Safety of Long-term Large Doses of Aspartame

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• **Safety of long-term administration of 75 mg/kg of aspartame per day was evaluated with the use of a randomized, double-blind, placebo-controlled, parallel-group design in 108 male and female volunteers aged 18 to 62 years. Subjects received either aspartame or placebo in capsule form three times daily for 24 weeks. No persistent changes over time were noted in either group in vital signs; body weight; results of standard laboratory tests; fasting blood levels of aspartame's constituent amino acids (aspartic acid and phenylalanine), other amino acids, and methanol; or blood formate levels and 24-hour urinary excretion of formate. There also were no statistically significant differences between groups in the number of subjects experiencing symptoms or in the number of symptoms per subject. These results further document the safety of the long-term consumption of aspartame at doses equivalent to the amount of aspartame in approximately 10 L of beverage per day.**

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The nutritive sweetener aspartame (NutraSweet, The NutraSweet Co, Deerfield, Ill) was approved by the Food and Drug Administration (FDA) for use as a tabletop sweetener and for inclusion in dry foods in 1981[1] and subsequently for use in carbonated soft drinks in 1983.[2] Aspartame is the methyl ester of the dipeptide L-aspartyl-L-phenylalanine (Fig 1). During the absorption process it is rapidly and completely metabolized to its constituent amino acids (aspartic acid and phenylalanine) and methanol, which enter the portal circulation.[3] Liberated methanol is metabolized in the liver to formate and carbon dioxide.

Safety concerns raised about this food additive both before and since FDA approval include the possibility that increases in blood levels of phenylalanine might produce alterations of brain neurotransmitter activity, resulting in functional or behavioral changes.[2,4] Another concern has been for possible adverse health effects of methanol and formate. Formate is of special concern since it appears to be primarily responsible for the metabolic acidosis and ocular toxic effects associated with methanol intoxication.[5,6]

On the basis of a review of evidence from more than 100 studies in animals and humans, the American Medical Association's Council on Scientific Affairs in 1985 agreed with the FDA and other regulatory agencies around the world that "consumption of aspartame by normal humans is safe and is not associated with serious adverse health effects."[7] The purpose of this controlled study was to assess further, in healthy adults, the safety of long-term doses of aspartame (75 mg/kg daily) that exceed the FDA's current acceptable daily intake (50 mg/kg)[8] and that constitute approximately 30 times the average amount of aspartame currently consumed on a daily basis by the general population across all age groups combined.[9]

The present study, preliminary results of which were published in abstract form,[10] was designed to scrutinize clinical evaluations and a comprehensive battery of biochemical factors that could potentially be affected by changes in the blood concentrations of aspartame's components, including plasma concentrations of component amino acids, blood concentrations of methanol and formate, and urinary concentration of formate. Previous long-term studies of aspartame ingestion carried out in healthy children and adolescents[11]; obese adults and children[12]; adults carrying the heterozygous trait for phenylketonuria[13]; and people with diabetes[14,15] failed to show any significant clinical or biochemical changes related to aspartame, including many of the same factors measured in this study. The possible effects of long-term aspartame administration on blood lipid levels has not been previously assessed.

SUBJECTS AND METHODS

Study Population

Subjects consisted of 108 healthy, paid volunteers, aged 18 years and over, primarily recruited from among University of Minnesota (Minneapolis) students, faculty, and staff. Written informed consent was obtained from each participant. Exclusion criteria included any of the following: phenylketonuria; any chronic disease detected by history, physical examination, or routine laboratory tests; body weight more than 20% outside of the normal range for height based on the 1983 Metropolitan Life Insurance tables; and long-term use of any medication (including vitamin supplements) except for oral contraceptives or replacement estrogen. All women included in the study were required at entry to be postmenopausal, to have been surgically sterilized, to be taking oral contraceptives, or to have had an intrauterine device in place for at least 6 months to prevent the possibility of pregnancy. Subjects agreed not to make any major changes in usual eating, alcohol consumption, smoking, or exercise habits during the course of the study and not to consume foods or beverages containing aspartame.

Study Design

The study protocol as approved by the University of Minnesota's institutional review board for human research consisted of a randomized, double-blind, placebo-controlled, parallel-group design. Subjects were assigned by a computer-generated random schedule to receive either aspartame or placebo. Subjects in both groups were administered identical-appearing opaque white capsules in three divided doses each day along with meals over a 6-month period of observation.

Each aspartame capsule contained 300 mg of the sweetener. A sample of the aspartame lot used was analyzed and found to be 98% pure aspartame (α-aspartylphenylalanine methyl ester). Other components included 0.17% α-aspartylphenylalanine, 0.56% aspartylphenylalanine diketopiperazine, 0.32% β-aspartylphenylalanine methyl ester, and small quantities of other conversion products, such as β-aspartylphenylalanine and phenylalanine methyl ester. The total daily dose administered to each subject receiving the active preparation was approximately 75 mg/kg. This dose is 1.5 times the FDA's current acceptable daily intake (50 mg/kg). Placebo capsules con-

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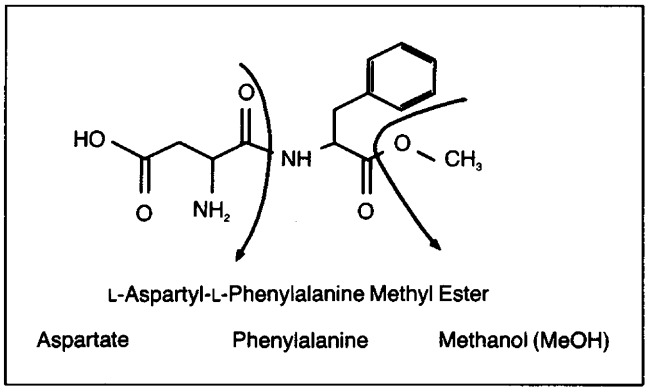


Fig 1.—Chemical structure and metabolism of aspartame.

Table 1.—Time Schedule for Laboratory Tests

Test	Time, wk: Baseline	3	6	9	12	15	18	21	24
Clinical battery	X	X	X	X	X		X		X
Plasma lipids	X	X	X	X	X		X		X
Plasma amino acids	X	X	X		X		X		X
Blood methyl alcohol	X		X		X		X		X
Serum folate	X								X
Blood formate	X		X		X		X		X
24-h urine formate, creatinine, calcium	X		X		X				X

tained 300 mg of microcrystalline cellulose and 0.9 mg of silicon dioxide.

To monitor subject compliance, unused capsules were returned and capsule counts were done at each 3-week visit. Subjects were given a 28-day supply of capsules at each visit. Except for three subjects who stopped taking capsules for 1, 6, and 19 days, there were no noteworthy breaches of compliance. The missing treatment days for these three subjects composed only a small part of the total treatment period. Thus, their data were not compromised.

Subjects were studied in a clinic in the University of Minnesota's Health Sciences Complex every 3 weeks (±1 week) for 24 weeks. A medical history and physical examination were performed at baseline (within 1 week of study initiation) and at the last clinic visit. Body weight (measured with the subjects lightly clothed but with coats and shoes off) and vital signs (respiratory rate, sitting radial pulse rate, and sitting and standing blood pressure determinations) were performed at each visit. Blood pressure was measured in the right arm with a random-zero muddler mercury sphygmomanometer (Hawsley and Sons Ltd, Hawsley, England) and appropriate-sized cuff in the sitting position after at least 5 minutes of rest and again after 2 minutes of standing. Disappearance of the Korotkoff sounds was used for the diastolic readings. The time of day of the clinic visits was kept relatively constant for each subject. Subjects also were asked at each visit about any unusual symptoms since the previous visit. Elicited symptoms were classified on the basis of the World Health Organization terminology.[16]

Laboratory Studies

Clinical Laboratory Test Battery.—Laboratory studies were performed according to the schedule shown in Table 1. These studies were intended to be comprehensive, with special emphasis on the products of aspartame metabolism, ie, aspartic acid, phenylalanine, and methanol. A battery of clinical laboratory tests (performed by Lufkin Medical Laboratories, Minneapolis, a division of Smith Kline-Bio-Science Laboratories), performed at baseline and repeated six

Table 2.—Range of Normal Laboratory Values

Substance	Low	High
Hematology		
Hemoglobin, g/L		
Male	140	160
Female	125	150
Hematocrit		
Male	0.42	0.52
Female	0.37	0.47
Red blood cells, $\times 10^{12}$/L		
Male	4.50	5.50
Female	4.00	5.00
White blood cells, $\times 10^{9}$/L		
Male	4.5	11.0
Female	4.5	10.0
Neutrophils	0.36	0.66
Bands	0	0.08
Lymphocytes	0	0.40
Monocytes	0	0.04
Eosinophils	0	0.05
Basophils	0	0.02
Clinical Chemistry		
Calcium, mmol/L		
Male	2.22	2.69
Female	2.17	2.67
Phosphorus, mmol/L	0.71	1.49
Creatinine, μmol/L	44	177
Uric acid, μmol/L		
Male	178	541
Female	149	482
Total protein, g/L	58	76
Albumin, g/L	36	53
Total bilirubin, μmol/L		
Male	0	27
Female	0	24
Alkaline phosphatase, U/L	0	250
Lactate dehydrogenase, U/L	0	221
Aspartate aminotransferase, U/L	0	55
Alanine aminotransferase, U/L	0	36
Sodium, mmol/L	135	149
Potassium, mmol/L	3.5	5.5
Carbon dioxide, mmol/L	22	34
Chloride, mmol/L	95	112
Glucose, mmol/L	3.9	6.7
Serum urea nitrogen, mmol/L	2.9	11.8
Creatinine phosphokinase, U/L		
Male	0	301
Female	0	236

times during the study, included urinalysis; a complete blood cell count, including a platelet count; and an 18-factor serum chemistry battery (glucose, serum urea nitrogen, creatinine, uric acid, total bilirubin, alkaline phosphatase, lactate dehydrogenase, aspartate and alanine aminotransferase, creatine phosphokinase, total protein, albumin, calcium (total and ionized), inorganic phosphorus, sodium, potassium, carbon dioxide, and chloride). The normal ranges of values for these tests are included in Table 2. For these blood tests, as well as those described below, venous blood was drawn from an antecubital vein after at least a 12-hour fast, except for water.

Plasma Lipid Analyses.—The same schedule as for the clinical laboratory testing was used for blood lipid assessments (Table 1). Blood collection and preparation procedure and laboratory quality control precautions have been described previously.[17] Lipid analyses

Table 3.—Subject Characteristics by Group

Group	n	Mean ± SEM		Race, No.		
		Age, y	Weight, kg	W	B	Other
Aspartame						
Male	23	31.3 ± 2.0	74.0 ± 1.5	21	1	1
Female	30	31.4 ± 1.8	61.3 ± 1.6	29	1	0
Total	53	31.4 ± 1.3	66.8 ± 1.4	50	2	1
Placebo						
Male	28	26.8 ± 1.3	75.4 ± 2.0	24	2	2
Female	27	32.4 ± 2.4	63.7 ± 1.9	27	0	0
Total	55	29.5 ± 1.4	69.6 ± 1.6	51	2	2

were performed in the University of Minnesota Lipid Research Clinic Core Laboratory. Plasma total cholesterol and triglyceride levels were determined by the Lipid Research Clinic's AutoAnalyzer II (Technicon, Tarrytown, NY) methods.[18] Plasma high-density lipoprotein cholesterol was measured by the AutoAnalyzer II method after precipitation of very-low-density lipoproteins and low-density lipoproteins by the Lipid Research Clinic's heparin and manganese chloride method.[18] Plasma very-low-density lipoprotein and low-density lipoprotein cholesterol were determined by the Lipid Research Clinic's standard β-quantification procedure involving ultracentifugation.[18]

Metabolic Studies.—*Plasma Amino Acids.*—Heparinized blood for amino acid assays was obtained in the fasting state at least 12 hours after the last dose of aspartame. Plasma was immediately separated from cellular elements by low-speed centrifugation and stored at −20°C. Frozen plasma specimens were packed in dry ice and shipped to the analytical laboratory at the University of Texas Medical Branch at Galveston (D.K.R.) for analyses. Proteins were precipitated with 5% sulfosalicylic acid in lithium citrate buffer (pH, 2.2; 0.2 mol/L) and removed by centrifugation (17 000*g* for 15 minutes). The resulting supernatant then was passed through a 0.2-μm syringe filter, and the filtrate was stored frozen at −20°C until analyzed. The concentrations of 21 amino acids (excluding tryptophan) were measured by means of an amino acid analyzer (Beckman 6300; Beckman Instruments, Palo Alto, Calif) with an on-line computing integrator (SP4200; Spectra-Physics, San Jose, Calif). Plasma total tryptophan level was determined by the fluorometric technique of Denckla and Dewey[19] as modified by Bloxam and Warren.[20] The ratios of plasma phenylalanine to the sum of the plasma concentrations of other large neutral amino acids (LNAAs) sharing the blood-brain transport site were calculated as described by Fernstrom and Wurtman.[21] The LNAAs included in the ratio were isoleucine, leucine, phenylalanine, tryptophan, tyrosine, methionine, and valine. In addition, ratios of the concentrations of each of the other LNAAs to the sum of the remaining six were calculated.

Blood Methanol and Formate.—Blood for folate determinations was allowed to clot for 30 minutes at room temperature, and serum was separated by centrifugation (1000 g) and stored at −20°C. Serum samples were shipped in dry ice to Searle Laboratories (Skokie, Ill), where folate level was determined by radioassay (Bio-Rad Quanta-phase; Richmond, Calif). Blood methanol and formate levels were determined by one of us (T.R.T.) on frozen specimens shipped to his laboratory at the University of Iowa, Iowa City. Blood methanol level was determined by the gas chromatographic method of Baker et al.[22] Blood formate was assayed by the method of Makar and Tephly[23]; this quantitative spectrophotofluorometric measurement involves the use of bacterial formate dehydrogenase coupled with resazurin to form a fluorophore.

Urinary Studies.—Twenty-four–hour urine specimens were maintained by participants in containers of ice during collection. A 5-mL aliquot of each specimen was transferred to a plastic screw-top tube, frozen immediately, stored at −20°C, and shipped on dry ice to one of us (T.R.T.) for assay of formate.[23] A 20-mL aliquot of each collection was analyzed locally for 24-hour creatinine[24] and calcium excretion by the Technicon RA 1000 method.

Data Analyses

Argus Computing Inc (Shawnee Mission, Kan) was responsible for data entry. The double-blind condition was maintained by the computing company, the investigators, and the study sponsor until the data set was entered and finalized. Only at that time was the sealed code opened and entered into the database. Data analyses were performed by Biometric Research Institute Inc (Arlington, Va).

Safety variables possessing distributions consistent with parametric analysis were compared by means of Student's *t* tests or repeated-measures analysis of variance to assess treatment group homogeneity and group differences at baseline and over time.

For nominal safety variables not meeting the assumptions for parametric testing analysis, the χ^2 test or Fisher's Exact Test[25] was used to assess treatment group differences in changes over time from baseline levels. All statistical testing was done at the two-tailed, $\alpha \leq .05$ level of significance.

The coded adverse experiences fell into 72 terms in 14 World Health Organization (Geneva, Switzerland) organ-systems categories. When sufficient data were present to warrant statistical analysis, the incidences for each term were compared with the use of the χ^2 test or Fisher's Exact Test.[25] In addition, the Mann-Whitney *U* test[26] was used to detect differences in number of reports per patient of an effect, and the Kolmogorov-Smirnov test[27] was used to test for differences in timing of a particular effect.

This study design provides sensitivity to detect the following changes in the safety parameters with a statistical power of 80%[26]: 0.9 kg for body weight, 6 mm Hg for diastolic blood pressure, 0.015 for hematocrit, 4 U/L for aspartate aminotransferase, 1.8 mmol/L for serum sodium, 0.3 mmol/L for serum cholesterol, 3.6 μmol/L for serum creatinine, 5.9 nmol/L for serum folate, 0.13 mmol/L for blood formate, 0.17 mmol/L for urine formate, 5 μmol/L for plasma phenylalanine, and 0.012 for plasma phenylalanine/LNAA ratio.

RESULTS

Fifty-three of the 108 subjects enrolled in the study were randomly assigned to the aspartame group and 55 to the placebo group. Subjects' characteristics at baseline by group assignment are shown in Table 3. About 94% of these subjects were white. The subjects' ages ranged from 18 to 62 years, with a mean of 31.4 ± 1.3 (SEM) years for the aspartame group and 29.5 ± 1.4 years for the placebo group. Fifty-seven of the subjects were women and 51 were men, with a slight plurality of women in the aspartame group and of men in the placebo group. Mean heights for the two groups were similar (171 cm), while the mean body weight for the placebo group was slightly higher than that for the aspartame group. Mean baseline sitting blood pressure level was 114.1 ± 1.6/70.7 ± 1.6 mm Hg for the aspartame group as compared with 116.6 ± 1.5/73.8 ± 1.3 mm Hg for the placebo group. During the course of the study there were no significant changes for either group in body weight (Fig 2), sitting or standing blood pressure levels, pulse rate, or respiratory rate.

Of the 108 original volunteers, 101 (94%) successfully completed the 24-week study. Four of the seven subjects prematurely terminated from the study were in the placebo group. Reasons for the premature termination of these four subjects were as follows: having to move from the area (two subjects), difficulty in swallowing capsules (one subject), and an episode of severe gastroenteritis after a death in the family (one subject). Of the three subjects in the aspartame group who were prematurely terminated from the study, two were dropped within the first few weeks because of difficulty in

swallowing the capsules. The third, a 37-year-old white man with a history of bronchial asthma, was dropped from the study at week 6 because of repeated multiple symptoms lasting for hours after each capsule ingestion, including throbbing headaches, nausea, and malaise. There were no associated laboratory abnormalities. After withdrawal from the study, the subject reported occasional consumption of aspartame-sweetened food and beverages without experiencing any side effects. He also continued to experience about 10 to 12 severe, prolonged headaches per year unrelated to aspartame ingestion.

After completion of the study and uncoding of the double-blind condition, this subject agreed to participate in a rechallenge with aspartame and placebo capsules, with the use of a random-order, double-blind, placebo-controlled, multiple-dose design. During a 9-day period, the subject received 25-mg/kg doses of aspartame or placebo in capsules twice daily in the clinic with standardized meals at breakfast and lunch. A total of 17 doses, 8 placebo and 9 aspartame, were ingested. The total daily aspartame intake (50 mg/kg) was equivalent to that contained in more than 6 L of an aspartame-sweetened beverage.

No complaints were reported after 7 placebo doses and 6 aspartame doses. Stomach gas accompanied by belching was reported after 1 placebo dose and 2 aspartame doses. Headaches were reported after 2 consecutive aspartame doses during a 2-day period. The initial headache began 5½ hours after the noon dose of aspartame and 3 hours after the patient underwent dental work. The following morning's dose was omitted because the headache was still present. The headache returned shortly after the noon dose on that day, and in retrospect this may have reflected the diminishing analgesic effect of ibuprofen taken earlier that day in response to the initial headache. Subsequent rechallenges with aspartame and placebo, still under blinded conditions, did not yield any further complaints.

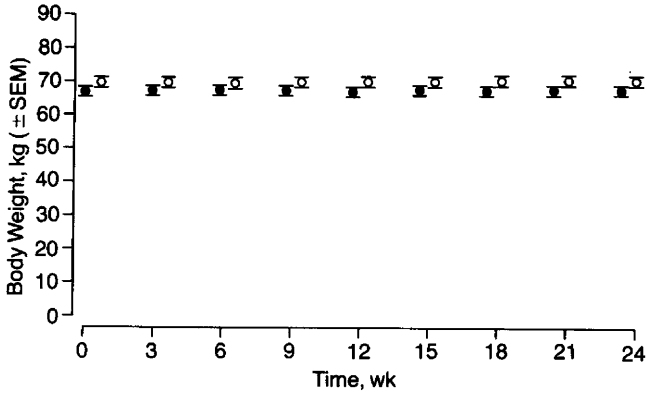


Fig 2.—Changes in body weight during the course of the study for aspartame (closed symbols) and placebo (open symbols) groups.

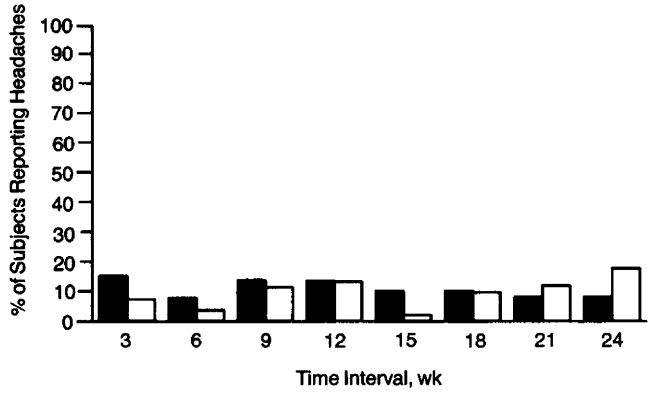
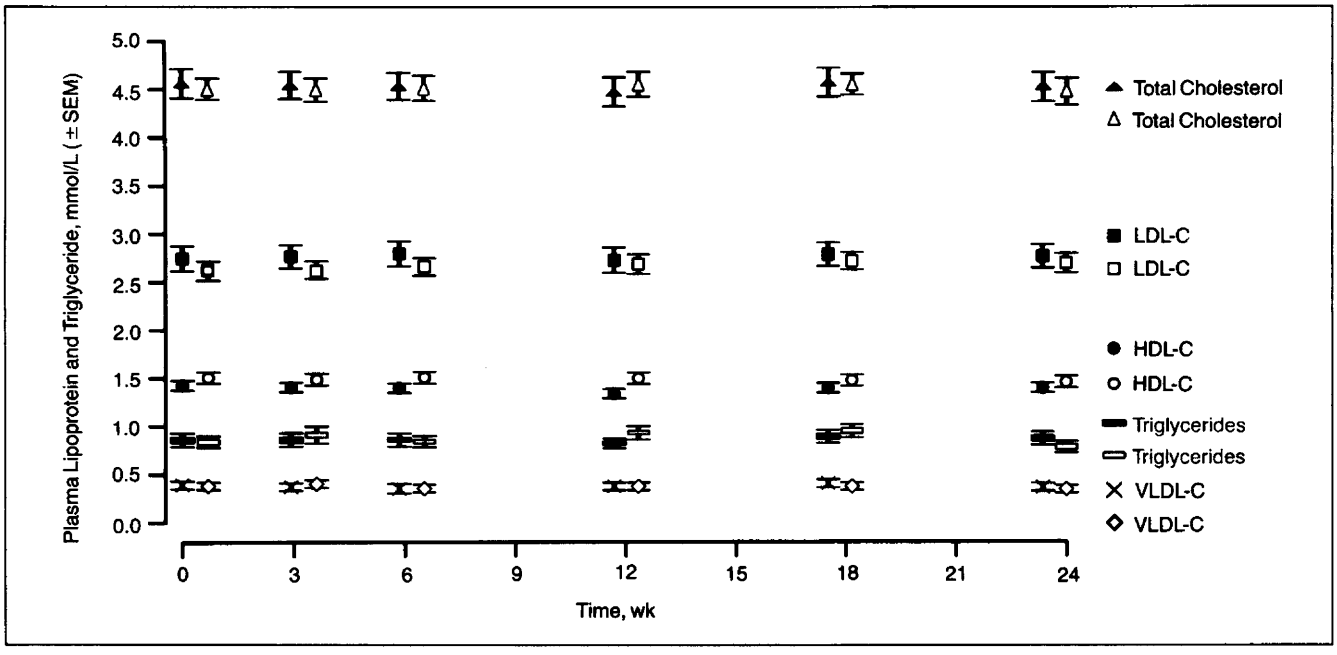


Fig 3.—Comparison of the incidence rate of headaches between the aspartame (solid bars) and placebo (open bars) groups.

Fig 4.—Changes in mean levels of plasma lipid and lipoprotein during the 24-week study for the aspartame (closed symbols) and placebo (open symbols) groups. LDL-C indicates low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and VLDL-C, very-low-density lipoprotein cholesterol.



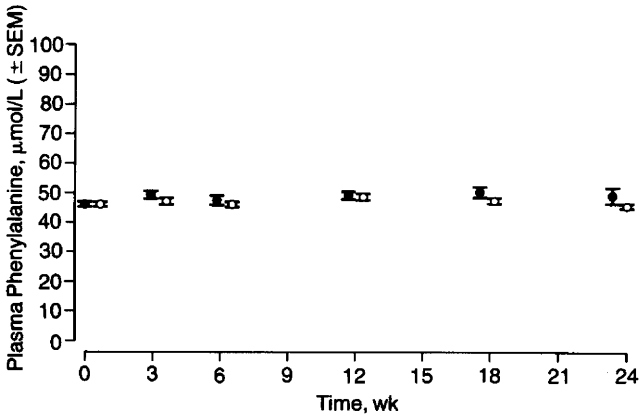


Fig 5.—Mean plasma phenylalanine concentrations over time in the aspartame (closed symbols) and placebo (open symbols) groups.

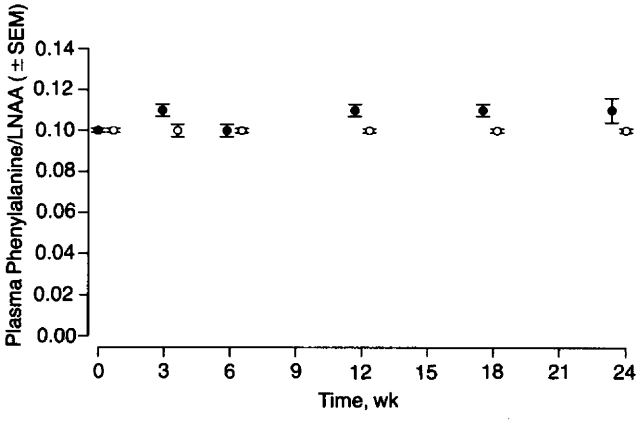


Fig 6.—Mean ratio of phenylalanine to the other large neutral amino acids (LNAAs) during the course of the study in the aspartame (closed symbols) and placebo (open symbols) groups.

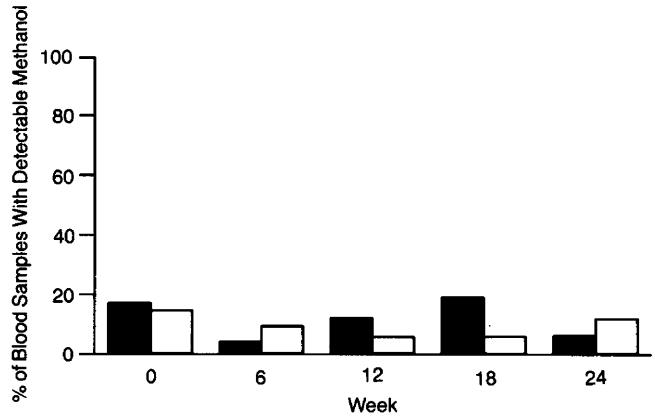


Fig 7.—Percentage of blood samples containing detectable concentrations of methanol during the course of the study for the aspartame (solid bars) and placebo (open bars) groups.

Of the 108 original subjects, 90 (83%) reported 72 different complaints, grouped into 14 World Health Organization organ-system categories, during the course of this 6-month study. There were a total of 193 symptoms in the aspartame group and 130 in the placebo group. Most of the reported symptoms were mild or moderate. There were no visual complaints in either group or ocular findings on physical examination. The most common complaints in each group were headaches, upper respiratory tract symptoms, and abdominal pain. No consistent pattern of occurrence was noted in these or in any other symptoms, nor were there any statistically significant differences between groups in number of subjects experiencing these symptoms or in number of symptoms per subject.

There was no statistically significant difference in the incidence of headaches (the most commonly reported complaint) in the aspartame group compared with the placebo group. Figure 3 shows the frequency of headaches for each group during the course of the study. The pattern displayed here of sporadic fluctuations in incidence of headache in each group was similar to that seen with the other common symptoms.

Similarly, during the course of the study there were only sporadic abnormalities in each group in hematologic and clinical chemistry test results, with no persistent pattern of abnormalities noted in any subject. Furthermore, there were no significant differences between groups in the number of subjects experiencing any clinical laboratory abnormalities, nor any clinically significant mean changes from baseline in any of the laboratory tests at any time point. Abnormalities on routine urinalysis also were uncommon in both groups. There were no statistically significant differences between groups in any of the urinalysis measures. No significant changes occurred in levels of any of the plasma lipid and lipoprotein cholesterol measures during the course of the study (Fig 4).

Mean fasting plasma concentrations of aspartic acid and phenylalanine (the two amino acid constituents of aspartame) showed no statistically significant differences from baseline in either group over time (Fig 5). Furthermore, there were no significant differences in concentrations of these amino acids at any of the time points or in their maximum and minimum concentrations. Analysis of the other 20 amino acids also failed to reveal any consistent pattern of changes in either group. The only statistically significant changes in amino acid levels were slightly higher plasma tyrosine levels at weeks 3 and 24 for the aspartame than for the placebo group ($P<.05$); however, these observed values were well within the normal range for these amino acids. The ratio of phenylalanine to the other LNAAs was not altered in either group over the course of the study (Fig 6). Only leucine at weeks 3 and 6 and tyrosine at week 3 had LNAA ratios that were significantly different between the two groups ($P<.05$).

Most of the blood methanol concentrations were below the detectable level of the method used (0.31 mmol/L).[22] Therefore, the numbers of subjects with detectable levels at each time period were compared for the aspartame and control groups. Figure 7 gives the number of subjects with detectable methanol levels at each time period measured. The numbers were similar in each group. The highest individual blood methanol values found were 1.0 mmol/L for one person in the aspartame group and 0.84 mmol/L for one person in the control group; both values were well below toxic levels.

Folate, involved in the metabolism of one-carbon fragments, was measured in the serum at baseline and week 24, and the level remained unchanged in both groups. Similarly, no statistically significant difference between groups was found in mean serum folate levels or in changes from baseline levels over time.

Twenty-four–hour urine specimens were used to determine whether one-carbon units derived from aspartame would ac-

Table 4.—Urinary Formate Levels (Mean ± SEM) in Individuals Receiving Aspartame or Placebo*

	Group	
	Aspartame	Placebo
Control†		
Formate	39.2 ± 4.1	51.4 ± 9.8
n	53	53
Week 6		
Formate	50.3 ± 3.7	42.8 ± 4.1
n	49	53
Week 12		
Formate	46.5 ± 7.0	34.1 ± 2.9
n	49	53
Week 24		
Formate	47.1 ± 3.5	41.9 ± 3.4
n	50	49

*Values represent the amount of formate divided by the amount of creatinine present in a 24-hour urine sample. They are expressed as micromoles of formate per millimole of creatinine. Aspartame was given in a daily dose of 75 mg/kg. Values of n differ during the different time periods because not all subjects made all the scheduled visits.

†Control values represent the ratio of formate to creatinine obtained before administration of aspartame or placebo.

cumulate in the urine as increased amounts of excreted urinary formate during long-term aspartame administration. No significant differences between groups were observed in either mean 24-hour concentrations of urinary formate or in the urinary formate to creatinine ratio (Table 4), indicating no significant increase in formate formation during long-term aspartame use. A relatively large variability in formate excretion was found in both groups as expressed by the SEM. In addition, no significant changes were noted in mean 24-hour urinary calcium excretion or in the urinary calcium to creatinine ratio in either group over the course of the study.

COMMENT

In this double-blind, placebo-controlled study, aspartame was administered in capsules to conceal its sweetness in three divided daily doses of 25 mg/kg each. For a 70-kg person, the daily aspartame intake in this study was equivalent to consumption of approximately 10 L/d of an aspartame-sweetened carbonated beverage. This level of consumption exceeds by about 30-fold the estimated mean daily intake of Americans of all ages.[9]

The possibility that elevated concentrations of the individual amino acid constituents of aspartame may have toxic effects on the central nervous system led previous investigators to study the effects of short-term, large doses of aspartame on concentrations of phenylalanine and aspartic acid. Stegink et al[28] demonstrated that even with acute bolus doses of aspartame as high as 200 mg/kg, increases in plasma aspartic acid and phenylalanine concentrations remain well below those associated with toxic effects. An objective of this study was to assess the effects of long-term aspartame ingestion on the baseline or "trough" plasma levels of phenylalanine and aspartic acid. Therefore, blood samples were drawn just before morning doses of aspartame (at least 12 hours after the last consumption of capsules containing either aspartame or placebo), as this time period would reflect any accumulation or alteration in baseline levels of these amino acids. There also was interest in the phenylalanine/LNAA ratios, since an increase in this ratio was associated with increased brain phenylalanine uptake in rats,[29] which was accompanied in some, but not all, studies with alterations in the concentration of brain neurotransmitter substances derived from phenylalanine and tyrosine.[29-31] However, we found with long-term aspartame consumption no evidence of alterations in phenylalanine, aspartic acid, or other amino acid concentrations nor of any consistent alterations in the ratio of each individual LNAA to the sum of the other six LNAAs.

We also were concerned about whether long-term ingestion of high doses of aspartame leads to accumulation in body fluids of methanol released during aspartame metabolism and whether its potentially toxic metabolic by-product, formate, accumulates due to inadequate metabolism. No significant increase was found in mean blood methanol concentrations at any time point. Only a small number of individuals in both groups had an occasional blood methanol level above the detectable limit at any time, and none were in the toxic range. Our results indicate that healthy individuals adequately metabolize methanol even when aspartame is ingested over a long term in large quantities.

The finding of this study of no significant increases in levels of formate in the blood or in 24-hour urine collections due to long-term aspartame administration is important. Theoretically, one might expect that long-term administration of aspartame would increase urinary formate levels through the conversion of one-carbon fragments to formate. The large variability in formate levels, observed both within and between individuals in both groups, has previously been reported in the absence of aspartame intake.[32] Individual variation in rate of endogenous protein and lipid demethylation as well as in dietary source of methyl groups is suspected as a contributing factor in this variability. However, more work is needed to clarify this issue.

Clinical assessments and a battery of clinical laboratory tests also failed to reveal any evidence of significant metabolic changes or organ toxic effects as a result of long-term administration of large doses of aspartame. Absence of any significant change in mean body weight of participants of either sex in the aspartame group is of special interest in light of controversy about the possible effects of aspartame on appetite and body weight. Blundell and Hill[33] reported that aspartame consumption may lead to a "loss of control of appetite," ie, a "paradoxical effect." Stellman and Garfinkel[34] also alleged that users of artificial sweeteners are more likely to gain weight than are nonusers. In contrast, other investigators reported that very large doses of aspartame had no effect on short-term energy intake or hunger compared with the results with placebo.[35] Long-term tolerance studies (up to 27 weeks in length) failed to find weight changes in subjects receiving aspartame, in agreement with our results.[11-15] Furthermore, Kanders et al[36] conducted a study in which obese individuals were monitored during a weight-loss program with and without aspartame. There was clinically significant weight loss in both groups, with slightly greater weight loss in women in the aspartame group. Taken together, these studies argue against a "paradoxical effect" of aspartame on appetite.

The absence of changes in pulse rate and blood pressure levels with long-term intake of aspartame supports the absence of increased catecholamine synthesis from phenylalanine and tyrosine with long-term aspartame use. Blood pressure levels during this study were of particular interest, since

a study in spontaneously hypertensive rats suggested a blood pressure–lowering effect with aspartame administered intraperitoneally.[37] However, this effect was not observed when the study was repeated with spontaneously hypertensive rats given aspartame intraperitoneally or orally.[38]

The high incidence of symptoms volunteered by study subjects in both groups and their varied nature reflects the relatively long duration of the study and perhaps the power of suggestibility. The absence of a systematic pattern of symptoms in the aspartame group is in agreement with a review of anecdotal complaints from the US Centers for Disease Control (Atlanta, Ga).[39] In that study, 231 consumer complaints allegedly associated with use of products containing aspartame were evaluated, and headaches, abdominal pain, and nausea were found to be among the most commonly reported symptoms. This is in agreement with the findings in both groups in our study. Upper respiratory tract symptoms, commonly reported by both groups in our study, were not mentioned in the report of the US Centers for Disease Control, since consumers evidently do not attribute coldlike symptoms to aspartame ingestion. In another relevant study, Schiffman et al[40] performed a double-blind crossover trial of challenges with short-term administration of 30 mg/kg of aspartame vs placebo via capsules in 40 people who had previously reported headaches after consuming products containing aspartame. The incidence rate of headaches after aspartame (35%) was not statistically significantly different from that after placebo (45%). The absence of group differences in complaints after aspartame ingestion in the Schiffman et al[40] study and in ours does not rule out the possibility of individual idiosyncratic responses to aspartame. This was initially thought to be a possible cause of the symptoms in the single subject prematurely discontinued from our aspartame group. However, the subject subsequently consumed aspartame-containing foods and beverages without side effects, and the symptom complex could not be reproduced in a subsequent double-blind rechallenge study.

On the basis of our findings and those of others previously cited, it is concluded that aspartame consumed daily at doses equivalent to those contained in approximately 10 L of aspartame-containing beverage is not associated with any significant changes in clinical measures or adverse experiences in healthy adults.

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