

Circadian Variations in Plasma and Erythrocyte Glutamate Concentrations in Adult Men Consuming a Diet with and without Added Monosodium Glutamate^{1,2}

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ABSTRACT This study evaluated the effect of monosodium glutamate (MSG) ingestion as a component of the diet on the 24-h variations in plasma and whole-blood glutamate (GLU) concentrations in healthy adult men. In the first arm of the study, subjects were given test meals without added MSG for 3 d. Protein and energy intakes of the subjects were 1.5 g and 40 kcal/(kg body weight·d), respectively. On d 3, blood samples were collected over the 24-h period. One week later, the same protocol was repeated, except that 100 mg/(kg body weight·d) MSG was added to the meals (15, 40 and 45 mg/kg body weight to breakfast, lunch and dinner, respectively). Both plasma and whole-blood samples were analyzed for free amino acids. Unlike large neutral amino acids, which experienced high peak plasma concentrations at 2100–2300 h, the circadian variations in plasma GLU concentrations were small, varying between 33 and 48 $\mu\text{mol/L}$ on days in which no MSG was fed, and between 32 and 53 $\mu\text{mol/L}$ on days in which MSG was added to the meals. In both trials, plasma GLU concentration increased ($P < 0.01$) after lunch and dinner, and decreased early in the morning ($P < 0.05$). Calculated erythrocyte GLU concentrations varied between 500 and 640 $\mu\text{mol/L}$, with or without MSG addition to the meals. The rather low plasma GLU concentrations over the 24-h period, despite high dietary intake of MSG, indicate that dietary MSG is metabolized very rapidly. *J. Nutr.* 130: 1002S–1004S, 2000.

KEY WORDS: • glutamic acid • monosodium glutamate • plasma • erythrocytes • circadian variation • diet • humans

The total blood concentration of amino acids in adult men is known to vary rhythmically in a 24-h period (Feigin et al. 1967). Individual amino acids in plasma also vary with a similar diurnal rhythm (Feigin et al. 1968, Wurtman et al. 1968). Chronic modification of the dietary protein content appears not to have a marked effect on the general pattern of such diurnal variations, although the mean 24-h concentrations of many amino acids rise after the ingestion of increasing amounts of protein (Feigin et al. 1971). Within a 24-h period, for example, when even small amounts of protein are included in the diet, the diurnal pattern for the plasma concentrations of the large, neutral amino acids rises in the afternoon, and peak diurnal values occur at ~2300 h (Fernstrom et al. 1979). In these (and other) studies, blood samples were collected at

rather long intervals, (3–6 h). In a more recent study in healthy adults fed a normal hospital diet, blood samples were collected every hour for 24 h (Eriksson et al. 1989). The results affirmed the presence of significant circadian rhythms in the plasma concentrations of the large neutral amino acids, but with greater acuity.

In all such previous studies, no careful examination appears to have been made of the diurnal variations in the plasma and red blood cell concentrations of glutamate (GLU).⁴ Moreover, no study has examined the effect of dietary monosodium glutamate (MSG) content on such variations. Plasma and erythrocyte GLU concentrations have been studied in normal adult subjects fed a high protein meal, with or without added MSG. However, GLU concentrations were measured for only 4–8 h after meal ingestion (Stegink et al. 1979 and 1982). In this paper, we therefore describe our recent findings on the diurnal variations in plasma and erythrocyte GLU concentrations in adult men given meals with and without added MSG (Tsai and Huang 1999).

SUBJECTS AND METHODS

Subjects. Healthy young men ($n = 10$; mean age, 24.9 ± 1.1 y) were recruited into the study from the medical school community, and provided informed consent.

¹ Presented at the International Symposium on Glutamate, October 12–14, 1998 at the Clinical Center for Rare Diseases *Aldo e Cele Daccò*, Mario Negri Institute for Pharmacological Research, Bergamo, Italy. The symposium was sponsored jointly by the Baylor College of Medicine, the Center for Nutrition at the University of Pittsburgh School of Medicine, the Monell Chemical Senses Center, the International Union of Food Science and Technology, and the Center for Human Nutrition; financial support was provided by the International Glutamate Technical Committee. The proceedings of the symposium are published as a supplement to *The Journal of Nutrition*. Editors for the symposium publication were John D. Fernstrom, the University of Pittsburgh School of Medicine, and Silvio Garattini, the Mario Negri Institute for Pharmacological Research.

² Supported in part by a grant-in-aid from the International Glutamate Technical Committee.

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⁴ Abbreviations used: Hct, hematocrit; GLU, glutamate; MSG, monosodium glutamate.

Diet. The menu of the diet was designed to follow normal Taiwanese dietary customs. It consisted of three meals and two light snacks each day, and provided ~40 kcal/(kg body weight · d) energy and 1.5 g kcal/(kg body weight · d) protein. Of the total energy provided, 15% derived from protein, 55% from carbohydrate and 30% from fat. The light snacks provided ~1.6 kcal/kg body weight, consisting mainly of carbohydrate. Vitamins and minerals were supplied daily as a supplement to meet recommended dietary allowances. The calculated GLU content of the proteins contained in the diet was 18.4% (wt/wt). A subject who weighed 60 kg would thus have ingested ~16.5 g of protein-bound glutamate each day.

Experimental design. In the first arm of the study, the subjects consumed the test diet without added MSG for 3 d. All meals and snacks were provided daily on an outpatient basis at the nutrition unit of our department, at 0745, 1215 and 1800 h; light snacks were provided, and the subjects were instructed to consume them at 1500 and 2130 h. The subjects were instructed not to consume any other foods during the 3-d period. On the evening of d 2, subjects were admitted to a ward of our university hospital, where they spent the night. On d 3, meals were served at 0745, 1215 and 1800 h, and light snacks were eaten at 1500 and 2130 h. The meals were eaten within ~15–30 min, and light snacks within ~5–10 min. Blood samples (5 mL) were obtained from forearm veins at 0730, 0900, 1000, 1200, 1345, 1445, 1730, 1930, 2100, 2300, 0200, 0500 and 0730 h via an indwelling catheter. The subjects were allowed light activities and went to bed after blood collection at 2300 h. A similar trial was carried out again 1 wk later, but 100 mg/(kg body weight · d) MSG was added to the meals (15, 40 and 45 mg/kg body weight MSG were added to breakfast, lunch and dinner meals, respectively).

Analytical methods. For whole-blood analysis, 1 mL heparinized blood was diluted with 1 mL distilled water and completely hemolyzed by twice-repeated freezing and thawing. An aliquot of either plasma or the hemolysate was deproteinized with 50% sulfosalicylic acid. The supernatant obtained after centrifugation at 18,000 × g for 20 min at 4°C was stored at -70°C until analysis (no longer than 4 mo). Just before analysis, the supernatant was diluted with LiS buffer containing an internal standard of S-2-aminoethyl-L-cysteine, and then filtered through Durapore-PVDF membrane (Millipore, Milford, MA). The amino acid concentration of the filtrate was determined by ion-exchange chromatography using a Beckman 6300 Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA). Beckman System Gold software was used for peak identification and integration. The concentrations of the free amino acids in erythrocytes (E) were calculated from the whole blood (WB) and plasma (P) concentrations using the following formula [with the hematocrit (Hct) expressed as a fraction]: $(E) = [(WB) - (1 - Hct) \times (P)]/Hct$.

Statistical analysis. The results were expressed as means ± SD. Multiple comparisons among mean values were made using repeated-measures ANOVA followed by a Student's *t* test using the Bonferroni correction (SAS/STAT Version 6.03; SAS Institute, Cary, NC). Comparisons of data within each subject at each sampling time were made using the nonparametric Wilcoxon matched pairs test. For all tests, *P* < 0.05 was considered significant.

RESULTS AND DISCUSSION

The mean plasma GLU concentration of the 10 subjects before breakfast was $33.2 \pm 15.4 \mu\text{mol/L}$ in the first trial (meals without added MSG) and $32.3 \pm 13.1 \mu\text{mol/L}$ in the second trial (meals containing added MSG). This concentration was higher than that of another dicarboxylic amino acid, aspartic acid (5.3–5.4 $\mu\text{mol/L}$), but much lower than that of glutamine (561–589 $\mu\text{mol/L}$). Figure 1 shows the diurnal variations in plasma and erythrocyte glutamate concentrations observed under the two treatment conditions. ANOVA revealed no significant difference in the pattern of the two glutamate concentration curves (i.e., no treatment or interaction effects).

Plasma GLU concentrations increased 1–2 h after meals (with or without added MSG), especially after lunch and

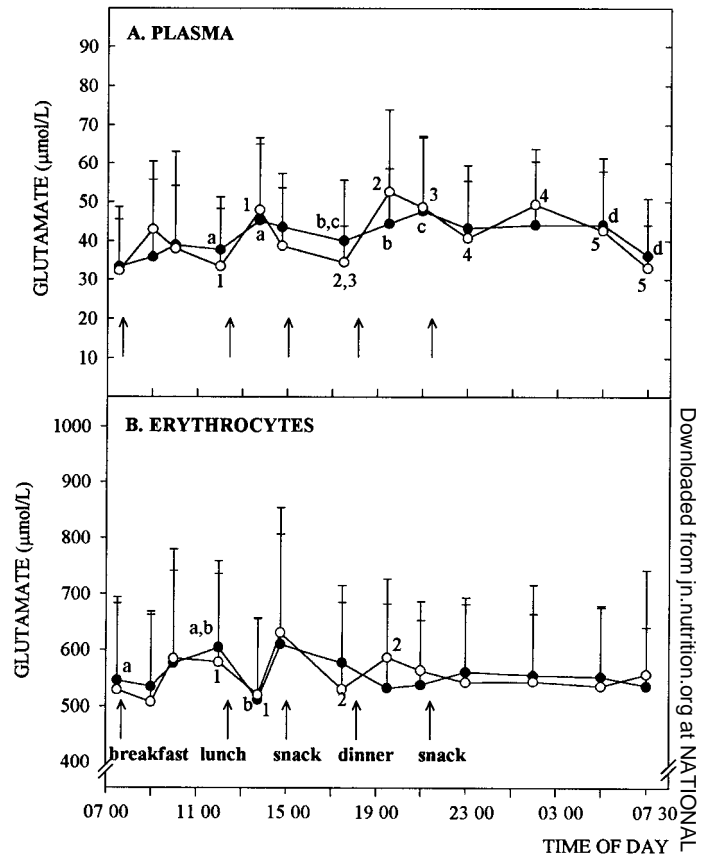


FIGURE 1 Circadian variations in the concentration of glutamate (GLU) in plasma (panel A), and erythrocytes (panel B) in 10 adult men ingesting test a diet without (-●-●-) or with (-○-○-) added monosodium glutamate (MSG) [100 mg/(kg · d)]. Concentrations are expressed in $\mu\text{mol/L}$; vertical bars represent SD. The arrows indicate the time of ingesting meals. The concentrations at different time points with the same letters (for -●-●-) or numbers (for -○-○-) are significantly different (*P* < 0.05); (from Tsai and Huang 1999).

dinner (*P* < 0.01). When meals contained added MSG, plasma GLU concentrations also were increased moderately (but significantly, *P* < 0.05) between 0000 and 0200 h. Plasma GLU concentrations were generally maintained within a narrow range during the 24-h period, but demonstrated a significant circadian rhythmicity, as analyzed by repeated-measures ANOVA (i.e., a time effect: *P* < 0.01). Presumably, these variations reflect changes occurring in GLU absorption and metabolism throughout the 24-h period (Stegink et al. 1979).

In contrast to plasma, erythrocyte GLU concentrations were very high (~500–650 $\mu\text{mol/L}$), and although meals had some effects on erythrocyte GLU concentrations during the daytime, these concentrations were remarkably constant between 2100 and 0700 h.

The circadian variation pattern of plasma glutamate is rather unique. In contrast to many other amino acids (e.g., the large neutral amino acids), GLU does not show a markedly high peak around 2100 to 2300 h. Although the dietary intake of GLU is the largest among all amino acids, the plasma GLU concentration is one of the lowest. This fact suggests that its metabolism in humans is very rapid. Consistent with this notion, Reeds et al. (1996) recently reported in pigs that enteral GLU is metabolized almost completely during absorp-

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LITERATURE CITED

- Eriksson, T., Voog, L., Walinder, J. & Eriksson, T. E. (1989) Diurnal rhythm in absolute and relative concentrations of large neutral amino acids in human plasma. *J. Psychiatr. Res.* 23: 241-249.
- Feigin, R. D., Klainer, A. S. & Beisel, W. R. (1967) Circadian periodicity of blood amino acids in adult man. *Nature (Lond.)* 215: 512-514.
- Feigin, R. D., Klainer, A. S. & Beisel, W. R. (1968) Factors affecting circadian periodicity of blood amino acids in man. *Metab. Clin. Exp.* 17: 764-775.
- Feigin, R. D., Beisel, W. R. & Wannemacher, R. W., Jr. (1971) Rhythmicity of plasma amino acids and relation to dietary intake. *Am. J. Clin. Nutr.* 24: 329-341.
- Fernstrom, J. D., Wurtman, R. J., Hammarstrom-Wiklund, B., Rand, W. M., Munro, H. N. & Davidson, C. S. (1979) Diurnal variations in plasma concentrations of tryptophan, tyrosine, and other neutral amino acids: effect of dietary protein intake. *Am. J. Clin. Nutr.* 32: 1912-1922.
- Reeds, P. J., Burrin, D. G., Jahoor, F., Wykes, L., Henry, J. & Frazer, E. M. (1996) Enteral glutamate is almost completely metabolized in first pass by the gastrointestinal tract of infant pigs. *Am. J. Physiol.* 270: E413-E418.
- Stegink, L. D., Filer, L. J., Jr. & Baker, G. L. (1982) Plasma and erythrocyte amino acid levels in normal adult subjects fed a high protein meal with and without added monosodium glutamate. *J. Nutr.* 112: 1953-1960.
- Stegink, L. D., Reynolds, W. A., Filer, L. J., Jr., Baker, G. L., Daabees, T. T. & Pitkin, R. M. (1979) Comparative metabolism of glutamate in the mouse, monkey, and man. In: *Glutamic Acid: Advances in Biochemistry and Physiology* (Filer, L. J., Jr., Garattini, S., Kare, M. R., Reynolds, W. A. & Wurtman, R. J., eds.), pp. 85-102. Raven Press, New York, NY.
- Tsai, P.-J. & Huang, P.-C. (1999) Circadian variations in plasma and erythrocyte concentrations of glutamate, glutamine and alanine in men on a diet without and with added monosodium glutamate. *Metabolism* 48: 1455-1460.
- Wurtman, R. J., Rose, C. M., Chou, C. & Larin, F. F. (1968) Daily rhythms in the concentrations of various amino acids in human plasma. *N. Engl. J. Med.* 279: 171-175.