Oral arginine does not stimulate an increase in insulin concentration but delays glucose disposal¹–³

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ABSTRACT

Background: Ingested protein increases circulating insulin concentrations. Several years ago it was also determined that an intravenously administered mixture of 10 essential amino acids stimulated insulin secretion. Of these, arginine was the most potent. The effect was synergistic with administered glucose.

Objective: Because the amounts of amino acid administered intravenously were very large and because ingested arginine is partially metabolized in the intestinal mucosa, we were interested in determining whether orally administered arginine stimulates a rise in circulating insulin concentration and whether arginine affects the glucose-induced rise in insulin concentration.

Design: Nine healthy subjects (4 women and 5 men aged 21–52 y) ingested 1 mmol arginine/kg lean body mass, 1 mmol arginine/kg lean body mass + 25 g glucose, 25 g glucose alone, and water only, in random order on separate occasions, at 0800. Blood samples were obtained at baseline and at 10-min intervals over the next 2 h and were assayed for glucose, insulin, glucagon, and amino acid concentrations. The half-time for gastric emptying was determined by scintigraphy.

Results: Unlike with intravenous administration, ingested arginine did not stimulate a rise in insulin concentration. The glucagon concentration was increased. Arginine attenuated and prolonged the glucose rise when it was ingested with glucose. Gastric emptying time was similar after ingestion of glucose alone or arginine plus glucose.

Conclusion: Arginine, in an amount likely to be ingested in a high-protein meal, does not stimulate insulin secretion but attenuates the increase in glucose when given with glucose. Am J Clin Nutr 2002;76:1016–22.

KEY WORDS Protein, amino acids, glucagon, glucagon resistance, insulin, glucose tolerance, gastric emptying, arginine

INTRODUCTION

It has been known for many years that ingestion of protein stimulates insulin secretion. In healthy young subjects the insulin area response to 50 g ingested beef protein was ~28% as great as the response to 50 g ingested glucose (1). However, in subjects with untreated type 2 diabetes the insulin response to ingestion of 50 g beef protein was greater and was equal to the response stimulated by 50 g glucose. In addition, there was a synergistic insulin response when both were ingested simultaneously (2). Synergism did not occur in healthy subjects.

Subsequently, we studied 7 different types of protein and found that all were effective in synergistically stimulating an increase in insulin concentration in subjects with type 2 diabetes when ingested with glucose (3). However, some proteins were more potent than others. This suggested that the specific amino acid composition of the proteins was important in determining insulin secretion. On the basis of these data, we became interested in the insulin response to ingestion of individual amino acids. We have begun studying nondiabetic subjects. We determined the response to arginine first because it is considered to be the prototypical amino acid secretagogue.

SUBJECTS AND METHODS

Nine healthy subjects were studied (4 women and 5 men aged 21–52 y). The mean body mass was 75 kg (range: 58.5–88.7 kg). The mean lean body mass was 61 kg (range: 48–72 kg). The mean body mass index (in kg/m²) was 25.9 ± 0.5 (range: 24.1–28.2). Thyroid, renal, and liver function test results were normal. The subjects were nondiabetic based on the National Diabetes Data Group criteria (4). Written, informed consent was obtained from all subjects, and the study was approved by the Department of Veterans Affairs Medical Center and the University of Minnesota Committees on Human Subjects.

The subjects were studied in the Special Diagnostic and Treatment Unit after they had fasted 12 h overnight. An indwelling catheter was placed into a forearm vein and flushed with 0.9% saline. Baseline blood samples were obtained at 0730, 0740, and 0750. At 0800 the subjects ingested, in random order and on separate occasions, 1 mmol arginine/kg lean body mass, 1 mmol arginine/kg lean body mass + 25 g glucose, 25 g glucose, and water only. Glucose was given as Glutol (Paddock Laboratories, Inc, Minneapolis), a d-glucose solution (25 g/45 mL). Arginine was given as L-arginine in water (Ajinomoto, USA, Inc, Raleigh, NC).

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Blood was obtained at 10-min intervals over the subsequent 2-h period. Plasma or serum was assayed for glucose, insulin, glucagon, and amino acids, including arginine.

The plasma glucose concentration was determined by a glucose oxidase method with the use of a Beckman glucose analyzer with an oxygen electrode (Beckman Instruments, Fullerton, CA). Serum immunoreactive insulin was determined by a standard double-antibody radioimmunoassay method with kits produced by Endotech (Louisville). Glucagon was determined by radioimmunoassay with 35K antiserum purchased from Health Science Center (Dallas). Individual amino acids were determined by HPLC in the laboratory of KS Nair (Rochester, MN).

The half-life for gastric emptying was determined by scintigraphy, several months after the blood studies. [99mTc]Diethylene-triamine pentaacetic acid (500 μCi; Mallinckrodt Corporate, Maryland Heights, MO) was added to a glucose or a glucose + arginine solution. The total volume of the ingested solution was 60 mL. The subjects drank the solution within 60 s. Imaging was begun immediately after ingestion with the subject standing in front of a large-field-of-view gamma camera with a low-energy, all-purpose parallel-hole collimator. Anterior images were obtained for 60 s at 10-min intervals for 1 h. Images were obtained over the 140-keV photopeak by using 20% energy windows. Images were obtained for 60 s at 10-min intervals for 1 h. Digital images were stored in a computer for data processing. Data processing was performed with the ADAC Laboratories Pegasys system (Milpitas, CA). Time-activity curves were plotted from the gastric region of interest and the half-time for gastric emptying was determined. Each subject was studied twice, receiving glucose alone on one occasion and arginine + glucose on a separate occasion; thus each served as his or her own control. Lean body mass was determined by bioelectric impedance with an RJL Systems instrument (Clinton Town, MI) (5).

To quantify the circulating amino acid, glucose, insulin, and glucagon responses to the test substances, the areas under the curves were calculated by using the trapezoidal rule (6). The areas under the curve were calculated with the use of the initial fasting value of the respective hormone or metabolite as baseline and were measured over the 2-h period after the ingestion of the test substance. Statistics were determined by repeated-measures analysis of variance with the PRISM computer program (version 2.1; Graphpad, San Diego), followed by post hoc Tukey’s tests for multiple comparisons. The criterion of significance was set at a P value of ≤ 0.05. Data are presented as means ± SEMs.

RESULTS

The mean amount of arginine given was 10.6 g (range: 8.4–12.5 g). This was based on 1 mmol/kg lean body mass. The latter ranged from 48 to 72 kg. The mean fasting arginine concentration for all studies was 261 ± 22 μmol/L with a range of 237–280 μmol/L (Figure 1). After the ingestion of water only or glucose only, the plasma arginine concentration tended to decrease slightly. After the ingestion of arginine, the plasma arginine concentration increased from a baseline of 237 ± 37 μmol/L to a peak of 388 ± 60 μmol/L at 60 min, after which it decreased only slightly. At the end of the study, it was still elevated (347 μmol/L). When glucose was ingested with arginine, the response was markedly attenuated compared with arginine ingestion alone. The arginine concentration was elevated at the 100-min time point only (323 μmol/L).

The 2-h integrated arginine area response to arginine was significantly greater than the responses to water alone, glucose alone, and arginine + glucose (P ≤ 0.05) (Figure 1). The area responses to water and glucose were not significantly different from one another but were significantly smaller than the response to arginine + glucose (P ≤ 0.05).

Other plasma amino acids were measured at each time point, as in Figure 1. However, only the 0-, 60-, and 120-min data are presented here, for brevity. The plasma alanine concentration decreased 120 min after ingestion of water or glucose but was essentially unchanged after ingestion of arginine or of arginine + glucose (Table 1). The net area response after arginine ingestion was significantly greater than that after water or glucose ingestion (P < 0.05). The plasma glutamine concentration tended to decrease after ingestion of glucose, but the decrease was not significant. The histidine concentration also tended to decrease 120 min after ingestion of glucose. The branched-chain amino acids (isoleucine, leucine, and valine) and threonine decreased at 120 min after ingestion of glucose and arginine + glucose but remained essentially unchanged after ingestion of water only or arginine. The isoleucine and valine net area responses were significantly greater (P < 0.05) after arginine than after glucose ingestion. The plasma serine concentration decreased 120 min after ingestion of glucose. The serine net area response was significantly greater (P < 0.05) after arginine than after glucose ingestion. Plasma glutamate, glycine, lysine, methionine, phenylalanine, and tyrosine concentrations also
were measured, but they did not change significantly (data not shown).

The mean fasting plasma glucose concentration was 4.3 ± 0.2 mmol/L (77 ± 3 mg/dL) (Figure 2). After ingestion of water only, the plasma glucose concentration remained stable. After ingestion of arginine, the glucose concentration also was stable. After ingestion of glucose, the glucose concentration increased, reached a maximum of 6.9 mmol/L (124 mg/dL) at 30 min, and returned to baseline at 70 min. When arginine was ingested with glucose, the rise in plasma glucose concentration was attenuated by ~60%. However, the glucose concentration did not return to baseline and was still modestly elevated at the 120-min time point (Figure 2).

The 2-h integrated glucose area response to arginine + glucose was slightly but not significantly smaller than the response to glucose alone (Figure 2). One of the reviewers of the present article requested that the area response be calculated over a 70-min period. The comparative net areas for 70 min compared with 120 min are as follows: for water alone, 0.4 compared with −0.3; for glucose alone, 26 compared with 25; for arginine, −0.3 compared with 1.9; and for arginine + glucose, 14.5 compared with 21.6 units·h⁻¹.

The mean fasting serum insulin concentration was 48 ± 6 pmol/L (8.1 ± 1 μU/mL) (Figure 3). Arginine ingestion did not stimulate an increase in insulin concentration. After glucose ingestion, there was a rapid rise in insulin concentration, which corresponded with the rise in glucose concentration. When arginine was ingested with glucose, the insulin response was attenuated and prolonged. However, the increase was only 29% smaller than that after glucose ingestion. Thereafter, the insulin concentration decreased more rapidly than did the glucose concentration (Figure 3).

The mean insulin area response to arginine + glucose was essentially identical to the response to glucose alone (Figure 3). As expected, the insulin area response to arginine alone was similar to that for water. The reviewer also requested that glucose-insulin area ratios be calculated. These were as follows for 70 compared with 120 min, respectively: for glucose, 0.5 and 0.43; for arginine + glucose, 0.33 and 0.38.
Arginine stimulated a modest increase in glucagon concentration when ingested alone (Figure 4). With water ingestion, there was a small decrease in glucagon concentration. After glucose ingestion there was a further modest decrease. When glucose was ingested with arginine, the glucagon concentration also decreased and was similar to that when only water was ingested.

The glucagon area response to ingestion of arginine alone was positive, as expected, and was different from that resulting from the ingestion of water, glucose, or the combination of arginine + glucose (Figure 4). None of the latter 3 were statistically different from one another.

The times required for 50% of the radioactivity to leave the stomach (half-life) were 23.6 ± 3.6 min after glucose ingestion (range: 8–40 min) and 24.0 ± 3.2 min after arginine + glucose ingestion (range: 4–36 min).

DISCUSSION

Floyd et al (7) first showed that arginine given intravenously increased the circulating insulin concentration. In addition, of the 7 essential amino acids infused, arginine was the most potent in this regard. Subsequently, intravenous arginine was used in most in vivo studies as a representative of a nonglucose insulin secretagogue.

In previous studies, as in the studies by Floyd et al, large amounts of arginine were infused and the resulting concentration is likely to have been unphysiologically high. The amount of arginine given intravenously to stimulate insulin secretion generally is ≥30 g, infused over a 30-min period (8–15). This regimen for arginine administration has been reported to increase the plasma arginine concentration ≥90-fold (15, 16). Even infusion ≤0.52 mg · kg⁻¹ · min⁻¹ over 30 min (1.1 g/70 kg body wt) has been reported to increase the arginine concentration 5-fold (17).

Rapidly infused boluses of ≤0.1–0.3 g were also reported to stimulate a rise in insulin and glucagon concentrations. The maximal response occurred at a dose of 5 g (18). A bolus ingestion of arginine stimulates largely first-phase insulin secretion. To study both phases of insulin secretion (first phase and second phase), a primed, continuous arginine-infusion approach was used. The dose at which half the maximal response (ED₅₀) was observed for first-phase insulin secretion was reported to occur at an arginine concentration of 0.7 mmol/L. For second-phase secretion, it was 2.7 mmol/L in euglycemic subjects (19). These data clearly indicated a difference in sensitivity of first-phase and second-phase insulin secretion to a rise in arginine concentration. However, the preinfusion arginine concentrations were not given, making
interpretation in a physiologic context somewhat difficult. There is a considerable variation in reported arginine concentrations in subjects who fasted overnight (range: 46 to \( \approx 300 \) \( \mu \text{mol/L} \)) (20, 21). In any case, all of the ED\(_{50} \) values reported were much greater than the observed arginine concentrations in the present study.

Floyd et al (9) also reported that arginine, infused with glucose, resulted in a synergistic effect on insulin concentration; i.e., the increase in the insulin concentration when both arginine and glucose were given was far greater than the sum of the individual responses. The effect of raising the glucose concentration was subsequently shown to be caused by an increase in the arginine-stimulated maximal rate of insulin secretion and not by a glucose-induced sensitization of the \( \beta \) cell to an increase in arginine concentration (19).

Arginine has been assumed to be an important contributor to stimulation of insulin secretion by ingested proteins. Pek et al (22) reported that ingestion of 30 g arginine resulted in an increase in insulin concentration and that the increase was \( \approx 30\% \) of the increase after ingestion of a 30-g mixture of 10 essential amino acids. They also reported that ingestion of 30 g arginine resulted in an increase in insulin concentration that was \( \approx 30\% \) of that found with ingestion of 100 g protein given as lean beef. Thirty grams is equal to 3.4 mmol arginine/kg lean body mass. Because arginine makes up \( \approx 6\% \) of the amino acids in a typical protein such as beef (23), this amount would be similar to the arginine contained in \( \approx 2.5 \) kg (5.5 lb) beef. Given the slow digestion of proteins (24, 25), even the ingestion of this large amount of protein is not likely to raise the arginine concentration in the peripheral circulation to anywhere near that produced by the intravenous infusion of arginine used by others or by the ingestion of 30 g arginine.

In addition, enterocytes extensively metabolize arginine (26). As a consequence, only \( \approx 30–40\% \) of ingested arginine appears in the splanchnic circulation, according to one study (27); only \( \approx 20\% \) of a 10-g dose ingested orally is bioavailable, according to another (16). Arginine also is removed by splanchnic tissues, presumably largely by the liver, because an infusion of arginine resulted in an increase in urea production. In overnight-fasted subjects, the fractional extraction was 13%. In subjects fasted for 60 h, it was 37% (15). Nevertheless, intraduodenal installation of arginine (15 g over 40 min) resulted in a small rise in insulin concentration. This was attributed in part to a stimulated release of incretin hormones (28).

We were interested in the effect of the ingestion of a high but attainable dietary amount of arginine. We chose a dose of 1 mmol/kg lean body mass, or \( \approx 10 \) g in a typical 70-kg young man. This is the amount of arginine in 1.7 lb (0.78 kg) beef, a very large—but not unusual—amount offered in some restaurants in the United States and Argentina. Because administered glucose was reported to synergistically increase an arginine-stimulated rise in insulin concentration (9), arginine also was ingested with glucose by the same subjects on a separate occasion.

The amount of arginine ingested in the present study only raised the peripheral plasma arginine concentration by \( \approx 64\% \) in the absence of glucose and \( \approx 27\% \) when ingested with glucose (Figure 1). This increase, as a percentage of baseline, is in the range reported in subjects during the daytime hours when they were ingesting a “normal diet” (\( \approx 44\% \)), the content of which was not specified (16). It is less than the increase after ingestion of 10 g arginine (\( \approx 3.3\)-fold) reported in the same study. Neither the size nor the sex of the subjects was indicated.

In the present study, the administered arginine did not stimulate an increase in insulin concentration when ingested alone, nor did it synergize in stimulating insulin secretion when ingested with glucose. Thus, an increase in plasma arginine of 27–64% was not effective in stimulating insulin secretion. This makes it unlikely that arginine in an amount that reaches the peripheral circulation after a large protein meal is an independent, physiologically significant insulin secretagogue. It does not rule out a contribution to insulin secretion when absorbed with other amino acids.

The stimulation of insulin secretion by arginine has been reported to be dependent on the ambient glucose concentration both in vitro (29, 30) and in vivo (18, 19, 31). Higher glucose concentrations potentiated the insulin response. Whether this occurred in the present study is uncertain. A confounding factor in interpreting the effect of arginine in stimulating insulin secretion when ingested with glucose was the much smaller rise in arginine concentration when arginine was ingested with glucose than when arginine was ingested alone. Another possibility is that arginine actually stimulated more insulin secretion but that it also resulted in an increased extraction of insulin by the liver and thus an increased peripheral insulin concentration was not observed. We consider this unlikely because the insulin response closely mimics the glucose response.

When glucose was ingested independently of arginine, the arginine concentration did not change (Figure 1). Thus, it is unlikely that the glucose ingested with arginine was accelerating the arginine removal rate. The simultaneous ingestion of glucose with the arginine could have reduced the arginine absorption rate or accelerated its metabolism by enteral cells. Whether a larger amount of arginine or glucose ingestion, or both, would change the results remains to be determined.

The attenuation in the rise in serum glucose concentration and the prolongation of this attenuated rise when arginine was ingested with glucose was an unexpected finding (Figure 2). This suggested that the ingested arginine was delaying gastric emptying. However, gastric emptying, as determined by transport of technetium out of the stomach after glucose ingestion, was unaffected by the addition of arginine. Thus, the mechanism by which arginine modifies the glucose serum rise remains to be determined. Presumably it is affecting bowel motility or is directly affecting mucosal transport of the glucose, or both.

Arginine is the direct precursor of nitric oxide. Nitric oxide was reported to be an important regulator of gastrointestinal motility (32). It also was reported to have a regulatory role in esophageal and gall bladder motility (33, 34). Intravenous or intragastric administration of large amounts of arginine was reported to delay gastric emptying in humans (35, 36) and dogs (37). However, this effect also is likely to be caused by a pharmacologic effect of the ingested arginine and not by a physiologic response. We are not aware of data indicating an effect of arginine administration on bowel motility or transport of glucose out of the gut lumen.

In contrast to the lack of effect on insulin concentration in the present study, the ingested arginine clearly increased the peripheral circulating glucagon concentration. The increase in glucagon concentration was not associated with an increase in glucose concentration even though the increase in glucagon concentration in the portal vein must have been much greater than in the peripheral circulation (38). In addition, different immunoreactive species of polypeptides in the circulation are known to be detected with the assay used (39). Only the 3500 M\(_s\) species represents true glucagon and is likely to represent the major species increased.
after arginine administration. Thus, the molar increase of true glucagon is likely to have been greater than indicated by the assay. Presumably the liver did not respond to the rise in glucagon concentration with an increased output of glucose. In the absence of a rise in insulin concentration, it is unlikely that both an increased glucose production and a corresponding increase in glucose removal rate—i.e., an increased glucose turnover rate—were present. However, a direct effect of arginine to increase the glucose removal rate cannot be ruled out (40, 41). If the liver did not respond, then the reason for this lack of response to the relatively large increase in glucagon concentration remains to be explained (glucagon resistance?). The observed modification in the glucagon rise when arginine was ingested with glucose was as expected because insulin and probably glucose inhibit glucagon secretion (see reference 42 and its references).

In summary, an amount of arginine that should be present in a large beef protein meal, when ingested either alone or with glucose, does not increase the serum insulin concentration. However, it does stimulate an increase in glucagon. Arginine, when ingested with glucose, attenuates and prolongs the glucose and the insulin rise when compared with glucose ingestion alone. This attenuation is not caused by delayed gastric emptying. The mechanism remains to be determined. Overall, the present data indicate that the amount of arginine absorbed after ingestion of a mixed meal is not likely to contribute significantly to insulin secretion.

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