Plasma, Erythrocyte and Human Milk Levels of Free Amino Acids in Lactating Women Administered Aspartame or Lactose

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ABSTRACT Aspartame is a dipeptide (L-aspartyl-L-phenylalanyl methyl ester) with a sweetening potential of 180 to 200 times that of sucrose. Questions have been raised about the potential toxic effects of its constituent amino acids when the compound is ingested in large amounts by lactating women. Plasma, erythrocyte and milk levels of free amino acids were measured in six normal female subjects with established lactation after oral administration of either aspartame or lactose at 50 mg/kg body weight in a cross-over design. No significant change in plasma or erythrocyte aspartate levels was noted following aspartame or lactose administration. Plasma phenylalanine levels increased approximately fourfold over fasting values 45 minutes after aspartame loading (P < 0.001), and returned to baseline by 4 hours. Erythrocyte phenylalanine levels showed similar changes. No statistically significant difference in milk aspartate or phenylalanine levels between aspartame and lactose loading were noted following aspartame or lactose administration. Plasma phenylalanine levels increased approximately fourfold over fasting values 45 minutes after aspartame loading (P ≤ 0.001), and returned to baseline by 4 hours. Erythrocyte phenylalanine levels showed similar changes. No statistically significant difference in milk aspartate or phenylalanine levels between aspartame and lactose loading were noted following aspartame or lactose administration. Plasma phenylalanine levels increased approximately fourfold over fasting values 45 minutes after aspartame loading (P ≤ 0.001), and returned to baseline by 4 hours. Erythrocyte phenylalanine levels showed similar changes. No statistically significant difference in milk aspartate or phenylalanine levels between aspartame and lactose loading were noted following aspartame or lactose administration. Plasma phenylalanine levels increased approximately fourfold over fasting values 45 minutes after aspartame loading (P ≤ 0.001), and returned to baseline by 4 hours. Erythrocyte phenylalanine levels showed similar changes. No statistically significant difference in milk aspartate or phenylalanine levels between aspartame and lactose loading were noted following aspartame or lactose administration. Plasma phenylalanine levels increased approximately fourfold over fasting values 45 minutes after aspartame loading (P ≤ 0.001), and returned to baseline by 4 hours. Erythrocyte phenylalanine levels showed similar changes. No statistically significant difference in milk aspartate or phenylalanine levels between aspartame and lactose loading were noted following aspartame or lactose administration. Plasma phenylalanine levels increased approximately fourfold over fasting values 45 minutes after aspartame loading (P ≤ 0.001), and returned to baseline by 4 hours. Erythrocyte phenylalanine levels showed similar changes. No statistically significant difference in milk aspartate or phenylalanine levels between aspartame and lactose loading were noted following aspartame or lactose administration. Plasma phenylalanine levels increased approximately fourfold over fasting values 45 minutes after aspartame loading (P ≤ 0.001), and returned to baseline by 4 hours. Erythrocyte phenylalanine levels showed similar changes. No statistically significant difference in milk aspartate or phenylalanine levels between aspartame and lactose loading were noted following aspartame or lactose administration.

INDEXING KEY WORDS aspartame • phenylalanine • aspartate • lactation

In the United States, approximately 50% of women breast feed their newborn infants at the time of hospital discharge (1). Current data indicate that this practice will continue or increase slightly. About 40% of breast feeding mothers, in this country, continue to breast feed their infants to 6 months of age (1). Thus, it is important to determine the effect of food additives on human milk.

Although plasma free glutamate and aspartate levels are low in humans, concentrations of these amino acids in milk and tissues are high (2). We have previously tested the effect of oral glutamate administration on the concentration of glutamate in human milk. Six gram loads of monosodium glutamate (MSG) administered orally to lactating women elevated mean plasma glutamate levels from 4.4 μmoles/100 ml to levels of 31 μmoles/100 ml. De-

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spite this sevenfold increase in plasma levels, milk glutamate levels were not significantly increased (3, 4). Recently, aspartame (L-aspartyl-L-phenylalanyl methyl ester), a new dipeptide sweetener, was proposed as a food additive. Because of concerns about the potential toxic effects of its component amino acids, questions were raised about the safety of aspartame (5, 6).

The present study was designed to determine if aspartame ingestion would have a significant effect on blood and milk amino acid levels in lactating women. We have previously reported that aspartame administration to normal adult subjects at 34 mg/kg body weight (the projected 99.9th percentile of daily ingestion) increased plasma and erythrocyte phenylalanine levels to those seen in the post-prandial state. This dose had no effect upon plasma and erythrocyte aspartate levels (7). Because of the lack of effect of 34 mg/kg body weight, a higher dose (50 mg/kg body weight) was chosen for these studies.

**MATERIALS AND METHODS**

Six healthy women with well established lactation were studied. The proposed study was fully explained to each subject, and informed, written consent was obtained. The protocol for the study was reviewed and approved by the Committee on Research Involving Human Subjects of the University of Iowa. The women ranged in age from 20 to 29 years, with a mean ± so of 26 ± 3 years. Their weights ranged from 45 to 63.6 kg, with a mean ± so of 55.2 ± 6.42 kg. All women had established lactation patterns, with the length of lactation ranging from 42 to 159 days, with a mean ± so of 98 ± 39 days.

The women were studied after oral administration of aspartame or lactose (50 mg/kg body weight) in a randomized cross-over design. An interval of at least 2 weeks separated each segment of the cross-over studies. Aspartame or lactose was dissolved in 300 ml cold orange juice and administered to the subjects at 0800 hours following an overnight fast. The subjects were fasted for an additional 4 hours following administration of the test dose, however they were allowed a normal diet after this time. Blood samples (3 ml) for amino acid analyses were obtained at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3 and 4 hours after administration of each test load through an indwelling catheter with a heparin lock, placed in an arm vein. Breast milk samples were collected at 0, 1, 2, 3, 4, 8, 12 and 24 hours after loading. Each woman collected milk by manual expression. Samples were obtained from each breast, with a total of 10 to 15 ml collected. The women were allowed to suckle their infants at will. All women suckled their infant one time during the 4-hour blood study period.

Blood samples for amino acid analyses were centrifuged immediately to separate plasma and erythrocytes. The plasma was deproteinized with sulfosalicylic acid (7) and either analyzed immediately, or stored at -70° to prevent loss of glutamine and cystine (8, 9). Erythrocyte and milk samples were prepared as previously described (3, 7). Amino acid analyses were carried out on automated amino acid analyzers.

Initial statistical analysis consisted of the paired t-test for data at each time point (10). The data were also tested by analysis of variance using orthogonal polynomials. The plasma, milk and erythrocyte amino acid data for each woman were curve-fitted by orthogonal polynomials into the mean, linear, quadratic and cubic time-effect scores for each treatment (11). A randomized block design was used to test if the mean, linear, quadratic and cubic time-effect scores differed. The randomized block analysis is equivalent to performing a paired t-test on the curves, where two treatments were given.

**RESULTS**

Figure 1 shows the response of plasma aspartate, asparagine, glutamate and glutamine levels to both aspartame and lactose loading. No significant difference between aspartame administration and lactose administration was noted in mean plasma aspartate, asparagine, glutamate or glutamine levels.

Figure 2 shows plasma phenylalanine and tyrosine levels in these subjects. As expected, plasma phenylalanine levels in-
increased significantly over baseline levels \((P < 0.001)\), reaching peak values of 16.2 ± 4.9 µmoles/100 ml after aspartame ingestion, but were not affected by lactose loading. Peak plasma phenylalanine levels observed at this dose were higher than values noted after aspartame ingestion at 34 mg/kg \((7)\). Plasma tyrosine levels also increased significantly \((P = 0.001)\) after aspartame loading, reflecting conversion of phenylalanine to tyrosine. Tyrosine levels decreased slightly \((P = 0.01)\) after lactose loading compared to zero time levels, following the pattern reported previously after aspartate loading \((7)\).

Plasma levels of alanine and proline increased significantly over zero time levels \((P < 0.02)\) after both aspartame and lactose loads \((\text{fig. 3})\). This most likely is due to either the orange juice vehicle, or the stress of blood sampling \((12)\). Similar effects were seen after both aspartame (34 mg/kg) and aspartate (13 mg/kg body weight) loading in previous studies \((7)\).

Plasma levels of the branched chain amino acids decreased significantly from baseline levels \((\text{leucine}, P = 0.001; \text{isoleucine}, P = 0.01; \text{and valine}, P = 0.002)\) after loading in both groups \((\text{fig. 4})\). Plasma levels returned to fasting values at 4 hours. This decrease presumably reflects insulin release secondary to the carbohydrate content of the orange juice \((13)\). No differences were noted between the two treatments for all other amino acids.

It has been suggested that under some circumstances, certain amino acids are transported in the erythrocyte to a greater extent than in plasma \((14-19)\). Since aspartame had no effect on plasma aspartate levels, erythrocyte levels of free amino acids were measured to assure that aspartate was not carried by the red cell. As shown in figure 5, erythrocyte glutamate, glutamine, aspartate and asparagine levels were unchanged after either aspartame or lactose administration. Erythrocyte phenylalanine and tyrosine levels \((\text{fig. 6})\) decreased significantly after lactose loading as compared to baseline levels \((P = 0.01)\). Erythrocyte phenylalanine, but not tyrosine levels, increased significantly \((P = \ldots)\)
Fig. 3 Mean (±SD) plasma alanine and proline levels in six lactating women administered aspartame (O—O) or lactose (••••••) at 50 mg/kg body weight.

0.001) after aspartame loading. The increase in phenylalanine levels was expected in view of the increased plasma levels noted.

Human milk aspartate and glutamate levels in these subjects are shown in figure 7. During the 4 hour fasting period after aspartame loading, milk glutamate levels increased from 109 to 120 μmoles/100 ml, while milk aspartate levels increased from 2.3 to 4.8 μmoles/100 ml. Differences in levels of these amino acids were not statistically significant using the paired t-test analysis. However, when the data were subjected to curve analysis using orthogonal polynomials, milk aspartate levels were significantly higher during both the immediate 4 hour post-absorptive period (P = 0.034, mean time-effect scores using orthogonal polynomials did not differ significantly. When milk data for the entire 24 hour study post-dosing period were analyzed by analysis of variance using orthogonal polynomials, no statistically significant differences were noted. No differences in milk glutamate levels were noted with any statistical analysis.

Milk phenylalanine and tyrosine levels are shown in figure 8. The differences in milk phenylalanine and tyrosine levels between test substances were not significant using paired t-test analysis. However, when the data were subjected to curve analysis using orthogonal polynomials, milk phenylalanine levels were significantly higher during both the immediate 4 hour post-absorptive period (P = 0.034, mean

Fig. 4 Mean (±SD) plasma valine, leucine and isoleucine values in six lactating women administered aspartame (O—O) or lactose (••••••) at 50 mg/kg body weight.
only) and during the overall 24 hour period (P = 0.02, mean only). However, linear, quadratic and cubic time-effect scores showed no significant effects. Milk tyrosine levels increased significantly (P = 0.018) in the immediate 4 hour post-absorptive period after aspartame loading compared to lactose (orthogonal polynomials, linear only), but did not differ significantly when data for the entire 24 hour period were analyzed.

However, glutamate, aspartate, phenylalanine and tyrosine levels in milk samples collected postprandially (8 and 12 hours) were similar to those noted 4 hours after aspartame loading. The 8 hour milk samples were obtained approximately 3 hours after the subjects luncheon meal, while the 12 hour samples were obtained approxi-

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**Fig. 5** Mean (±sd) erythrocyte glutamine (GLN), glutamate (GLU), asparagine (ASN) and aspartate (ASP) levels in six lactating women administered aspartame (O—O) or lactose (●—●) at 50 mg/kg body weight.

**Fig. 6** Mean (±sd) erythrocyte tyrosine and phenylalanine levels in six lactating women administered aspartame (O—O) or lactose (●—●) at 50 mg/kg body weight.

**Fig. 7** Mean (±sd) milk free glutamate and aspartate levels in six lactating women administered aspartame (O—O) or lactose (●—●) at 50 mg/kg body weight.
Phenylalanine -- — Tyrosine

**Fig. 8** Mean (±SD) milk free phenylalanine and tyrosine levels in six lactating women administered aspartame (○--○) or lactose (●—●) at 50 mg/kg body weight.

Immediately 2 hours after the evening meal.

No free aspartame or aspartyl-phenylalanine were detected in the plasma, milk or erythrocytes of the subjects at any time. The limit of detection was 0.5 μmoles/100 ml. These data are consistent with animal studies indicating hydrolysis of aspartame either in the intestinal lumen (20, 21), or in intestinal mucosal cells after uptake (22) with subsequent release of aspartame components (aspartate, phenylalanine and methanol) to the portal blood.

**DISCUSSION**

The dose of aspartame given in this study is considerably beyond the total daily projected intake of this compound under any but abuse conditions. We have previously pointed out (7) that daily intake of aspartame would approximate 7.5 to 8.5 mg/kg/day if all usual sucrose intake (17% of energy) were replaced by aspartame. Similar calculations indicate that an aspartame intake of 34 mg/kg/day represents the 99.9th percentile of projected daily intake. The aspartame load used in the present study is approximately 1.5 times this level.

The results of the present study demonstrate rapid metabolism of the aspartate portion of aspartame. Neither plasma nor erythrocyte aspartate levels increased after aspartame loading. Similarly, there was no evidence for the accumulation of amino acids that might be derived from aspartate (asparagine, glutamine, glutamate).

Plasma phenylalanine and tyrosine levels increased after aspartame administration. In contrast, levels of these amino acids remained constant or decreased slightly after lactose loading. Mean peak plasma phenylalanine values for subjects given aspartame at 50 mg/kg were higher, and the area under the plasma phenylalanine concentration-time curve greater than noted in subjects given 34 mg/kg (fig. 9) (7). However, these plasma phenylalanine levels were only slightly above those found postprandially (12 ± 2 μmoles/100 ml) in young infants or adults (23, 24). The phenylalanine levels observed are far below peak levels routinely noted (78 ± 45 μmoles/100 ml) in subjects tested for the

**Fig. 9** Mean (±SEM) plasma phenylalanine levels in adult subjects administered lactose at 50 mg/kg (○—○), aspartame at 34 mg/kg body weight (●—●), or aspartame at 50 mg/kg body weight (○—○).
heterozygous condition of phenylketonuria (25). In children with classical phenylketonuria, elevated plasma phenylalanine levels are associated with mental retardation. However, plasma phenylalanine levels are much higher, and vary between 120 to 300 μmoles/100 ml (20 to 50 mg/100 ml) continually (26–28).

The data in figure 7 indicate that aspartame administration at 50 mg/kg body weight has only a very small effect upon milk aspartate levels when compared to values noted after lactose loading. Although a small, statistically significant increase in aspartate time-effect scores was noted by the linear orthogonal polynomial method for the immediate 4 hour post-absorptive period, no significant difference was noted over the entire 24 hour period by any method of analysis. Aspartame loading also had a small but significant effect upon milk phenylalanine and tyrosine levels when the data were analyzed by the orthogonal polynomial method.

However, the biological importance of these small differences is minimal. In these studies, the subjects fasted until after the 4 hour milk sample was obtained. After that time they were allowed their usual meal pattern. The levels of phenylalanine and tyrosine after aspartame loading were no greater than the values noted in the postprandial milk samples (8 and 12 hours) following ingestion of meals. Furthermore, the phenylalanine and tyrosine levels noted in the milk samples obtained after aspartame loading were similar to those observed, but not published in a previous study of lactating women (3).

The slightly increased quantities of phenylalanine and tyrosine present in human milk after aspartame loading would have little impact upon the total levels of these amino acids ingested by the infant. This can be demonstrated by calculating the daily intake of selected amino acids by the 3.5 kg breast-fed infant (table 1). Such infants, fed ad libitum, have a mean milk intake of 164 ml/kg/day (29). This level provides approximately 110 kcal/kg body weight/day. The levels of various free amino acids in pooled human milk have been reported by Stegink (2), while the total (free plus protein-bound) glutamate, aspartate, phenylalanine and tyrosine content have been reported by Macy et al. (30).

However, the total glutamate values (230 mg/100 ml) reported by Macy et al. (30) include both glutamine and glutamate while total aspartate values (116 mg/100 ml) include both aspartate and asparagine, since the values were obtained after acid hydrolysis. When the data of Macy et al. (30) are corrected for the quantities of free glutamate (20.7 mg/100 ml), glutamine (6.5 mg/100 ml), asparagine (15 mg/100 ml) and aspartate (0.5 mg/100 ml) in human milk (2), the total protein-bound glutamate plus glutamine content of human milk is 203 mg/100 ml, while the total protein-bound aspartate plus asparagine content is 114 mg/100 ml.

The approximate quantities of glutamine and asparagine which contribute to the "total protein bound glutamate" and "total protein bound aspartate" can be calculated using the data of Jukes et al. (31). These data indicate that 55% of the total aspartate released from a typical protein upon acid hydrolysis arises from aspartate, while 45% arises from asparagine. Similarly, 58% of the total glutamate resulting from acid hydrolysis of a protein arises from glut-
mate, while 42% comes from glutamine. Using these correction factors, we can calculate the protein-bound glutamate content of human milk as 115 mg/100 ml and protein-bound aspartate content as 63 mg/100 ml.

From these data we can calculate the quantity of free and protein-bound amino acids ingested daily by the breast-fed infant (table 1). These calculations in turn allow us to evaluate the potential effect of the small changes noted in milk composition after aspartame loading at these levels. If we assume the lactating mother ingests sufficient aspartame to increase her milk phenylalanine level during the entire 24 hour period, to the extent reported here for the 4-hour sampling period, milk phenylalanine levels would increase by about 1.8 μmoles/100 ml. This increase would provide the breast-fed infant with an additional 3.1 μmoles of phenylalanine/kg/day (0.51 mg/kg/day). This must be compared to the infants normal intake of this essential amino acid of 79 mg/kg/day (table 1). Thus, even high doses of aspartame would not meaningfully affect the infant’s phenylalanine intake.

These calculations can also be made for aspartate intake. The small increase in aspartate levels noted in human milk after aspartame loading, although not statistically significant, would result in the ingestion of an additional 4.6 μmoles of aspartate/kg/day (0.77 mg/kg/day). This is only a trace quantity compared to the approximately 105 mg of aspartate ingested per kg/day from human milk.

Similar calculations for tyrosine and glutamate indicate that tyrosine intake might increase by 1.71 μmoles/kg/day (0.31 mg) and glutamate by 11.0 μmoles/kg/day (1.6 mg). Neither increase would meaningfully affect total daily intake.

In conclusion, aspartate loading of lactating women at a level of 50 mg/kg body weight resulted in: (a) no change in blood aspartate levels; (b) increased plasma phenylalanine levels to high postprandial levels; and (c) a small but statistically significant increase in breast milk aspartate, phenylalanine and tyrosine levels, increasing their values from the fasting range into the postprandial range.

LITERATURE CITED

PLASMA, ERYTHROCYTE AND HUMAN MILK LEVELS