Glutamate ingestion: the plasma and muscle free amino acid pools of resting humans

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Graham, T. E., V. Sgro, D. Friars, and M. J. Gibala. Glutamate ingestion: the plasma and muscle free amino acid pools of resting humans. Am. J. Physiol. Endocrinol. Metab. 278: E83–E89, 2000.—Monosodium glutamate (MSG) ingestion is known to increase plasma glutamate concentration, and MSG infusion stimulates insulin secretion. We investigated the impact of MSG ingestion on both the plasma and intramuscular amino acid pools. Nine postprandial adults ingested MSG (150 mg/kg) and rested for 105 min. Venous blood was sampled preingestion and then every 15 min; vastus lateralis muscle biopsies were taken preingestion and at 45, 75, and 105 min postingestion. Venous plasma glutamate and aspartate concentrations increased (P ≤ 0.05) ~700–800 and 300–400%, respectively, after 30–45 min. Although several other plasma amino acids increased modestly, the rise in glutamate accounted for ~80% of the increase in total plasma amino acids. In addition, plasma insulin increased threefold after 15 min; this occurred before a significant increase in plasma glutamate, indicating a feed-forward stimulation from the gastrointestinal tract. The intramuscular amino acid pool was remarkably constant, with only glutamate increasing (P ≤ 0.05) by 3.56 mmol/kg dry wt. By 105 min, the plasma and muscle amino acids had returned to resting concentrations. This increase in muscle glutamate concentration could account for ~40% of the MSG ingested; we propose that resting skeletal muscle is a major sink for the glutamate and metabolizes it to aspartate, alanine, and glutamine. Resting skeletal muscle normally releases glutamine and alanine in large quantities; they can represent from 50–100% of the amino acid efflux in the fasted and fed states, respectively, whereas glutamate is the dominant amino acid that is taken up by skeletal muscle (8, 9, 11).

Oral administration of modest amounts of glutamate has been shown (3, 14) to result in a large portion of the amino acid being oxidized by the splanchic bed on the first pass. However, ingestion of larger doses of monosodium glutamate (MSG; Refs. 18–21) produced marked increases in plasma glutamate and aspartate concentrations for 1–2 h. Recently, it has been shown (6) that glutamate can stimulate insulin secretion in rats at plasma glutamate concentrations that are achieved in humans by oral ingestion of MSG. Thus glutamate administration could indirectly affect many aspects of metabolism. Stegink et al. (20) found that MSG administration to newborn pigs did not alter the intramuscular concentrations of aspartate, glutamate, glutamine, or alanine and concluded that the liver was the most likely site for the metabolism of the glutamate. However, subsequently, they (21) found that plasma responses differed both between species and with developmental position.

The studies of MSG ingestion or infusion in humans are limited in that often only a few plasma amino acids (usually glutamate and aspartate) have been measured and muscle amino acids have never been determined. In clinical trials, Thomassen et al. (22) administered bolus infusions of MSG to patients with coronary heart disease and observed transient (5–10 min) increases in insulin and glucose as well as in glutamate and alanine and a decrease in free fatty acids (FFA). In apparent contradiction to the study (20) of muscle amino acids in the newborn pig, the leg [arteriovenous (a-v)] glutamate difference increased 460% in the humans. This suggests that human skeletal muscle could very well be an important metabolic site of the glutamate load. However, the study was not conducted in a metabolic steady-state condition, it was not established if there was a disturbance in the rest of the plasma free amino acid pool, and the muscle pool was not investigated.

The present study examined the plasma and intramuscular responses in the free amino acid pool to the ingestion of MSG in healthy, resting adult humans. Plasma insulin, glucose, and glycerol were monitored to evaluate if there was a general disturbance of metabolism. It was hypothesized that the ingestion would...
result in an elevation of both the plasma and intramuscular concentrations of glutamate, aspartate, glutamine, and alanine, whereas there would be little or no disturbance in the remaining amino acids. In addition, there would be an increase in plasma insulin directly associated with the rise in plasma glutamate and a corresponding decline in blood glucose and glycerol.

METHODS

Nine healthy adults [8 men and 1 woman, mean age 26 yr (range 19–49 yr), mean weight 76.9 kg (range 59–97.5 kg)] volunteered to participate in the study after being informed both verbally and in writing of the purpose and risks of the study. The experimental protocol was approved by the Human Ethics Committee of the University of Guelph. A medical doctor was in attendance during all trials.

The subjects refrained from all prolonged and/or strenuous physical activity for 24 h before the experiment and reported to the laboratory at least 6 h postprandial. A small Teflon catheter was inserted into an antecubital vein and kept patent with a normal saline infusion. After this, both thighs were prepared for muscle biopsies; after a local anesthetic was injected into the skin and underlying fascia, two small incisions were made over the lateral aspect of each thigh, one for each of four biopsies from the vastus lateralis. The incisions were covered with a sterile dressing until a biopsy was required.

The subject remained either seated or lying for the entire experiment. A blood sample (5 ml) and a muscle biopsy were taken, and then the subject ingested a MSG solution (150 mg/kg body wt of monosodium L-glutamate dissolved in water at 4.2 ml/kg body wt). This time was referred to as time 0. Subsequently, muscle biopsies were taken at 45, 75, and 105 min (alternating the leg sampled) and blood samples (5 ml) were taken every 15 min. The muscle samples were immediately frozen in liquid N₂ and stored at −80°C. The blood samples were placed in heparinized tubes, and then 200 µl were transferred into 1 ml of 0.6 M perchloric acid and the supernatant was stored at −20°C for glucose (5) and glycerol (13) analysis. The remaining blood was centrifuged, and the plasma was stored at −80°C for insulin with a radioimmunoassay kit (Coat-A-Count: DPC, Los Angeles, CA) and amino acid analysis (12). The muscle samples were freeze-dried and powdered to dissect out nonmuscle elements. Subsequently, these were extracted and analyzed for amino acids as previously described (10) by the Waters picotag system.

Calculations and statistics. For those amino acids that increased significantly, the peak value and time of occurrence were determined for each subject and these were averaged for all subjects. The sum of all amino acids was calculated and referred to as total amino acids, the sum of the essential amino acids threonine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, and lysine was referred to as total essential amino acids, and the sum of valine, leucine, and isoleucine was referred to as branched-chain amino acids. All data are presented as means ± SE. The data were analyzed by a one-way ANOVA over time with repeated measures, and when significance was identified, a Tukey's post hoc test was employed. Differences were accepted as significant at a probability level of P ≤ 0.05.

RESULTS

Plasma amino acids. The subjects did not experience any serious side effects of the MSG ingestion, but several did have transient headaches. In every subject, the plasma glutamate concentration rose rapidly with the maximum occurring either 30 or 45 min after ingestion (Fig. 1A). The peak increase (the mean peak increase was 437 µM) represented a 700–800% increase above the initial concentration. The concentration declined rapidly, and at 90 min it was not significantly elevated over the initial level. Only two other amino acids had major increases in plasma concentration; aspartate increased 3–400%, reached a maximum at 30 min, and declined slowly until it was not different from the initial value at 105 min (Fig. 2A). Similarly, taurine doubled at 45 min and was significantly elevated until 75 min (Table 1). The absolute magnitude of increases in these latter two amino acids was only 20–30 µM, i.e., more than an order of magnitude less than that of plasma glutamate. The total amino acid concentration (Table 1) followed a temporal response similar to these three amino acids, increasing significantly after 30 min, with a peak increase of 540 µM at 30–45 min. Thus the changes in plasma glutamate
There were small (11–36 µM), but significant, differences in plasma glycine, proline, leucine, and lysine between their respective values at 15 min and those in the last 30–45 min of the trial. This appeared to be due to both a small, nonsignificant rise in their concentrations at 15 min and a modest decline late in the study. Glutamine concentration had a significant increase (75 µM) at 45 and 75 min compared with time 0. In addition, tyrosine, methionine, asparagine, and the essential amino acids each had a small (8, 5, 5, and 52 µM, respectively), significant decline compared with their initial and/or 15-min concentrations, and these generally occurred at 60–90 min. There were no significant changes in the plasma concentrations of serine, histidine, threonine, alanine, arginine, valine, isoleucine, phenylalanine, tryptophan, ornithine, or the branched-chain amino acids (data not shown).

Muscle amino acids. The muscle glutamate concentration increased significantly by 45 and 75 min; these two values were also significantly different from each other (Fig. 1B). The mean peak increase was 3.56 mmol/kg dry wt, and the time for the peak increase varied considerably among the subjects.

The modest increase in muscle aspartate (Fig. 2B) was not significant. There were no other changes in the intramuscular free amino acid pool; Table 2 summarizes these data for those amino acids that had changed significantly in the plasma pool.

Insulin, glucose, and glycerol. Insulin concentration increased significantly within 15 min of MSG ingestion and remained elevated until 90 min (Fig. 3). However, it is noteworthy that the increase in insulin concentration was greatest at 15 min, i.e., before the plasma glutamate concentration had even changed significantly (Fig. 1). Despite this rise in insulin, the blood glucose concentration remained very stable, with mean values ranging from 3.11 ± 0.15 to 3.28 ± 0.15 mM. Blood glycerol concentration tended (P = 0.16) to decline during the period of elevated insulin from an initial concentration of 251 ± 64 to 170 ± 24 µM at 60 min before returning to 218 ± 28 µM at the end of the trial.

Table 1. Plasma amino acids

<table>
<thead>
<tr>
<th>Amino Acid, µM</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>105</th>
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<tbody>
<tr>
<td>Gln</td>
<td>489 ± 42.3*</td>
<td>535 ± 41.5†</td>
<td>546 ± 42.8</td>
<td>563 ± 47.2†</td>
<td>543 ± 51.9†</td>
<td>563 ± 50.4†</td>
<td>520 ± 42.8†</td>
<td>517 ± 43.7†</td>
</tr>
<tr>
<td>Tau</td>
<td>37 ± 2.7*</td>
<td>44 ± 3.3</td>
<td>53 ± 4.2†§</td>
<td>61 ± 4.3</td>
<td>58 ± 4.4†</td>
<td>59 ± 6.7†</td>
<td>49 ± 3.9†</td>
<td>43 ± 4.4§</td>
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<tr>
<td>Gly</td>
<td>192 ± 14.7†</td>
<td>212 ± 15.8*</td>
<td>196 ± 11.9†</td>
<td>190 ± 13.1†</td>
<td>182 ± 15.2†</td>
<td>179 ± 14.1†</td>
<td>174 ± 14.3†</td>
<td>178 ± 12.1†</td>
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<tr>
<td>Pro</td>
<td>169 ± 22.4†</td>
<td>183 ± 26.3*</td>
<td>166 ± 25.1†</td>
<td>169 ± 26.8†</td>
<td>159 ± 23.7†</td>
<td>168 ± 25.8†</td>
<td>158 ± 23.5†</td>
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<td>Leu</td>
<td>103 ± 9.3†</td>
<td>108 ± 10.1*</td>
<td>105 ± 11.1†</td>
<td>105 ± 11.6†</td>
<td>97 ± 8.0†</td>
<td>101 ± 10.2†</td>
<td>99 ± 9.0†</td>
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<tr>
<td>Lys</td>
<td>140 ± 9.3†</td>
<td>148 ± 10.9*</td>
<td>145 ± 9.7†</td>
<td>143 ± 10.6†</td>
<td>137 ± 9.9†</td>
<td>140 ± 8.8†</td>
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<td>Tyr</td>
<td>66 ± 3.7*</td>
<td>67 ± 3.8*</td>
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<td>64 ± 3.4†</td>
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<tr>
<td>Met</td>
<td>28 ± 1.3*</td>
<td>29 ± 2.3*</td>
<td>28 ± 2.0†</td>
<td>27 ± 0.7†</td>
<td>26 ± 1.9†</td>
<td>26 ± 1.1†</td>
<td>24 ± 1.1†</td>
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<td>Asn</td>
<td>55 ± 3.7†</td>
<td>59 ± 4.7*</td>
<td>58 ± 4.7†</td>
<td>59 ± 5.2†</td>
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<td>57 ± 5.1†</td>
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<tr>
<td>EAA</td>
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<td>697 ± 41.2†</td>
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<td>664 ± 39.3†</td>
<td>677 ± 37.9†</td>
<td>656 ± 35.9†</td>
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<td>BCAA</td>
<td>396 ± 31.4*</td>
<td>390 ± 27.1*</td>
<td>388 ± 27.0*</td>
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<td>369 ± 23.7*</td>
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<tr>
<td>TAA</td>
<td>2,387 ± 91.2*</td>
<td>2,584 ± 111.0†</td>
<td>2,917 ± 83.3*</td>
<td>2,935 ± 93.8*</td>
<td>2,621 ± 120.8¶</td>
<td>2,652 ± 122.7¶</td>
<td>2,407 ± 95.1*</td>
<td>2,371 ± 96.2†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Values with same symbols are not statistically different from each other. EAA, essential amino acids; BCAA, branched-chain amino acids; TAA, total amino acids.
Fig. 3. Plasma insulin response to MSG ingestion. Figure is organized the same as in Fig.1. Data with same letter are not significantly different from each other.
One limitation of the present study is that plasma rather than whole blood amino acids were measured. Aoki et al. (2) demonstrated that the erythrocytes could be a major glutamate sink. However, Stegink et al. (19, 21) found that there was no change in erythrocyte glutamate concentration in response to MSG ingestion despite a large increase in plasma glutamate.

There was little or no disturbance in plasma alanine in the present study, but there were significant increases in plasma aspartate and glutamine concentrations. The former followed a very similar time course to that of plasma glutamate, whereas the increase in glutamine only occurred at 45 and 75 min. The sources of the aspartate and glutamine could not be established in the present study; however, human skeletal muscle appears to have been a major sink for the glutamate load (see below), and aspartate and glutamine can only be synthesized from glutamate (1). Figure 4 summarizes the interactions of these amino acids in muscle. These various reactions are all near equilibrium (9, 11, 17), and thus an increase in intramuscular glutamate should cause increases in aspartate, glutamine, and alanine production. In the present study, we found no disturbance in either the plasma or muscle alanine concentration. As discussed below, Thomassen et al. (22) presented indirect evidence that increased plasma glutamate resulted in an increased muscle uptake of glutamate but no increase in alanine release. Alanine production requires pyruvate, and resting muscle primarily metabolizes fats; thus pyruvate availability might have limited alanine formation.

We are aware of only two previous investigations of intramuscular amino acids after glutamate administration. As noted above, Stegink et al. (20) found no changes in the free amino acid pool (including glutamate) in newborn pig muscle when the plasma glutamate was increased to a concentration similar to that in the present study. Beaunoyer et al. (4) found that a massive intravenous MSG infusion (plasma glutamate increased from 23 to 10,733 µM) increased muscle glutamate concentration in resting horses. Surprisingly, despite this massive increase in plasma glutamate, there were no changes in the only other intramuscular amino acids measured (aspartate, alanine, and glutamine). The intramuscular glutamate only increased 2 mmol/kg wet wt, perhaps because the plasma change was fairly transient. Although this increase is compatible with the current data, one must question his or her data because the intramuscular amino acid data for the placebo condition are all much lower than have been reported by others (16) for horse muscle.

The present study demonstrated that the ingestion of MSG was successful in increasing the intramuscular free glutamate pool and maintaining it at an elevated concentration for at least 70 min. A maximum possible increase in intramuscular glutamate can be estimated, assuming that all of the MSG was absorbed, was not extracted by the splanchnic bed, and was equally distributed to all skeletal muscles. The dose of MSG for a 70-kg subject would be 60 mmol, and the body composition should be 40% muscle (i.e., 28 kg wet wt of muscle). If all of this glutamate entered the skeletal muscle, the increase in intramuscular free glutamate would be 2 mmol/kg wet wt or 8–9 mmol/kg dry wt. In the present study, the average net increase in intramuscular glutamate was 3.56 mmol/kg dry wt within 1 h. This does not include any glutamate that was metabolized to aspartate, ammonia, and glutamine. Undoubtedly, the splanchnic tissues metabolize a considerable portion of the ingested glutamate. Nevertheless, this demonstrates that MSG ingestion does result in a significant increase in the intramuscular glutamate pool and resting muscle is a major tissue in plasma glutamate clearance accounting for ~40% of the glutamate load. Rennie (17) reported that glutamate transport in muscle has a low capacity (maximum velocity of 80 nmol·g⁻¹·min⁻¹), and thus one could question whether it is reasonable that muscle could take up this quantity of glutamate.

We have measured glutamate uptake for the human quadriceps of 2–3 and 5–15 nmol·g wet wt⁻¹·min⁻¹ at rest and during prolonged exercise, respectively (9, 11). The net increase in muscle glutamate in the present study would require a net uptake of ~10–15 nmol·g wet wt⁻¹·min⁻¹.

Further support for the theory that muscle extracts considerable portions of the glutamate comes from the work of Thomassen et al. (22), who also studied resting, human skeletal muscle after MSG administration. However, their data are very limited in that they
investigated cardiac patients and infused the MSG rapidly such that plasma glutamate was elevated for only 6 min. The dose of MSG varied between patients, and they only measured two amino acids. Nevertheless, they did find a rapid, 460% widening of the leg (a-v) difference for glutamate (blood flow was not determined) during these few minutes, suggesting that muscle was extracting large amounts of glutamate. In agreement with the lack of change in plasma alanine in the present study, they found no change in the leg (a-v) for alanine. Unfortunately, they did not measure the (a-v) difference for glutamine, aspartate, or ammonia. Thomassen et al. (22) also found that insulin concentration rose rapidly (from 9 to 34 µU/ml) and concluded that plasma glutamate was a potent stimulus for insulin secretion. Similarly, Bertrand et al. (6) demonstrated that intravenous administration of glutamate to rats produced a dose-dependent increase in insulin that was blocked if the ionotropic α-amin0-3-hydroxy-5-methylisoxazole-4-propionic acid receptors of the pancreas were antagonized, suggesting that this excitatory amino acid receptor was mediating the increase in insulin secretion. Certainly, glutamate can act in this fashion and it is an important excitatory neurotransmitter that is linked to a variety of neuroendocrine functions (7, 15). Our data are consistent with these previous investigations; the insulin rose independent of any changes in blood glucose. The increase (from 4 to 11 µU/ml) in the present study was more modest than that in the previous studies (6, 22) with the glutamate infusion, but rapid, occurring within 15 min postinjection. Although the increase in plasma glutamate was large and reasonably fast after oral ingestion, there was no significant change in plasma glutamate after 15 min when the insulin level had already reached its maximum. Thus it is possible that feed-forward mechanisms from the gastrointestinal system are also a factor. Our data do not allow us to resolve the relative importance of a glutamate-related gastrointestinal stimulation compared with direct action of circulating glutamate on insulin secretion.

The increase in insulin may promote muscle clearance of the glutamate as Aoki et al. (2) found that insulin promoted glutamate uptake in the human forearm. However, the concentration of insulin in that study was an order of magnitude larger than that in the present investigation. Furthermore, Rennie (17) reported that although both glutamate and aspartate share the Xac transporter, it is not insulin dependent.

The rise in insulin appeared to be independent of blood glucose. It would be expected to inhibit adipose tissue lipolysis. Thomassen et al. (22) did find a reduced serum free fatty acid concentration with glutamate infusion. In the present study, the glutamate and insulin changes were less dramatic, and although it was not significant, there was a trend in the serum glycerol data to decline during the period of elevated insulin.

In summary, when resting humans ingest a large dose of MSG there is a rise in plasma glutamate that is large, peaks within 30–45 min, and is accompanied by a similar relative rise in plasma aspartate. This is accompanied and preceded by a rise in plasma insulin. The peak insulin concentration occurs within 15 min postingestion and before a significant increase in glutamate and aspartate, suggesting that insulin secretion is stimulated, in part, by a feed-forward mechanism from the gastrointestinal tract. The responses of these amino acids and of insulin are complete within 75–90 min. There are modest increases in several other plasma amino acids. Within the muscle, there is a slower increase in muscle glutamate, but no changes occurred in the rest of the intramuscular free amino acid pool. These data support the hypothesis that resting muscle takes up a major portion of the ingested glutamate, which is transaminated to aspartate and then released.

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