusion

In view of all the scientific evidence what can be concluded about the effects of phyto-oestrogens on cognitive function? When looking at the dementia data, at first sight one may be tempted to conclude that phyto-oestrogens are harmful. However, when taking a closer look we would be inclined to see a possible positive effect of phyto-oestrogens on the incidence of dementia. The limited value of observational studies such as the KAME and the HAAS has already been explained. The results for Alzheimer's disease in the monkey studies are very promising and the incidence of AD is lower in countries (such as Japan) with a high intake of phyto-oestrogens. These results contradict the evidence from the KAME and the HAAS study.

For the effects of phyto-oestrogens on vascular dementia we have even less evidence, but extensive research has been done on the more general effects of phyto-oestrogens on vascular function and heart disease. There is increasing evidence that consumption of soya protein as a replacement for animal protein lowers cholesterol levels (Anderson et al., 1995), and this has led the American Heart Association to release a statement that it is prudent to recommend including soya protein foods in a diet low in saturated fat and cholesterol to promote heart health in 2000 (Erdman, 2000). The US Food and Drug Administration previously, on October 26, 1999,

approved the use of the marketing claim that diets low in saturated fats and cholesterol that include 25 grams of soya protein a day may reduce the risk of heart disease (Food and Drug Administration 1999). The lipid-lowering effect of soya protein is at least partially ascribed to the isoflavones in soya, since soya protein without isoflavones appears to be less effective (Crouse et al., 1999). But soya extracts containing isoflavones do not seem to have a lipid-lowering effect. It has been noted that they may provide other cardiovascular benefits, such as improved arterial compliance and aorta stiffness (van der Schouw et al., 2001). If phyto-oestrogens are able to improve vascular functions and reduce the risk of heart disease, it is very reasonable to assume that this effect can be extended to the vascular bed in the brain and thus the incidence of vascular dementia. Therefore it is quite possible that soya phyto-oestrogens may have a beneficial effect on both the incidence and the severity of vascular dementia.

The results from the studies looking at cognitive function and the short-term effect of phyto-oestrogens are less conflicting. All rat studies show a positive effect of phyto-oestrogens on cognitive function, especially on visual spatial memory and orientation as tested with maze experiments. Circulating androgens in male rats can counteract this effect. The animal studies have also found a positive effect on the biomarkers important for cognitive function. Two of the human studies also found a positive effect of phyto-oestrogen supplementation on cognition, especially on verbal memory, sustained concentration and mental flexibility. The other large trial found no effect on cognitive function. The main differences between these two studies are the age of the participants and the duration of the intervention. The two trials that did find an effect comprised relatively young participants (students and women aged 51–65 respectively), while the other trial enrolled women aged 60–75. The trial involving students will not be considered here because of the limitations noted above. However, the phenomenon that perimenopausal women are benefiting from phyto-oestrogens while postmenopausal women are not is very similar to what is seen in the trials with HRT. In these trials, HRT was most effective in women in the perimenopause and in women suffering from menopausal complaints as compared to postmenopausal women. There are several possible explanations for this. Phyto-oestrogens have been shown to relieve hot flushes in symptomatic women. One can imagine that an improvement of symptoms can result in better concentration and thus better results on cognitive tests. However, the investigators in the trial with the younger participants used questionnaires to measure symptoms and they saw no significant differences between the two groups after the intervention. Another explanation could be that there is a down regulation of oestrogen receptors in the brain that starts after menopause making phyto-oestrogens (or HRT) less effective the longer the time after menopause. The other main difference between the two studies was the duration of intervention, 12 weeks for the trial that found an effect versus 12 months

in the trial that did not find an effect. It could be that there is an initial positive effect of phyto-oestrogens on cognition but after a while the body finds a new equilibrium and the effect fades away. The duration of the intervention in the HRT-trials focussing on cognition was always very short, 21 days to 3 months with one exception of an intervention of 6 months. This could make an interesting project for future research.

Altogether it seems that women can benefit from the consumption of phyto-oestrogens, especially soon after the menopause. Phyto-oestrogens can improve cognitive function – verbal memory, concentration and mental flexibility are predominantly beneficially affected. This is a short-term effect that can be seen within three months after the incorporation of phyto-oestrogens in the diet, but whether the effect remains constant over time or slowly fades away after the brain has adapted to this new diet remains to be elucidated. In the long run, the effect of phyto-oestrogens on the incidence of Alzheimer's disease (AD) is debatable. But in view of the evidence discussed earlier in this chapter it could be possible that phyto-oestrogens are capable of reducing the incidence of AD. On vascular dementia the data are scant but less conflicting. In view of the evidence for a positive effect of phyto-oestrogens on vascular and heart disease, phyto-oestrogens might reduce both the incidence and severity of vascular dementia. The promising results from research done so far warrant future investigation especially on the long-term effects of phyto-oestrogens on the incidence of dementia and the sub-groups that might benefit most from the introduction of phyto-oestrogens in their diets.

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6

Ginseng

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6.1 Introduction

The term 'Ginseng' is believed to originate from the Chinese names 'Jin-chen, Jen-chen or Schinseng' and refers to 22 different perennial herbs found in the family *Araliaceae* and the genus *Panax*. The term *Panax* is derived from a Greek work meaning 'cure-all'. The ginseng plant is also known by the botanical name, *Panaceae*, and is a smooth herbaceous perennial that is related to ivy (Hedera). Ginseng is indigenous to Korea, China (*Panax ginseng* C.A. Meyer), the Himalayas (*Panax pseudo-ginseng*), Vietnam (*Panax vietnamensis*), Japan (*Panax japonicus*) and North America (*Panax quinquefolium*) and has a long history of medicinal use. Ginseng is commonly referred to as either Asian ginseng (from China and Korea) or North American ginseng. In addition, very valuable wild specimens of ginseng, (e.g. san-tchi ginseng) are found in India, Nepal and Myanmar with characteristically long growing periods. Siberian or Russian ginseng comes from an entirely different plant, (e.g. *Eleutherococcus senticosus*) and is commonly known as eleuthero ginseng or 'taiga root' and contains eleuthrosides and not the ginsenosides present in *Panax ginseng*.

Ginseng is regarded as an adaptogenic, stimulant herb with aphrodisiac properties.[1,2] It is a primary ingredient in traditional Chinese medicines where it is used in combination with others to restore vital energy, especially in the elderly and in individuals who are weakened by illness. Recent reports concerning ginsenosides have shown these substances to have a propensity to promote general health and well-being and to assist with the prevention of disease. This is the basis for the role of ginseng to stimulate

'yin' (e.g. the cooling force) and 'yang' (e.g. the hot force), an ancient concept from Asian cultures that explains the balance in body cells and systems required to promote general health and well-being. Traditional Chinese medicine utilizes the ability of Asian ginseng to stimulate the 'yang', which heats and energizes the body. The practitioners have prescribed Asian ginseng to counter the effects of ageing, cold climates, stress, and hormonal changes for the purpose of alleviating such conditions as depression and asthma, as well as for heart, liver, nervous system, digestive and circulatory system problems.[3,4] Gradual use is recommended and is thought to result in stronger overall health. Conversely, North American ginseng is believed to have the opposite effect; it stimulates the 'yin' and thereby has a cooling effect on the body. North American ginseng is thought to be useful for those in warmer climates, the young, the elderly and those with high blood pressure, diabetes, heart and lung problems.[3] Native populations in North America have also used wild North American ginseng as part of their traditional medicinal practice.[3,4] Contemporary uses of ginseng, especially in North America, include formulations in herbal supplements and nutraceutical or functional food usages. These are based on specific health claims that focus on immunological, anti-cancer, metabolic and neurological effects.

6.2 Chemistry of ginseng

Chemical analysis of ginseng has shown the presence of many different components ranging from simple organic acids (e.g. maltol (3-hydroxyl-2,methyl-r-pyrone)), vitamins (e.g. vitamins A and B_{12}), sugars (e.g. glucose), inorganic salts (e.g. sodium, magnesium and the trace elements vanadium, selenium and fluorine) to more complex constituents such as sterols (e.g. β-sitosterol), oligopeptides (e.g. peptidoglycans), polysaccharides (e.g. ginsenan), volatile oils and saponins. Primary active components of ginseng are generally recognized to be a group of 31 different triterpene saponins, also referred to as ginsenoside, which varies in content and relative proportions among different species of ginseng.[1,2] Ginsenosides share a similar basic structure consisting of a gonane steroid nucleus having 17 carbon atoms arranged in four rings (Fig. 6.1). Ginsenosides are named Rx according to their mobility on thin-layer chromatography plates and migration is related to the hydrophobic properties of the compounds (Fig. 6.2). Differences in structure, which include the type, position and number of sugar moieties attached by glycosidic bonds at different parts of the rings can characteristically influence biological response.[5–7] Based on these structural differences, ginsenosides are classified into two main groups with bioactive interest and various sub-types and quantity found in different ginseng varieties. They are the 20(S)-protopanaxadiol and 20(S)-protopanaxatriol groups (Table 6.1).

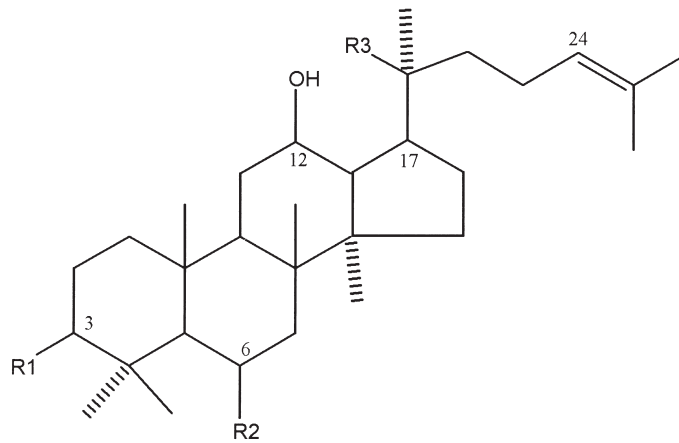

Fig. 6.1 Ginsenoside basic structure consisting of gonane steroid nucleus having 17 carbon atoms arranged in four rings. Individual ginsenosides differ by attachments of molecules at regions R1–R3.

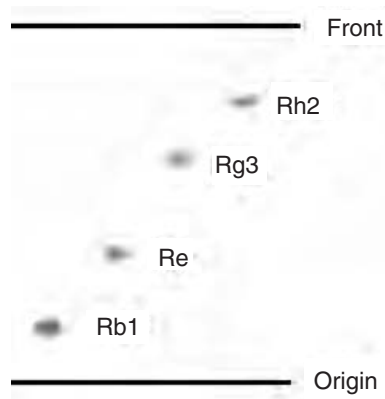

Fig. 6.2 Thin-layer chromatogram of four ginsenoside standards. Ginsenosides were chromatographed in chloroform–methanol–water (63:35:10, v/v/v) lower phase on Fischerbrand silica gel G glass backed plates (20 × 20 cm, 250 microns) (Fisher Scientific, Chicago, IL) and visualized by spraying with sulphuric acid (10% H_2SO_4) in ethanol and heated at 125 °C for 4 minutes. Pure ginsenoside standards Rb1 and Re were obtained from Sigma (St Louis, MO), Rg3 and Rh2 were obtained from Bioherb (Changchun, China). The plate was scanned using Biorad GS-670 densitometer.

6.3 Detection and extraction of bioactive components from ginseng

An important aspect of the use of plant extracts is the characterization and determination of the bioactive properties and constituents. It is generally thought that most of the bioactivities associated with ginseng are derived

Table 6.1 Selected ginsenosides of 20 (S)-protopanaxadiol and 20 (S)-protopanaxatriol classifications. Abbreviations refer to the following: Af: arabinofuranose, Ap: arabinopyranose, G: glucopyranose, R: rhamnopyranose, X: xylopyranose. R1: region 1, R2: region 2, R3 region 3 (see Fig. 6.1)

20(S)-Protopanaxadiol				20(S)-Protopanaxatriol			
	R1	R2	R3		R1	R2	R3
Rb1	O–G–G	–H	O–G–G	Re	O–H	O–G–R	O–G
Rb2	O–G–G	–H	O–G–Ap	Rf	O–H	O–G–G	O–H
Rb3	O–G–G	–H	O–G–X	Rg1	O–H	O–G	O–G
Rc	O–G–G	–H	O–G–Af	Rg2	O–H	O–G–R	O–H
Rd	O–G–G	–H	O–G	Rh1	O–H	O–G	O–H
Rg3	O–G–G	–H	O–H				
Rh2	O–G	–H	O–H				
Aglycone	O–H	–	O–H	Aglycone	O–H	O–H	O–H

from the ginsenoside content.[2] Techniques used for isolation and detection of ginsenosides vary and this has contributed to some of the inconsistencies concerning ginsenoside content and specific composition. For the quantitative analysis of ginsenosides, total ginsenosides can be measured colourimetrically[61–63] and identified by thin layer chromatography (TLC),[61] high performance liquid chromatography (HPLC),[64–66] and high performance liquid chromatography/mass spectrometry (MS).[67–68] A reversed phase HPLC system using acetonitrile-water on C_{18} columns allows the separation of the major and minor ginsenosides.[64] Quantitative determination of ginsenosides in ginseng extracts follows their extraction and separation from interfering components. Many different extraction solvents have been used to extract ginsenosides. Various preparations and combinations of methanol/water, ethanol/water, chloroform, n-butanol[69] and high temperature[70] have been employed. Ginseng root, leaf, flower, stem and seed all contain various levels of ginsenosides.[71]

6.4 Bioactivity and metabolism of ginseng extracts

Many research studies conducted in animal or clinical trials have included the use of a ginseng extract, rather than testing the activity of isolated components. This event has raised the need for more clinical efficacy and safety testing of ginseng products that are standardized for the bioactive components. The fate of ginseng after consumption is central to understanding biological functions. Pharmocokinetic studies conducted in rats have reported 23% absorption of ginsenoside (Rb1) after a period of 2.5 h.[14] Recovery of Rb1 was noted in the liver (0.25% dose), heart (<0.1% dose) and in most of the material recovered in the small intestine. Very little of the original material was recovered in the faecal material (<1% dose). These results

suggest that either metabolic or bacterial transformation of ginsenosides occurs in the intestinal system. Alcoholic tinctures of ginseng may provide relatively greater bioactivity of active components than powder preparations, due to the potentially improved solubility and thus availability from cell wall structure components of the plant. Human bacteria collected from faecal material have been reported to hydrolyze ginsenosides Rb1 and Rb2 to specific metabolites[15] that may be at least in part absorbed. Bacterial metabolites of ginseng have also been reported to have antigenotoxic properties.[16]

6.5 Anti-stress and cognitive performance properties

Ginseng has been demonstrated to have pharmacological effects on cardiovascular, immune, endocrine, and central nervous systems, which contribute improved health and vitality. Specific activities include possible stress reduction and memory enhancement,[8] antioxidant,[2,9,10] glycemic effects[11] and anti-tumourgenicity.[12,13]

Ginseng extracts typically made from solvent extraction or powdering of ginseng root have been shown to have bioactive properties with diverse modes of actions. Improved performance in behavioural testing in experimental animals was seen after treatment, possibly by increasing dopamine and norepinephrine release.[17] Liu et al.[8] examined the effects of North American ginseng and ginsenoside Rb1 on the electrophysiological properties of Na^+ channels relative to a voltage-dependent Na^+ channel blocker, lidocaine. Both a hot water extract of North American ginseng as well as Rb1 produced a voltage-dependent reversible attenuation of transient current in brain Na^+ channels which corresponded to similar shifts to a more hyperpolarized potential commonly observed with lidocaine. Neuronal damage that occurs during episodes of ischaemia has been associated with abnormal Na^+ fluxes and so agents that block voltage-dependent Na^+ channels can provide cytoprotection during cerebral ischaemia. Additional evidence of the neurological effects of ginseng have been reported from a placebo-controlled, double-blind crossover design where 20 participants were given 200, 400 and 600 mg of a standardized ginseng extract or placebo on separate days.[18] Cognitive performance was assessed both before treatment and up to 6 h after treatment. Results indicated that both quality of memory and secondary memory factors were significantly improved with the administration of a 400 mg dose of ginseng at all test times.

6.6 Immunological and antioxidant properties

Ginseng extracts have been shown to have varying immune stimulatory properties measured in polymorphonuclear leukocytes (human white blood

cells) *in vitro*.[19] Immune system enhancements in man have been reported for ginseng.[20] Capsules of the extract (100 mg) taken for 12 weeks with vaccination reduced the frequency of influenza from 42 cases for the placebo group to 15 for the ginseng treated group.[20] Other recent evidence of ginseng effects on the immune system have been shown with the maintenance of CD4+ T cell counts in HIV patients. The administration of Korean red ginseng to HIV-1-infected patients was shown to delay the resistance to active anti-retroviral drug therapy, a condition which greatly reduces the success of drug (e.g. zidovudine [ZDV]) therapy in the treatment of HIV infections.[21] Patients taking Korean red ginseng with ZDV therapy took significantly longer (e.g. 34 months) to develop drug-resistant mutant strains than control patients (e.g. 7 months). The active component of ginseng responsible for improved immune function may not be confined to ginsenosides but may also include polysaccharides. Interleukin-8, a cytokine which exerts chemotaxis on neutrophils, T-cells and basophils can be induced by ginseng root, an induction which is accompanied by increased IL-8 mRNA expression.[22] A similar *in vitro* increased TNF-α response to human blood monocytes cultured with ginseng extracts has also been reported.[75]

A number of *in vitro* studies have demonstrated antioxidant properties of ginseng extracts. They showed activity in scavenging stable free radical (e.g. 1,1-diphenyl-2-picrylhydrazyl (DPPH))[10,23] and carbon-centred free radicals (2,2′-azobis(2-amindinopropane) dihydrochloride (AAPH).[23,24] Metal-ion induced lipid peroxidation,[9,10] which includes protection against LDL oxidation,[24] has also been reported. Ginseng extracts have also been shown to affect the body antioxidant defence mechanism by increasing the hepatic gluthathione peroxidase activity, and superoxide dismutase in rats receiving ginseng extract for three months.[25] Ginseng extracts (G115), after artificial gastric digestion, exhibited a greater effect on pulmonary vasodilation and protection from free radical injury than artificially gastric-digested ginsenosides Rb1 and Rg1.[26] This effect on vasodilation and protection from free radicals may be mediated through the production of nitric oxide (NO). The activity of inducible nitric oxide synthase (iNOS) is associated with inflammation, cellular tissue damage, hypotension and different neurodegenerative disorders. Therefore, a ginseng-modulating effect on nitric oxide synthase isoenzyme activity, especially the inhibition of inducible nitric oxide synthase (iNOS) under conditions of stress and inflammation, would provide beneficial effects on blood flow and heart muscle contractility.

6.7 Bioactivity of specific ginsenosides

Ginsenosides, grouped by the structure of the aglycone, may have different and diverse effects both *in vivo* and in cell systems.[27] The

20(S)-protopanaxatriol group has shown a greater effect on relaxation in the rat aorta when compared to the 20(S)-protopanaxadiol group of ginsenosides.[28]

Ginsenoside Rg1 has been specifically shown to have a greater effect in endothelium-dependent relaxation in the rat aorta than has Rb1 and to have a corresponding increase in NO release.[28] A complex mixture of ginsenosides after a single injection resulted in endogenous production of NO in serum and urine of rats and stimulated activity of NO synthase in whole rat kidney.[29] Release of NO by ginseng may lie at the heart of the antioxidant effects of the extract.[2] It is through modulation of NO release that ginseng may exhibit an aphrodisiac action[2] through vasodilation ability.

Several ginsenosides have shown diverse bioactivity, and show direct cytotoxic and growth inhibition of tumour cells. Specific ginsenosides can induce apoptosis, effect cell proliferation[7,30–33] and suppress sister chromatid exchanges.[34] Ginsenosides, detected from the gut as intestinal metabolites, have also been shown to exhibit similar effects.[15,35–37] Ginsenosides also increase humorally mediated immune response and can induce differentiation in stem, melanoma, monocytes (leukaemia) cells and increase expression of enzymes involved in quenching free radicals.[38–40]

Specifically, ginsenoside Rh2 exhibits a strong effect on proliferation, cytotoxicity, and induction of apoptosis by effecting cell signalling compared to other ginsenosides. Rh2 can suppress proliferation in a number of human cancer cells, including breast, prostate, hepatic, stem and intestinal, and in animal cell lines.[30–33] The precise mechanism of the Rh2 effect on cells is unclear. However, there is evidence for Rh2 induction of apoptosis by effecting complex mechanisms that involve the activation and deactivation of cell signalling proteins. Apoptosis induction by Rh2 is linked to the activation of caspase-3 protease, which is involved in apoptosis, via a Bcl-2 insensitive pathway.[30] Rh2 also induced apoptosis in rat C6 gliomal cells by generation of reactive oxygen species (ROS).[32] Rh2 induced apoptosis by activating cyclin A-associated-cyclin-dependent kinase 2 (cyclin A-Cdk2) by cleavage of $p21^{WAF1/CIP1}$ in SK-HEP-1 cells (hepatoma cells).[41] The $p21^{WAF1/CIP1}$ inhibitor is transcriptionally regulated by p53 tumour suppressor, and is important in the G1/S phase DNA-damage checkpoint control machinery. G1/S cell cycle was blocked by Rh2 in cultured murine cells by modulation of Cdk2 activity although it is unclear if Cdk2 was directly or indirectly effected.[42] Furthermore, Rh2 blocked the cell cycle of SK-HEP-1 cells at the G1/S boundary by inducing the expression of $p27^{kip1}$ which has an inhibitory effect on cyclin E/Cdk2.[31] In MCF-7 human breast cancer cells Rh2 inhibited growth in an irreversible, concentration-dependent manner, induced a G1 arrest in the cell cycle, up regulated the expression of Cdk inhibitor $p21^{WAF1/CIP1}$ and reduced protein levels of cyclin D.[33]

Ginsenoside Rg3 has a structure similar to that of Rh2, but with the addition of a glucose moiety shows signs of anti-proliferative effects on a

prostate cancer cell line but at substantial higher concentration than Rh2.[7] Rh2 has an inhibitory effect with doses generally below 15 μM whereas Rg3 shows an LC_{50} greater then 500 μM.[7] At higher doses, Rg3 had similar effects on cell cycle inhibition and caspase 3 apoptosis as shown with Rh2.[7,30–32,42]

Ginsenosides Rh1 and Rh2 inhibited cellular proliferation of NIH 3TC mouse fibroblast cell line; Rh2 was slightly more potent than Rh1.[5] These ginsenosides also effected the cell signal cascade and thus cell proliferation by inhibiting phospholipase C and diacyl glycerol which is a second messenger for protein kinase C activation.[5]

Ginsenosides of the same classification such as the 20(S)-protopanaxadiol group but containing differing side chains may effect cell signalling and induce cell cycle arrest and apoptosis by different processes.[43] Ginsenosides, based on the similarities to steroids, are thought to act on the plasma membrane[44] and here may act on specific membrane proteins or penetrate the plasma membrane and initiate genomic effects and as steroid hormones become potent signalling molecules.[44] The intracellular steroid binding proteins may be the potential targets of ginsenosides. Steroids are known to interact with receptors, forming complexes and to influence gene expression.[38] The presence of ginsenosides has been detected within cells particularly in the nucleus. The uptake of Rh2 at a dose of 12 μM was detected in cultured B16 melanoma cells after 6 hours of exposure and reached a plateau of 3 nmol/10^6.[45] Ginsenosides have been suggested to be a functional ligand of the glucocorticoid receptor (GR).[46] Transfection with human GR treatment of B16 cells with Rh1 and Rh2 caused an increase in translocation (chromosome rearrangement) of GR similar to dexamethasone, a synthetic glucocorticoid.[38] The activity of Rh1 and Rh2 to bind with the GR was blocked by RU486, a glucocorticoid antagonist, suggesting further glucocorticoid activity.[38] Ginsenoside also may involve the GR and possess steroid-like activity. When compared to dexamethasone, Rg1 showed a similar dose-dependent impact on GR activity but Rg1 needed two to three times the concentration to get a similar effect as dexamethasone in FT02B cells and was also inhibited by RU486.[46] Rg1 was shown to down-regulate GR resembling second messenger cAMP.[47]

Ginsenosides Rh1 and Rh2 have been reported to induce differentiation of mouse B16 melanoma cells and F9 terato-carcinoma cells (stem cells) into parietal endoderm cells.[38] Further reports suggest Rh2 caused differentiation of B16 cells by the flattening of cells and increased cell-to-cell adhesiveness. It was incorporated in the membrane whereas Rh1 did not have the same effect.[40] Removal of Rh2 did not cause the growth rate to recover after 4 days[6] suggesting an effect in the membrane on the inside of the cells. Although Rh2 and Rh1 differ in structure by attachment of a glucose moiety at either C-3 or C-6 respectively, Rh1 did not inhibit the growth of B16 cells at a concentration over 100 μM compared to an Rh2 dose over 15 μM which caused complete inhibition of growth.[6]

6.8 Non-ginsenoside bioactivity

Other bioactive constituents of ginseng may either alone or in combination with ginsenosides manifest pharmacological effects. Acidic polysaccharides, from the ethanol insoluble fraction of ginseng root possess bioactivity.[48–50] In mice, the acidic polysaccharides (Ginsan) showed immuno-stimulator ability by activating macrophages to produce nitrogen intermediates, induced proliferation of T cells and B cells, and reduced proliferation of cultured cancer cells.[50] Furthermore, acidic polysaccharide (Ginsan) inhibited B16-F10 melanoma cells and induced production of cytokines[49] critical to an effective immune response. Four acid polysaccharides extracted from ginseng (C.A. Meyer) induced differing bioactivities. One component increased interleuklin-8 (IL-8), which is chemotactic for neutrophils and basophils but not monocytes, produced from human monocytes through IL-8 mRNA expression.[48]

Panaxans from Asian ginseng and quinquefolans from North American ginseng are examples of peptidoglycans that have potential hypoglycemic effects in mice.[51–53] Petroleum ether extracts of ginseng, containing a lipid-soluble fraction, possess anti-proliferation and cell cycle blockage capabilities.[54] In three renal carcinoma cell lines, partially purified petroleum ether extracts inhibited proliferation in a dose-dependent manner and blocked cell cycle progression at G1/S phase.[54] Furthermore, petroleum ether extracts of ginseng inhibited the aggregation of human platelets that were induced by thrombin, generated in blood clotting that acts on fibrinogen to produce fibrin, in a dose-dependent manner.[55] Also petroleum ether extracts caused elevated levels of cGMP, a second-messenger generated by guanylyl cyclase involved in nitric oxide release, and inhibited thromboxane A_2 (TxA2) production,[55] which when released induces platelet aggregation and release and causes arteriolar constriction.

6.9 The safety of ginseng

The very long-standing history of use of ginseng in both Asian and North American cultures is an indication of the safety of this herb.[56] There are few reported cases of ginseng toxicity or side effects when taken at recommended dosages. A careful evaluation of many of these reports has been published by Vogler et al.[57] Early animal studies, conducted in dogs, reported no adverse effect of ginseng on body weight or blood chemistry[58] following chronic exposure to a standardized ginseng extract that was given orally up to 15 mg kg^{-1} per day for 90 days.[58] In mice,[59] the LD_{50} for ginseng ranges from 10 to 30 g kg^{-1} and a lethal oral dose of purified ginseng is as high as 5 g kg^{-1} body weight.[58] A 'Ginseng Abuse Syndrome' characterized by Siegal[60] in 10% of 133 ginseng users was compromised by the fact that those experiencing the symptoms of overstimulation (e.g., hypertension/palpita-

tions, insomnia, gastrointestinal upset, menstrual abnormalities and breast tenderness) were similar to those of very high intakes of caffeine. Moreover, many of these individuals were taking very large amounts of ginseng that accumulated to a daily intake of 15 g (using 30–500 mg capsules). Notwithstanding these exceptional examples, caution is required for a combined intake of ginseng with various medications that include phenelzine sulphate (a monoamine oxidase inhibitor) and warfarin (blood thinner).

6.10 Quality control and use in food

Ginseng is listed in many pharmacopoeias from such diverse countries as Austria, China, France, Japan, Russia and Switzerland. A growing number of products containing ginseng is available on the nutraceutical market. Standardized ginseng extracts are available as capsules and as powders. Since there are many factors that influence the quality of ginseng products standardization of the composition (e.g. ginsenoside content) is important. The minimal content of ginsenoside (e.g. based on Rg1 content) required in ginseng products is 1.5–2% in such countries as France, Germany and Switzerland. Moreover, there are at least six individual ginsenosides that comprise the standard of reference (e.g. Rb1, Rb2, Rg1, Rc, Rd, and Re) for ginseng extract standardization. A study published in the *Lancet*,[72] with 54 different commercial ginseng products identified the propensity for fraud or adulteration, because 60% of all products tested contained very small amounts of ginsenoside and 25% had none at all.

Ginseng is marketed commercially as fresh, white or red ginseng. These classifications are based on the procedures used to prepare the product for the consumer. Fresh ginseng is not dried and whereas white ginseng constitutes peeled, fresh ginseng root the production of red ginseng involves steaming and drying the fresh ginseng for extended time periods. More recent studies have shown that heat-processing ginseng at high temperatures results in enhanced biological activity, especially in improved chemoprevention (increased cytotoxicity to cancer cells)[73] and enhanced vasorelaxation.[74]

A number of different product formats have been used to deliver ginseng constituents to the consumer. Tinctures, made with 40% water and 60% alcohol, provide a soluble form for the ginsenosides which may facilitate absorption. Moreover, in the processing of ginseng root, the use of alcohol will prevent enzymatic browning or degradation of the active components. In comparison, powdered extract, a very common form of ginseng, requires drying to remove moisture and this process can result in potential loss of the active components.

Ginseng extracts have been combined with tea leaves, fruit beverages, coffee, soft drinks and beer. Ginseng has also been found in other herbal cocktails often containing *Ginkgo biloba*. Ginseng teas derived from

different plant parts represent a tonic which with the application of heat in the boiling process will facilitate release of other ginseng constituents such as polysaccharides with bioactive properties. Other important food usages of ginseng include the traditional practice of adding ginseng along with other herbs to soups.

6.11 Future trends

Ginsenosides found in ginseng have bioactive properties and specific effects may be attributed to individual ginsenosides. Pure standards have measurable properties and modes of action can be elucidated but there is less certainty about the bioactivities of ginseng extracts or ginseng products. Standardization of all ginseng nutraceutical products is essential for consumer confidence and the sustained success of both the nutraceutical and functional food industries. To this end there is a very great need to understand further the complex chemical composition of this ancient herb that goes beyond the many specific ginsenosides known to us presently. Further chemical analysis of the composition of ginsenosides, quinquefolans and ginsenans, in addition to other presently unidentified bioactive compounds, in combination with both animal and human clinical studies that employ standardized extracts, is required before the herbal industry can predict confidently the basis for variability of efficacy and safety of ginseng products.

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7

Ginkgo biloba and Alzheimer's disease

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7.1 Introduction

Alzheimer's disease, one of the most devastating neurological disorders, affects approximately 15 million persons worldwide. The cost to the US health system of dealing with the 4 million patients with Alzheimer's is estimated at $80 billion a year, compared to around $3.8 billion to treat the UK's 500 000 patients annually. At present, extracts of ginkgo leaves are used extensively for the treatment of memory disorders associated with ageing, including Alzheimer's disease and vascular dementia. *Ginkgo biloba* is one of the most promising complementary remedies in dementia and alternative medicine (CAM) therapy that is often perceived as a cheaper, less costly alternative than conventional medicine. In the United States alone, nearly 11 million people took ginkgo extract in 1997 (Giese, 1999).

Ginkgo (*Ginkgo biloba* L.), also known as maidenhair tree, of the Ginkgoacea family, is a dioecious tree up to 30 m tall, with a bole circumference of up to 9 m. It is believed to be native to China. The name of the genus *Ginkgo* derives from a mis-translation of the Japanese name, *Yin-Kwo*, meaning 'silver fruit'. The leaves are deciduous, alternate or in clusters of 3–5 on short stems or petioles, fan-shaped, thickened at the margins, 5–10 cm across and bilobed, hence the species name *biloba*. The seed is yellow, round, about 2.5 cm long, with bad-smelling pulp surrounding the thin-shelled white nut that contains an edible sweet kernel.

The leaf extract of *Ginkgo biloba* is one of the oldest natural therapeutic agents still used today. The *Ginkgo biloba* tree has long been part of the traditional Chinese pharmacopeia, first cited as a medicinal agent about 5000 years ago. It was first mentioned in Chinese herbals around the

fourteenth century AD, for its 'fruit', that was consumed raw or cooked. In traditional Chinese medicine, ginkgo seeds are prescribed as a remedy against asthma, cough, bladder inflammation, blennorrhagia and alcohol abuse. Anticarcinogenic and vermifugal properties have also been claimed for the raw nuts. The use of ginkgo leaves in traditional Chinese medicine to treat cardiovascular disorders and asthma dates back to AD 1550. In the modern Chinese pharmacopeia, ginkgo is still highly regarded and inhalation of a decoction of the leaves is widely used to alleviate asthma and bronchitis. Ginkgo leaves are considered effective in the treatment of asthma, pneumonic tuberculosis, leukorrhea and disturbed spermatogenesis. They also stimulate blood circulation and have pain-relieving properties. The boiled leaves are still used against chilblains, which may be explained by its vasoactive properties demonstrated by modern pharmacology. The pharmacological effects of *Ginkgo biloba* extract involve vascular, rheological, metabolic and immunological mechanisms.

7.2 Chemistry

Ginkgo is one of the most extensively studied plants in herbal medicine. Investigations of the chemical constituents of *Ginkgo biloba* date back to 1818 and have recently been reviewed by Hasler (2000) and Mazza and Oomah (2000). The composition of all parts of the ginkgo plant, the root bark, wood, the sarcotesta and the seeds have been studied to a lesser extent than the leaves that are most commonly used for phytomedicines in the Western World. More than a hundred compounds identified in ginkgo leaves are ubiquitous in the leaves of higher plants, with the exception of certain flavonoids and the unique terpene trilactones. Compounds in ginkgo that have been extensively studied due to their documented biological activity include the ginkgolides, flavonoids and alkylphenols such as ginkgolic acids.

7.2.1 Ginkgolides

Bioactive terpenoids of *Ginkgo biloba* are represented by a group of diterpenes differing in the number of hydroxyl groups (ginkgolides) and a sesquiterpene (bilobalide). Ginkgolides, occurring naturally in the roots, root barks and leaves of *Ginkgo biloba*, are characterized as 20 carbon cage molecules with a t-butyl group and six, five-membered rings A to F including a spironane, a tetrahydrofuran ring and three lactone rings (Maruyama et al., 1967 a–d). The ginkgolides GA, GB, GC, GM and GJ (Fig. 7.1) differ only in the number and positions of the three lactone rings and the hydroxyl groups on the C_1, C_3 and C_7 of the spironane framework. These ginkgolides also known as BN52020, BN52021, BN52022, BN52023 and BN52024, respectively, in Institut Henri Beaufour (IHB) internal nomenclature, rep-

Ginkgolide	IHB nomenclature	R_1	R_2	R_3
A	BN 52020	OH	H	H
B	BN 52021	OH	OH	H
C	BN 52022	OH	OH	OH
J	BN 52024	OH	H	OH
M	BN 52023	H	OH	OH
synthetic	BN 50580	OH	OMe	H
synthetic	BN 50585	OH	OEt	H

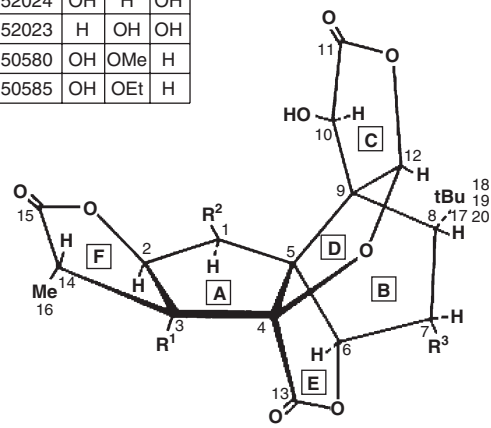


Fig. 7.1 Structure of ginkgolides.

resent the four platelet activating factor (PAF) antagonists initially characterized in *Ginkgo biloba* leaf extract EGb 761.

Among the ginkgolides, GB is the most active PAF antagonist and this activity is reinforced significantly when GB is combined with GA and GC. A standardized mixture of ginkgolides A, B and C in the 2:2:1 ratio, known as BN52063 is the first drug recognized as a potent PAF antagonist in humans (Guinot et al., 1988). Stable derivatives of ginkgolide B, 1-methoxy and 1-ethoxy have also been synthesized (Fig. 7.1).

The root bark of ginkgo trees is an abundant source of ginkgolides, yielding equal concentrations (0.01%) of GA and GB, 0.02% GC and 0.0002% GM after extraction and multiple purification steps (Nakanishi, 1988). Ginkgolide C (GC) is also the most abundant ginkgolide in leaves, the organ of choice for large-scale extraction of ginkgolides and ginkgo leaf extract EGb 761 (Mauri et al., 1999) (Table 7.1). Seasonal and environmental variations observed in the ginkgolide content of leaves are a major concern for industrial quality control of ginkgo products. The content of single ginkgolides has been found to vary by a factor of more than 100 between leaves of different origin (Beek et al., 1991). This can lead to significant variations in terpene lactone contents from 4 to 11% observed in *Ginkgo biloba* products on the US market (Kressman et al., 2002).

Bilobalide, a sesquiterpene lactone, bears a striking structural resemblance to the ginkgolides and is believed to be a degraded ginkgolide (loss of carbon 2, 3, 14, 15 and 16) devoid of the spironane unit and the

Table 7.1 Main components of *Ginkgo biloba* leaf and of EGb 761 extract

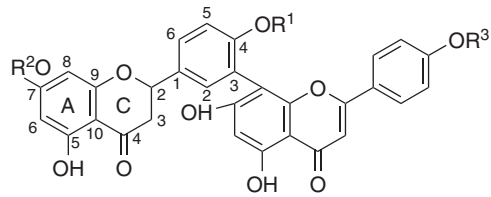
Constituents	Leaf	EGb 761
Flavonol glycosides (%)	0.5–3.5	21.6–26.4
Terpene trilactones (%)	0.065–0.4	5.4–6.6
Ginkgolides (%)		
Ginkgolide A (GA)	0.046	1.49
Ginkgolide B (GB)	0.038	0.58
Ginkgolide C (GC)	0.075	2.57
Ginkgolide J (GJ)	0.015	0.73
Bilobalide (%)	0.05–0.8	2.6–3.2
Proanthocyanidins (%)	4–12	7–9.5
Ginkgolic acid (ppm)	0.88–12.43	<5

tetrahydrofuran cycle (Nakanishi, 1988). Among the constituents of *Ginkgo biloba*, bilobalide has the most potent anti-apoptotic capacity and is recognized for its neuroprotective effects (Chandrasekaran et al., 2001). Hence, bilobalide from ginkgo leaf extract may be one of the compounds partly responsible for the inhibition of toxicity and cell death induced by beta-amyloid peptides (Bastianetto et al., 2000a). Bilobalide and GA are present in similar concentrations in the leaves and extracts thereof (Mauri et al., 1999), although variation in concentrations is known to occur due to the type of extraction used (Yang et al., 2002).

7.2.2 Flavonoids

Flavonoids constitute one of the major groups of constituents of *Ginkgo biloba* extract EGb 761. The flavonoid fraction of the extract has been found to block β-amyloid-induced events, i.e. the accumulation of reactive oxygen species and apoptosis, that relates to the neuroprotective effects of EGb 761 against AD (Bastianetto et al., 2000a). These protective and rescuing abilities of EGb 761 have been attributed to the antioxidant properties of the flavonoids and their ability to inhibit nitric oxide-stimulated protein kinase activity (Bastianetto et al., 2000b). The thirty or more flavonoids isolated from ginkgo leaves consist of flavones, flavonol diglycosides and flavonol (proanthocyanidins).

Seven dimeric flavones, so called biflavones (Fig. 7.2), of the amentoflavone-type are well characterized in *G. biloba.* The four commonest of these dimeric flavones are: bilobetin, ginkgetin, isoginkgetin and sciadopitsin. *Ginkgo biloba* is the first plant from which ginkgetin was isolated. The biflavones bear the same 3′, 8″-linked nuclei with one to three methoxy substitutes (Fig. 7.2). In contrast to the ubiquitous flavonol aglycones, the biflavones are important compounds in the identification of



1	Amentoflavone	$R^1 = R^2 = R^3 = H$
2	Bilobetin	$R^1 = Me, R^2 = R^3 = H$
3	Ginkgetin	$R^1 = R^2 = Me, R^3 = H$
4	Isoginkgétin	$R^1 = R^3 = Me, R^2 = H$
5	Sciadopitysin	$R^1 = R^2 = R^3 = Me$

Fig. 7.2 Structure of biflavones.

ginkgo leaves and leaf extracts. The concentration of biflavones in ginkgo leaves and extracts varies from 0.4 to 1.9% and from 0.047 to 1.68%, respectively (Beek, 2002) (Table 7.1). The biflavones are generally removed from special extracts EGb 761 and LI 1370 (Lichter Parma, Berlin) and are therefore not detected (Sticher et al., 2000). The biflavonoids were previously thought not to possess any clear pharmacological activities and therefore not needed for standardization but recently, free radical scavenging properties, antioxidant, antiarthritic and analgesic activities of biflavones have been reported (Joyeux et al., 1995; Kim, 2001; Kim et al., 1999).

Two different groups of flavonol glycosides, the rutinosides and a unique group of glycosides characteristic of ginkgo, the bilosides, occur in ginkgo leaves. The 22 glycosides in ginkgo leaves and extracts occur most commonly as derivatives of quercetin, kaempferol and isorhamnetin, with the aglycones themselves present only in very low concentrations. Hence the aglycone content, resulting from the acid hydrolysis of the flavonol glycosides, is commonly expressed in terms of the three aglycones quercetin, kaempferol and isorhamnetin due to their abundance. In the leaves, the aglycones generally occur in the ratio of 1:2.8:1.5 of isorhamnetin: kaempferol: quercetin. However, in commercial ginkgo extracts, this ratio changes with a prevalence of quercetin (Sticher et al., 2000; Yang et al., 2002). Ginkgo leaves and dry leaves from the commercial market have aglycone contents (w/w) of 0.2 to 1.4% and 0.2 to 0.4%, respectively, corresponding to calculated flavonol glycoside content of 0.5 to 3.5% and 0.5 to 1% w/w, respectively.

Proanthocyanidins of ginkgo leaves (4–12% dry wt) and standardized extract (7% w/w) are believed to contribute significantly to the beneficial effects of ginkgo leaf extracts. Four monomers (catechin (C), epicatechin (EC), gallocatechin (G) and epigallocatechin (EG)), as well as four dimers (EC-C, C-C, GC-C, EG-C) have been identified in ginkgo leaves (Stafford

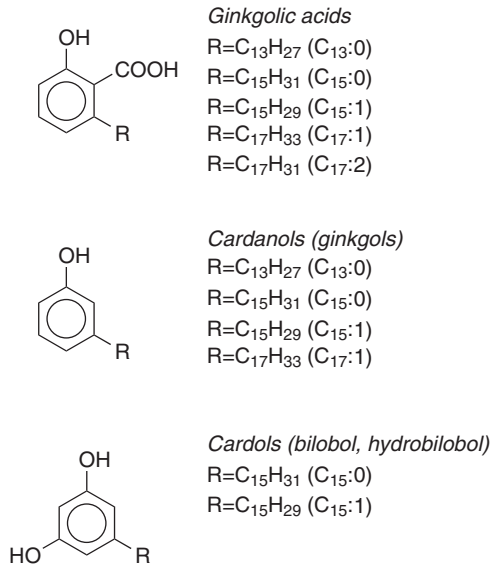


Fig. 7.3 Structure of ginkgolic acids, cardanols and cardols.

et al., 1986). However, in the leaves, the polymeric structures of procyanidin and prodelphinidin prevail over the dimeric and monomeric structures.

7.2.3 Alkylphenols

Three classes of alkylphenols, ginkgolic acids (2-hydroxy-6-alkylbenzoic acids, 6-alkylsalicilic acids or anacardic acids), ginkgols (3-alkylphenols or cardanols) and bilobols (5-alkylresorcinols or cardols) are present in various parts of *Ginkgo biloba* including the leaves (Fig. 7.3). The alkyl residue varies from 13 to 17 carbon side chains with zero to two double bonds in the Z configuration (Beek, 2002). Ginkgolic acids occur in approximately 10 and 200 times higher concentration than ginkgols and bilobols, respectively, and have the highest allergenic potential (Beek and Wintermans, 2001). The concentration of bilobols varies from 27 to 87 ppm in leaves (Zarnowska et al., 2000) while that of total alkylphenols is limited to 5 or 10 ppm by most suppliers of ginkgo extract. During the production of the standardized ginkgo extract EGb 761, alkylphenols are largely eliminated as water insoluble compounds from the primary acetone extract. The presence of these compounds is considered undesirable in ginkgo extracts due to their known contact allergenic, cytotoxic, mutagenic and slight neurotoxic effects. Strong lethal embryotoxic effects at 64 ppm in a hen's egg test and immunotoxic effect of ginkgolic acids resulting in enlargement of the popliteal lymph nodes in mice have recently been observed (Baron-

Ruppert and Luepke, 2001; Koch et al., 2000). However, ginkgolic acids in particular have several beneficial biological activities including antimicrobial, molluscicidal and antitumoral activities that can lead to reduced risk of various diseases such as hyperglycemia, diabetes, myocardial infarction and high blood pressure (Mazza and Oomah, 2000).

7.2.4 Other constituents

Ginkgo biloba leaves contain significant amounts of carboxylic acids. A level of 13% in the standardized extract EGb 761 has been reported (Beek, 2002). The non-phenolic acids group consists mainly of ascorbic, D-glucaric, quinic and shikimic acids, with the content of the latter at 1.9% based on dried ginkgo leaves. p-Coumaric acid (0.25 mg/g of leaves) is the major phenolic acid while procatechuic acid predominates amongst the free phenolic acids (19.7 µg/g of fresh leaves) (Ellnain-Wojtaszek, 1997; Ellnain-Wojtaszek et al., 2001). A rare nitrogen-containing phenolic acid, 6-hydroxykynurenic acid, occurs in ginkgo leaves (1–2 mg/g of fresh leaves) (Beek, 2002). 6-Hydroxykynurenic acid may have clinical relevance under conditions that compromise the integrity of the blood-brain barrier by acting as a glutamate mediator for synaptic responses (Weber et al., 2001).

Polyprenols consisting of a large number of isoprene units are present in ginkgo leaves mostly as acetates at an average content of 1.5%. They are absent in standardized ginkgo extracts due to their removal during the manufacturing process. Antivitamin B6, 4-O-methylpyridoxine, has been detected in leaves (2–5 µg/fresh leaf) (Beek, 2000).

7.3 Functional effects

The wide use of *Ginkgo biloba* extracts for the treatment of memory disorders associated with ageing, including Alzheimer's disease and vascular dementia, especially in Germany, is based on the pharmacological action resulting from the combined activities of several active principles (primarily, the flavonoids and terpenoids). This 'polyvalent' (terminology coined by DeFeudis, 1991) action explains the vaso- and tissue-protective and cognition-enhancing benefits of the *Ginkgo biloba* extract (Beek et al., 1998). Use of most ginkgo preparations is based on their antioxidant properties and their action on the peripheral blood circulation with the aim of preventing chronic degenerative diseases typical of ageing (Table 7.2).

The dimeric flavonoids are particularly strong vasoactive agents (Bombardelli et al., 2000). They are particularly active due to their mechanism of action: inhibiting histamine release from mast cells and cyclic-AMP-phosphodiesterase, thereby enhancing the effects of prostaglandin I2 or prostacyclin, mediators of vasoprotection and thrombosis. Biflavones from *Ginkgo biloba*, with the exception of sciadopitysin, stimulate lipolysis

Table 7.2 *Ginkgo biloba* constituents and their pharmacological effects

Substances	Activities
Flavonol glycosides	Antioxidant, vasokinetic, antiinflammatory
Dimeric flavonoids	Vasokinetic, antiinflammatory, antiphosphodiesterasic
Ginkgolides	Antiinflammatory, antiallergenic, anti-PAF
Bilobalide	Antiinflammatory, antimicrobial
Proanthocyanidins	Antioxidant, venotonic, vasoprotectant
Ginkgols and ginkgolic acids	Allergenic properties (should be absent from extracts)
Other aromatic acids	Antioxidant

in adipocytes and skin microcirculation (Dell'Agli and Bosisio, 2002). Amentoflavone and quercetin are the principal components of *Ginkgo biloba* extract exhibiting the positive chronotropic and ionotropic actions that affect cardiac function on isolated rat atria (Kubota et al., 2002). Amentoflavone also exhibits anti-inflammatory activity with dual inhibitory activity of group II phospholipase A2 and cyclooxygenase (COX) (Kim et al., 1998). Ginkgetin, another *Ginkgo biloba* biflavone, may be a potential antiarthritic agent with analgesic activity since it strongly reduces arthritic inflammation in an animal model of rat adjuvent-induced arthritis (Kim et al., 1999). It protects skin inflammation by down-regulating COX-2 induction (Kwak et al., 2002). The biflavones and some flavonoids from *Ginkgo biloba* have antilipoperoxidant, antinecrotic and scavenging properties (Joyeux et al., 1995) and exhibit potent antimicrobial activities against *Clostridium perfringens* and *E. coli* without inhibiting human intestinal bacteria (Lee and Kim, 2002).

The terpenoid constituents of *Ginkgo biloba* extract, especially ginkgolide A, exhibit a cardioprotective effect that involves a direct free scavenging mechanism. The terpenoid constituents and the flavonoid metabolites of EGb 761 act in a complementary manner to protect against myocardial ischemia-reperfusion injury (Liebgott et al., 2000). Ginkgolide B stimulates a rapid and weak production of oxygen species in human polymorphonuclear leukocytes and directly activates platelet-activating factor receptors (Lenoir et al., 2002). Both ginkgolide A and bilobalide elevate glutathione-transferase (a detoxifying enzyme) activity in mouse liver (Sasaki et al., 2002). Ginkgolides A and B and bilobalide contribute to the selective inhibitory effect of EGb 761 on nitric oxide synthase expression without affecting the vasodilatory effects (Cheung et al., 2001).

Ginkgo biloba extract demonstrates anti-ischemic properties attributable to the terpenoid fraction, mainly due to the presence of bilobalide. It allows mitochondria to maintain their respiratory activity under ischemic conditions as long as oxygen is present, thus delaying the onset of ischemia-

induced damage (Janssens et al., 1999). Bilobalide may be beneficial in the treatment of brain hypoxia and/or neuronal hyperactivity since it inhibits the glutamatergic excitotoxic membrane breakdown both *in vitro* and *in vivo* (Weichel et al., 1999). It also possesses anticonvulsant activity since it modulates gamma-aminobutyric acid (GABA)-related neuronal transmission. The anticonvulsant effect is due to the elevation of GABA levels, possibly through potentiation of glutamic acid decarboxylase activity of bilobalide (Sasaki et al., 1999). Bilobalide protects beta-amyloid peptide fragment-35 (Aβ25–35)-induced PC-12 cell cytotoxicity by inhibiting elevated lipid peroxidation (Zhou et al., 2000). Protection of PC-12 cells by bilobalide may be due to increased activity of reactive oxygen species-scavenging enzymes such as superoxide dismutase and catalase (Song et al., 2000), or blockage of reactive oxygen species-induced apoptosis (Ahlemeyer et al., 1999; Zhou and Zhu, 2000).

7.4 Role in managing Alzheimer's disease

The constituents of *Ginkgo biloba* contributing to its neuroprotective effects are generally thought to be the ginkgolides and bilobalides within the terpene fraction and to the flavonoids. They appear to act synergistically. *Ginkgo biloba* special extract preparations are used as antidementia drugs or nootropics that improve disturbance of higher interactive noetic functions and impairment of vigilance. Standardized extracts of ginkgo leaves have been used since 1986 for the treatment of slight to moderate forms of senile dementia (AD or MID) (DeFeudis, 1991). On the grounds of proven clinical efficiency, dry extracts of *Ginkgo biloba* leaves SeGb, EGb 761 and LI 1370 have been approved by the German federal health authority for the symptomatic treatment of impaired cerebral function in dementia syndromes within a treatment plan. Their main indications are primary degenerative dementia, vascular dementia and their mixed forms. The clinical efficacy of *Ginkgo biloba* extracts EGb 761 and LI 1370 in dementia of the Alzheimer type and vascular dementia, as well as age-associated impairment of cerebral function, has been clearly demonstrated (Schultz et al., 2000). The principal clinical trials concerning the therapeutic applications of *Ginkgo biloba* extract in managing Alzheimer's disease have been extensively reviewed (Darlington et al., 2000; Mazza and Oomah, 2000; Schultz et al., 2000). Several new clinically controlled studies confirming the efficacy of *Ginkgo biloba* extract in neuropathology have appeared since publication of the above cited reviews. These new studies are summarized in Table 7.3 and briefly discussed below.

Le Bars et al., (2002) recently evaluated the therapeutic effect of *Ginkgo biloba* extract EGb 761 in Alzheimer's disease based on the severity of cognitive impairment. This involved a 52 week randomized, double-blind, and placebo-controlled, parallel-group multicenter study with 120 mg of

Table 7.3 Double-blind, placebo-controlled, randomized trials of *Ginkgo biloba* extract for Alzheimer's disease

Authors	Patients entered (analyzed)	Dosage and duration	Outcome
Le Bars et al., 2002	236	120 mg for 52 weeks	Improvement in cognitive performance and social functioning regardless of the stage of dementia.
Dongen et al., 2000	214	240 mg (high) or 160 mg (usual) daily for 24 weeks	Treatment not effective for older people with mild to moderate dementia or age-associated memory impairment.
Le Bars et al., 2000	309 (244)	120 mg (40 mg t.i.d.) for 26 weeks	Improvement in cognitive assessment, daily living and social behavior.
Le Bars et al., 1997	309 (202)	120 mg daily for 52 weeks	Improvement in cognitive and geriatric ratings and social functioning of demented patients for 6 to 12 months.
Maurer et al., 1997	20	240 mg per day for 3 months	Improvement in attention and memory, according to SKI test; effective in mild to moderate dementia.
Kanowski et al., 1996	216 (156)	240 mg oral dose daily for 24 weeks	Effective as a therapy for cognitive performance for presenile and senile dementia.
Haase et al., 1996	40	4 days per week for 4 weeks	Improvement in psychopathology and cognitive performance by short term intravenous infusion therapy.

EGb 761. A data set collected for 236 patients (137 women, 99 men; average age 68), was stratified, based on Mini-Mental State Examination (MMSE) score, into mild (MMSE > 23), moderate (MMSE < 24) and severe (MMSE < 15) stages of dementia. The results of this retrospective study indicated that EGb 761 favorably affected cognitive performance ($p = 0.02$) and social functioning ($p = 0.001$), especially in the group of patients with very mild to moderate dementia. The difference between EGb 761 and placebo, on the outcome measure of cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog), was comparable to the drug–placebo differences observed with cholinesterase inhibitor drugs, rivastigmine (6–12 mg/day), tacrine (160 mg/day) and donepzil (10 mg/day) (Le Bars et al., 2002).

The same data set collected during the 52 week multicenter study referred to above was used to perform an intent-to-treat (ITT) analysis of the efficacy of 26 weeks treatment with a 120 mg (40 mg t.i.d.) of EGb 761 in patients with mild to severely impaired dementia (Le Bars et al., 2000). Although 309 patients diagnosed with uncomplicated Alzheimer's disease or multi-infarct dementia according to ICED-10 (WHO, 1991) and DSM-III-R (APA, 1987) criteria, were included in the ITT analysis, only 244 patients (76% for placebo and 73% for EGb 761) actually reached the 26 week stage. Patients receiving EGb 761 treatment improved on cognitive assessment, daily living and social behavior evaluated by ADAS-Cog, Geriatric Evaluation by Relative Rating Instrument (GERRI), and Clinical Global Impression of Change (CHIC), respectively.

A similar 24 week randomized, double blind, placebo-controlled, parallel-group multicenter trial was conducted by Dongen et al. (2000). In the southern part of the Netherlands, 214 elderly people with mild to moderate dementia or age-associated memory impairment were recruited from 39 homes. The participants were allocated randomly to treatment with EGb 761 (2 tablets per day, total dosage of either 240-high dose [n = 73] or 160-usual dose [n = 79] mg/day) or placebo (0 mg/day, n = 4). Outcome measures included neuropsychological testing (digital memory span, trail-making speed, and verbal learning), clinical assessment (presence and severity of geriatric symptoms, depressive mood, self-perceived health and memory status) and behavioral assessment. These were evaluated after 12 and 24 weeks of intervention. An intent-to-treat analysis showed that after 12 weeks of treatment, the group receiving ginkgo performed slightly better with regard to self-reported activities of daily life. However, analysis for the entire 24 week period showed no detectable benefit from ginkgo. This suggests that contrary to previous trials, ginkgo was not effective as a treatment for older people with mild to moderate dementia or age-related cognitive decline.

The two prospective studies (Le Bars et al., 2000, 2002) discussed above were based on a core 52 week multicenter trial designed to assess the efficacy and safety of EGb 761 in Alzheimer's disease and multi-infarct

dementia (Le Bars et al., 1997). This study, conducted at 6 research centers in the USA enrolled 327 mildly to severely demented outpatients including 251 individuals with Alzheimer's disease or multi-infarct dementia, without other significant medical conditions. Patients were randomly assigned to treatment with EGb 761 (120 mg/day) or placebo. Complete assessment of the primary outcome measures, ADAS-Cog, GERRI and CHIC tests, were performed at baseline, 12, 26 and 52 weeks. The intent-to-treat analysis involved 309 (155 for EGb 761 and 154 placebo), 283 (142 for EGb 761 and 141 placebo), 202 (97 for EGb 761 and 105 placebo) and 137 (78 for EGb 761 and 59 placebo) at baseline, 12, 26 and 52 weeks end-point analysis, respectively. Results of analysis for the 52 week point showed that EGb 761 was safe, capable of stabilizing and improving the cognitive performance and social functioning of dementia patients substantially for 6 months to a year. The changes induced by EGb 761 measured objectively by the ADAS-Cog were of sufficient magnitude to be recognized by the caregivers in the GERRI test (Le Bars et al., 1997).

The effectiveness of special *Ginkgo biloba* extract EGb 761 in mild to moderate dementia of the Alzheimer type was confirmed by a very small double-blind, randomized, placebo-controlled parallel-group clinical trial involving 20 outpatients undergoing oral treatment (240 mg/day of EGb 761) for 3 months; Maurer et al., 1997). EGb 761 treatment significantly ($p < 0.013$) improved attention and memory, psychometric efficacy, psychopathology and dynamic functional levels as assessed by the sum score of the Syndrom-Kurz test (SKT), psychometric tests (trail making test, ADAS-Cog, CHIC), and electrophysiological investigations (EEG topography), respectively.

The positive therapeutic risk-benefit of *Ginkgo biloba* extract EGb 761 for the treatment of outpatients with Alzheimer's disease and multi-infarct dementia was clearly validated by a clinical study carried out in a total of 41 study centers in Berlin and Karlsruhe in 1990, 1991, and 1992 (Kanowski et al., 1996). A total of 222 outpatients, at least 55 years of age, suffering from either Alzheimer's or multi-infarct dementia of mild to moderate severity were included in the study. After a 4 week run-in period, 216 outpatients included in the randomized 24 week trial, received either a daily oral dose of EGb 761 (240 mg) or placebo. The confirmatory efficacy analysis was performed on 156 outpatients (52 women, 27 men, average age 70 for EGb 761; 53 women, 24 men, average age 66–72 for placebo) who completed the double-blind treatment period in accordance with the study protocol. *Ginkgo biloba* extract EGb 761 showed significant ($p < 0.005$) therapeutic effect evaluated multi-dimensionally for psychopathological assessment (Clinical Global Impressions – CGI), patients' attention and memory (SKT) and behavioral assessment of daily life activities by the Nurnberger Alters-Beobachtungsskala (NAB).

Haase et al. (1996) demonstrated the beneficial effect of *Ginkgo biloba* extract EGb 761 administered intravenously for a short time in patients

with moderate dementia. In this placebo-controlled, randomized, double-blind clinical trial, infusions of either EGb 761 or placebo were administered 4 days per week for 4 weeks in 40 patients (mean age of 68 years) with moderate dementia (Alzheimer, vascular, or mixed type). *Ginkgo biloba* treatment significantly ($p < 0.05$) improved the behavioral, psychopathologic and psychometric outcome of patients as assessed by NAB, CCIG and the Kurztest fur Allgemeine Intelligenz (KAI), respectively. The most prominent symptom of illness and depression also decreased with EGb 761 therapy thereby confirming the clinical benefit of *Ginkgo biloba* in improving psychopathological and cognitive performance in patients with moderate dementia.

In addition to the numerous double-blind and randomized controlled trials with *Ginkgo biloba* extracts dating back to 1984, five meta-analyses of clinical studies have been performed with *Ginkgo biloba* relating to Alzheimer's disease, dementia and cerebral performance, where *Ginkgo biloba* has generally been found to have significant positive clinical effects (Table 7.4). Recently, Ernst and Pittler (1999) systematically reviewed the clinical evidence of *Ginkgo biloba* preparations as a symptomatic treatment for dementia. Computerized literature searches were performed to identify all double-blind, randomized, placebo-controlled trials assessing clinical end-points of *Ginkgo biloba* extract as a treatment for dementia. Data were extracted from Medline, Embase, Biosis and the Cochrane Library without restrictions regarding the language of publication in a standardized, predefined fashion, independently by both authors. Nine double-blind, randomized, placebo-controlled trials that met the inclusion criteria were reviewed. These studies of varying methodological quality collectively suggest that *Ginkgo biloba* extract is more effective for dementia than is the placebo; however, a few, generally mild, adverse effects were reported.

Flint and van Reekum (1998) made a qualitative review of randomized, double-blind, placebo-controlled trials of medications used to treat cognitive deficits, disease progression, agitation, psychosis, or depression in Alzheimer's disease (AD). A computerized search of Medline was used to identify relevant literature published during the period 1968 to 1998. Key words used in the search were 'randomized controlled trials,' 'dementia' and 'Alzheimer's disease'. Donepezil and *Ginkgo biloba*, two of the four agents currently available in Canada to treat the cognitive deficits of AD, were associated with a statistically significant but clinically modest improvement in cognitive function in a substantial minority of patients with mild to moderate AD. This indicates that selected medications can be used to treat cognitive deficits, disease progression, agitation, psychosis and depression in AD. However, considerable heterogeneity was observed in patients' responses to these medications.

The effect of treatment with *Ginkgo biloba* extract on objective measures of cognitive function in patients with AD has been determined based on a formal review of the current literature (Oken et al., 1998). According

Table 7.4 Meta-analyses of clinical studies with *Ginkgo biloba* extract

Authors	Type of study	Disease/diagnosis	Assessment
Ernst and Pittler, 1999	Computerized literature searches for randomized controlled trials to March 1998	Dementia	9 studies out of 18 double-blind, randomized, placebo-controlled trials met the inclusion/exclusion criteria. Collectively, the trials suggest that *Ginkgo biloba* is effective in delaying the clinical deterioration of patients with dementia.
Flint and van Reekum, 1998	Literature review 1968–1998 of randomized double-blind placebo-controlled trials	Alzheimer/cognitive deficits agitation, psychosis, or depression in AD	Inconclusive data. GB associated with a significant but clinically modest improvement in cognitive function.
Oken et al., 1998	Review of 50 studies	Cognitive function in Alzheimer's disease	Small but significant effect of 3–6 months treatment with 120–240 mg of GB extract on cognitive function in AD.
Hopfenmuller, 1994	Review of 11 controlled clinical trials	Cerebrovascular insufficiency	7 studies confirmed effectiveness of GB vs placebo based on total scores of clinical symptoms.
Kleijnen and Knipschild, 1992	Review of 40 trials	Cerebral insufficiency	8 well-performed trials met the inclusion/exclusion criteria and confirmed the effectiveness of GB in cerebral insufficiency.

AD – Alzheimer's disease
GB – *Ginkgo biloba* extract

to these authors, only 4 of the 50 studies examined met all inclusion criteria of clear diagnoses of dementia and AD. In total, there were 212 subjects in each of the placebo and ginkgo treatment groups. Quantitative analysis of the literature showed a small but significant effect of 3- to 6-month treatment with 120 to 240 mg of *Ginkgo biloba* extract on objective measures of cognitive function in AD. The drug had no significant adverse effects in formal clinical trials but two cases reported bleeding as a complication.

Eleven placebo-controlled, randomized, double-blind, clinical trials were evaluated in a meta-analysis in order to prove the effectiveness of the *Ginkgo biloba* special extract LI 1370 (Kaveri forte, 150 mg extract/day) in patients with cerebrovascular insufficiency in old age (Hopfenmüller, 1994). The biometrical requirements for the quality of the studies were the basic criteria for the performance of clinical drug tests. Seven of the eleven studies were comparable with regard to diagnoses, inclusion and exclusion criteria as well as methodology and therefore statistical meta-analysis could be performed for them, analyzing the parameters 'single symptoms', total score of clinical symptoms and 'global effectiveness'. For all analyzed single symptoms significant differences could be concluded, indicating the superiority of *Ginkgo biloba* compared to placebo. The analysis of the total score of clinical symptoms from all relevant studies indicated that seven studies confirmed that *Ginkgo biloba* was better than the placebo, while only one study was inconclusive.

Kleijnen and Knipschild (1992) by means of a critical review sought evidence from controlled trials in humans of the efficacy of *Ginkgo biloba* extracts in treating cerebral insufficiency. The methodological quality of 40 trials on ginkgo and cerebral insufficiency was assessed using a list of predefined criteria of good methodology and the outcome interpreted in relation to their quality. A comparison of the quality was made with trials of codergocrine, which is registered for the same indication. There were 8 well-performed trials out of a total of 40. Shortcomings were limited numbers of patients included and incomplete description of randomization procedures, patient characteristics, effect measurement and data presentation. In no trial was double-blindness checked. Virtually all trials when ginkgo extract (120 mg/day) was given for at least 4 to 6 weeks reported positive results. Furthermore, *Ginkgo biloba* extract EGb 761 has been found to be equally effective as second generation cholinesterase inhibitors (donepezil, rivastigmine and metrifonate) in the treatment of mild to moderate Alzheimer's dementia, based on the results of 6 published studies (Wettstein, 2000).

Several clinical trials involving *Ginkgo biloba* sponsored by the National Center for Complementary and Alternative Medicine (NCCAM) are currently being investigated and two of them (Phase 3) relate to Alzheimer's disease. The first trial – a *Ginkgo biloba* prevention trial in older individuals – involves 3000 participants who will be studied in a multicenter, randomized trial of 240 mg of *Ginkgo biloba* compared to a placebo in healthy

men and women, median age of 80, for 6 years. The primary end point of this study is dementia, specifically Alzheimer's disease. Secondary end points will include the incidence of vascular disease, changes in cognitive function scores over time, total mortality and changes in functional status. The second trial – preventing cognitive decline with alternative therapies – is a small, 42 months study of the effect of standardized *Ginkgo biloba* extract on preventing or delaying cognitive decline in people age 85 years or older. This randomized, placebo-controlled, double blind trial involves 200 elderly cognitive healthy subjects. It is a pilot study set up to demonstrate the disease-modifying effect of *Ginkgo biloba* on the brain, as an alternative or complementary therapy, due to its antioxidant activity.

In the United Kingdom, a systematic review of complementary and alternative treatments and a placebo-controlled clinical trial investigating the potential of *Ginkgo biloba* for early stage dementia in primary care is currently in progress. This study will evaluate the efficacy (improvement in memory and quality of life) of *Ginkgo biloba* administered at home for 26 weeks to individuals with early dementia. Results of this study will be compared to those of similar trials conducted in a clinical setting in order to detect the magnitude of the additional effect (the Hawthorne effect) due to the added attention provided during clinical trials. This will hopefully provide more acceptance for the use of *Ginkgo biloba* in general medical practice. The Imperial College School of Medicine and the Royal London Homeopathic Hospital are supervising this research jointly.

7.5 Safety issues

Ginkgo biloba standardized extract is remarkably safe based on subchronic and chronic toxicity in rodents (Bilia, 2002) and in humans (Mazza and Oomah, 2000). No mutagenicity, carcinogenicity, teratogenicity or embryotoxicity have been demonstrated for the extract. Tolerability in 98% of the clinical studies is good or very good and only in a few instances (less than 0.001%) have gastrointestinal upset, headaches, and dizziness been reported (Bilia, 2002). New users who take doses in excess of 300 mg/day of *Ginkgo biloba* extract or some patients with poor blood flow to the brain may experience a mild transient headache or dizziness for the first two days of use.

One important safety aspect, herb–drug interaction, has until recently been under-researched and is currently receiving a lot of attention. Interactions between herb products and drugs are based on the same pharmacokinetic and pharmacodynamic principles as drug–drug interactions (Scott and Elmer, 2002). An overview of suspected *Ginkgo biloba*–drug reactions, based on published case reports, is presented in Table 7.5. *Ginkgo biloba* has recently been linked to increased risk of precipitating epileptic seizures in the elderly, based on two case reports (Granger, 2001) (Table 7.5). In both

Table 7.5 *Ginkgo biloba* – drug interactions

Drug	Reported interactions	Reference
Aspirin	Spontaneous hyphema	Rosenblatt and Mindel (1997)
Insulin	Hyperglycemia	Kudolo (2001)
Paracetamol and engotamine/caffeine	Bilateral subdural hematoma	Rowin and Lewis (1996)
Seizure threshold lowering drugs	Might induce seizure	Granger (2001)
Thiazide diuretic	Hypertension	De Smet and D'Arcy (1996)
Trazodone	Coma	Galluzi et al. (2000)
Warfarin or other non steroidal, anti-inflammatory drugs	Intracerebral hemorrhage	Matthews (1998)
Anticoagulants/ antiplatelet drugs	Increased risk of bleeding	Gruenwald et al. (1998)
Fluoxetine and Buspirone (selective serotonin reuptake inhibitors)	Hypomania	Spinella and Eaton (2002)

cases (a 78 year old male with mild cognitive impairment and an 84 year old female with severe dementia), sodium valproate (an anti-epileptic medication, 1200 and 1600 mg/day, respectively), aspirin and other medications (temazepan, rampiril, rivastigmine and thioridazine) were being taken concurrently with *Ginkgo biloba* tablets, 120 mg daily for 12 to 14 days. Both patients became seizure-free after discontinuing the *Ginkgo biloba* remedy. It has been suggested that the flavonoids of *Ginkgo biloba* may potentiate the epileptogenic actions since the flavonoids exhibit GABA-ergic activity and act as partial agonists at benzodiazepine binding sites (Granger, 2001). The potential increased effect of anticoagulants due to ginkgo is reflected in one case of over-anticoagulation and brain hemorrhage in a patient on warfarin, one case of bleeding in the eye while on aspirin and one case of hypertension in a patient on a thiazide diuretic (Ernst, 2000). The bilateral subdural hematoma due to intake of paracetamol and ergotamine and ginkgo may not be interaction but due to ginkgo alone since subarachnoid hemorrage and subdural hematoma have been reported with the use of ginkgo alone (Fugh-Berman, 2000). A case report of a patient with a history of mild traumatic brain injury and resulting depression experiencing hypomania after adding St John's wort and *Ginkgo biloba* to her regimen of fluozetine and buspirone, which remitted after discontinuation of the herbal medicines (Spinella and Eaton, 2002), illustrates the complexity of the

herb–drug interactions. It should be noted that reports of adverse events and ginkgo–drug interactions are rare and probably reflect the benign nature of ginkgo when used wisely. Nevertheless, caution is recommended when using ginkgo especially for individuals with bleeding disorders, diabetes and epilepsy; patients should discontinue use at least two weeks prior to elective surgical procedures.

The use of ginkgo is not recommended for individuals taking anticlotting medication, including regular aspirin use, or tiazide diuretic since it may raise blood pressure and cause spontaneous and excessive bleeding. Mixing ginkgo with Ticlopidine (Ticlid), Clopidogrel (Plavix), Dipyridamole (Persantine) and Warfarin (Coumadin) should be avoided.

The current trend in researching the herb–drug interaction as it relates to *Ginkgo biloba* is illustrated by two prospective clinical trials sponsored by the National Center for Complementary and Alternative Medicine. In the first non-randomized, single group study, effects of concurrently administered herbs including *Ginkgo biloba* on the metabolism of the enzyme-specific probe drug substrates alprazolam and the over-the-counter cough suppressant, dextromethorphan, will be investigated in normal volunteers by evaluating the plasma and urine concentration of these agents and their respective metabolites. The information from this study will be used to predict potential herb–drug interactions with many prescription drugs commonly used by the elderly. The second interventional, randomized, double-blind, placebo-controlled, crossover study, will examine the effect of *Ginkgo biloba* extract on the efficacy of three classes of diabetic medications – glucotrol, glucophage and actose. This study will also examine the effect of *Ginkgo biloba* on pancreatic function in the elderly to determine the potential for pancreatic dysfunction and development of insulinopenia. The first and second studies are currently recruiting patients and slated to be completed by May 2003 and 2004, respectively.

The presence of colchicine (26 μg/tablet) in commercial ginkgo tablet and its potential for affecting the viability of fetuses in women using those supplements during pregnancy (Petty et al., 2001) has sparked a controversy in the manufacture and quality of dietary herbal supplements, especially for the consumers. However, this report has been highly contested in the press and the latest report by Seo (2002) clearly proves the absence of colchicine (at a detection limit of 0.01 μg by HPLC) in ginkgo leaves and extracts, which is reassuring for the safety of these products.

7.6 Conclusion

Alzheimer's disease is the most common cause of dementia and accounts for 50–60% and even up to 80% of all cases. Globally, 18 million people have dementia out of which 11 million people (66%) live in developing countries. By 2020, the number of people affected with dementia is

expected to have increased particularly markedly in the most rapidly developing and populous regions, China, India and Latin America, which are witnessing a rapid demographic ageing. By 2025 there will be about 34 million people with dementia in the world, about 70% of whom will be in developing countries. Worldwide, it is estimated that 22 million individuals will develop Alzheimer's disease by the year 2025. In the United States, one in ten persons over 65 and nearly half of those over 85 have Alzheimer's disease. At the time of writing, four million Americans have Alzheimer's disease and by 2050, 14 million people will be affected unless a cure or prevention is found. The Alzheimer's Society believes that careful planning for the future is needed now. Therefore, alleviation of this disease through prevention becomes essential.

Although the etiology of Alzheimer's disease is not completely unraveled yet, a half dozen types of therapies are being explored (Table 7.6) most of which are aimed at blocking the formation or aggregation of amyloid or accelerating its clearance. Many major pharmaceutical companies including Bristol-Myers Squibb are attempting to inhibit amyloid production with either β-or δ-secretase inhibitors. The disruption of Aβ aggregation is another therapeutic option that has been successful in mouse trials and is undergoing a Phase I human clinical trial. Preliminary results are expected soon from Neurochem Alzhemed. Estrogen replacement therapy in post-menopausal women has been found to be beneficial in reducing the risk of developing Alzheimer's disease by 50%. Hormone therapy and anti-inflammatories are more effective when taken prophylactically since they are believed to inhibit amyloid accumulation resulting in modulation of Alzheimer's disease risk. Immune therapy, anti-inflammatory drugs and cholesterol-lowering drugs are also therapies that have potential merit. Clinicians currently use compounds such as tacrine hydrochloride (Cognes), donepezil hydrochloride (Aricept), rivastigmine tartrate (Exelon), and galantimine hydrobromide (Reminyl) to moderate symptoms of Alzheimer's disease. These medications inhibit the metabolic breakdown of the neurotransmitter acetylcholine, which may improve the cognitive abilities of patients with Alzheimer's disease.

The only FDA-approved treatments for Alzheimer's disease are tacrine, donepezil, rivastigmine and galantamine, each of which boosts levels of acetylcholine, the chemical messenger involved in memory. Use of these drugs, as well as vitamin E and *Ginkgo biloba* is allowed. The NIA (National Institute of Ageing) is currently supporting 17 Alzheimer's disease clinical trials, seven of which are large-scale cognitive impairment and Alzheimer's disease prevention trials. Many of the agents being tested in these trials, including ginkgo, have been suggested as possible interventions based on long-term epidemiological and molecular studies. The trials are testing a variety of agents, such as aspirin, antioxidants such as vitamin E, combined folate/B6/B12 supplementation, anti-inflammatory drugs and *Ginkgo biloba* to determine if they will slow the rate of cognitive decline

Table 7.6 Potential therapies for Alzheimer's disease (AD)

Mode of action	Therapy development	Present status/References
Secretase inhibition	Bristol-Meyers Squibb-secretase inhibition	Phase I clinical trial
Presenilin function/dysfunction	Knocking out the presenilin gene PS1 enhances memory storage in adult mice	*Neuron*, 32, 911, 2001
Disruption of amyloid-β(Aβ) peptide aggregation	Neurochem **Alzhmed** mimics the glycosaminoglycan moieties of proteoglycans and blocks and delays fibril formation	Phase I human clinical trial – preliminary results available in April
Inhibition of amyloid accumulation by hormone treatment	Estrogen diminishes Aβ generation in cultured neurons. Hormone suppression therapy for prostate cancer in men indicates connection between sex hormones and AD	*Nat. Med.* 4, 1998 *J. Am. Med. Assoc.*, 285, 2195, 2001
Metal chelation	Antibiotic **clioquinol** is a chelator with high binding capacity for zinc and other metals. It competes with amyloid for metal ions resulting in break up of the amyloid plaque	Phase II clinical trial, University of Melbourne, Australia
Immune therapy	Elan CorP's antiplaque option of vaccination with synthetic Aβ42. Treatment reduces or eliminates existing deposits and inhibits further plaque formation in transgenic mice. Human trial unsuccessful due to serious central nervous system inflammation	*Nature*, 400, 173, 1999 Phase II human clinical trial suspended

Protective effect of nonsteroidal anti-inflammatory drugs (NSAIDs)	NSAID, diclofenac (100 mg per day), ibuprofen (about 1200 mg per day) lowers the risk of AD if taken before onset of disease	*N. Engl. J. Med.*, 345, 1515, 2001
Reduction of toxic Aβ peptide production	NSAID including ibuprofen, indomethacin and sulindac sulfide that can cut Aβ42 in humans	*Nature*, 414, 212, 2001 Human clinical trial to start soon.
Cucurmin	Antioxidant activity	*J. Neurosci.* 21, 8370, 2001
Anti-inflammatory action	Immune Network is using dapsone, an old leprosy drug with anti-inflammatory activity in slowing the progression of AD	Phase II clinical trial
Nutritional supplements	Vitamin E *Ginkgo biloba* Mindset Bio-Pharmaceuticals Oxigon (indole-3-pronionic acid) Folic acid and vitamins B_6 and B_{12} lower homocysteine levels	Disease Prevention Trials 1998–2006 (National Institute of Ageing) *N. Engl. J. Med.*, 346, 476, 2002
Cholesterol lowering drug-statins	Andrx Corp lovastaton may lower amyloid-β levels in blood streams	Completed study
Slow cognitive decline	Merz Pharma memantine to slow cognitive decline in AD	Marketing stage
Control plaque formation	ReGen Therapeutics is developing colostinin to stabilize and improve cognitive function in AD patients	Clinical trial underway

or prevent the onset of Alzheimer's disease. The trial with ginkgo started in 2000 and will be completed through 2006. The expected increase in the incidence of Alzheimer's disease worldwide provides an opportunity for the use of *Ginkgo biloba* as an inexpensive globally accessible prophylactic in preventing this disease with limited side effects.

7.7 References

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8

Functional ingredients in sports drinks

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8.1 Introduction: challenges of athletic performance

To delay the onset of fatigue and to improve performance, several strategies are open to athletes. The most effective of these is clearly a programme of systematic and intensive training, but at the highest levels of sport all the competitors are highly trained and highly motivated. Nutritional interventions offer a low risk strategy for performance improvement, and this accounts for the attention that most serious performers devote to their dietary strategies. Devising an effective nutrition programme requires an understanding of the athlete's nutrition goals and of the dietary strategies that will allow these goals to be achieved. An effective programme will identify the potential limitations to performance and implement a comprehensive strategy that includes training, nutrition, psychological issues and other factors.

The limitations to exercise performance clearly depend on the exercise activity itself. In short duration, high intensity exercise, the limitations are quite different from those that apply during prolonged exercise. Where the exercise duration is less than about 30–40 minutes, however, there is little scope for nutritional intervention during the event itself. In exercise lasting longer than this, and certainly when the duration exceeds one hour, there is scope for the ingestion of foods and fluids that can influence performance.[1] Dehydration and substrate depletion are major factors in fatigue during prolonged exercise, and Below et al. (1993) showed that provision of carbohydrate and fluid have independent and additive effects on performance.[2] Sports drinks, which can supply substrate in the form of carbohydrate as well as water to replace sweat losses, have a clear role to

play during any activity where fatigue is likely to influence the outcome. This applies as much to team games, where skill generally deteriorates in the later stages of the game, as to endurance running or cycling events. The optimum type and concentration of energy substrates and the presence or otherwise of electrolytes and other components gives an infinite number of possible formulations. Many sports drinks now contain exotic herbal and other components that offer prospects of improved performance.

For the athlete in training, consumption of sports drinks before, during and after the training session offers potential benefits in terms of improved performance in training that will enhance the physiological and biochemical adaptations taking place. There is also a need in training to promote recovery between training sessions and to reduce some of the potentially negative consequences of hard training, such as oxidative damage to cells and tissues or compromised immune function that might lead to an increased susceptibility to minor illnesses and infections.

Clearly, there is not a single sports drink formulation that will meet the needs of all athletes in all situations. Commercial formulations are inevitably a compromise, designed to meet the needs of most athletes in most situations. Inevitably, this means that there may be some drinks that are not ideal for some individuals or for some situations, and the athlete should be aware of the factors that will influence their efficacy so that the best choice can be made.

8.2 Formulation of sports drinks: carbohydrate content

Sports drinks should be formulated to be effective in improving performance, and their efficacy will be determined by the solute content and composition. Taste is also an important consideration in encouraging intake and commercial formulations must strike a balance between these sometimes conflicting aims. The major factors that can be manipulated to alter the functional characteristics of sports drinks are the carbohydrate content, the electrolyte content, the osmolality and the flavouring components.

8.2.1 Effects of carbohydrate ingestion

Carbohydrate ingested during exercise will enter the blood glucose pool, if it is absorbed from the gastrointestinal tract; if performance is limited by the availability of carbohydrate, from either the endogenous liver or muscle glycogen stores, then exercise capacity should be improved when carbohydrate is consumed. Many studies have shown that the ingestion of glucose during prolonged intense exercise will prevent the development of hypoglycaemia by maintaining or raising the circulating glucose concentration.[3–5] In prolonged exercise, performance, which was measured in most of the early studies as the time for which a fixed power output can be sustained,

is improved by the addition of an energy source in the form of carbohydrate; more recent studies have used a variety of different experimental models and have confirmed that this improvement in performance seems to apply also to other exercise models. Beneficial effects of carbohydrate ingestion are seen during cycling as well as during running.[6,7] This ergogenic effect may be related to a sparing of the body's limited muscle glycogen stores by the oxidation of the ingested carbohydrate.[5,8] Other studies, however, have failed to show a glycogen-sparing effect of carbohydrate ingested during prolonged exercise, and the consensus view is probably that there is little or no sparing of muscle glycogen utilization, although liver glucose release is slowed.[9,10] The primary benefit of ingested carbohydrate is probably its role in supplementing the endogenous stores in the later stages of exercise.[11] It is clear from tracer studies that a substantial part of the carbohydrate ingested during exercise is available for oxidation, but there appears to be an upper limit of about 1 gram per minute to the rate at which ingested carbohydrate can be oxidized, even when much larger amounts are ingested.[12] Recent work from Coyle's laboratory suggests that ingestion of a commercial sports drink was more effective in attenuating the progressive decline in peak power generating capacity that occurred during a prolonged (122 minute) exercise session in the heat than was the ingestion of the same volume of plain water.[13] Ingestion of the carbohydrate alone gave the same response as the placebo trial. This supports the idea that maintenance of hydration should be the first priority during prolonged exercise in the heat.

8.2.2 Role in water uptake

As well as providing an energy substrate for the working muscles, the addition of carbohydrate to ingested drinks will promote water absorption in the small intestine, provided the concentration is not too high. Because of the role of sugars and sodium in promoting water uptake in the small intestine, it is sometimes difficult to separate the effects of water replacement from those of substrate and electrolyte replacement when CHO-electrolyte solutions are ingested. Below et al. (1993) showed that ingestion of carbohydrate and water had separate and additive effects on performance capacity, and concluded that ingestion of dilute carbohydrate solutions would optimize performance.[2] Most reviews of the available literature have come to the same conclusion.[14–17]

In most of the early studies, the ingested carbohydrate was in the form of glucose, but the type of carbohydrate does not appear to be critical, and glucose, sucrose and oligosaccharides have all been shown to be effective in maintaining the blood glucose concentration when ingested during prolonged exercise and in improving endurance capacity.[17] There are theoretical advantages in the use of sugars other than glucose. Substitution of glucose polymers for glucose will allow an increased carbohydrate content

without an increased osmolality, but the available evidence suggests that the use of glucose polymers rather than free glucose does not alter the blood glucose response or the effect on exercise performance.[18–22] Similar effects are seen with the feeding of sucrose or mixtures of sugars.[23–26] Taste issues may be more important than other qualities, as high glucose concentrations may be perceived as being too sweet.

8.2.3 Nature of the carbohydrate involved

Some studies have suggested that orally ingested long chain glucose polymers are more readily used by the muscles during exercise than are glucose or fructose,[27] but others have found no difference in the oxidation rates of ingested glucose or glucose polymer.[28,29] Massicotte et al. (1989) also found that ingested fructose was less readily oxidized than glucose or glucose polymers.[28] Mixtures of glucose and fructose in equal amounts seem to have some advantages: when ingested in combination there is an increased total exogenous carbohydrate oxidation.[30] Fructose in high concentrations is generally best avoided on account of the risk of gastrointestinal upset. The argument advanced in favour of the ingestion of fructose during exercise, namely that it empties rapidly from the stomach and provides a readily available energy source but does not stimulate insulin release and consequent inhibition of fatty acid mobilization, is in any case not well founded: insulin secretion is suppressed during exercise. These studies have been reviewed earlier.[17] There may be benefits in including a number of different carbohydrates, including free glucose, sucrose and maltodextrin: this has taste implications which may influence the amount consumed, and, by limiting the osmolality and providing a number of transportable solutes, may maximize the rate of sugar and water absorption in the small intestine.[31]

8.2.4 Carbohydrate concentration

The optimum concentration of sugar to be added to drinks will depend on individual circumstances. High carbohydrate concentrations delay gastric emptying, thus reducing the amount of fluid that is available for absorption, but increase the rate of carbohydrate delivery. If the concentration is high enough to result in a markedly hypertonic solution, a transient net secretion of water into the intestine will result, and this will actually increase the danger of dehydration. High sugar concentrations (>10%) may also increase the risk of gastrointestinal disturbances.[32] Where the primary need is to supply an energy source during exercise, increasing the sugar content of drinks will increase the delivery of carbohydrate to the site of absorption in the small intestine. Beyond a certain limit, however, simply increasing carbohydrate intake will not continue to increase the rate of oxidation of ingested carbohydrate, although it is not entirely clear where the limit lies.[12] Dilute glucose–electrolyte solutions may also be as effective in

improving performance as are more concentrated solutions,[32] and adding as little as 90 mmol/l glucose (1.6%) may improve endurance performance as effectively as more concentrated solutions do.[33]

8.3 Formulation of sports drinks: osmolality

It has become common to refer to carbohydrate–electrolyte sports drinks as isotonic drinks, as though the tonicity was their most important characteristic. The osmolality of ingested fluids is important because this can influence both the rates of gastric emptying and of intestinal water flux: both of these processes together will determine the effectiveness of rehydration fluids at delivering water for rehydration. An increasing osmolality of the gastric contents will tend to delay emptying, and increasing the carbohydrate or electrolyte content of sports drinks will generally result in an increased osmolality. The composition of the drinks and the nature of the solutes are, however, of greater importance than is the osmolality itself.[17]

Although osmolality is identified as an important factor influencing the rate of gastric emptying of liquid meals, there seems to be rather little effect of variations in the concentration of sodium or potassium on the emptying rate, even when this substantially changes the test meal osmolality.[29] The effect of increasing osmolality is most consistently observed when nutrient-containing solutions are examined, and the most significant factor influencing the rate of gastric emptying is the energy density.[34,35] Substitution of glucose polymers for free glucose will result in a decreased osmolality for the same carbohydrate content, and this may be one way of maximizing availability of carbohydrate without compromising fluid uptake. This has led to the inclusion of glucose polymers of varying chain length in the formulation of sports drinks. Vist and Maughan (1995) have shown that there is an acceleration of gastric emptying when glucose polymer solutions are substituted for free glucose solutions with the same energy density: at low (about 40 g/l) concentrations, this effect is small, but it becomes appreciable at higher (180 g/l) concentrations; where the osmolality is the same (as in the 40 g/l glucose solution and 180 g/l polymer solution), the energy density is shown to be of far greater significance in determining the rate of gastric emptying.[36] This effect may therefore be important when large amounts of energy must be replaced after exercise, but is unlikely to be a major factor during exercise where more dilute drinks are taken.

Water absorption occurs largely in the proximal segment of the small intestine, and, although water movement is itself a passive process driven by local osmotic gradients, is closely linked to the active transport of solute. Osmolality plays a key role in the flux of water across the upper part of the small intestine. Net flux is determined largely by the osmotic gradient between the luminal contents and intracellular fluid of the cells lining the intestine. Absorption of glucose is an active, energy-consuming process

linked to the transport of sodium. The rate of glucose uptake is dependent on the luminal concentrations of glucose and sodium, and dilute glucose–electrolyte solutions with an osmolality which is slightly hypotonic with respect to plasma will maximize the rate of water uptake.[37] Solutions with a very high glucose concentration will not necessarily promote an increased glucose uptake relative to more dilute solutions, but, because of their high osmolality, will cause a net movement of fluid into the intestinal lumen.[38] This results in an effective loss of body water and will exacerbate any pre-existing dehydration with potentially negative implications for exercise performance. Other sugars, such as sucrose or glucose polymers can be substituted for glucose without impairing glucose or water uptake, and may help by increasing the total transportable substrate without increasing osmolality.[39–41] In contrast, iso-energetic solutions of fructose and glucose are isosmotic, and the absorption of fructose is not an active process in man: it is absorbed less rapidly than glucose and promotes less water uptake.[42] The use of different sugars which are absorbed by different mechanisms and which might thus promote increased water uptake is supported by recent evidence from an intestinal perfusion study.[31]

Most of the popular sports drinks are formulated to have an osmolality close to that of body fluids,[43] and are promoted as isotonic drinks, but there is good evidence that hypotonic solutions may be more effective when rapid rehydration is desired.[37] Although it is argued that a higher osmolality is inevitable when adequate amounts of carbohydrate are to be included in sports drinks, the optimum amount of carbohydrate necessary to improve exercise performance has not been clearly established.

8.4 Formulation of sports drinks: electrolyte composition and concentration

8.4.1 Role of sodium

The available evidence indicates that the only electrolyte that should be added to drinks consumed during exercise is sodium, which is usually added in the form of sodium chloride.[17] Sodium will stimulate sugar and water uptake in the small intestine and will help to maintain extracellular fluid volume. Most soft drinks of the cola or lemonade variety contain virtually no sodium (1–2 mmol/l); sports drinks commonly contain about 10–30 mmol/l; oral rehydration solutions intended for use in the treatment of diarrhoea-induced dehydration, which may be fatal, have higher sodium concentrations, in the range 30–90 mmol/l. A high sodium content, although it may stimulate jejunal absorption of glucose and water, tends to make drinks unpalatable, and it is important that drinks intended for ingestion during or after exercise should have a pleasant taste in order to stimulate consumption.

When the exercise duration exceeds 3–4 h, sweat losses are likely to be high, especially in warm climates. If circumstances permit the ingestion of large volumes of fluid, there may be advantages in adding sodium to drinks to avoid the danger of dilutional hyponatraemia. Physicians dealing with individuals in distress at the end of long distance races have become accustomed to dealing with hyperthermia and hypernatraemia associated with dehydration, but it has become clear that a small number of individuals at the end of very prolonged events may be suffering from hyponatraemia in conjunction with either hyperhydration or dehydration.[44–46] All the reported cases have been associated with ultramarathon or prolonged triathlon events; most of the cases have occurred in events lasting in excess of 8 h, and there are few reports of cases where the exercise duration is less than 4 h. Noakes et al. (1985) reported 4 cases of exercise-induced hyponatraemia; race times were between 7 and 10 h, and post-race serum sodium concentrations were between 115 and 125 mmol/l.[44] Estimated fluid intakes were between 6 and 12 l, and consisted of water or drinks containing low levels of electrolytes; estimated total sodium chloride intake during the race was 20–40 mmol. Frizell et al. (1986) reported even more astonishing fluid intakes of 20–24 l of fluids (an intake of almost 2.5 l/h sustained for a period of many hours, which is in excess of the maximum gastric emptying rate that has been reported) with a mean sodium content of only 5–10 mmol/l in two runners who collapsed after an ultramarathon run and who were found to be hyponatraemic (serum sodium concentration 118–123 mmol/l).[47] These reports indicate that some supplementation with sodium salts may be required in extremely prolonged events where large sweat losses can be expected and where it is possible to consume large volumes of fluid. Most CHO–electrolyte sports drinks intended for consumption during prolonged exercise contain sodium at a concentration of about 10–30 mmol/l, which is rather lower than the normal sweat sodium concentration (Table 8.1). While the formulation of these drinks might represent a reasonable strategy for providing substrates and water (although it can be

Table 8.1 Approximate concentration, in mmol/l, of the major electrolytes present in sweat, plasma and in intracellular (muscle) water in humans

	Plasma	Sweat	Intracellular
Sodium	137–144	40–80	10
Potassium	3.5–4.9	4–8	148
Calcium	4.4–5.2	3–4	0–2
Magnesium	1.5–2.1	1–4	30–40
Chloride	100–108	30–70	2

See Maughan (1994)[17] for further details: the values are collated from a variety of sources

argued that a higher sodium concentration would enhance water uptake and that a higher carbohydrate content would increase substrate provision), these recommendations may not be appropriate in all circumstances. Palatability may be an issue at higher sodium concentrations, but it is possible, with a suitable choice of electrolytes, to formulate drinks with a high (up to 100 mmol/l) sodium concentration that are not unpalatable.

8.4.2 Post-exercise recovery

Restoration of fluid and electrolyte balance after exercise is an important part of the recovery process, especially if another training session or competition must follow after a short time interval. Several studies have investigated the effects of ingestion of water or of commercially available drinks on restoration of fluid balance after exercise-induced dehydration. Costill and Sparks (1973) showed that ingestion of a glucose–electrolyte solution after dehydration resulted in a greater restoration of plasma volume than did plain water: a higher urine output was observed on the water trial.[48] Gonzalez-Alonso et al. (1992) have recently confirmed that a dilute carbohydrate–electrolyte solution (60 g/l carbohydrate, 20 mmol/l Na^+, 3 mmol/l K^+) is more effective in promoting post-exercise rehydration than either plain water or a low-electrolyte diet cola: the difference between the drinks was primarily a result of differences in the volume of urine produced.[49] Similar results were obtained by Nielsen et al. (1986).[50] In none of these studies, however, could the mechanism of the action be identified, but the authors did establish that, because of the high urine flow that ensued, even drinking large volumes of electrolyte-free drinks did not allow subjects to remain in positive fluid balance for more than a very short time.

If large volumes of plain water are consumed rapidly after exercise-induced dehydration, a marked fall in plasma osmolality and in the plasma sodium concentration ensues, and both of these effects will stimulate urine output.[51,52] Ingestion of plain water also removes the drive to drink by causing plasma osmolality and sodium concentration to fall. This makes it difficult to achieve complete rehydration when fluid intake is on a volitional basis.

The importance of the addition of sodium to rehydration fluids was systematically evaluated by Maughan and Leiper (1995) who dehydrated subjects by the equivalent of 2% (w/w) of body mass by intermittent exercise in the heat: subjects then ingested a volume equal to 150% of the mass loss of one of the test drinks over a 60 min period and were followed for a further 6 h.[53] The test drinks contained 0, 25, 50, or 100 mmol/l sodium. Urine output over the subsequent few hours was inversely proportional to the sodium content of the ingested fluid: only when the sodium content exceeded 50 mmol/l were the subjects in positive sodium balance, and only then did they remain in positive fluid balance throughout the recovery period.

These observations were confirmed in a further study that systematically varied the volume of fluid ingested as well as the sodium content of rehydration drinks administered after induced hypohydration.[54] Even drinking large volumes (twice the sweat loss) did not allow subjects to remain in positive fluid balance for more than 2 h when the sodium content of the drinks was low (20 mmol/l): increasing the sodium content to 60 mmol/l, however, allowed subjects to remain well hydrated when volumes equal to 1.5 times or twice the sweat loss were ingested.

It is clear from these studies that rehydration after exercise can only be achieved if the sodium lost in sweat is replaced as well as the water, and it might be suggested that rehydration drinks should have a sodium concentration similar to that of sweat. However, the sodium content of sweat varies widely (Table 8.1), and no single formulation will meet this requirement for all individuals in all situations. The upper end of the normal range for sodium concentration (80 mmol/l), however, is similar to the sodium concentration of many commercially produced oral rehydration solutions (ORS) intended for use in the treatment of diarrhoea-induced dehydration, and some of these are not unpalatable. The ORS recommended by the World Health Organization for rehydration in cases of severe diarrhoea has a sodium content of 90 mmol/l, reflecting the high sodium losses which may occur in this condition.[55] By contrast, the sodium content of most sports drinks is in the range of 10–30 mmol/l and is even lower in some cases; most commonly consumed soft drinks contain virtually no sodium and these drinks are therefore unsuitable when the need for rehydration is paramount. The problem with high sodium concentrations is that this may exert a negative effect on taste, resulting in a reduced consumption.

8.4.3 Role of potassium

Various authors have speculated that inclusion of potassium, which has a concentration in the intracellular space of about 150 mmol/l compared with the low (10 mmol/l) concentration of sodium, would enhance the replacement of intracellular water after exercise and thus promote rehydration.[56] There has been limited experimental investigation of this, but inclusion of potassium may be as effective as sodium in retaining water ingested after exercise-induced dehydration.[57] Addition of either ion will significantly increase the fraction of the ingested fluid which is retained, but, when the volume of fluid ingested is equal to that lost during the exercise period, there is no additive effect of including both ions as would be expected if they acted independently on different body fluid compartments.

Potassium is normally present in commercial sports drinks in concentrations similar to those in plasma and in sweat (Table 8.1), but there is little evidence to support its inclusion. Although there is some loss of potassium in sweat (about 3–7 mmol/l),[58] an increase in the circulating potassium concentration is the normal response to exercise: increasing this further by

ingestion of potassium does not seem useful. The concentration of potassium in sports drinks (about 3–6 mmol/l, or about 0.12–0.24 g/l) is close to that present in sweat, but the amounts are small compared with the total daily intake of potassium for normal sedentary adults in the UK (about 3.2 g).[59] Replacement of losses will normally be achieved after exercise from the potassium present in foods.

8.4.4 Role of magnesium

Commercially available sports drinks intended for use by athletes in training and competition are generally rather similar in their electrolyte content, suggesting a consensus, at least among the manufacturers, as to the requirements for electrolyte replacement. There is some debate as to the need to add magnesium: this is added in some countries (e.g. Germany) to products that are sold elsewhere without added magnesium. In spite of the commonly held belief that exercise-induced cramp is associated with a falling plasma magnesium concentration, there is little or no experimental evidence to substantiate this belief.[60] A slight decrease in the plasma magnesium concentration is generally observed during exercise, but this seems to be the result of a redistribution of the body magnesium stores, and there seems to be no good reason for its addition to drinks consumed during exercise (for review, see Maughan, 1994).[17]

8.5 Formulation of sports drinks: flavouring components

Taste is an important factor influencing the consumption of fluids. The thirst mechanism is rather insensitive and will not stimulate drinking behaviour until some degree of dehydration has been incurred.[61] This absence of a drive to drink is reflected in the rather small volumes of fluid that are typically consumed during exercise: in endurance running events, voluntary intake seldom exceeds about 0.5 l/h.[62] Sweat losses normally exceed this, even in cool conditions and a fluid deficit is therefore almost inevitable. Several factors will influence palatability, and the addition of a variety of flavours has been shown to increase fluid intake relative to that ingested when only plain water is available. Hubbard et al. (1984) and Szlyk (1989) found that the addition of flavourings resulted in an increased consumption (by about 50%) of fluid during prolonged exercise.[63,64] More recently, Bar-Or and Wilk (1996) have shown that the fluid intake during exercise of children presented with a variety of flavoured drinks is very much influenced by taste preference: under the conditions of this study, sufficient fluid to offset sweat losses was ingested only when a grape-flavoured beverage was available.[65] In many of these studies, the addition of carbohydrates and/or electrolytes accompanied the flavouring agent, and the results must be interpreted with some degree of caution.

Given the need to add electrolytes to fluids intended to maximize the effectiveness of rehydration, there are clearly palatability issues that influence the formulation. Effective post-exercise rehydration requires replacement of electrolyte losses as well as the ingestion of a volume of fluid in excess of the volume of sweat loss.[54] When sweat electrolyte losses are high, replacement with drinks with a high sodium content can result in an unpalatable product. This can be alleviated to a large degree by substituting other anions for the chloride that is normally added. The addition of carbohydrate has a major impact on taste and mouth feel, and a variety of different sugars with different taste characteristics can be added.

8.6 Future trends: other active ingredients

As our understanding of the factors that limit exercise performance is extended, so opportunities arise for novel nutritional interventions. It seems unlikely that the basic composition of the sports drink will change greatly, but some recognition of the need for different drinks in different situations and for different individuals may lead to alternative formulations.

There is already a growing trend for the formulation of sports drinks to be modified to include other components which might affect the functional characteristics of the drink. This raises many important issues, including not only efficacy, but also safety, stability and palatability.[66] Many of the drinks aimed at the active individual include a range of vitamins and minerals, but it is widely agreed that these are not generally necessary. There is also little convincing evidence for beneficial effects of the addition of purported ergogenic compounds such as taurine, ginseng or aspartate. There is good experimental evidence to support the use of caffeine, and some evidence that the addition of glycerol, protein and amino acids may confer benefits in some situations.

8.6.1 Glycerol

The difficulties of achieving an adequate fluid intake during many competitive sports events and the growing recognition of the negative effects of hypohydration have led to an increasing emphasis on ensuring an optimum hydration status before exercise begins. Given the small volumes that it is normally possible to consume during exercise, there would seem to be benefits from ingesting fluid before exercise begins. Ingestion of large volumes of fluid, however, is likely to invoke a diuretic response, which is undesirable in the pre-exercise period. Water balance is largely controlled by monitoring of the circulating osmolality: this in turn is determined largely by the plasma sodium concentration, as sodium is the major circulating osmotically active particle, and the osmoreceptor may be in the form

of a sodium receptor. Changes in osmolality cause alterations in output of anti-diuretic hormone (ADH) by the pituitary, and ADH controls the reabsorption of water by the distal tubule of the nephron and by the collecting duct, thus determining the urine flow.

Some degree of temporary hypervolaemia and hyperhydration results when drinks with high sodium concentrations are ingested, and there may be benefits of this blood volume expansion for the endurance athlete.[67] The acute effect of ingestion of saline solutions is, however, transient and is rapidly corrected by the appropriate renal response. Attempts have been made to induce a state of relative hyperhydration prior to exercise by administration of glycerol solutions: ingestion of glycerol results in a marked rise in the extracellular glycerol concentration, and thus of osmolality.[68] This has the effect of increasing total body water, but results in an increase in plasma osmolality, and so may be considered to result in a relative hypohydration, even though total body water is increased.[69] The elevation of the osmolality of the extracellular space may result in water movements from the intracellular space, and cell dehydration, resulting in tissue shrinkage, is a well-recognized consequence of the administration of large amounts of glycerol.

Several studies have recently reported that ingestion of glycerol together with water can elevate the plasma osmolality and increase the total body water content, and that there may be benefits for thermoregulation and exercise performance.[70] Lyons et al. (1990) gave subjects glycerol plus water or water alone 2.5 h prior to a 90 min exercise test at 60% of VO_{2max} in a hot (42 °C) dry (25% rh) environment.[71] The addition of glycerol decreased urine output over the trial and resulted in an increased sweat rate and a smaller rise in rectal temperature during the exercise period: there was also a tendency, although not statistically significant, for heart rate to be lower on the glycerol trial. Freund et al. (1995) have also reported an enhanced fluid retention and reduced renal flow when glycerol was added to drinks ingested at rest, and proposed that this effect might be mediated by an effect on ADH output.[72]

There have also been some reports of improvements in the ability to perform prolonged exercise after glycerol administration.[73,74] Montner et al. (1996) found that prior administration of glycerol (1.2 g/kg) plus water resulted in significant improvements in time to exhaustion compared with a water alone trial in two exercise tests lasting about 90 min: the improved exercise performance was associated with a lower heart rate during exercise and a smaller rise in core temperature.[73] Anderson et al. (2001) found a lower heart rate and core temperature during 90 min of exercise in the heat after ingestion of a glycerol solution compared to a water-only control: power output in a 15 min cycle ride which followed was higher on the glycerol trial.[74] Scheett et al. (2001) have also shown that addition of glycerol to fluids ingested after exercise-induced dehydration improves exercise performance in an exercise test carried out 3 h later.[75]

The recommendation that endurance athletes competing in the heat should ingest glycerol and water prior to exercise, or that glycerol should be added to drinks, might be premature at this stage, but the evidence of benefits is accumulating. Further developments in this area are awaited with interest.

8.6.2 Protein and amino acids

Protein is not a major fuel for oxidative energy supply during exercise, but there is an increased rate of protein oxidation during prolonged exercise. It has generally been assumed, however, that there is no reason to add protein to drinks intended for consumption before, during or immediately after exercise. In the recovery period, muscle glycogen synthesis is a priority, but synthesis of new proteins should perhaps be seen as being of equal or even greater importance. Because little attention has been paid to this area, it is not at present apparent what factors may be manipulated to influence these processes.

Protein synthesis is stimulated for some time after exercise, and this reduces the size of the free amino acid pool within the muscle cells. These amino acids provide the essential building blocks for incorporation into new proteins, and a reduced concentration in turn restricts the rate of synthesis of new proteins. Administration of as little as 6 g essential amino acids after a bout of strength training exercise can increase the rate of protein synthesis.[76] If this effect persists in the longer term, the net effect is an improvement in muscle protein balance.

It is increasingly being recognized that cell volume is an important regulator of metabolic processes,[77,78] and there may be opportunities to manipulate this to promote synthesis of both proteins and glycogen in the post-exercise period. During and after exercise there may be large changes in cell volume, secondary to osmotic pressure changes caused by metabolic activity, hydrostatic pressure changes, or by sweat loss. It is well known that alterations in cell volume induced by changes in osmolality alter the rate of glycogen synthesis in skeletal muscle.[79] Amino acid transport into muscles is also affected by changes in cell volume induced by manipulation of the trans-membrane osmotic gradient: skeletal muscle uptake of glutamine is stimulated by cell swelling and inhibited by cell shrinkage,[80] and the intracellular glutamine concentration appears to play an important role in a number of processes, including protein and glycogen synthesis.[81]

The full significance of these findings for the post-exercise recovery process and the roles they play in adaptation to a training programme remain to be established. Manipulation of fluid and electrolyte balance and the ingestion of a variety of osmotically active substances or their precursors offer potential for optimizing the effectiveness of a training regimen. There are clearly implications for the formulation of sports drinks designed to promote recovery and enhance adaptations to training.

8.6.3 Branched chain amino acids

Although the subjective sensations of fatigue that accompany prolonged exercise are generally considered to be the result of events occurring in the muscles or in the cardiovascular system, there is growing evidence that the signals that arise in the periphery are modulated by events occurring within the central nervous system: this is generally referred to as the central fatigue hypothesis.[82] This hypothesis proposes that an increased brain serotonin (5-hydroxytryptamine, 5-HT) concentration is associated with the onset of fatigue: increases in brain 5-HT can result from an increase in the transport of the precursor tryptophan (Trp) from the plasma across the blood–brain barrier. Increasing the plasma concentration of the branched chain amino acids (BCAA), which are competitive inhibitors of Trp uptake, can reduce brain 5-HT accumulation, and these observations have led to suggestions that BCAA should be added to drinks intended for consumption during prolonged exercise.[82]

Although the evidence supporting a role of 5-HT in the fatigue process seems strong,[83] attempts to improve performance by the administration of BCAA during exercise have not generally been successful. One study did show beneficial effects in a subgroup of marathon runners who ingested drinks containing BCAA during the race,[84] but these results have not been reproduced under controlled laboratory conditions.[85–87] In the study by Verger et al. (1994) which involved treadmill-running rats, BCAA feeding resulted in a shorter exercise time to fatigue.[87] In spite of these rather unpromising findings, at least one sports drink which contains added branched chain amino acids is on sale.

8.6.4 Glutamine and antioxidants

Moderate exercise levels seem to be associated with a reduced risk of minor illness and infection, but there is some evidence that athletes in hard training are at increased risk of opportunistic infections, especially those of the upper respiratory tract.[88] Although minor in themselves, these may be sufficient to interrupt training. Failure to recover fully between training sessions also leads to a condition of chronic fatigue, and although this condition is not well defined, it is well recognized and is characterized by underperformance.[89] Damage to tissues caused by an increased level of free radical generation during and after exercise (in part from increased rate of aerobic metabolism and in part from release by neutrophilic leucocytes during phagocytosis) has been proposed as one of the factors in incomplete recovery, leading to suggestions that an increased dietary intake of antioxidant nutrients may confer some protection.

A variety of nutritional interventions have been proposed to enhance immune function, to increase the antioxidant defence mechanisms and to improve the resistance of the athlete to illness. Glutamine is used by the cells of the immune system and hard exercise causes a fall in the plasma

glutamine level: from here it is only a short step to propose that glutamine supplementation may enhance immune function.[90] Unfortunately, the available evidence does not support this suggestion at the present time, even though glutamine is currently being sold to athletes.[91] A more effective dietary strategy to enhance immunity may be to ensure adequate dietary carbohydrate intake. This has the effect of minimizing any rise in plasma levels of stress hormones (cortisol, catecholamines and growth hormone) known to have a negative effect on immunity, and is likely to be the most successful nutritional strategy.[92] More recent data suggest that ingestion of drinks containing glutamine or protein during and after exercise does abolish the post-exercise fall in the plasma glutamine concentration but has no effect on markers of immune function.[93]

Although there is good evidence that the delayed onset muscle soreness and damage that are experienced after hard exercise may be mediated at least in part by the release of free radicals, it is less clear that supplementation of the endogenous defence mechanisms with an increased intake of antioxidant nutrients will have any effect on these processes. Supplementation with antioxidants may reduce some of the markers of free radical mediated muscle damage, but this alone is not sufficient justification to recommend the use of dietary supplements, and there is no evidence of performance benefits for athletes.[94] Again, a number of products, including sports drinks, are on sale with these ingredients added.

8.6.5 Caffeine

The beneficial effects of caffeine on exercise performance may arise from any one of several different mechanisms. Caffeine has metabolic effects by virtue of its ability to stimulate lipolysis and thus spare muscle glycogen stores,[95] but it also has a variety of effects of the contractility of muscle and on the central nervous system.[96,97] The use of caffeine is not prohibited in sport, but there is a limit to the amount that may be taken by athletes in competition: any individual whose urine contains caffeine at a level of more than 12 mg/l is guilty of a doping offence and is liable to be banned from competition. In spite of this, however, caffeine is present in some commercially available sports drinks (it is also a component in tea, coffee, cola and 'energy drinks'). There is good evidence of performance enhancing effects of caffeine at doses less than those that are normally required to exceed the urine concentration threshold, so this may be some justification for the presence of caffeine in these drinks.

Kovacs et al. (1998) tested sports drink formulations containing varying does of caffeine and found that performance of a time trial lasting about 1 h was improved when doses of 225 mg or 320 mg were included in the drinks, but that a dose of 150 mg did not result in a performance that was better than that after taking the sports drink itself.[98] The 150 mg dose did result in a better performance than when water was drunk. These low doses would

not be likely to cause an athlete to fail a drugs test, and the results of this study do support a role for the addition of caffeine to sports drinks.

8.7 Sources of further information and advice

A variety of paper and electronic resources provide information on sports drinks. Many have a commercial bias, but some of the commercially funded websites are nonetheless valuable resources. A recent publication devoted specifically to the properties of sports drinks and to the effects of ingesting them provides a broad introduction to the topic.[99] Most textbooks on sports nutrition have one or more chapters devoted to issues relating to hydration and sports drinks and recent texts include those by Burke and Deakin (2000) and Maughan (2000).[100,101] There are also numerous reviews of the scientific evidence relating to specific aspects of the topic in the published literature and these are referred to throughout the text of this chapter as appropriate.

Of the available web-based sources of information, that published by the Gatorade Sports Science Institute (GSSI, www.gssiweb.com) is both extensive and reliable. Most of this information is also published in paper format.

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9

Pharmacological functions of green tea polyphenols

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9.1 Introduction

Green tea is a simple refreshing beverage consumed by millions of people around the world, and its medicinal properties have been recognized for many centuries. It was even referred to in Chinese literature written in 200 BC as the remedy for various illnesses (Hu, 1986). However, until recently its medicinal properties have not been appreciated in the modern world. Currently green tea is provoking great interest within the medical community because of mounting scientific evidence on its powerful antioxidant activity in preventing various diseases.

The scientific evidence suggests that this simple beverage contains large quantities of low molecular weight polyphenols (Table 9.1), that are found to be more potent antioxidants and free-radical scavengers than are vitamin C or vitamin E (Rice-Evans et al., 1996). The green tea polyphenols are mainly composed of six kinds of catechins (also known as tannins), epigallocatechin gallate (EGCg), epigallocatechin (EGC), epicatechin gallate (ECg), epicatechin (EC), gallocatechin (GC) and catechin (C). Among the six, EGCg is the main component available in large quantities in either crude or refined green tea extracts (Table 9.2). The chemical structures of these polyphenols indicate that they belong to a class of flavan-3-ol, which is composed of C_{15} (fifteen carbon atoms) compounds; their derivatives are composed of two phenolic nuclei (A and B rings) connected by three carbon units (2, 3 and 4) of C ring (Fig. 9.1). The significance of these compounds in medical use is their ability to react with various substances. The reactions of polyphenols with substances such as soybean lipoxygenase (Sekiya et al., 1984), caffeine (Martin et al., 1986; Murayama et al., 1991),

Table 9.1 Composition of green tea (% of dry weight)

Components	%
Proteins	24.0
Carbohydrates	45.8
Lipids	4.6
Polyphenols	13.0
Caffeine	2.3
Ash	5.4
Vitamins A and B	0.02
Vitamin C	0.25

Anonymous (1996), *Standard tables of food composition in Japan*, Tokyo, Resource Council of Science and Technology, 198–9

Table 9.2 Composition of polyphenols in crude (hot water infusion) and refined (Sunphenon® 100S) green tea extract

Name of the catechin		% of dry extract	
		Crude	Refined
(−) – Epigallocatechin gallate	(EGCg)	10.0	31.5
(−) – Epigallocatechin	(EGC)	8.9	17.3
(+) – Gallocatechin	(GC)	3.3	9.4
(−) – Epicatechin	(EC)	4.1	5.9
(−) – Epicatechin gallate	(ECg)	3.9	5.1
(−) – Catechin	(C)	1.9	2.8

cystine (Richard et al., 1991) and methylmercaptan (Yasuda and Arakawa, 1995) indicated that their reactions mainly involve conjugation at the 3′ and 4′ positions on the B ring. It has been suggested that green tea polyphenols act as a powerful radical scavenger through these chemical reactions in preventing various diseases.

In the last two decades, various *in vitro* and *in vivo* studies have indicated the antimicrobial and antioxidant properties of green tea polyphenols. Recent clinical studies have confirmed their antioxidant properties in preventing various ailments such as cardiovascular diseases, cancer, renal failure and allergy. In addition to this they were found to be powerful in the suppression of growth and enzyme synthesis of various pathogenic bacteria and viruses that are harmful to humans. In this chapter, the pharmacological functions of green tea polyphenols are classified into antibacterial, antiviral and antioxidant activities. We also discuss their functions in relation to the above disorders with an emphasis on clinical results.

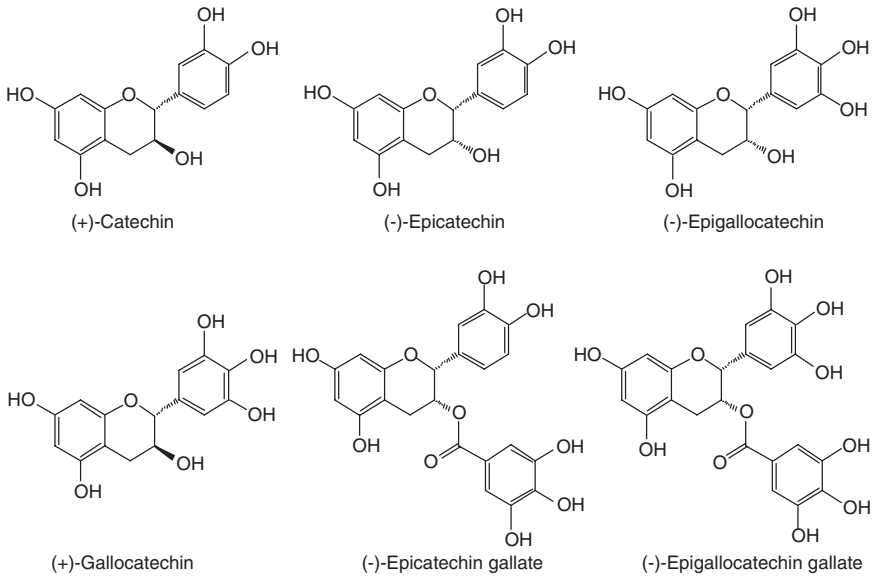

Fig. 9.1 The structures of green tea polyphenols.

9.2 Antibacterial activity

Green tea polyphenols have very strong antibacterial activity against various pathogens (Juneja et al., 2000). In this section, antibacterial activities against oral bacteria, intestinal bacteria and foodborne bacteria that are relevant to human health are discussed.

9.2.1 Oral care

The benefits of green tea consumption on oral care were first noticed in the middle of the 1970s in Japan, when a reduction in the rate of dental caries with the consumption of green tea was observed in school children (Onisi et al., 1981a; Onisi 1985). Similar studies in China also linked the consumption of green tea with lowered incidence of tooth decay (Jin et al., 1991). The effect of green tea in the prevention of dental caries was once thought to be due to fluorides contained in the tea leaves. Later it had been noticed that the tea infusion was more effective in decreasing dental caries than the application of the fluoride solution itself (Onisi et al., 1981b; Yu et al., 1992). Further studies *in vitro* and *in vivo* pointed out that polyphenols present in green tea are the real functional compounds in the suppression of tooth decay. Detailed studies suggested that the antibacterial activity of green tea polyphenols against cariogenic bacteria is the basis for the prevention of tooth decay (Muroi and Kubo, 1993). Green tea polyphenols

Table 9.3 Minimum inhibitory concentrations (MIC: $\mu g\,ml^{-1}$) of green tea polyphenols on several pathogenic oral bacteria. The inhibition of the bacterial growth was observed in sensitive meat extract agar medium

Bacterial species and strains	Green tea polyphenols					
	C	EC	GC	EGC	ECg	EGCg
Streptococcus mutans						
MT8148	>1000	>1000	250	250	1000	500
IFO 13955	>1000	>1000	250	250	>1000	500
Streptococcus sobrinus						
6715DP	>1000	>1000	250	250	>1000	500
Porphyromonas gingivalis						
381	1000	1000	1000	1000	1000	500
ATCC 33277	1000	1000	1000	1000	1000	250
GAI	1000	1000	1000	1000	1000	500

Sakanaka et al., 1997

were also recognized as a powerful deodorant in the alleviation of bad oral odor and therefore improved oral health (Chu and Juneja, 1998).

Prevention of tooth decay

The main causes of tooth decay are dental caries and periodontal diseases that are induced by oral microflora. Among the several hundreds of microorganisms indigenous to oral cavities, *Streptococcus mutans* is predominantly cariogenic (Hamada and Slade, 1980; Loesche, 1986). The growth and cariogenicity of this bacterium depend on the availability of sucrose from food. It synthesizes water-insoluble glucans (plaque formation) catalyzed by glucosyltransferase (GTase) in the presence of sucrose and in this way adheres strongly to the tooth surface causing tooth decay (Hamada and Slade, 1980). *In vitro* studies on the green tea polyphenols were found to inhibit the growth of this bacterium, glucan synthesis and the cellular adherence of cariogenic *S. mutans* (Hattori et al., 1990; Otake et al., 1991; Sakanaka et al., 1989, 1990, 1992). The minimum inhibitory concentrations (MIC) of green tea polyphenols on the growth of *Streptococcus* species were in the range of 250 to $1000\,\mu g\,ml^{-1}$ (Table 9.3). In another study, Sakanaka et al. (1990) reported that glucan synthesis by the above bacteria was strongly inhibited by ECg and EGCg of green tea extract. They also found that even as little as 25–$30\,\mu g\,ml^{-1}$ of ECg and EGCg could completely inhibit glucan synthesis and a concentration of $50\,\mu g\,ml^{-1}$ of both components could completely inhibit adherence of the bacterial cells.

Oiwa et al. (1993) and Terajima et al. (1997) conducted clinical studies with human volunteers to examine the effect of Sunphenon® (a commercial product of green tea polyphenols, Taiyo Kagaku Co., Ltd., Japan) on plaque formation. In these studies, the volunteers were asked to rinse their

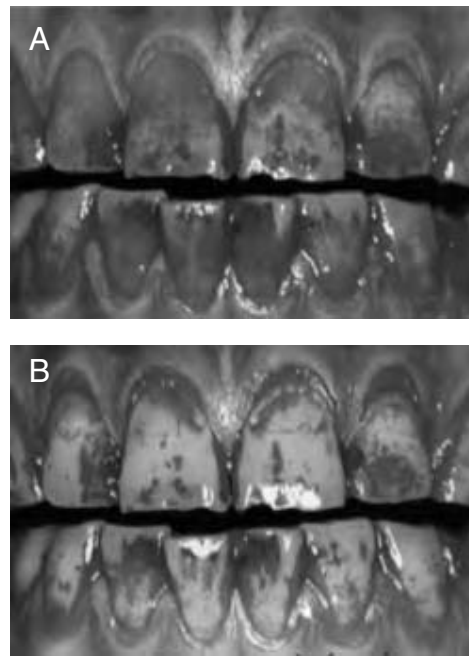

Fig. 9.2 Effect of green tea polyphenols on dental caries plaque formation. A, without and B, with the application of green tea polyphenols as mouth wash.

teeth either with water or with a solution containing 0.05–0.5% of green tea polyphenols for 20 seconds. They did this three times a day after meals for three consecutive days. They were forbidden to brush their teeth during the test period. After these treatments, dental plaque formation was identified by staining with prospec dye and photographed. In a visual comparison, a significant inhibition of plaque formation was noticed in those volunteers administered with green tea polyphenol (Fig. 9.2). In a continuation of the study, the same volunteers were divided into four groups and administered with 0.05, 0.1, 0.2 and 0.5% green tea polyphenols for another three days in the same procedure. Photographs were taken to calculate the inhibition rate of plaque formation. As shown in Table 9.4, dental plaque formation decreased in the volunteers who rinsed their teeth with green tea polyphenols. The inhibition rate of plaque formation was 30–43% in the test groups (Sakanaka et al., 1997) and so the green tea polyphenols are recognized as an effective inhibitor of plaque formation in humans.

Green tea polyphenols are also effective in the inhibition of growth and adherence of another bacterium, *Porphyromonas gingivalis*, which causes periodontal disease (Kakuda et al., 1994; Sakanaka et al., 1996). The adherence of *P. gingivalis* to oral epithelial cells is the initial step in the pathogenesis of periodontitis. *In vitro* experiments showed that Sunphenon® at

Table 9.4 Effect of green tea polyphenols on dental plaque formation in men

Polyphenols in mouth wash solution (%)	Inhibition rate (%)
0.05	41.0
0.1	33.9
0.2	30.6
0.5	43.1

Sakanaka et al., 1997

concentrations of 0.1 mg ml^{-1} and above strongly inhibited the adherence of *P. gingivalis* to epithelial cells (Sakanaka et al., 1996). EGCg, the active component of green tea polyphenols, suppressed adherence of the bacterium onto human buccal epithelial cells at a concentration of 250–500 μg ml^{-1} (Table 9.3). Clinical tests have revealed a substantial decrease in levels of this bacterium with the application of green tea in periodontitis patients (Nawashiro et al., 1996).

Collagenase, which is produced by *P. gingivalis*, breaks down collagen in the gums, weakens the periodontal pocket and eventually recedes the gums leading to gingival and periodontal diseases. The application of Sunphenon® almost completely inhibited the activity of collagenase at concentration of 50 μg ml^{-1}, and the enzyme activity was completely inhibited at 100 μg ml^{-1} (Juneja et al., 2000).

One may expect that the effectiveness of the polyphenols as anticariogenic agents may depend on the duration of their availability at minimum inhibitory concentrations in saliva. The minimum inhibitory concentrations of catechins to cariogenic *Streptococci* and other related bacteria are 250–500 μg ml^{-1} or less (Sakanaka et al., 1989). Among the catechins, EGCg, ECg and GCg are effective inhibitors of both *S. mutans* and *P. gingivitis* (Sakanaka et al., 1990, 1996). These components have shown the enzyme-inhibitory effect even at 25–30 μg ml^{-1} or less (Sakanaka et al., 1990). Tsuchiya et al. (1997) analyzed the constituents of human saliva after the intake of green tea extract at a concentration of 5 mg ml^{-1}. They found that catechins at such concentrations were retained in saliva for up to 60 min after the intake of green tea extract (Table 9.5). These results suggest that green tea polyphenols can be used as effective natural anticariogenic agents.

Prevention of halitosis

In modern life, diversified food and drink intake induces several kinds of oral odor or halitosis (Chu and Juneja, 1998). The odor is mainly caused by the proteins of epithelial organization, connective tissues, food residues and

Table 9.5 Green tea polyphenols concentrations ($\mu g\,ml^{-1}$) in saliva after mouthwash with green tea solution ($5.0\,mg\,ml^{-1}$)

Time after mouth wash (min)	Green tea polyphenols concentrations ($\mu g\,ml^{-1}$)					
	C	EC	GC	EGC	ECg	EGCg
1	11.9	20.5	26.7	38.2	43.4	165.1
10	3.41	7.42	9.58	17.6	15.4	52.1
30	1.75	4.34	6.41	10.2	7.57	24.7
60	1.65	2.8	5.39	7.02	5.24	16.1

Tsuchiya et al., 1997

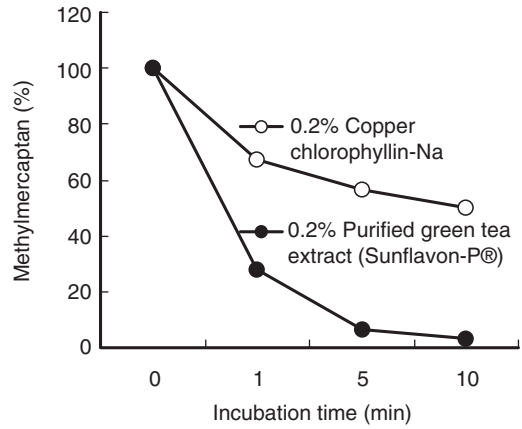

Fig. 9.3 Effect of purified green tea extract (Sunflavon P®) on the inhibition of oral odor in comparison with copper chlorophyllin-Na, a normal deodorant.

bacteria in the mouth. The proteins are dissolved by enzymes to produce odorous volatile sulfide substances. Among several odorous sulfide gases, methylmercaptan (CH_3SH) has a strong relationship with oral odor (Ui et al., 1991).

The components of green tea polyphenols were reported to have strong deodorant activity against CH_3SH (Ui et al., 1991). The purified green tea extract (Sunflavon-P®) showed stronger deodorant activity in the suppression of oral CH_3SH content than copper chlorophyllin-Na, a common deodorant (Fig. 9.3). Other natural substances from different plant extracts also possess deodorizing activity; most of them are polyphenols and phenolic derivates (Tokita et al., 1984; Yasuda and Ui, 1992). Among the six tea catechins, EGCg had the strongest deodorizing activity against CH_3SH (Ui et al., 1991). The deodorizing mechanism of polyphenols against CH_3SH is

considered to involve the hydrogen bonds between phenolic hydroxyl groups and the thiol group (Yasuda and Arakawa, 1995). The order of deodorizing activity of green tea polyphenols was EGCg > EGC > ECg > GA > EC (Ui et al., 1991; Yasuda and Arakawa, 1995), which is similar to the order of molar intensity for antioxidant activity of these catechins (Matsuzaki and Hara, 1985; Rice-Evans et al., 1996). This suggests a link between deodorant and antioxidant activities of these catechins.

9.2.2 Harmonizing enteric microflora

Hundreds of microbial species, millions in cell counts, are native to the intestine. These microbes are involved in various physiological functions, of which the majority are beneficial to the human body, but some are very harmful (Okubo and Juneja, 1997). Most of the species constitute lactic acid bacteria, such as certain *Bifidobacterium* and *Lactobacillus* genera that play a significant role in metabolism (digestion), host-defense against infection, ageing and immunopotentiation (Hentges, 1983; Mitsuoka, 1984) and so these species of bacteria are generally considered beneficial. On the other hand, some harmful bacteria belonging to certain clostridial species, *Clostridium perfringens* and *C. difficile* are found and these are closely linked to intestinal diseases and tumor growth (Bokkenheuser, 1983; Gary and Sherwood, 1984; Goldman, 1983). It is therefore necessary to maintain a balance among these microflora.

The composition of gut microflora is largely affected by diet and by age (McCarthy and Salyers, 1988; Rowland et al., 1985; Salyers and Leedle, 1983). A healthy diet with sufficient oligosaccharides and dietary fiber was found to promote useful bacteria and suppress the harmful ones. Similarly, green tea polyphenols with antibacterial activity exhibited a favorable influence on the composition of the intestinal microflora.

In vitro studies on the green tea polyphenols have shown a selective, growth inhibitory activity against various harmful *Clostridia* species, while having little effect on other bacteria (Ahn et al., 1990a, b). All the catechins except EGC of green tea polyphenols have some growth inhibitory effect on *C. perfringens* (Table 9.6). However, EGCg and ECg have the highest degree of inhibitory effect on both *C. difficile* and *C. perfringens*. (Ahn et al., 1991). These results suggest a certain relationship between the structures of polyphenols and the growth inhibitory effect. The gallate moiety linked by an ester linkage in the polyphenol molecules seems to be related to bacterial growth inhibition activity.

Okubo et al. (1992) conducted a clinical study to investigate the effect of green tea polyphenols (in the form of Sunphenon®) on the growth of the human intestinal microflora. Eighty healthy volunteers were administered with 0.4 g polyphenols after each meal (i.e., 3 times a day) for 8 weeks in the following schedules. In the first two weeks (weeks 1 and 2) the volunteers did not take the polyphenols, the following two weeks (weeks 3

Table 9.6 Growth inhibitory activity of tea polyphenols against *C. difficile* ATCC-9689 and *C. perfringens* ATCC-13124

Polyphenols	*C. difficile*	*C. perfringens*
C	*	+
EC	–	+
GC	–	+
ECg	++	++
ECG	–	–
EGCg	++	++

*, no inhibition; +, inhibition and ++, strongest inhibition
Ahn et al., 1991

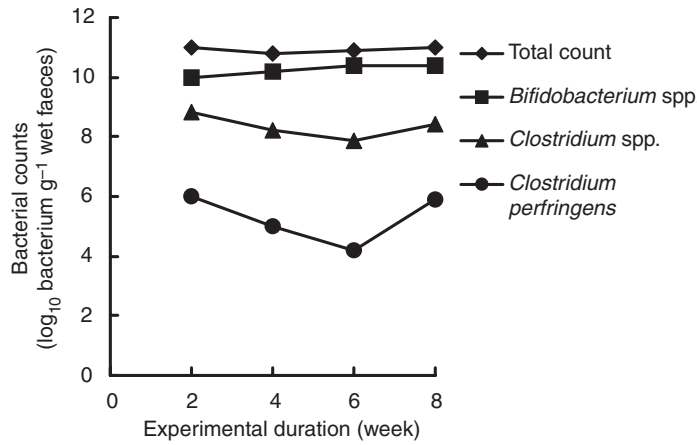

Fig. 9.4 The changes in the intestinal microflora during administration (2 to 6 weeks) and post administration (6 to 8 weeks) of green tea polyphenols in human volunteers.

and 4) and the next two weeks (weeks 5 and 6) they took the polyphenols and the last two weeks (weeks 7 and 8) they again did not take the polyphenols. The composition of different species of bacteria in the faeces was examined at the end of each test period (after every two weeks). The results were inconsistent with those of *in vitro* studies. The data indicated a decrease in the bacterial populations of *Clostridium* spp. and an increase in the populations of *Bifidobacterium* spp. with intake of polyphenols (Fig. 9.4). This evidence suggests that the intake of green tea polyphenols may have an inhibitory effect on the growth of harmful *Clostridia* species. Colon cancer patients are often reported to have a high percentage composition of *Clostridia* and a low percentage of *Bifidobacteria*. Green tea polyphe-

nols, having the selective growth inhibitory effect on *Clostridia* and promoting effect on *Bifidobacteria*, may be helpful in regulating the balance of these bacteria in the intestine of colon cancer patients.

9.2.3 Prevention of foodborne bacterial infections

The delivery of food materials from production to consumer involves a series of steps in processing and storage. During these steps, the food may be exposed to different pathogenic bacterial contamination which may cause serious illness. The green tea polyphenols exhibited a strong antibacterial activity against several of those foodborne bacteria (Juneja et al., 2000; Sakanaka et al., 1997). Thermophilic spore-forming *Bacillus stearothermophilus*, a virulent bacterium resistant to high temperatures can spoil soft drinks stored in vending machines and Sakanaka et al. (2000) found green tea polyphenols to be very effective in inhibiting the growth of this bacterium. Similarly, the growth of several microbial and psychrophilic bacteria in ice-stored fish has been diminished with the application of polyphenols (Noriyuki et al., 2001). It has also been reported that polyphenols can inhibit the release of verotoxins from enterohemorrhagic *Escherichia coli* O157:H7 (Sugita-konishi et al., 1999). It was noticed that green tea polyphenols could inhibit *E. coli* infection in mice (Isogai et al., 1998) and the minimum inhibitory concentration for this organism was $<250\,\mu g\,ml^{-1}$ (Hara-kudo et al., 2001). A list of minimum inhibitory concentrations of green tea polyphenols on the growth of different foodborne bacteria of fish and animal origin is given in Table 9.7 (Sakanaka et al., 1997).

9.3 Antiviral activity

Besides their powerful antibacterial activity, green tea polyphenols have also exhibited strong activity against viral infections.

9.3.1 Prevention of viral infection

Nakayama et al. (1990) investigated the effect of polyphenols on the infectiousness of influenza A virus and influenza B virus in Madin-Darby canine kidney (MDCK). They observed a significant decrease in infection by the virus proportional to the concentration of green tea extract (Table 9.8). Nakayama et al. (1993) discovered that EGCg prevented the adsorption of the virus to MDCK. The catechin binds with hemagglutinin of influenza virus, inhibits adsorption to MDCK cells, and thus blocks infection.

The green tea polyphenols have also shown significant antiviral activity against rotavirus (Ebina, 1991; Hatta et al., 1989; Mukoyama et al., 1991), enterovirus (Mukoyama et al., 1991), *Vaccinia* virus, *Herpes simplex* virus, *Coxsackie* virus B6 and polio 1 virus (John and Mukundan, 1979). In all of

Table 9.7 Minimum inhibition concentration (MIC) of green tea extract on the growth of foodborne pathogens of fish and meat origin

Host and microorganisms	MIC ($\mu g\,ml^{-1}$)
Fish origin	
Ayu	
Vibrio anguillarum species	100–200
Vibrio spp.	200
Crawfish	
Vibrio spp.	50–400
Yellowfish	
Streptococcus spp.	700–900
Pasteurella piscicida spp.	100–200
Eel	
Edwardsiella tarda spp.	300–400
Vibrio spp.	200
Salmon	
Vibrio spp.	100–200
Meat origin	
Cattle	
Salmonella spp.	4000
Psedomonas spp.	500
Staphylococcus spp.	100–1000
Escherichia coli spp.	>4000
Pig	
Salmonella spp.	4000
Escherichia coli spp.	4000
Chicken	
Salmonella spp.	4000

Juneja et al., 2000

Table 9.8 Inhibition of influenza viruses A and B by green tea extract. Virus was mixed for 60 min with tea extract before adsorption to the MDCK cells. Inhibition of virus activity indicated by the reduction in plaque formation

Concentration of green tea extract ($\mu g\,ml^{-1}$)	Reduction in plaque formation (%)	
	Virus A	Virus B
0.05	78.5	59.4
0.1	87.0	80.0
0.2	98.8	94.0
0.4	100.0	98.6
0.8	100.0	100.0
1.0	100.0	100.0

Nakayama et al., 1990

these studies, EGCg was found to be an active component in the antiviral activity. The polyphenols, EGCg and ECg, were found to inhibit HIV (human immuno-deficiency virus), reverse transcriptase and cellular DNA and RNA polymerase (Nakane and Ono, 1990; Ono and Nakane, 1991). The minimum concentration of these components for 50% inhibition of HIV-reverse transcriptase was in the range of 0.01–0.02 $\mu g\,ml^{-1}$. It is likely that treatments for these viral infections could be developed from green tea polyphenols.

9.4 Antioxidant functions

It is well known that the free active radicals such as superoxide (O_2^-), hydroxyl radical (–OH) and radicals derived from hydrogen peroxide (H_2O_2) are very closely related to injury of cell membranes and DNA (Elias and Cohen, 1977). These free radicals were also found to be involved in ageing and in the initiation of several diseases. For example, diseases such as cancer, cardiovascular diseases and arthritis have a free radical component (Ames, 1983). There is no system to eliminate free radicals in our organs or tissues, physiological antioxidant protection is invoked as one of the major defense mechanisms in fighting free radical-induced, mediated and promoted disorders. This means that the most significant function of the antioxidants is believed to be disease prevention.

Vitamin C, vitamin E and β–carotene are well-known antioxidants. They appear to maintain the proper functioning of the immune system (Anon, 1990). Recently, the green tea polyphenols, particularly ECg and EGCg, were found to have the greatest activity of a number of antioxidants (Rice-Evans et al., 1996), when compared with other natural substances (Fig. 9.5). The efficacy of antioxidant activity of green tea polyphenols has been related to their chemical structures (Jovanovic et al., 1995; Rice-Evans et al., 1996; van Acker et al., 1996). Since antioxidant activity corresponds broadly with structures having the greatest number of hydroxyl groups, the green tea polyphenols (Fig. 9.1) with three –OH groups in the B ring (such as gallocatechins) and three –OH groups in the C ring (such as catechin gallates) have an advantage over others in scavenging the free radicals (Rice-Evans et al., 1996).

9.4.1 Prevention of cardiovascular diseases

A large percentage of human mortality occurs from cardiovascular disorders, which are mainly caused by atherosclerosis. Such cases are characterized by local thickening of the intema or innermost part of the arteries. A popular theory for the cause of atherosclerosis is that oxidation of low-density lipoprotein (LDL) leads to uptake via macrophage scavenger receptors in the arterial wall (Ross, 1993; Steinberg et al., 1989). It

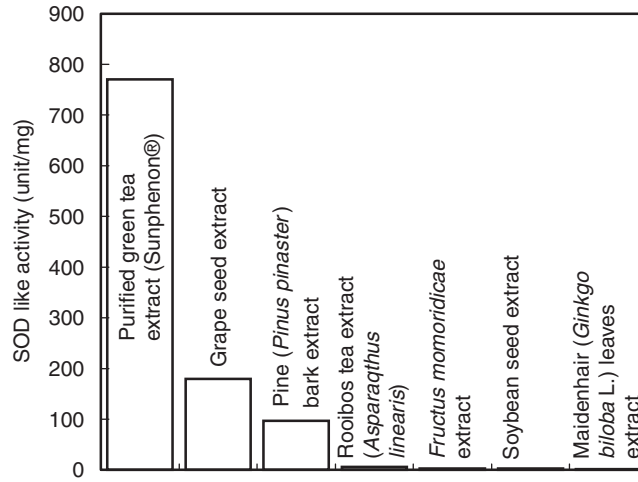

Fig. 9.5 Physiological antioxidant activity of various natural substances compared with purified green tea extract (Sunphenon®).

Table 9.9 Effect of green tea extract and its components on Cu-induced LDL peroxidation. LDL peroxidation is estimated fluorometrically as thiobarbituric acid (TBA)-reactive substances after 4 hours of incubation

Component concentration ($\mu g\,ml^{-1}$)	TBA-reactive substances ($nmol\,MDA\,ml^{-1}$)				
	0	0.1	0.5	2.5	25.0
Green tea extract	0.41	0.38	0.33	0.28	0.26
Polyphenols	0.42	0.29	0.25	0.23	0.22
Caffeine	0.42	0.40	0.40	0.38	0.37
Theanine	0.38	0.35	0.34	0.32	0.31

Yokozawa and Dong, 1997

therefore appears likely that substances capable of counteracting LDL oxidation would be of potential therapeutic interest. As antioxidants and scavengers of free radicals, green tea polyphenols were found to be a suitable natural substance to counter oxidation of LDL.

Yokozowa and Dong (1997) have tested the effect of green tea components *in vivo* on the oxidation of LDL which was time-dependently oxidized by copper (II) sulphate, leading to peroxidation, the extent of which was estimated fluorometrically as thiobarbituric acid (TBA)-reactive substances. The researchers observed a concentration-dependent decrease of LDL peroxidation with green tea components such as polyphenols and theanine (a major amino acid in green tea). However, caffeine did not influence peroxidation (Table 9.9). Miura et al. (1995) also found a similar effect of EGCg on copper-induced lipid peroxidation.

Table 9.10 Antioxidative activity of green tea polyphenols (final concentration, 1000 μM) compared with control and α-tocopherol and determined by the rabbit erythrocyte 'ghost' system

Component	Lipid peroxidation (%)
Control	100
α-tocopherol	24.1
C	29.8
EC	27.8
GC	36.3
EGC	27.8
ECg	17.1
EGCg	16.2

Ramarathnam et al., 1995

In another study, Ramarathnam et al. (1995) examined the effect of green tea polyphenols on lipid peroxidation in rabbit erythrocyte (red blood cell) membrane 'ghosts'. They observed substantial reduction of lipid peroxidation with different components of green tea polyphenols. Of the four catechins evaluated, ECg and EGCg produced strongest protection against lipid peroxidation, and are more active than the standard antioxidant α-tocopherol (Table 9.10).

Laboratory studies on animals suggest that green tea extracts suppress absorption of cholesterol from the digestive tract (Chisaka et al., 1988; Ikeda et al., 1992), and reduce the serum level of total cholesterol (Ali et al., 1990; Chisaka et al., 1988; Matsuda et al., 1986). A cross-sectional epidemiological study with Japanese men reported a significant inverse relationship between green tea consumption and serum levels of total cholesterol and triglycerides, as well as a positive association between green tea drinking and high density lipoprotein (HDL) cholesterol levels (Imai and Nakachi, 1995). These studies indicate the positive effects of green tea polyphenols on the management of cholesterol and indirectly on the prevention of cardiovascular diseases.

9.4.2 Prevention of cancer

The anticarcinogenic property of green tea polyphenols was first identified in the 1980s by Khan et al. (1988) and Wang et al. (1989a,b). Since then, extensive laboratory and epidemiological research has shown that green tea polyphenols can protect against a variety of cancer types (Table 9.11). Most of those studies were conducted either *in vitro* or *in vivo* using tissues or different organs or mice, respectively. Experimental evidence suggests that the polyphenolic antioxidants present in green tea inhibit cancer initiation

Table 9.11 List of publications referring to the anticarcinogenic properties of green tea polyphenols

Affected organ	Carcinogen	Reference
Skin	PAH, UVB	Katiyar et al. 1992, 1993a, 1999, 2000, 2001
	DMBA/TPA	Yang and Wang 1993;
	Benzoyl peroxide/TPA	Mukhtar et al. 1994; Huang et al. 1992
	4-NQO/TPA	Wang et al. 1989b, 1991, 1992a, 1994
Lung	B(a)P, NDEA, NNK	Wang et al. 1992b,c; Katiyar 1993b,c; Xu et al. 1992; Shi et al. 1994
Colon	Azoxymethane,	Yamane et al. 1991, 1995;
	DMH,	Inagake et al. 1995;
	MNNG	Narasiwa and Fukaura 1993
Prostate	Androgen, testosterone,	Gupta et al. 1999a,b, 2000; Liao and Hipakka 1995
Breast	–	Liao et al. 1995
Mammary gland	DMBA	Hirose et al. 1994
Esophagus	NMBA, Nitrososarcosine	Xu and Han 1990; Gao et al. 1990
Duodenum	ENNG	Fujita et al. 1989
Liver	Aflatoxin B_1, NDEA	Chen et al. 1987; Li 1991
Pancreas	N-nitroso-bis (2-oxypropyl)-amine	Harada et al. 1991

and its subsequent development. Much of the cancer chemopreventive effects of green tea have been attributed to the major polyphenolic constituent EGCg (Katiyar and Mukhtar, 1996). The polyphenols, in particular EGCg, seem to affect a variety of targets and pathways, and thus may be responsible for the exceptionally high cancer chemopreventive efficacy of these compounds. The targets and pathways modulated by polyphenols are:

1. MAPK, ERK2, JNK1.
2. Urokinase.
3. Apoptosis and cell cycle.
4. Protein tyrosine kinase (PTK) and ornithine decarboxylase (ODC) activities (Ahmad et al., 1997; Ahmad and Mukhtar, 1999).

Recently, Jankun et al. (1997) proved that the EGCg could effectively reduce the incidence of cancer and the size of tumors in humans. They identified that EGCg could fit well into the cavities formed by His 57, Asp 102,

Table 9.12 The intake of green tea extract or a single polyphenol 'EGCg' on urinary methylguanidine excretion during adenine-induced renal failure in rats

Substance concentration (mg day^{-1})	Methylguanidine in urine (μg ml^{-1}) 0	0.5	1.0	2.0
Green tea extract	71.9	58.0	62.6	44.7
Polyphenol EGCg	76.8	63.8	45.4	35.7

Yokozawa et al., 1992

Ser 195, Arg 35 and Arg A37 on the surface of urokinase and thereby suppress its carcinogenic enzyme activity.

9.4.3 Prevention of renal failure

Renal failure, a functional disorder of the kidney, occurs with the accumulation of highly oxidative uremic toxins; hence the symptoms of uremia, a high oxidative stress condition, were usually observed in renal failure patients (Fillit et al., 1981; Flament et al., 1986; Giardini et al., 1984; Kuroda et al., 1985). Among the various uremic toxins, methylguanidine was found to be the most pertinent in uremia. Methylguanidine is produced from creatinine by the hydroxyl radical (Ienaga et al., 1991; Nakamura et al., 1991; Yokozawa et al., 1991a,b and 1992). Green tea polyphenols known for their active free radical-scavenging activity inhibited the production of methylguanidine and thus alleviated renal failure both in animals (Sakanaka and Kim, 1997; Yokozawa et al., 1992, 1994, 1996a) and in humans (Sakanaka and Kim, 1997; Yokozawa et al., 1996b).

In rats, Yokozawa et al. (1992) examined the effect of green tea polyphenols on adenine-induced renal failure. They examined urinary methylguanidine excretion as an index of scavenging reaction. They administered rats with different doses of green tea polyphenols (as Sunphenon®) orally for 14 days after adenine administration for 20 days. A dose-dependent decrease in methylguanidine excretion was observed (Table 9.12), which was about 40% lower compared to control (administration of 2 mg green tea extract). A further decrease (about 50%) was noticed with the administration of exclusively EGCg at the rate of 0.5 mg day^{-1}. These results suggested that EGCg, the active component of green tea polyphenols, has very high scavenger activity against free radicals. In a clinical study, the effect of green tea polyphenols on the concentrations of serum creatinine and methylguanidine was observed in 50 dialysis patients (Yokozawa et al., 1996b). There was an 8% decrease in creatinine and 20% decrease in methylguanidine after 5 months' treatment (Fig. 9.6). These findings suggest

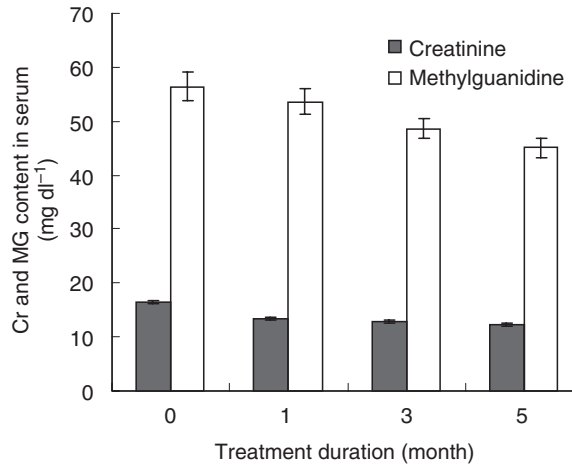

Fig. 9.6 Effect of green tea polyphenol on serum creatinine and methylguanidine contents in dialysis patients.

that green tea polyphenols have significant pharmacological effects in ameliorating the state of uremia in dialysis patients.

Green tea polyphenols have also relieved the pain and complications associated with renal failure. The post-dialysis pains in different parts of the body were relieved by the intake of green tea polyphenols (Yokozawa et al., 1996b). The application of polyphenols improved the glomerular function (Oura and Yokozawa, 1990; Yokozawa et al., 1993) and relieved renal hypertension (Yokozawa et al., 1994) by inhibiting mesangial cell proliferation and renal blood circulation, respectively. The 'antinephropathic' activity of green tea was confirmed by its powerful antioxidant activity against those free radicals that are involved in the complications of renal failure (Yokozawa et al., 1996a).

9.4.4 Prevention of allergy

Individuals are becoming increasingly susceptible to allergens – in particular to those in food and those that are airborne. Among the four types of classified allergies (Coombs and Gell, 1968), Type I allergy is most common in which people suffer from immediate hypersensitive reaction to allergens. The allergic reactions include a series of events; production of allergen specific IgE, its binding to Fc&RI receptor on the surface of mast cells or basophils, cross-linking of IgE by newly absorbed allergens, and release of chemical mediators such as histamine and leukotrienes (LT) from mast cells (Kawabe et al., 1987; Plaut and Zimmerman, 1993). Inhibition of any of these sequential steps may ease allergic symptoms. Compounds such as

Table 9.13 Effect of dietary green tea polyphenols (Sunphenon®) and fats on calcium ionophore A23187 induced histamine and leukotrienes (LT) released from rat peritoneal exudates cells (PEC)

Diet type	Chemical mediator release (ng 10^{-6} PEC cells)	
	Histamine	Leukotrienes
Without polyphenol		
Safflower oil	630	30.0
Perilla oil	609	13.0
Palm oil	769	27.0
With polyphenol		
Safflower oil	637	11.3
Perilla oil	595	8.7
Palm oil	821	20.0

Matsuo et al., 2000

antihistamine and LT synthesis inhibitors have shown antiallergic properties by inhibiting one of the above specific reactions.

Until recently there have been no specific studies that examined the exclusive effect of green tea polyphenols on allergy. However, studies have indicated that various polyphenols normally found in tea such as EGCg, ECg and EGC have inhibited LT release (Matsuo et al., 1996; Yamada et al., 1999) and histamine release (Maeda et al., 1989; Matsuo et al., 1997; Yamashita et al., 2000). These polyphenols were also found to inhibit allergies induced by Df-protease (Noguchi et al., 1999) and hyaluronidase (Maeda et al., 1990). Recently, Matsuo et al. (2000) have examined the combined effect of dietary green tea polyphenols and fats on the calcium ionophore A23187-induced release of chemical mediators (histamine and LT) in peritoneal exudates cells (PEC) of rats. They found that diets with a combination of green tea polyphenols have significantly inhibited LT release from PEC (Table 9.13). However, they did not find any effect on the release of histamine. These results suggest that the green tea polyphenols have a pharmacological effect in alleviating Type-I allergy by inhibiting LT production.

9.5 Conclusions

The secret behind the longevity and low incidence of certain diseases in the people of Japan might be linked to their consumption of green tea. Scientific evidence suggests that this simple beverage, rich in low molecular-weight polyphenols, protects the body from various ailments. Green tea

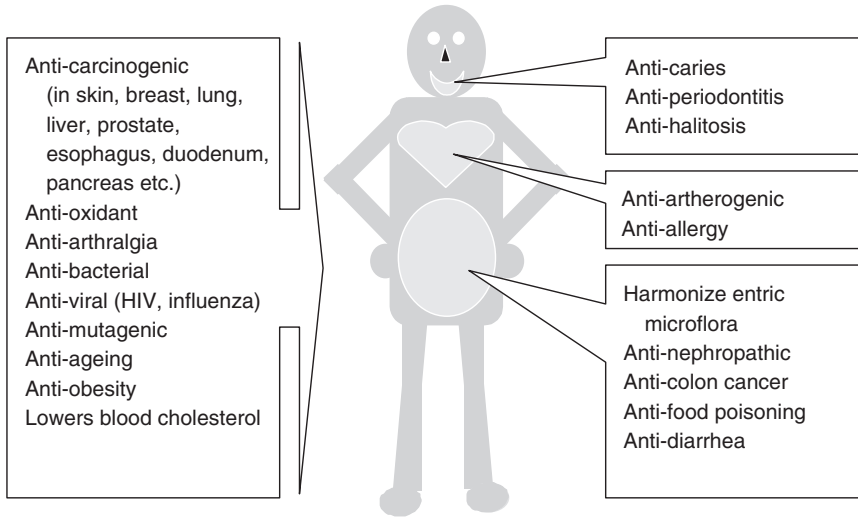

Fig. 9.7 Various pharmacological functions of green tea polyphenols in the human body.

polyphenols have powerful antioxidant and radical scavenger properties against disease-causing free radical species. In addition to this, green tea polyphenols also showed strong antimicrobial activity against various bacterial and viral infections.

Lately, several clinical studies showed various pharmacological functions of green tea polyphenols in humans (Fig. 9.7). These include 1) an improvement in oral health by suppression of those bacteria that cause dental caries, periodontal diseases and halitosis, 2) harmonization of enteric microflora by suppression of disease-induced bacteria, 3) prevention of foodborne bacterial and viral infections, 4) antiartherogenicity by regulating the oxidation of LDL, 5) potential anticarcinogenicity by suppression of various cancer-induced pathways, 6) antinephropathicity by easing the renal failure complications, and 7) antiallergicity by suppression of Type-1 allergic reactions.

The quest to find more pharmacological benefits of green tea extract has not yet ended. New findings suggest that the green tea polyphenols are effective in retarding cataracts (Thiagarajan et al., 2001), muscle necrosis (Buetler et al., 2002), obesity (Bell and Goodrick, 2002), liver damage (Arteel et al., 2002) and various cancer types (Yang et al., 2002). An increasing number of research findings are being published at approximately between 150 and 200 each year, indicating the vast physiological and pharmacological uses of green tea polyphenols. The trend in current research is largely focussed on the function of green tea polyphenols on lifestyle related disease, such as various types of cancer, cardiovascular disease, HIV, allergy and obesity and on ageing agents.

Green tea polyphenols are strong prophylactic and therapeutic agents, and could therefore offer the food industry an excellent opportunity for application as functional food ingredients in many processed foods. The green tea polyphenols, Sunphenon® and Sunkatol® from Taiyo Kagaku Co., Ltd., Japan, have been used widely in such products as chewing gum, candies, caramels, jelly beans and beverages in Japan and other countries. Several pharmacological, physiological functions and safety aspects of these polyphenols have been well tested around the world and have been reported in a number of articles and also published in a book, *Chemistry and Application of Green Tea* (CRC Press). In 1993, Sunphenon® was approved as a 'Food for Specific Health Use' (FOSHU) in Japan. The safe green tea polyphenols are a potential natural healthy ingredient for a good diet.

9.6 References

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10

Caffeine, mental performance and mood

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10.1 Introduction

The inclusion of a chapter on caffeine in a book on functional foods would come as no surprise to most people. The popular image, and indeed the current view of most nutrition experts, is that caffeine is helpful to mental performance and mood. Consequently, it may be a surprise to many people that such beliefs are not consistent with current scientific evidence. The main aim of this chapter is to examine findings that help to explain the basis for the radical revision in understanding that has emerged recently concerning caffeine, mental performance and mood.

The present chapter provides an overview of selected background information about caffeine, including the main sources and consumption patterns of the drug; its chemistry, pharmacology, and main biological mechanism of action; and caffeine physical dependence. This is followed by separate reviews of literature concerning caffeine-induced effects on mental performance and mood, with an emphasis on key methodical issues. Specific attention is given to the recently articulated Caffeine Deprivation Hypothesis (described below) as the basis for an integrated understanding of caffeine's biobehavioural actions. The chapter concludes with a consideration of future trends, sources of further information, and a summary of the main conclusions.

10.2 Sources of caffeine and consumption patterns

The main sources of caffeine are coffee, tea, and caffeine soft drinks, including the more recently developed 'energy' drinks. The presence of caffeine

in coffee and tea beverages is due to the presence of the drug in the plants from which these beverages are derived. The chances of survival for caffeine-bearing plants are increased because the drug is unpalatable to a host of potential herbivorous predators. The caffeine in sodas and energy drinks may also derive, in part, from plants (e.g. cacao, cola nut, maté, guarana), although manufacturers also add refined caffeine to such drinks.

The widespread consumption of caffeine is a relatively recent development in history. Throughout most of human history, caffeine was accessible to relatively few people in relatively few geographical regions. European colonization in the sixteenth and seventeenth centuries ultimately resulted in the introduction of coffee and tea to the many parts of the world in which caffeine foods and beverages had been unavailable previously (James, 1991). This dissemination has been so successful that caffeine is now the most widely consumed psychoactive substance in the world. More than 80% of people worldwide consume caffeine daily, and its use transcends almost every social barrier, including age, gender, geography, and culture (James, 1997a).

Globally, coffee is the main source of caffeine. Tea, however, is consumed more widely than coffee, but qualifies as the second main source of caffeine because it is generally lower than coffee in caffeine content (Graham, 1984). On average, tea beverages have about one-half to two-thirds of the caffeine concentration of coffee (James, 1991). Approximately 90% of dietary caffeine is consumed as coffee and tea, with the remaining 10% being consumed mostly as cola soft drinks (Gilbert et al., 1976; Graham, 1978). Although still only accounting for a relatively small percentage of overall caffeine consumed, energy drinks are an increasingly significant source, especially amongst young people. Some prescription and nonprescription medications contain caffeine, as do chocolate and chocolate-flavoured drinks. However, these sources generally account for a small fraction of consumed caffeine. In the developed countries, per capita intake varies from about 200 to 400 mg per day or approximately 2 to 6 cups of coffee or tea per day (Gilbert, 1984).

10.3 Chemistry and pharmacology

Caffeine is one of a family of purine derivative methylated xanthines often referred to as *methylxanthines* or merely *xanthines*. At room temperature, caffeine is a white odourless powder with a bitter taste (Vitzthum, 1976). Caffeine was first isolated from green coffee beans in 1820 by Ferdinand Runge in Germany. In 1827, Oudry discovered a substance in tea which he called ‘thein’, but which was later found to be identical to caffeine. Subsequently, caffeine was also found to be present in maté, in 1843, and in cola nuts, in 1865 (Arnaud, 1984). Figure 10.1 shows the structure of caffeine (1, 3, 7-trimethylxanthine), the most common of the methylxanthines in

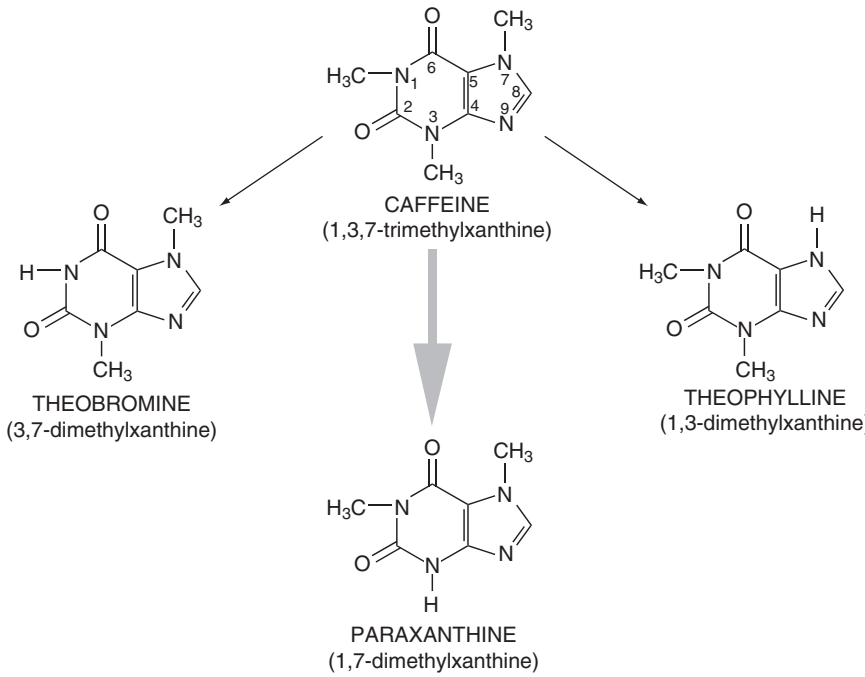

Fig. 10.1 The structure of caffeine (1, 3, 7-trimethylxanthine), and its dimethylated metabolites in humans: theobromine (3, 7-dimethylxanthine), theophylline (1, 3-dimethylxanthine), and paraxanthine (1, 7-dimethylxanthine). Arrow width indicates relative proportions of the metabolites in plasma (Adapted from James (1997a)).

nature. Figure 10.1 also shows the structure of the three dimethylxanthine isomers of caffeine, theobromine (3, 7-dimethylxanthine), theophylline (1, 3-dimethylxanthine), and paraxanthine (1, 7-dimethylxanthine). It appears that theobromine is an immediate precursor in caffeine biosynthesis in coffee (Looser et al., 1974; Roberts and Waller, 1979), tea (Suzuki and Takahashi, 1976a, 1976b, 1976c), and cacao (Aleo et al., 1982; Keifer et al., 1983), whereas all three dimethylxanthines are primary metabolic products of caffeine in humans (see Fig. 10.1).

Following oral ingestion, caffeine is rapidly absorbed from the gastrointestinal tract into the bloodstream (Arnaud, 1987; Blanchard and Sawers, 1983). Approximately 90% of the caffeine contained in a cup of coffee is cleared from the stomach within 20 minutes (Chvasta and Cooke, 1971) and peak plasma concentration is typically reached within about 40–60 minutes (Rall, 1990). Once ingested, caffeine is readily distributed throughout the entire body. Concentrations in blood are highly correlated with those found in the brain, saliva, breast milk, semen, amniotic fluid, and foetal tissue

(James, 1997a). The drug has an elimination half-life of about 5 hours in adults (Pfeifer and Notari, 1988) and typical consumption patterns result in plasma concentrations that remain at pharmacologically active levels for most waking hours. In adults, caffeine is virtually completely transformed by the liver, with less than 2% of the ingested compound being recoverable in urine (Arnaud, 1987; Somani and Gupta, 1988). For present purposes, it should be noted that although caffeine is a familiar constituent of the diets of people throughout the world, caffeine is a drug, not a food. Caffeine has no nutritional value, although other constituents (e.g. sugar, milk) in the beverages and foods that contain caffeine may have that property. More importantly, caffeine is a drug, because rather than aiding the normal functions of cells (as is the case for food) caffeine disrupts normal functions of the body. This is evidenced by a consideration of the main biological mechanism of action of caffeine.

10.4 Biological mechanism of action and dependence

Caffeine exerts a variety of pharmacological actions at diverse sites, both centrally and peripherally, which are generally believed to be mostly due to competitive blockade of adenosine receptors (Dunwiddie and Masino, 2001). Adenosine is a neuromodulator that acts on specific cell-surface receptors distributed throughout the body (Bush et al., 1989; Marangos and Boulenger, 1985; Schiffman and Warwick, 1989; Watt et al., 1989). While it is generally accepted that most of the effects of caffeine are due primarily to antagonism of endogenous adenosine (Smits et al., 1989; Snyder and Sklar, 1984), some effects may also be mediated by catecholamines and possibly by the renin-angiotensin system (Burghardt et al., 1982). Because the two compounds, caffeine and adenosine, have similar molecular structures, caffeine has the potential to occupy adenosine receptor sites, thereby blocking the regulatory effects of adenosine. Some of the main actions of adenosine, which generally functions to inhibit physiological activity, are summarised in Table 10.1. By blockading adenosine receptors, caffeine has broadly 'stimulant' effects (Biaggioni et al., 1991; Carter et al., 1995; Franchetti et al., 1994; LeBlanc and Soucy, 1994).

Chronic exposure to a drug (i.e., frequent or habitual use) can lead to physical dependence, characterized by the appearance of a syndrome of behavioural, physiological, and subjective disruption that is provoked by abrupt withdrawal of the drug (abstinence) after a period of chronic use. Caffeine withdrawal effects have been demonstrated in animals (Carroll et al., 1989; Holtzman, 1983; Sinton and Petitjean, 1989) and in humans (e.g. Griffiths and Mumford, 1995; Griffiths and Woodson, 1988a, b). Although incompletely understood, the mechanism responsible for caffeine physical dependence is believed to involve adenosine. Habitual use of caffeine may result in an increased number of adenosine receptors and/or enhanced

Table 10.1 Some acute biological effects of adenosine[a,b]

Biological system	Effect
Central nervous	Decreased transmitter release, sedation
Cardiovascular	Dilates cerebral and coronary blood vessels
Renal	Antidiuresis
Respiratory	Bronchoconstriction
Gastrointestinal	Inhibition of acid secretion
Metabolic	Inhibition of lipolysis

[a] These effects are broadly opposite to those of caffeine
[b] Adapted from James (1997a)

receptor affinity, resulting in hypersensitivity to adenosine after abrupt withdrawal of the drug (Biaggioni et al., 1991; Paul et al., 1993; von Borstel and Wurtman, 1982; von Borstel et al., 1983). In humans, headache, sleepiness, and lethargy are the most frequent symptoms of caffeine withdrawal (Evans and Griffiths, 1999; Griffiths et al., 1990; Hughes et al., 1991; Lane, 1997; Lane and Phillips-Bute, 1998; Phillips-Bute and Lane, 1998; Silverman et al., 1992; R. Smith, 1987; Streufert et al., 1995; van Dusseldorp and Katan, 1990). Cessation of as little as 100 mg (one cup of coffee) per day, and possibly considerably less (e.g., Lieberman et al., 1987; Smit and Rogers, 2000) can produce symptoms, which may begin within about 12 to 16 hours, peak at around 24 to 48 hours, and persist for up to one week (Griffiths et al., 1990; Hughes et al., 1992; Hughes et al., 1993). Caffeine physical dependence has practical implications in a number of everyday situations. For example, a pattern of weekend caffeine withdrawal headache has been reported in persons whose consumption of caffeine during weekends is less than during the week (Couturier et al., 1992). In addition, several studies have reported caffeine withdrawal headache being prompted by caffeine abstinence in connection with surgery and other medical procedures (Fennelly et al., 1991; Galletly et al., 1989; Nikolajsen et al., 1994).

10.5 Psychomotor performance

Interest in the psychopharmacology of caffeine can be traced to at least as long ago as the early part of the last century (Hollingworth, 1912a, b). Over the intervening period, a large and diverse literature on the subject has emerged. However, coherence within that literature is undermined by the fact that, as with psychopharmacology in general (Parrott, 1991a, b, c), there

has been little standardization of measurements of caffeine-induced behavioural effects. Even the grouping of studies for review purposes is difficult, as the absence of methodological standardization means that categorization of findings is largely arbitrary. Nevertheless, certain trends in the literature are discernible, and these are the focus of this review.

One trend suggested by the literature is that greater consistency is to be found in relation to simple motor activity than to higher level cognitive processes. Such is the case in relation to what would appear to be one of the simplest motor functions of all, namely, tremor. Numerous studies have reported that caffeine increases tremor or 'psychomotor agitation' (Gilliland and Bullock, 1984), particularly as measured by decreased hand steadiness (Chait and Griffiths, 1983; Franks et al., 1975; Ghoneim et al., 1986; Gilliland and Nelson, 1939; Hull, 1935; James, 1990; Lehmann and Csank, 1957; Loke et al., 1985; Richardson et al., 1995; Smith et al., 1977; Thornton et al., 1939; Wharrad et al., 1985). Apart from suggesting that caffeine might have the potential to disrupt activities requiring precise motor control, the practical implications of caffeine-induced tremor are unclear. More complex behaviours have yielded less consistent findings. Results have been inconsistent even for tapping (e.g. rapid depressions of a telegraph key), a behaviour which superficially, at least, would appear to be as simple a 'voluntary' act as any. There have been reports of increased tapping rate following caffeine (Fagan et al., 1988; Hollingworth, 1912a; Horst et al., 1934a; Horst et al., 1934b; Thornton et al., 1939), no effect (Alder et al., 1950; Flory and Gilbert, 1943; Rogers and Dernoncourt, 1998), and decreased rate (Gilliland and Nelson, 1939).

Similarly, studies of reaction time have produced mixed results. Some studies have reported reduced (i.e., improved) reaction times after caffeine (Cheney, 1935, 1936; Gilliland and Nelson, 1939; Smith et al., 1977; Thornton et al., 1939), others have reported increased reaction times (Eddy and Downs, 1928; Hawk, 1929; Schilling, 1921), and still others reported no change (Alder et al., 1950; Lehmann and Csank, 1957; Seashore and Ivy, 1953). The use of different caffeine doses in different studies may explain some of the inconsistencies, with the suggestion that reaction time might be enhanced by doses that are neither too small nor too large. For example, Roache and Griffiths (1987) found that reaction time was improved more by a 400 mg dose of caffeine than by either 200 or 600 mg. Even so, findings are inconsistent. For example, Richardson et al. (1995) found that 70 mg of caffeine reduced reaction time, whereas 250 mg had no effect. One explanation for the inconsistent results is that reaction time, and hence measures of reaction time, are not as simple as might appear. Distinctions are sometimes made between a number of components, including simple reaction time, movement time, and choice reaction time, with each component possibly being affected differently by caffeine. Furthermore, reaction time is subject to influence by a variety of other factors, which may or may not

interact with caffeine, including the sense modality involved (e.g. auditory, visual), stimulus intensity, and the performer's pre-caffeine level of arousal (Estler, 1982; Wenzel and Rutledge, 1962).

10.6 Cognitive performance

There is a long history of scientific interest in the effects of caffeine on cognitive functions, as measured by a wide variety of tasks involving a host of different information processing demands, including memory span (Cattell, 1930), arithmetic addition (Barmack, 1940; Gilliland and Nelson, 1939), numerical reasoning (Franks et al., 1975; Lienert and Huber, 1966), reading speed and comprehension (Flory and Gilbert, 1943), learning of nonsense syllables (Hull, 1935), and solving of chess problems (Holck, 1933). As with psychomotor performance, one consequence of this variation in measurements of cognitive performance is that systematic comparison between studies has been impeded. To the extent that reasonable comparisons can be made, they reveal a high level of inconsistent findings. While methodological diversity has provided researchers with a seemingly endless source of possible reasons for explaining discrepancies between their own and others' findings, no particular set of differences between studies provides an obvious explanation for the numerous inconsistencies.

One popular explanation of the many null results is that these could have been due to studies having insufficient statistical power, with a commensurately high risk of Type 2 error (failing to observe a real difference), attributable primarily to small sample size and proportionately large measurement error. Conversely, it should be noted that the overwhelming majority of studies employed multiple measurements, with each being treated as separate dependent variables, often in the absence of appropriate statistical methods for controlling experiment error (e.g. multivariate analysis of variance, Bonferroni adjustment). As such, it can be argued that an unacceptably high risk of Type 1 error (concluding chance differences to be real) would have existed in many studies that reported caffeine-induced enhancement of performance.

In addition to statistical concerns, inconsistencies in findings might also have occurred because of confounding due to differing inherent features of tests measuring different functions. There is evidence that performance on tasks involving relatively simple cognitive functions that are repetitive, protracted and boring might show greater sensitivity to caffeine than performance on tasks involving higher-level cognitive processes that are inherently arousing, engaging and challenging. For example, Phillips-Bute and Lane (1998) found no changes in performance due to caffeine when performance was measured using multiple brief psychomotor and cognitive tasks. Overall testing time for the battery was 15 minutes, with most of the individual tasks running for only 2 minutes. In a subsequent study, however,

the same authors reported significant caffeine-induced effects on a single visual monitoring vigilance task lasting 30 minutes (Lane and Phillips-Bute, 1998). The authors speculated that the difference in findings was due to task differences, whereby participants in the earlier study were able to 'push themselves to overcome the deficits they felt' when performing the short-duration tasks.

Inconsistencies in the findings of different studies might also have occurred because of confounding due to individual differences, especially in relation to participant level of arousal. There is a general principle, embodied in the Yerkes–Dodson (1908) function, which states that the relationship between physiological arousal and performance efficiency takes the form of an inverted U. That is, performance tends to be optimal at intermediate levels of arousal, and less than optimal when persons are under-aroused (e.g. bored, fatigued) or over-aroused (e.g. anxious, stressed). Caffeine has provided interest because of its putative membership of a class of drugs (including amphetamines and nicotine) said to have stimulant properties (e.g. Humphreys and Revelle, 1984). However, among the many reports of caffeine-induced enhancement of performance, there have been studies involving participants who were not fatigued. Indeed, studies of the effects of caffeine in persons in varying states of fatigue has led to the claim that caffeine is exquisitely suited for use as a general performance enhancing agent (e.g. Smith et al., 1993a; Smith et al., 1992; Smith et al., 1999). Such claims, however, are open to challenge. To examine the question we must first consider the implications of a recently articulated alternative hypothesis concerning the main biobehavioural effects of caffeine, and to integrate the findings from studies of caffeine and performance with studies of caffeine and mood.

10.7 The Caffeine Deprivation (Withdrawal Relief) Hypothesis

The findings reviewed above suggest that the performance-enhancing effects of caffeine are not as striking as is sometimes presumed. Nevertheless, if taken at face value, the many studies reporting some measure of caffeine-induced effects would tend to suggest that caffeine can have performance-enhancing properties, albeit modest and unstable. In recent years, however, even this qualified confirmation of caffeine-induced performance enhancement has been seriously, if not fatally, challenged. This challenge comes in the form of what has been referred to as the Caffeine Deprivation Hypothesis or the Withdrawal Relief Hypothesis.

It has come to light that the large majority of studies conducted over the many decades of research into caffeine and performance contained a methodological flaw that had long gone essentially unnoticed. This flaw derives from the almost universal adoption of a classic research design, the placebo-controlled drug trial. Here, the effects of the compound of interest

(e.g. a therapeutic drug) are compared to the effects of an inert compound (placebo), with both drug and placebo being administered under double-blind conditions. In 1994, James drew attention to the fact that (a) most people are caffeine consumers, and (b) placebo-controlled studies of caffeine (as with drug trials in general) typically require participants to abstain from the drug under examination (caffeine in this instance) for a period immediately preceding the trial. Because caffeine abstinence produces a variety of withdrawal effects (as outlined above) improvements in performance following the experimental administration of caffeine may simply reflect restoration of performance that was degraded during the pre-trial period of caffeine abstinence. It happens that this problem is not unique to the study of caffeine and human performance. A similar flaw has also been identified in relation to psychopharmacological research on nicotine (Heishman et al., 1993; Heishman and Henningfield, 1994; Hughes, 1991).

James (1994a, 1995) illustrated the general problem as pertains to caffeine by reference to a series of studies by A. P. Smith and colleagues (Smith et al., 1990; Smith et al., 1991; Smith et al., 1992; Smith et al., 1993a). These authors claimed to have demonstrated 'great' and 'global benefits' of caffeine, and had proposed their experimental approach as a model that should be emulated by others. For example, Smith et al. (1993a) examined 24 student volunteers who were tested under three conditions: decaffeinated coffee with 1.5 or 3 mg/kg caffeine added, decaffeinated coffee only, and fruit juice. Measurements were taken during two 8.5 hour 'shifts' (one during the day and one overnight), and included a number of tests of psychomotor performance. On certain indices, performance was better and participants reported increased alertness during both the day and night after caffeine (both doses) than after either decaffeinated coffee or juice, with no difference between the two caffeine-free conditions. On the basis of these results, the authors concluded that caffeine enhances performance. However, this interpretation is open to challenge, because participants were all 'moderate coffee drinkers' (2 to 4 cups per day). There is a question that neither this study, nor the scores of others like it, can answer: Was the superior performance observed during the caffeine condition due to actual enhancement by caffeine, or were the observed differences between the conditions due to performance and alertness being degraded by caffeine withdrawal in the two caffeine-free conditions? If the latter, the apparent superior performance during the caffeine condition is explained as a restoration of 'normal' performance due to reversal of withdrawal symptoms following re-ingestion of caffeine.

Interestingly, Smith et al. (1993a) may have anticipated this problem, because they asserted that the withdrawal effects of caffeine are 'extremely small' in persons who habitually consume moderate amounts. This assertion is contrary to the extensive literature showing that caffeine withdrawal has significant adverse effects (e.g. Griffiths and Mumford, 1995; Griffiths and Woodson, 1988a, b). In addition to the headache and other dysphoric

symptoms (i.e., mental or subjective discomfort) mentioned above, drowsiness, irritability, and impaired concentration are also frequent (Hughes et al., 1991; Silverman et al., 1992; van Dusseldorp and Katan, 1990). Moreover, considering the severity of the dysphoric effects reported by some individuals (e.g. 'extreme' and 'totally incapacitating'; Griffiths and Mumford, 1995), it would be surprising if performance impairments were found to be nothing more than 'extremely small'. Indeed, Smith et al. (1993a) cited no evidence in support of their assertion. Conversely, although relatively few studies had examined the effects of caffeine withdrawal on performance (compared to the large number that had examined dysphoric effects), withdrawal-induced performance impairments had been reported at the time that the debate was initiated in relation to a variety of psychomotor, vigilance, and cognitive tasks (Bruce et al., 1991; Griffiths et al., 1986; Horst et al., 1934a, b; Hughes et al., 1991; Mitchell and Redman, 1992; Rizzo et al., 1988; Silverman et al., 1992).

Subsequent empirical studies have provided further confirmation of the validity of the Caffeine Deprivation Hypothesis. For example, James (1998) employed a character-recognition task designed to measure speed and accuracy of information processing involving varying demands on short-term memory in male and female caffeine consumers, who participated in four consecutive one week experimental conditions. Each condition involved ingestion of placebo or caffeine (the approximate equivalent of 1 cup of coffee) three times daily for six days followed by a seventh ('challenge') day of placebo or caffeine ingestion, as summarized in Table 10.2. Saliva samples taken daily confirmed the integrity of the study in that samples taken during abstinence periods contained negligible amounts of caffeine, whereas pharmacologically significant caffeine levels were detected during caffeine periods of the study. The four conditions were designed to induce alternating periods of caffeine exposure and abstinence, which for the first time enabled the acute and chronic effects of caffeine to

Table 10.2 Summary of the double-blind placebo-controlled crossover protocol incorporating alternating periods of caffeine exposure and abstinence[a]

Week	Run-in days (days 1–6)	'Challenge' (day 7)	Effect revealed by challenge	Condition (abbrev.)
1	Placebo	Placebo	'Abstinence'	PP
2	Placebo	Caffeine	'Acute Challenge'	PC
3	Caffeine	Placebo	'Withdrawal'	CP
4	Caffeine	Caffeine	'Habitual use'	CC

PP = Placebo ingested for 6 days followed by 1 day of placebo challenge; PC = 6 days of placebo followed by 1 day of caffeine challenge; CP = 6 days of caffeine followed by 1 day of placebo challenge; CC = 6 days of caffeine followed by 1 day of caffeine challenge

[a] Design originally described by James (1998)

be examined and compared in the one experiment, while also controlling for potential confounding due to tolerance and withdrawal effects associated with habitual consumption. No evidence was found that caffeine improved performance, either in the context of acute or habitual use (no significant difference between the PP, PC and CC conditions in Table 10.2), whereas performance was significantly impaired when caffeine was withdrawn abruptly following habitual use (CP condition in Table 10.2). Had the study not included controls for habitual exposure and abstinence, results would have given the appearance of caffeine-induced enhancement of performance (CC condition significantly superior to CP condition). In reality, habitual caffeine (CC) was no different from either habitual abstinence (PP) or acute exposure (PC), indicating no benefits due to either acute or chronic exposure to caffeine. Conversely, the acute withdrawal condition (CP) showed significant impairment. That is, the results were consistent in all key respects with the Caffeine Deprivation Hypothesis.

10.8 Mood

In contrast to human psychomotor and cognitive performance, which are amenable to objective measurement, mood states are inherently subjective, and are measurable only by self-report. Despite the subjective nature of mood, however, studies of caffeine and mood have been more consistent, in both measurement approaches and findings, than studies of caffeine and performance. Although a wide range of mood states has been examined, measurements in most studies have involved the use of Likert or visual analogue scales, and most have employed substantially overlapping mood categories. In particular, many studies have employed the Profile of Mood States (POMS) (McNair, Lorr and Droppleman, 1971) or variants of it. Consistent with caffeine's putative status as a 'stimulant', there has been particular interest in mood states related to subjectively experienced arousal (e.g. alert-tired, relaxed-tense, awake-sleepy).

Frequently, caffeine-induced effects on performance and mood have been examined in the same study. However, results have often been inconsistent, with some studies reporting effects for both performance and mood, while others have reported effects for performance or mood but not both sets of outcome variables. Only infrequently has attention been given to underlying mechanisms and how these might be related. For example, to the extent that caffeine affects mood, it is conceivable that observed performance effects could be an indirect effect of changed mood rather than the result of the drug acting directly on information processing centres in the brain. As with studies of caffeine and performance, there have been numerous reports of caffeine-induced 'improvements' in mood (e.g. Bruce et al., 1986; Chait and Johanson, 1988; Griffiths and Woodson, 1988a, b; Griffiths et al., 1990; Lieberman et al., 1987; Silverman and Griffiths, 1992;

Smith et al., 1992; Smith et al., 1993b; Smith et al., 1994; Smith et al., 1999; Stern et al., 1989; Warburton, 1995; Warburton et al., 2001). However, whether such changes in mood actually represent improvements is rarely considered closely. For example, increased arousal is sometimes reported as mood enhancement, when the subjective experience of the participant may actually have involved moving from a comfortable level of arousal to an aversive state of over arousal.

Notwithstanding frequent reports of enhanced mood, null findings have also been reported (e.g. King and Henry, 1992; Rush et al., 1994; Svensson et al., 1980). In addition, dysphoric effects have been reported, including increased ratings of anxiety, tension, anger/hostility and jitteriness (Chait and Griffiths, 1983; Charney et al., 1984; Evans and Griffiths, 1991; Loke, 1988; Loke et al., 1985; Oliveto et al., 1993; Richardson et al., 1995; Roache and Griffiths, 1987). Moreover, as with research on caffeine and performance, the overwhelming majority of studies of caffeine and mood have included multiple measurements. Consequently, the risk of Type 1 error was probably unacceptably high in the many studies that did not employ appropriate statistical methods to control error rates. Indeed, when Christensen, Miller and Johnson (1991) applied Bonferroni correction (a statistical operation designed to reduce the number of spurious findings) to the results of a study of caffeine-induced changes in mood, effects that had previously been apparent no longer reached the minimum conventional level of statistical significance.

It is particularly important, however, to note that studies of caffeine and mood have typically employed the same kinds of placebo-controlled designs as have generally been employed in studies of caffeine and performance. Consequently, the methodological shortcomings described in the preceding section also apply to studies of caffeine and mood. One early demonstration of abstinence-induced disturbances in mood was by Goldstein et al. (1969), who compared the effects of caffeine and placebo in two groups, one of which reported drinking no coffee while the other reported drinking five cups or more daily. In the experiment, before drinking coffee, habitual consumers reported feeling less alert, less active, less content/at ease, more sleepy and more irritable than the abstainers, but these differences were removed after caffeine was consumed. On placebo days, differences persisted, with the habitual consumers reporting more frequent headache and feeling more jittery/nervous/shaky than non-consumers.

Similarly, Richardson et al. (1995) found that dysphoric effects induced by caffeine withdrawal abated following caffeine ingestion. More recently, Lane and Phillips-Bute (1998) and Phillips-Bute and Lane (1998) employed a strategy that involved examining caffeine consumers at midday following mornings of caffeine intake or caffeine deprivation. Specifically, the effects of a brief period of deprivation (overnight and morning) were compared with caffeine ad lib (participants' usual intake) (Lane and Phillips-Bute,

1998) and a single caffeine dose of 250 mg (Phillips-Bute and Lane, 1998). Caffeine deprivation resulted in reports of decreased vigour and ability to work and increased fatigue, sleepiness and headache. These various findings confirm the Caffeine Deprivation Hypothesis by showing that caffeine ingestion reliably reinstates mood levels that have been adversely affected by caffeine deprivation (Goldstein et al., 1969; James, 1998; Lane and Phillips-Bute, 1998; Phillips-Bute and Lane, 1998; Richardson et al., 1995). Furthermore, in the study outlined above by James (1998), results indicated that caffeine ingestion not only re-instated mood in caffeine-deprived consumers, but that habitual exposure to caffeine was associated with reports of decreased alertness relative to sustained caffeine abstinence.

10.9 Reinforcing properties of caffeine

A further source of evidence in support of the Caffeine Deprivation Hypothesis is provided by studies of the reinforcing properties of caffeine in consumers who have been deprived of the drug. Several studies, using choice and/or self-administration procedures, have demonstrated caffeine reinforcement (e.g., Evans et al., 1994; Hughes et al., 1995; Rogers et al., 1995; Schuh and Griffiths, 1997; Silverman et al., 1994). For example, Evans et al. (1994) examined caffeine versus placebo in moderate consumers over a 24 week period. Each week consisted of three consecutive daily sessions (two sampling days followed by a choice day), during which participants were required to abstain from dietary sources of caffeine. On each sampling day, participants ingested one capsule every two hours over eight hours. Capsules contained placebo on one sampling day and caffeine on the other, each being associated with a different colour code. At the beginning of the choice day, participants chose one of the two colour-coded capsules they wished to consume on that day. They ingested one capsule, and thereafter ingested up to six additional capsules of the same code during the remainder of that day. Caffeine was chosen by a majority of subjects on a majority of occasions they were permitted to choose between caffeine and placebo. One important feature of this study was that participants maintained their usual caffeine intake on non-experimental days, suggesting that habitual consumption is a contributing factor in the development of caffeine reinforcement. Specifically, it appears that caffeine reinforcement is potentiated by the effects of caffeine withdrawal.

Consistent with the notion of withdrawal potentiated reinforcement, Hughes et al. (1995) found that the occurrence of withdrawal effects (e.g. increased headache and drowsiness) predicted caffeine reinforcement, as indicated by participants' choice of caffeine over placebo. Similarly, Rogers et al. (1995) investigated caffeine reinforcement by assessing changes in preference for a novel drink (fruit juices specially prepared so as to be distinctive in flavour and colour while also being palatable) consumed with

and without caffeine. Caffeine had no significant effects on drink preference (or mood) in participants whose habitual level of caffeine consumption was low. In moderate users, however, overnight withdrawal effects were alleviated by the drug, with participants showing a distinct preference for drinks containing caffeine. In a more recent study, Schuh and Griffiths (1997) found that choosing to ingest caffeine is more potently influenced by alleviation of aversive withdrawal effects than by any positive effects the drug may have.

Overall, studies of the reinforcing properties of caffeine show that the drug functions as a weak and unreliable *positive* reinforcer, a result that is consistent with the conclusion that caffeine produces little or no net benefit for performance and mood. In habitual consumers, however, the reinforcing properties of caffeine are markedly potentiated by periods of abstinence, even periods as brief as overnight. Under these circumstances, caffeine functions as a rather strong *negative* reinforcer, whereby aversive withdrawal effects are ameliorated by ingesting the drug. These findings indicate that withdrawal relief, rather than net enhancement of performance or mood, is a major factor contributing to the maintenance of habitual caffeine consumption. That is, the findings add considerable weight to the already abundant evidence in support of the Caffeine Deprivation Hypothesis.

10.10 Challenges to the Caffeine Deprivation Hypothesis

Despite compelling theoretical and empirical support for the Caffeine Deprivation Hypothesis, it has not won universal favour. Smith et al. (1999), for example, have argued against the Hypothesis, stating that studies have shown caffeine-induced effects in non-consumers 'where withdrawal is not an issue' (1999: 14), but the authors cite no empirical evidence in support of their claim. In any event, even if true, the claim by Smith et al. (1999) would pose little threat to key tenets of the Caffeine Deprivation Hypothesis, because caffeine use is self-selected, and performance differences between consumers and non-consumers may be due to factors other than (but associated with) caffeine use. Indeed, it is conceivable that caffeine has different effects on persons who choose to consume the drug and those who do not, which in turn may influence whether a person becomes a consumer. Thus, while individual differences between caffeine consumers and non-consumers (who represent a small minority) may be of interest, such information alone is evidence neither for nor against the Caffeine Deprivation Hypothesis.

Smith et al. (1999) also make the curious suggestion that caffeine should enhance performance and mood because of its acknowledged ability to disrupt sleep. Apart from other shortcomings, this claim involves a spurious line of reasoning. It is obvious that performance on typical psychomotor

and cognitive tasks must be superior in a person who is awake versus one who is asleep. Consequently, if caffeine disrupts sleep, a person who has ingested caffeine is likely to 'perform' better than someone whose sleep has not been disrupted. Before long, however, the well-being of the person whose sleep has been disrupted will suffer. Moreover, the claim contains within it the admission that caffeine does not produce net *benefits* in that whatever short-term advantage may accrue is achieved only at the cost of disruption to sleep.

In another claimed challenge to the Caffeine Deprivation Hypothesis, Warburton (1995) sought to determine whether improved performance is due to restoration of abstinence-induced impairment. One hour before each session, participants were pretreated with 75 mg caffeine (equivalent to a weak cup of coffee) in order that they would be 'without caffeine abstinence' at the time of testing in the laboratory. Performance was found to be enhanced when participants again ingested caffeine in a laboratory session. However, the claim that the study shows that caffeine has net performance-enhancing effects not attributable to restoration of impaired performance is unwarranted. Pretreatment might only have been partially effective in eliminating abstinence effects, and it is very unlikely to have eliminated abstinence uniformly between subjects.

A more recent study by Warburton et al. (2001), claiming that caffeine-induced enhanced performance and mood 'in individuals who could not have been in caffeine withdrawal' (2001: 322), is even less adequate as a test of the Caffeine Deprivation Hypothesis than the earlier study by the same group. Warburton et al. (2001) report two experiments involving moderate habitual consumers. In one experiment, participants were 'told they could consume their normal quantities [of caffeine] during the day' (2001: 324), but not to ingest any caffeine during the hour preceding their attendance at a laboratory session. In the second study, 'participants were not required to abstain from caffeinated beverages at all during the 12-hour period prior to testing' (2001: 324). No explanation is given of what this seemingly inconsequential difference in instructions was intended to achieve, nor why this purported key difference in instructions would warrant two separate experiments. More importantly, neither set of instructions is adequate to meet the investigators' objective of demonstrating net benefits of caffeine while controlling for caffeine withdrawal effects. It is entirely unclear how participants would have responded to the instructions, which in both experiments were vague and open to varying interpretations. Moreover, there is no way of knowing what levels of caffeine deprivation participants may or may not have been experiencing prior to testing, as no information (e.g., plasma or saliva caffeine levels) was given regarding level of compliance with either set of instructions.

Furthermore, it should be noted that the interpretation by Warburton et al. (2001) of the data they collected is directly contradictory to the findings of an earlier study by Robelin and Rogers (1998) that addressed similar

issues more systematically. In the Robelin and Rogers study, participants were administered placebo and/or caffeine in one of four combinations (placebo only, or 1, 2 or 3 spaced doses of caffeine) during the morning following overnight abstinence. Caffeine improved performance and mood relative to placebo alone, but by the same amount, irrespective of number of doses. Considering the adverse effects (e.g., fatigue) of overnight abstinence, the findings support the Caffeine Deprivation Hypothesis in suggesting that there is no net benefit from caffeine, only withdrawal relief. Finally, it should be noted that the criticisms by Warburton et al. (2001) of the Caffeine Deprivation Hypothesis may stem from a confused understanding of key methodological issues. For example, Warburton et al. cite a study by Smit and Rogers (2000) as providing evidence against the Hypothesis, but perusal of that study shows that this cannot be so. The Smit and Rogers (2000) study is important because it showed that performance and mood in caffeine consumers may be responsive to lower levels of caffeine (12.5, 25 and 50 mg) than realised hitherto. However, the study design was a variant of the classic placebo-controlled drug trial, which as explained above is incapable of unravelling the separate acute and chronic effects associated with dietary use. Indeed, because effects on performance were more marked in participants with a higher level of habitual caffeine intake, the Smit and Rogers (2000) findings are consistent with the Caffeine Deprivation Hypothesis.

When considering the literature on the effects of dietary caffeine on performance and mood, it would be difficult to exaggerate the extent of confusion and possible harm caused by the use of inadequate experimental methodology and misrepresentation of findings. In addition to the instances mentioned above, these anomalies are illustrated in the work of Horne and Reyner (2001) who assessed the 'beneficial' effects of a well-known brand of caffeine energy drink (*Red Bull*). The drink was administered double blind to 11 sleep-deprived young adults, whose performance was tested on a driving simulation task. The results were interpreted as showing that caffeine significantly improved performance (i.e., reduced the frequency of driving 'incidents' such as lane drift), a finding that has been used by the drink's manufacturer to promote the product. However, participants were moderate consumers ('2–4 cups daily'), and all presented for testing in a state of caffeine deprivation. Indeed, all were instructed to abstain from caffeine after 6.00 pm the evening before testing, which began at approximately 2.00 pm in the afternoon of the test day and finished approximately 3 hours later, thereby ensuring that testing coincided nicely with the full onset of withdrawal effects. Thus, rather than demonstrating enhanced performance due to caffeine ingestion, this study, like other similarly-designed studies, is better explained in terms of reversal of withdrawal effects following re-ingestion of caffeine. Indeed, instead of supporting the use of caffeine drinks when driving, the study provides an indication of the added risk of accident faced by sleepy drivers who are caffeine deprived, a risk

that can be removed most effectively by not being a caffeine consumer in the first place.

It is notable that Horne and Reyner (2001) appear to have anticipated the threat posed by the process of withdrawal relief to the integrity of their study, just as Smith et al. (1993a) appear to have done earlier (mentioned above). Horne and Reyner (2001) asserted that the participants in their study would not have experienced 'any caffeine withdrawal effects during the driving session' (2001: 84), and cited three sources in support of this assertion, namely, Bättig and Welzl (1993), Griffiths and Woodson (1988b) and James (1991). Notably, however, Griffiths and Woodson (1988b) and James (1991) provide substantial, if not conclusive, evidence of the likelihood of participants in the Horne and Reyner (2001) study experiencing pronounced withdrawal effects within the timeframe of that study. Although Bättig and Welzl (1993) refer to studies in which participants experienced 'few, if any, withdrawal symptoms' (1993: 245), the studies in question were by James (James et al., 1985; James et al., 1988) and others (e.g., Foxx and Rubinoff, 1979) who employed caffeine 'fading' procedures to assisted heavy caffeine consumers reduce their daily caffeine intake. The express purpose of caffeine fading, which involves reducing daily caffeine intake gradually over weeks or months, is to circumvent the aversive symptoms that are experienced when caffeine is withdrawn abruptly as in the Horne and Reyner (2001) study. Thus, the work of Horne and Reyner (2001) amply demonstrates that readers of caffeine literature must not only be critically perceptive to avoid being misled by experiments that are fatally flawed, but the same readers must also be on guard against pointedly erroneous claims that serve to conceal the flaws in such experiments.

10.11 Future trends

Considering the obvious interest amongst some investigators in challenging the Caffeine Deprivation Hypothesis, the level of ingenuity in doing so has been disappointing to date. At an empirical level, there are at least three alternative approaches that could be used to try to clarify the effects of caffeine on human performance and mood. One approach, advocated by Smith et al. (1999), is to administer caffeine to non-consumers (i.e. caffeine 'naive' persons) for whom the risk of caffeine withdrawal is absent because they have no recent history of caffeine use. However, as noted above, since these individuals represent a very small self-selected minority of the population, the generality of the findings would be questionable. A second approach is to 'pretreat' participants with caffeine in an attempt to remove caffeine withdrawal effects at the time laboratory testing is scheduled to occur. This approach was tried by Warburton (1995), but as explained above (and in more detail by James, 1997a), the study is a failure. The third, and most suitable, approach uses alternating phases of caffeine exposure and abstinence

with habitual caffeine consumers (James, 1998). This approach, or variants of it, will be the ultimate arbiter of our efforts to understand the effects of dietary caffeine on mental performance and mood. The approach also serves to illustrate the futility of persisting solely with studies such as those undertaken by Smith et al. (1999), Warburton (1995), Warburton et al. (2001) and Horne and Reyner (2001), which manipulate acute exposure to caffeine while ignoring the underlying effects of chronic exposure.

Ultimately, a comprehensive understanding of the effects of dietary caffeine on performance and mood can be achieved only by manipulating chronic exposure directly. Indeed, the main challenge to the Caffeine Deprivation Hypothesis does not relate to the flawed counter-assertions by Smith et al. (1999), Warburton (1995), Warburton et al. (2001), Kenemans et al. (1999) among others. The main challenge relates to the extent of supportive empirical evidence from studies that directly examine processes of chronic exposure. There is now a large body of evidence concerning the effects of acute caffeine exposure on mental performance and mood that provides extensive *indirect* support for the Caffeine Deprivation Hypothesis, and a comparatively small body of evidence concerning chronic caffeine exposure that provides strong *direct* support for the Hypothesis. More studies in the latter category are needed, studies that systematically compare the effects of protracted periods of abstinence (minimum of one week) with the effects of habitual caffeine use (again, minimum of one week). The experimental design employed by James (1998) represents one example, and replications of this, or alternative study designs that achieve the same objectives, are needed.

10.12 Conclusions

One implication of the evidence reviewed in this chapter is that caffeine is not a functional food. Firstly, as mentioned above, caffeine is not food, it is a drug. Secondly, when used chronically, as is the case for the overwhelming majority of consumers, evidence of net benefits is lacking. Indeed, considering the strength of evidence of likely harm due to dietary caffeine, consuming the drug regularly should be regarded as *dysfunctional*. One example of harm caused by dietary caffeine is physical dependence (including associated withdrawal effects) as outlined above. In addition, there are other notable adverse physical effects, such as increased risk of cardiovascular disease (e.g. James, 1997b), probable increased risk to the foetus when caffeine is consumed during pregnancy and adverse interactions when caffeine is consumed conjointly with some therapeutic drugs (James, 1991, 1997a).

Regarding mental performance and mood, the following may be concluded: Caffeine-induced improvements in mental performance and mood are substantially, if not wholly, attributable to withdrawal relief (Caffeine

Deprivation Hypothesis). Contrary to widespread opinion, dietary caffeine has not been shown to produce absolute improvements in either performance or mood. If such improvements do exist, they are modest at best, certainly smaller in magnitude than the decrements in performance and mood that accompany caffeine withdrawal. Hence, in practical terms, the caffeine consumers derive no net benefit from their habit. Such findings are damaging to the commercial interests of the producers of caffeine products, whose efforts to date have had a measure of success in undermining the integrity of caffeine research (James, 1994b, 2002a, b). Consumers can help to restore integrity to the scientific process by thinking critically whenever they receive information extolling biobehavioural benefits of caffeine and caffeine products.

10.13 Sources of further information

The production, distribution, and sale of caffeine products are multinational, multi-billion dollar enterprises. Public perception of caffeine-induced benefits is good for business, and public awareness of adverse effects is bad for business. Any recommendation regarding sources of further information for the student, researcher or other interested reader must be conveyed with a prominent 'health warning'. Searching the Worldwide Web is especially risky. There are several well-maintained Websites funded by the caffeine industry that exist for the sole purpose of disseminating misinformation about caffeine products. Typically, such sites are designed to create impressions of independence, respectability and public concern that conceal the true purpose of the sponsor, which is to protect and promote commercial interests. A common feature of such sites is the frequent reference to 'independent research' and 'scientific findings'. Unfortunately, the dissemination of commercially inspired misinformation does not end there and sadly, corporate interests have long succeeded in recruiting 'scientists from the ranks of the most prestigious academic institutions' (Yach and Bialous, 2001). In the process, scientific integrity has been undermined, and the content of the scientific literature distorted.

Using tactics similar to those employed by tobacco manufacturers, the caffeine industry has sought to manipulate the dissemination of scientific knowledge about caffeine and to influence the course of caffeine research (James, 1994b). Increases in the consumption of caffeine products over the past decade suggest that such tactics have had some success. Moreover, industry has succeeded not only in influencing individual scientists, but has also sought to influence the activities of pre-eminent international institutions, such as the World Health Organization. While these pernicious efforts are now being debated in the scientific literature, effective controls have yet to be implemented (e.g., James, 2002a, b, Yach and Bialous, 2001). Hence, before drawing any conclusions, readers must seek to bring to bear all the

powers of critical thinking they can muster, if they are to succeed in discerning biased, or simply misinformed, accounts about caffeine from the truly independent and informed.

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