CHAPTER II

REVIEW OF LITERATURE

With the expansion of the artificial sweetener market, concerns have arisen among consumers regarding the safety of artificial sweeteners and their possible long-term health effects. Over 20 years have elapsed since aspartame was approved by regulatory agencies as a sweetener and flavour enhancer. Since then aspartame has been consumed in more than 6000 products by hundreds of millions of people around the world who want to enjoy the sweet taste of sugar without the calories. The safety of aspartame and its metabolic constituents was evaluated through extensive toxicology studies in laboratory animals. Its safety was further tested through studies in several human subpopulations, including healthy infants, children, adolescents, and adults; obese individuals and diabetics. Nonetheless, additional research, including evaluations of possible associations between aspartame and headaches, seizures, behaviour, cognition, and mood as well as allergic-type reactions and its use by potentially sensitive subpopulations has continued after approval. Several scientific issues were raised and further evaluated in the postmarketing period, largely as a result of assertions of potential toxicity of its metabolic components.

Epidemiological studies

The centre of the debate on the safety of aspartame was the question of the potential carcinogenic risks associated with artificial
sweetener use. Very few epidemiological studies are available to evaluate the relationship between aspartame intake and the development of cancer.

In 1996, Olney *et al.* published an article on a possible link between the increase in the frequency of brain tumours in humans and the consumption of aspartame in the United States. Based on the data from the National Cancer Institute from 1975-1992, the authors concluded that there was a significant increase in the frequency of brain tumours in the mid-1980s, that is to say the period following aspartame came onto the market. The authors suggested that brain tumour incidence was increasing, and that increase was related to the introduction of aspartame into the market. The authors suggested that the incidence of brain tumours is due to nitrosation of aspartame, which is mutagenic.

A case-control study, conducted to examine the hypothesis that aspartame may act as a human brain carcinogen, examined aspartame consumption by patients with primary brain tumours and an age and sex matched control subjects (Gurney *et al.*, 1997). This case study comprises children in the United States who were diagnosed with a brain tumour between the ages of 0 and 19 during 1984 to 1991. Exposure of both the mother and the child to environmental and nutritional factors, including aspartame, had been collected by personal interviews with the mothers of the children prior to diagnosis. No evidence to support a causal association between increased consumption of aspartame by case children or by mothers of case children was found. There was no suggestion of a dose response based on years or frequency of consumption.
Lim *et al.* (2006) investigated prospectively, whether aspartame consumption is associated with the risk of hematopoietic cancers or gliomas (malignant brain cancer). This study examined men and women ages 50 to 71 years involved in the NIH-AARP Diet and Health Study cohort. Daily aspartame intake was derived from responses to a baseline self-administered food frequency questionnaire that queried consumption of four aspartame-containing beverages (soda, fruit drinks, sweetened iced tea, and aspartame added to hot coffee and tea). Histologically confirmed incident cancers were identified from eight state cancer registries. Multivariable-adjusted relative risks (RR) and 95% confidence intervals (CI) were estimated and adjusted for age, sex, ethnicity, body mass index, and history of diabetes. Higher levels of aspartame intake were not associated with the risk of overall hematopoietic cancer, glioma or their subtypes in men and women. It concludes that findings do not support the hypothesis that aspartame increases hematopoietic or brain cancer risk.

To provide information on the role of artificial sweeteners on the risk of cancer in humans, Gallus *et al.* (2007) evaluated data from a large and integrated network of case-control studies conducted in Italy. This work observed a lack of association between saccharin, aspartame and other sweeteners and the risk of several common neoplasms.

**Metabolism**

The intestinal absorption and metabolism of aspartame have been intensively studied in rodents, pigs, primates and humans
In all species examined, aspartame may be completely hydrolyzed by esterases and peptidases to phenylalanine, aspartic acid, and methanol in the gastrointestinal lumen and absorbed into the general circulation, or may be hydrolyzed to methanol and aspartylphenylalanine dipeptide. The dipeptide is absorbed into the gastrointestinal mucosa cells and then cleaved into phenylalanine and aspartic acid (Stegink, 1987). Phenylalanine enters the free amino acid pool from the portal blood after partial conversion to tyrosine by hepatic phenylalanine hydroxylase. Aspartic acid is metabolized within the enterocyte via transamination producing oxaloacetate, thereby reducing the concentration of aspartate entering the portal circulation and plasma free amino acid pool (Filer and Stegink, 1989). Aspartame can also be absorbed into the mucosal cells prior to hydrolysis, and be cleaved within the cell, to its three components, which then enter circulation (Matthews, 1984). Lipton et al. (1991) compared the absorption and metabolism of aspartame decomposition products using in situ perfusion of the jejunum in adult rats. The α-aspartylphenylalanine (α-AP) isomer was rapidly absorbed, but β-aspartylphenylalanine (β-AP) and diketopiperazine (DKP) were slowly absorbed and to a much lesser degree. Studies in animals and humans indicate that DKP is poorly absorbed, is not biotransformed by mammalian enzymes and is rapidly eliminated in the urine (Butchko et al., 2002). In addition, only α-aspartylphenylalanine was metabolized into amino acids by rat liver and intestinal mucosal cell homogenates (Kotsonis and Hjelle, 1996). Hooper et al. (1994) used microvillar
membrane preparations from human duodenum, jejunum, and ileum and from pig duodenum and kidney to evaluate the metabolism of aspartame, α-AP, β-aspartame and DKP by gastrointestinal tract aminopeptidases. Expectedly, this study demonstrated that aspartame and α-AP were rapidly metabolized by aminopeptidases, with aminopeptidase A being the major enzyme in the microvillar membranes involved with the metabolism of aspartame. β-AP and DKP were essentially resistant to hydrolysis in human and pig microvillar membrane preparations. Under dry conditions, the conversion of aspartame to DKP is slow (Homler, 1984).

Methanol is not subjected to metabolism within the enterocyte and rapidly enters the portal circulation. The methanol is oxidized in the liver to formaldehyde. Enzymes involved depends on the species: In the rat, the metabolism of methanol to formaldehyde is mediated though a catalase-peroxidase system, whereas in primates and humans, an alcohol dehydrogenase is responsible (Makar et.al, 1990). Formaldehyde is further oxidized to formic acid by formaldehyde dehydrogenase. Formic acid is ultimately converted to CO₂ and water, via the formation of 10-formyl tetrahydrofolate (Barceloux et.al, 2002). Humans are uniquely sensitive to methanol poisoning because of their low liver folate content (Johlin et.al, 1987). There are profound differences in the rate of formate oxidation in different species which determine their sensitivity to methanol (Mcmartin et.al, 1978; Eells et.al, 1983).
Chapter II

Metabolism of aspartame

Metanol metabolism
Neurotoxicity

Much interest regarding the safety of aspartame has related whether its consumption produce any neurological effects. The effect of aspartame consumption on the levels of its component amino acids was extensively investigated due to several potential effects of aspartic acid and phenylalanine. These include: early observations of adverse effects of subcutaneous injections of L-aspartic acid in neonatal mice; neurotoxicity observed in patients with the phenylketonuria (PKU) resulting in high plasma phenylalanine; and potential behavioral effects due to competitive inhibition of brain uptake of tryptophan, a precursor of serotonin (Groff and Gropper, 2000). Concerns have been voiced regarding possible effects of aspartame on brain amino acid levels and brain function (Olney, 1980).

Filer et al. (1983) had compared the effect of acute doses of aspartame on plasma amino acid levels in infants with that of the results obtained by adults, showed a dose dependent increase in plasma phenylalanine levels to the same levels observed in adults given the same dose. Plasma aspartic acid levels did not change in infants at low doses, but did rise when high dose was consumed. Similarly, erythrocyte amino acid responses were the same as adults, suggesting that infants absorb and metabolize aspartame in the same manner as adults. It is hypothesized that fluctuations in phenylalanine/LNAA transport to brain occur as a result of dietary manipulations or normal dietary variability. Romano et al. (1989) evaluated the effect of both acute and sub acute dosing of aspartame with and without carbohydrate, on plasma and brain LNAA and brain neurotransmitter concentrations in rats. Although
there were changes in plasma and brain amino acid concentrations in the acute dosing paradigm, there were no differences in brain LNAA concentrations after aspartame compared to aspartame plus carbohydrate in the subacute study.

Meanwhile, Yokogoshi et al. (1984) observed that aspartame consumption elevates brain levels of phenylalanine. Similar results were obtained with dose-response studies confirming that aspartame dose-dependently increased brain concentrations of phenylalanine and tyrosine, but not tryptophan (Fernstrom et al., 1983). Plasma phenylalanine levels increased dose-dependently to the same levels observed in adults given the same dose. Wurtman and Maher (1987) proposed that phenylalanine levels are reduced in the brain following the consumption of protein (although they rise in plasma), due to the competition for uptake by other LNAA at the transport site. In contrast, when phenylalanine is administered either as a pure amino acid or in aspartame, brain phenylalanine levels increased.

A number of single dose or short term animal studies have been done to determine whether plasma phenylalanine concentration increases secondary to aspartame loading may resulted in changes in concentration or turnover of monoamine neurotransmitters in whole brain or specific regions of the brain. Most of these studies were acute or sub acute dosing paradigms. Changes in neurotransmitters in specific areas of the brain following aspartame dosing of mice were assessed by Coulombe and Sharma (1986). Tissues from various regions of the brain were analysed for catecholamines, indole amines and metabolites. The greatest effect
was observed in the hypothalamus, where the concentrations of nor epinephrine and dopamine increased dose-dependently. In addition, in the hypothalamus, the concentration of 3-methoxy-4-hydroxy mandelic acid was increased in the highest dose group and the concentration of homovanillic acid was increased in medium dose. Significant increases in nor epinephrine in the medulla oblongata and corpus striatum were also observed.

Yokogoshi and Wurtman (1986) reported that nor epinephrine levels were increased in cerebral cortex and amygdala of rats with a single dose of aspartame compared with those of saline controls. Animals receiving oral aspartame also exhibited higher plasma tyrosine and phenylalanine. These findings are in contrast to the studies described by Reilly et. al (1989) in which administration of aspartame in the drinking water did not significantly alter serotonin, dopamine, or nor epinephrine in the cortex and hippocampus, and dopamine in the striatum.

Fernstrom et. al (1986) evaluated the effect of aspartame on the rate of tryptophan hydroxylation in the brain. Increased tryptophan hydroxylation followed a carbohydrate-rich meal added with different doses of aspartame, resulting in increased levels of serotonin. In a later study by Goerss et. al (2000) showed that striatal levels of serotonin and its metabolite, 5-hydroxyindoleacetic acid increased following the administration of aspartame. This study observed significantly decreased aggression as observed by the increased latencies to the first attack and decreased number of bites per session in experimental rats. It is suggested that the behavioural change observed in this study is a consequence of the increased
serotonin levels. Bergstrom et al. (2007) conducted an in vivo voltammetry study to measure evoked extracellular dopamine levels in the striatum. This work providing a real-time measurement of evoked extracellular dopamine levels in the striatum before and after the administration of a single high dose of aspartame, revealed a significant decline in evoked extracellular dopamine levels. This study suggests a role of phenylalanine competition for the LNAA carrier system for transport into the brain.

Aspartic acid is thought to play a role as an excitatory neurotransmitter in the central nervous system (Watkins, 1984). From an in vitro study, Pan-Hou et al. (1990) suggested that aspartame especially its aspartate component, inhibited glutamate binding to N-methyl-D-aspartate (NMDA) receptors in a dose dependent manner, altering the affinities for the receptors. Later, Hellali et al. (1996) observed increased brain aspartate concentrations in mice after an acute aspartame dosing. Fountain et al., (1992) evaluated the excitatory effect of aspartame in vitro using hippocampus slice preparation. Exposure of slices of the hippocampus to aspartame resulted in potentiation of the response of hippocampal CA1 pyramidal cells.

The potential of aspartame in producing neuronal damage in the central nervous system has been the subject of extensive debate for several decades. Reynolds et al. (1980) found hypothalamic lesions in neonatal mice when treated with aspartame at doses higher than 1g/kg. Another study by Finkelstein et al. (1988) reported that aspartame induces neuronal necrosis in young mice at similar doses. Lau et al. (2006) observed that a combination of aspartame
with quinoline yellow, a common food colour showed synergistic effects on neuronal cell differentiation by inhibiting the neurite outgrowth in the NB2a neuroblastoma cells in vitro, suggests a role of aspartame in neuronal cytotoxicity. Furthermore, Omar (2009) reported that chronic aspartame ingestion could result in marked morphological necrotic changes in Pyramidal cells in the frontal cortex. In addition to this Abd El-Samad (2010) evaluated the effect of aspartame in cerebellar cortex in rats and observed that Purkinje cells in the cerebellar cortex of aspartame group appeared deformed.

A few studies have implicated aspartame consumption in learning or memory. Potts et.al (1980) showed that administration of aspartame altered learning behaviour in male rats. Dow-Edwards et.al (1989) treated pregnant guinea pigs using a low dose of aspartame throughout gestation and demonstrated that aspartame-treated pups showed a disruption of odour-associative learning. LaBuda and Hale (2000) evaluated the combining effect of aspartame with ethanol on behaviour in male CD-1 mice, and found that neither aspartame alone nor in combination with ethanol affected anxiety-related behaviours. Later Christian et.al (2004) used a T-maze to test memory in rats receiving aspartame. The study has shown that chronic consumption of aspartame may affect the T-maze cognitive performance of male rats, promoting impairment in retention of learning behaviour. In addition muscarinic cholinergic receptor density was significantly higher in several areas of the brain of rats receiving aspartame compared to rats receiving only water. This study speculates that the increase in time to find a reward in the T-maze indicates that chronic
aspartame consumption results in memory loss and this was mediated by the change in cholinergic receptor density. Supporting this study, Collison et.al (2012) investigated the effects of chronic lifetime exposure to aspartame in mice using Morris Water Maze (MWM) test, and observed impairment in cognitive performance, most notably in males. Acetylcholine esterase is a key enzyme of the muscarinic cholinergic system, involved in learning and memory. It plays an essential role in acetylcholine mediated neurotransmission. Tsakiris et.al (2006) reported that in vitro incubation of human erythrocyte membranes in the presence of combinations of methanol, aspartic acid and phenylalanine resulted in a dose-dependent reduction of acetylcholine esterase activity. Furthermore, Simintzi et.al (2007) reported that in vitro incubation of rat hippocampal acetylcholine esterase in the presence of various concentrations of each or the sum of aspartame metabolites resulted in a dose-dependent reduction of acetylcholine esterase activity. These studies suggested that the free radical production and oxidative stress induced by methanol and phenylalanine derived from aspartame may contribute to the mechanism.

The brain is particularly sensitive to toxic compounds. Xenobiotic metabolic enzymes are of special interest as changes in their function could lead to an increased susceptibility of the organisms to the harmful effects of a variety of contaminants found in the environment and in food products. The effects of aspartame on rat brain xenobiotic-metabolizing enzymes were reported (Vences-Mejia et.al, 2006). This study demonstrated that chronic aspartame consumption lead to an increase of phase I metabolizing
enzymes (CYP P450 enzymes) in rat brain. Increases in activity were consistently found both in cerebrum and cerebellum for all seven enzymes, although not at the same levels. Recently Iyyaswamy and Rathinasamy (2012) investigated the effect of chronic aspartame administration on oxidative stress in the rat brain. There was a significant increase in lipid peroxidation (LPO) levels, superoxide dismutase, catalase and glutathione peroxidase activities with a significant decrease in GSH and protein thiol. Chronic exposure of aspartame resulted in detectable methanol in blood. It is hypothesized that methanol as such and its metabolites may be responsible for the generation of oxidative stress in brain regions.

Among the possible adverse effects of aspartame, researchers have paid particular attention to seizures. Several studies have suggested a relationship between the consumption of aspartame and the triggering of epileptic seizures. Case reports of seizures anecdotally associated with aspartame consumption (Wurtman, 1985; Eshel and Sarova-Pinhas, 1993) stimulated interest regarding whether aspartame may potentially be associated with seizures. The ability of aspartame to potentiate the induction of chemically or electrically induced seizures in rats was assessed as a measure of neurotoxicity (Guiso et al., 1988). Pinto and Maher (1988) observed that aspartame potentiate the pentylenetetrazole (PTZ) or fluorothyl induced seizures. Plasma levels of phenylalanine and tyrosine were increased, and the ratio of phenylalanine to large neutral amino acids was also increased. Using equimolar doses of phenylalanine, aspartic acid, and methanol, the authors demonstrated that the phenylalanine component mediates this activity. Diomede et al. (1991) demonstrated
interspecies differences in the susceptibility to PTZ-induced seizures in animals given aspartame. But certain other studies found no increase in seizures (Dailey \textit{et.al}, 1989; Sperber \textit{et.al}, 1995). The effect of aspartame on induction of seizures has been studied in primates also. Meldrum \textit{et.al} (1989) tested the effect of aspartame and phenylalanine on photically induced myoclonic stimulation in genetically photosensitive Baboons. Despite a 30-fold increase in the plasma phenylalanine to other neutral amino acids following administration of 1g/kg b.wt aspartame, there was no proconvulsant or anticonvulsant effect on any treated baboons.

A study by Van den Eeden \textit{et.al} (1994) assessed whether the consumption of aspartame is associated with headaches in a double-blind randomized crossover trial in subjects who self-reported sensitivity to aspartame. A significantly higher occurrence of self-reported headaches was reported following exposure to aspartame as compared to placebo. Hence it can be concluded that a small subset of the population is susceptible to aspartame-induced headaches.

\textbf{Carcinogenicity}

The role of aspartame on cancer risk has been widely debated over the last few decades. During this period Ishii \textit{et.al} (1981) conducted an aspartame carcinogenicity study in Wistar rats. No increase in the incidence of brain tumours was observed in the treated groups compared with the controls.

Soffritti \textit{et.al} (2005, 2006 and 2007) published three reports on carcinogenicity study in rats. In the first study (2005) they reported a
weight loss, reduced food consumption, and a greater incidence of lymphomas and leukaemias in the treated animals. In addition, it described a non statistically significant increase in brain tumours. Food consumption decreased significantly with increasing concentrations of aspartame. This study concludes that the increase in lymphomas and leukaemia’s could be related to methanol, a metabolite of aspartame.

In the second study Soffritti et al. (2006) conducted a mega experiment in rats from 8 weeks of age until natural death, demonstrated for the first time that aspartame is a multipotential carcinogenic agent capable of inducing an increased incidence of malignant tumour in males and females. This study figured out a dose-related increase in lymphomas/leukaemias, transitional cell carcinomas of the renal pelvis and ureter (dysplasias) in females and an increased incidence of malignant schwannomas of peripheral nerves in males. In a later study Soffritti et al. (2007) demonstrated that lifespan exposure to aspartame which begins during fetal life increases carcinogenicity. This study has demonstrated a significant increase in the incidence of lymphomas and leukaemias in males and females, and an increase in the incidence of mammary cancer in females.

In 2005, a carcinogenicity study on aspartame performed by the U.S. National Toxicology Program showed that genetically altered strains of mice, develop lymphomas or sarcomas, squamous cell papillomas/carcinomas of the fore stomach and brain tumours, with increased susceptibility and decreased latency (National Toxicology Program, 2005).
Genotoxicity

The genotoxic potential of aspartame has been extensively evaluated in microbial, cell culture and animal models. In the Ames/Salmonella typhimurium reversion assay, aspartame was found to be nonmutagenic at concentrations up to 5000 µg/plate in tester strains (Molinary, 1978). But Sheppard et al (1991, 1993) reported that aspartame was mutagenic in S. typhimurium strains following its nitrosation. Rencuzogullari et al (2004) reported a dose dependent genotoxicity in S. typhimurium strain TA98. Aspartame significantly induced chromosome aberrations, micronucleus formation and a decrease in mitotic index at all concentrations used in the study. Gebara et al (2003) reported that aspartame induced somatic segregation in diploid cells of Aspergillus nidulans, although this effect was not concentration-dependent. Durnev et al (1995) evaluated the potential clastogenic activity of aspartame by gavage to mice, observed no clastogenic activity. Karikas et al (1998) measured the direct molecular interaction of aspartame with DNA. A moderate DNA molecular interaction, expressed as almost 40% DNA peak exclusion was observed when aspartame was tested with DNA (0.05 mg/mL) at a final concentration of 0.25 mg/mL. Analogous effect was exhibited by phenylalanine (31.6%) at a final concentration of 0.25 mg/mL, whereas a 65.5% DNA peak exclusion was observed when aspartame reached the final concentration of 0.50 mg/mL. In conclusion these in vitro findings are of interest because aspartame along with its metabolites gave a measurable molecular interaction with DNA.
Sasaki et al. (2002) assessed the genotoxicity of aspartame along with 39 food additives using the comet assay of tissues including stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow from male ddY mice. This study found no changes in the comet lengths of nuclear DNA from tissues from aspartame-treated mice. Supporting this study Mukhopadhyay et al. (2000) stated that the combination of aspartame and acesulfame-K was not genotoxic in vivo. Gombos et al. (2007) investigated the effect of aspartame on gene expression alterations relating to oncogenes and a suppressor gene expression in different organs. Results showed considerable differences in the key oncogene and tumour suppressor gene expressions between treated and control groups. A considerable increase was detected in the expressions of h-ras and c-myc oncogenes and p53 tumour suppressor gene in all treated groups in the lymphoreticular, bone-marrow and kidney tissues. AlSuhaibani (2010) has investigated the genotoxicity of aspartame and its metabolic breakdown products (phenylalanine, aspartic acid and methanol) on bone marrow cells of mice. Treatment with aspartame dose dependently induced chromosome aberrations even at a concentration of 35 mg/kg b.wt and above. Elfatah et al. (2012) observed that administration of aspartame to mother rats and offsprings found a significant increase in chromosomal aberrations in liver and bone marrow cells and significant reduction in body weight.

**Hepatotoxicity**

Tutelyan et al. (1990) reported that aspartame at high dose did not substantially affect hepatic microsomal enzyme function. Later Hertelendy et al. (1993) conducted a randomized, placebo controlled
crossover study in individuals with chronic, alcoholic liver disease. Aspartame resulted in a significantly greater increase in plasma phenylalanine concentrations compared to skim (no-fat) milk and placebo. Recently, Iman (2011) investigated the effect of aspartame on lipid peroxidation and antioxidant status in liver and kidney of male albino rats for six weeks. This study found a significant increase in LPO levels in the liver tissue after 4 and 6 weeks of aspartame administration while there was a significant decrease in LPO level after 2 weeks. SOD activity significantly decreased in the liver tissue after 2, 4 and 6 weeks of treatment. Catalase activity significantly decreased in the liver tissue after 2 and 4 weeks of aspartame administration. GSH content was significantly decreased in the liver tissue after 2, 4 and 6 weeks which were accompanied by a significant increase in GST activity after 4 and 6 weeks of aspartame administration.

**Metabolic and biochemical toxicity**

Many studies have been done to evaluate the safety of aspartame on diabetes population. In a randomized, double-blind, placebo-controlled study, Nehrling et.al (1985) compared the effect of aspartame consumption with that of a placebo on glycemic control in Type 1 and Type 2 diabetes subjects. They concluded that daily consumption of aspartame did not affect fasting plasma glucose (FPG), 2-hour postprandial blood glucose or glycated hemoglobin levels. Okuno et.al (1986) studied the effect of 100 g of glucose or 500 mg of aspartame on blood glucose and insulin concentrations in normal subjects and Type 2 diabetics. After the oral glucose load, there were the expected increases in blood glucose and serum
insulin concentrations in proportion to the severity of fasting hyperglycemia. However in all groups, the ingestion of 500 mg of aspartame was associated with a decline in glycemia which was likely due to fasting, which occurred without observable changes in serum insulin or plasma glucagon concentrations. With ongoing interest in nutritional guidelines for diabetics, Colaguirí et al. (1989) undertook a double-blind, crossover study in subjects with type 2 diabetes for 6 weeks. The study compared the effect on several metabolic parameters by adding sucrose or an equivalent sweetening amount of aspartame to the usual meals, which were high in complex carbohydrates and fibre. Neither aspartame nor sucrose had statistically or clinically significant effects on glycemic control, lipids, glucose tolerance or insulin action in these individuals. Horwitz et al. (1988) compared the changes in plasma glucose, insulin and glucagon in normal and diabetic individuals. No differences were observed in peak plasma insulin, plasma glucose or glucagon levels. In 2007, Ferland et al. investigated the effect of aspartame on plasma glucose and insulin levels during acute exercise in men with type 2 diabetes. Contrary to all previous findings, the aspartame breakfast induced a similar rise in glucose and insulin levels at baseline as the sucrose meal. This study speculates that aspartame might have enhanced the cephalic phase of insulin secretion evoked by the recognition of the sweet taste, sight, smell, and expectation of food.

In addition, the effect of aspartame on plasma glucose and insulin concentrations has been evaluated in nondiabetic subjects in some studies. It has been suggested that sweetness may induce
cephalic-phase insulin release (CPIR), resulting in lowering of blood glucose concentrations with consequent increases in hunger (Rogers et al., 1988). In a randomized, double-blind study, Melchior et al. (1991) reported elevated plasma glucose concentrations after the consumption of chocolate drink sweetened with aspartame. Plasma insulin concentrations were also increased compared to fasting conditions. It is suggested that there is a possible cephalic insulin release effect caused by aspartame. In contrary to these findings Hartel et al. (1993) evaluated five different test solutions (containing aspartame, acesulfame-K, cyclamate, saccharin, or sucrose) and water in a multiple crossover study. The results showed glucose concentrations were within the normal range, and there were no significant differences in plasma insulin or blood glucose concentrations with the four high-intensity sweeteners compared to water. Supporting to this observation Abdallah et al. (1997) reported the results of a randomized, double-blind, placebo-controlled study to evaluate CPIR with oral exposure (sucking on the tongue) to sucrose, aspartame plus polydextrose or polydextrose as the placebo. Plasma glucose, insulin, and glucagon concentrations were not modified after aspartame or sucrose tablets, suggesting that sweet taste per se was not sufficient for eliciting CPIR.

Carlson and Shah (1989) evaluated the effect of aspartame and its constituent amino acids, aspartic acid and phenylalanine on serum concentrations of prolactin, cortisol, growth hormone and insulin and blood glucose concentrations in healthy individuals. Aspartame, aspartic acid, or phenylalanine (in equimolar amounts) did not change the magnitude and pattern of secretion of prolactin,
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cortisol, or growth hormone. The high dose of phenylalanine did modestly increase serum prolactin concentrations to a level expected after a high-protein mixed meal. There were some minor increases in serum glucose and decreases in serum insulin concentrations in all test groups, which were thought to be related to the postprandial alterations from breakfast eaten 3–5 h earlier. This study concludes that aspartame did not affect glycemic control. Wouassi et al. (1997) compared metabolic and hormonal responses during repeated, brief, intense exercise after administration of glucose or aspartame. Plasma lactate increased significantly after exercise, but no differences were observed between glucose and aspartame treatments. Plasma insulin concentrations decreased in both groups but exhibited a higher peak after glucose treatment than aspartame. Glucagon and epinephrine concentrations did not change significantly in either group. Recently Collison et al. (2012a) had examined the neonatal exposure to aspartame and monosodium glutamate (MSG) on glucose homeostasis. This study suggested that neonatal exposure to aspartame or a combination of MSG and aspartame, together with continued exposure to these additives can markedly influence glucose homeostasis in mice. Aspartame raised fasting blood glucose levels by 1.6-fold, whereas a combination of aspartame and MSG further raised fasting glucose to prediabetic levels in both genders. This study also observed a reduction in total cholesterol levels in both male and female mice following aspartame and MSG exposure, together with a lowering of triglyceride levels in female mice compared to control. Siegler et al. (2012) reported that despite a normal insulin response during the
ingestion period (at rest), a combination of aspartame and carbohydrate led to significantly lower serum insulin levels during exercise than when compared to carbohydrate alone.

Nguyen et al. (1998) evaluated the effect of single doses of aspartame and glucose on calcium and oxalate metabolism in a crossover study in healthy adults. They reported that urinary calcium excretion increased after both aspartame and glucose administration, which they related to increased calcemia and decreased phosphatemia. Aspartame did not alter plasma concentrations of glucose or insulin or urinary oxalate excretion. However, these findings from a single-dose study are contrary to those observed by Leon et al. (1989), who reported no differences between blood calcium concentrations or urinary calcium excretion after very high doses of aspartame compared to placebo or baseline. Beck et al. (2002) investigated the relationships between Neuropeptide Y (NPY) and aspartame ingestion and the consequences of the latter on food intake, body weight and composition. NPY concentrations were decreased in the hypothalamus particularly in arcuate nucleus of aspartame treated rats. The lower body weight and the change in body composition in aspartame rats could be related to the decreased effects of NPY on lipid and energy metabolism. Rajasekar et al. (2004) examined the rhythms of blood glucose, plasma cholesterol, and protein and serum aspartate transaminase (AST) in experimental rats. Results showed delays in glucose and total protein and advances in AST rhythms. Aspartate levels in brain tissues were increased significantly in aspartame treated animals when compared to
controls. It concludes that oral administration of aspartame might lead to increased levels of aspartate in the brain which could alter the characteristics of biochemical variables possibly by modulating the transmission in several areas/nuclei in the brain including retinohypothalamic tract (RHT) and suprachiasmatic nuclei (SCN). A recent study by Kim et al. (2011) investigated the short-term physiologic effect of aspartame and saccharin in lipid metabolism and inflammation in the presence or absence of a high cholesterol diet (HCD) in zebra fish. The aspartame group showed an increase in glucose and triglyceride level and decrease in total cholesterol. When fed with a HCD, aspartame-fed zebra fish showed acute and severe swimming defects with an increase in brain inflammation. In the absence of cholesterol, the aspartame group had an increase in the inflammatory response, which was correlated with increased infiltration of inflammatory cells and production of ROS in the liver and brain.

It has been hypothesized that if consumers replaced sugar in their diet with intense sweeteners, the proportion of calories derived from carbohydrates would be reduced. The majority of studies aimed at determining the influence of intense sweetener consumption on appetite, hunger and food intake have been conducted using aspartame and are of short-term duration. Blundell and Hill (1986) suggested that aspartame may stimulate appetite, speculating that the use of aspartame by certain individuals may lead to a loss of control over appetite and contribute to disordered patterns of eating. In another study Rogers et al. (1990) reported that aspartame ingested without tasting (in capsule form) inhibited hunger and food
intake. They speculated that this was due to a postingestive inhibitory effect of aspartame on appetite. Tordoff and Alleva (1990) investigated the effect of adding aspartame in a blinded manner to a normal diet in free-living normal weight subjects. There was a significant reduction in caloric intake in both females and males and decreased body weight of males during the period when aspartame-sweetened soda was consumed. Many other studies, using various research paradigms, have been done to evaluate the effect of aspartame on hunger/appetite and food intake. Replacing sucrose with aspartame in foods or beverages has not been shown to increase food intake or hunger in children (Anderson et al., 1989; Birch et al., 1989) food intake in normal (Rolls et al., 1990; Canty and Chan, 1991) or overweight men and women (Rodin, 1990; Drewnowski et al., 1994). Blackburn et al. (1997) undertook a randomized, controlled trial in obese women to investigate the effect of consuming or abstaining from aspartame-sweetened foods and beverages during a 16-week weight-loss program and a 2-year follow-up maintenance program. After 16 weeks, all subjects had lost 10% of their initial body weight, but more weight was lost in the aspartame group.

Reproductive toxicity

The effect of aspartame on reproduction was evaluated by many studies. Lennon et al. (1980) conducted a study in female Charles River rats to assess the effect of adding aspartame to the diet on lactation. Food consumption and body weights were significantly lower in rats fed with 7.5 and 14% aspartame diets throughout the experiment. Pup body weight was also significantly
reduced in these diet groups. Pup survival was significantly reduced in the highest dose group. Leme and Azoubel (2006) described morphometric alterations in the rats treated with aspartame, during the gestational period. The mean initial and final body weights of rats treated with aspartame was significantly reduced in relation to those of rats from the control group during the gestation period. Portela et.al (2007) evaluated possible alterations in maternal-fetal body weight, placental weight, length of the umbilical cord and in nuclei of fetal hepatocytes by means of kariometry after administration of aspartame at room temperature or heated to 40º C. There were reductions in mean placental and maternal-fetal weights, umbilical-cord length, and majority of kariometric parameters of the hepatocytes.