Lysine ingestion markedly attenuates the glucose response to ingested glucose without a change in insulin response

Dionysia Kalogeropoulou, Laura LaFave, Kelly Schweim, Mary C Gannon, and Frank Q Nuttall

ABSTRACT
Background: Ingested proteins are known to stimulate a rise in insulin and glucagon concentrations. In our effort to explain this effect, we have begun to measure the effect of individual amino acids.

Objectives: The objectives were to determine the effect of lysine ingestion on insulin and glucagon concentrations and whether the effect is moderated by glucose ingestion.

Design: Thirteen healthy subjects were studied on 4 occasions. Water, 25 g glucose, 1 mmol lysine/kg lean body mass, or lysine plus glucose was given on separate occasions at 0800 after a 12-h fast. Serum lysine, glucose, insulin, and glucagon were measured during a 2.5-h period. The amount of lysine provided was equivalent to that present in a 672-g (24-oz) steak.

Results: Lysine ingestion resulted in an ~3-fold increase in lysine concentration and a small decrease in glucose concentration. When lysine was ingested with glucose, the 2.5-h glucose area response was decreased by 44% (P < 0.02). Lysine alone increased the insulin area response modestly; the insulin increase when lysine was ingested with glucose was similar to that when only glucose was ingested. Lysine stimulated an increase in glucagon (P < 0.02), whereas glucose decreased glucagon.

Conclusions: Lysine ingestion results in a small decrease in serum glucose and an increase in glucagon and insulin concentrations. Lysine ingested with glucose dramatically attenuated the glucose-stimulated glucagon response, but there was no change in insulin response. Whether similar effects will be observed with more physiologic doses of lysine remains to be determined. Am J Clin Nutr 2009;90:1–7.

INTRODUCTION
Protein ingestion stimulates insulin secretion both in healthy individuals and in subjects with type 2 diabetes. In patients with type 2 diabetes, we have reported previously that ingestion of protein with glucose results in a synergistic increase in insulin area response and decreases the plasma glucose response compared with glucose ingestion without protein (1).

Subsequently, 7 different protein sources were tested (2). All tests resulted in an increased insulin response and thus a decreased glucose response. However, the responses observed were different for different proteins, which suggested that a difference in the amino acid composition of the proteins may be responsible for this variation in insulin secretion.

Therefore, we have been systematically evaluating the insulin and glucose responses to individual amino acids ingested with and without glucose. Healthy nondiabetic volunteers are being studied first. To date, insulin and glucose responses to ingestion of glycine (3), proline (4), arginine (5), phenylalanine (6), isoleucine (7), and leucine (8) individually have been reported. In this article, we describe the response to lysine ingestion.

SUBJECTS AND METHODS
Thirteen healthy subjects (6 men and 7 women) were studied between October 1999 and October 2008. All of the subjects gave informed consent before participating in the study, which was approved by the Department of Veterans Affairs Medical Center and the University of Minnesota Committees on Human Subjects. Volunteers did not have type 1 or type 2 diabetes mellitus according to the National Diabetes Data Group criteria (9). The mean age of the subjects was 30 y (range: 21–48 y). Their mean weight was 80 kg (range: 51–103 kg), with mean a body mass index (in kg/m²) of 26 (range: 21–39) and a mean lean body mass of 60 kg (range: 35–77 kg). Lean body mass was measured with a portable Bioelectrical Impedance Analyzer (RJL Systems Inc, Clinton Township, MI). Thyroid, liver, kidney function, and lipid profiles were within normal reference ranges.

Subjects were studied in the special diagnostic and treatment unit at the Minneapolis VA Medical Center, which is similar to a clinical research center. After a 12-h overnight fast, an indwelling catheter was placed into an antecubital vein and kept patent with intravenous saline. Baseline blood samples were obtained at 0730, 0740, and 0750. At 0800, subjects ingested water only on the first visit and then 1 of the 3 test meals in random order subsequently. All of the subjects were given all 4 test meals. Generally, the 4-d study was conducted over a 2-wk period. The meals consisted of 1 mmol lysine/kg lean body mass (mean: 11 g/61 mmol, with a range of 6 g/33 mmol to 13 g/72 mmol) or 1 mmol lysine/kg lean body mass plus 25 g glucose or

1 From the Endocrine, Metabolism & Nutrition Section, VA Medical Center (DK, LL, KS, MCG, and FQN), and the Departments of Medicine (MCG and FQN) and Food Science and Nutrition (MCG), University of Minnesota, Minneapolis, MN.
2 Supported in part by the Department of Veterans Affairs and the Minnesota Medical Foundation. The amino acids were kindly provided by Ajinomoto USA Inc.
3 Address correspondence to FQ Nuttall, VA Medical Center, One Veterans Drive, Room 111G, Minneapolis, MN 55417. E-mail: nutta001@umn.edu.
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25 g glucose alone. Glucose was given as Glutol (Paddock Laboratories Inc, Minneapolis, MN), a d-glucose solution (25 g/45 mL). Lysine was given as l-lysine in 100 mL of water (Ajinomoto USA Inc, Raleigh, NC). Blood was obtained every 10 min for 120 min and then at 150 min.

After the study period, subjects were asked to complete a satiety index. The satiety index consisted of the following 4 questions:
1) How strong is your desire to eat?
2) How hungry do you feel?
3) How full do you feel?
4) How much food do you think you can eat?
5) How pleasant was the test substance?

Answers were given on a linear scale of 1 to 100, with 1 being the least and 100 being the most. Subjects were then provided with a regular mixed meal with more food energy than the subjects could eat, and the amount of food energy (kcal) ingested was calculated.

Serum glucose concentrations were measured with a hexokinase method by using an Abbott Architect analyzer (Abbott Laboratories, Abbott Park, IL). Serum immunoreactive insulin was measured with an automated chemiluminescent assay on a DPC Immulite machine (Diagnostic Products Corp, Los Angeles, CA). Glucagon was measured by radioimmunoassay by using kits purchased from Linco Research (a subsidiary of Millipore Inc, Billerica, MA). α-Amino nitrogen (AAN) was measured with an ω-phthalaldehyde dye-binding method (Gusmer Enterprises Inc, Waupaca, WI). Individual amino acid concentrations were measured by HPLC using precolumn online derivatization with ω-phthalaldehyde and 3-mercaptopropionic acid and 9-fluorophthaldialdehyde dye-binding method (Gusmer Enterprises Inc, Waupaca, WI). The net integrated areas under the curve after test meals. Areas with different letters are statistically significantly different by Student’s t test (P < 0.05).

Sample size was determined using a computer program using the trap-ezoid rule (Veterans Information Systems and Technology Architecture; US Department of Veterans Affairs, Washington, DC).

Statistics were determined with Student’s t test for matched paired variates and the Wilcoxon signed rank test with the StatView 512+ program (Abacus Concepts, Calabasas, CA) for paired variates and the Wilcoxon signed rank test with the Bonferroni correction was applied for multiple comparisons. Data are presented as means ± SEMs.

RESULTS

The mean fasting lysine concentration was 248 ± 17 μM (Figure 1A). After lysine ingestion, the serum lysine concentration doubled at 30 min and continued to rise. It had increased by 2.9-fold at 150 min. Similarly, after lysine and glucose ingestion, the lysine concentration, after a 10-min delay, started increasing linearly at the same rate as with only lysine ingestion. After ingestion of glucose or water, the lysine concentration remained at baseline. The lysine area response was significantly increased after lysine and lysine plus glucose ingestion compared with water or glucose (P < 0.001; Figure 1B). The area response of lysine ingestion alone was not significantly different from the lysine plus glucose response (P = 0.25).

The mean fasting AAN concentration was 3.7 ± 0.05 mg/dL. After lysine or lysine plus glucose ingestion, the AAN concentration increased above the baseline in a similar manner (Figure 2A). The AAN concentration remained stable after 40 min. Glucose ingestion had a lowering effect on the AAN concentration. When glucose was ingested with lysine, the increase in AAN concentration was modestly attenuated compared with lysine ingestion alone. There was little variation in the AAN concentration after water ingestion. The AAN area response was significantly increased after lysine ingestion compared with glucose ingestion (P = 0.001) and water ingestion (P < 0.004; Figure 2B). There was no difference between the AAN area after lysine or lysine plus glucose (P = 0.19).

The mean fasting glucose was 82 ± 1 mg/dL. When glucose was ingested alone, the glucose concentration increased by 48 mg/dL at 40 min (Figure 3A). It returned to baseline at 120 min. After glucose plus lysine ingestion, the maximal glucose concentration reached was decreased by 35%. When lysine was ingested alone, the glucose concentration decreased below the baseline for 120 min and returned to the baseline at 150 min. After water ingestion, the glucose concentration remained stable.

After ingestion of lysine plus glucose, the glucose area response was decreased by 44% (P = 0.02) when compared with glucose ingestion alone (Figure 3B). After lysine ingestion
alone, the glucose area response also was significantly decreased when compared with water ingestion ($P = 0.02$).

The mean fasting insulin concentration was $7 \pm 0.5 \mu U/mL$ (Figure 4A). After glucose ingestion, the insulin concentration increased, reaching a maximum concentration of $34 \mu U/mL$ at 30 min. It then returned to baseline at 150 min. When lysine was ingested with glucose, the insulin concentration increased and reached a maximum of $38 \mu U/mL$ at 30 min and then gradually decreased. However, it still remained elevated at 150 min. Lysine ingestion alone resulted in a modest increase in insulin concentration, and this persisted throughout the 150 min of the experiment. The insulin concentration did not change after water ingestion.

The insulin area response was similar after glucose ingestion compared with the glucose plus lysine ($P = 0.6$; Figure 4B). After lysine ingestion, the insulin area response was higher than with the water. The difference did not reach statistical significance by $t$ test ($P = 0.08$), although it reached significance by the Wilcoxon signed rank test ($P = 0.02$).

The mean fasting glucagon concentration was $62 \pm 2 \text{ pg/mL}$. After glucose ingestion, the glucagon concentration decreased and remained below the baseline for the duration of the study (Figure 5A). Ingestion of glucose plus lysine also resulted in a decrease in the glucagon concentration, but the decrease was less than in those who ingested glucose alone. In contrast, when lysine was ingested alone, the glucagon concentration increased and remained elevated during the study.

As expected, after glucose ingestion the glucagon area response was negative (Figure 5B). It also was negative after water ingestion. After lysine ingestion, the glucagon area response was positive. When lysine was ingested with glucose, the glucagon response was less negative compared with the response to the glucose ingestion alone. However, the response to the ingestion of glucose and lysine together did not equal the sum of the responses to each meal individually, ie, lysine was less potent in increasing than glucose was in decreasing the response. The glucagon area response after lysine ingestion was statistically higher than the response after water ingestion ($P = 0.009$) as well as after glucose ingestion with lysine ($P = 0.02$) or glucose alone ($P = 0.004$).

A total of 91% of the satiety questionnaires was completed. The answers for all of the components for the satiety index were given on a scale of 1 to 100, with 1 being the least and 100 being the most. Because of multiple comparisons, a Bonferroni correction was applied. A $P$ value of 0.008 is required for statistical significance. The desire to eat after glucose plus lysine ($55 \pm 6$) and lysine ($55 \pm 6$) was less compared with water ($74 \pm 5$; $P < 0.02$ and 0.005, respectively). Similarly, the proposed intake after glucose plus lysine ($59 \pm 5$) and lysine ($61 \pm 5$) was less.
compared with water (75 ± 6; P < 0.02). In addition, the degree of hunger was decreased after glucose and lysine ingestion (52 ± 5) compared with water (69 ± 5; P = 0.02). Lysine was reported to have an unpleasant taste compared with glucose (32 ± 8 compared with 63 ± 4; P = 0.006) and water (53 ± 7; P < 0.09). Co-administration of glucose did not change the pleasantness score (34 ± 6).

Most subjects experienced mild gastrointestinal upset after lysine ingestion. One subject complained of abdominal discomfort lasting 2–3 h. Six subjects experienced 1 to 2 episodes of diarrhea in the afternoon after the lysine was ingested. Symptoms were mild and did not interfere with daily activities.

Subjects ate the breakfast provided only 83% of the time. Participants consumed a mean total energy of 600 kcal (range: 515–658 kcal). There was no difference in carbohydrate, protein or fat intake after either of the test meals.

**DISCUSSION**

Lysine, 1 of the 3 basic amino acids, is an indispensable amino acid. It undergoes transamination at the ε-amino group forming the metabolite, saccharopine. The ultimate final product of lysine catabolism is acetoacetyl-CoA, which enters the tricarboxylic acid cycle. Lysine also is an important precursor of carnitine. Conversion of lysine to carnitine is a multistep process beginning with methylation of the ε-amino group of a peptide-linked lysine, giving trimethyllysine. Hydrolysis of the proteins containing trimethyllysine provides the substrate for the subsequent conversion to carnitine.

The estimated average requirement for lysine in adults is 31 mg · kg⁻¹ · d⁻¹ (10), equivalent to 2.2 g/d for a 70-kg person. The mean daily intake of lysine is ≈5.3 g/d. Men between ages 50 and 71 had the highest intakes, 12.5 g/d (10). Lysine is found in high quantities in red meat [≈0.5 g/28 g (1 oz) of beefsteak] (11). The amount of lysine ingested by the subjects in the present study (≈11 g) corresponds to the amount in 672 g (24 oz) of beefsteak.

Intravenous administration of 30 g of lysine in healthy individuals resulted in marked insulin secretion. Lysine was the second most potent amino acid insulin secretagogue. Arginine was the most potent when given intravenously (12).

In another study, the effect of chronic ingestion of arginine with lysine supplementation on glucose tolerance was reported in
30 healthy individuals. Subjects received 66 mg each of L-arginine and L-lysine · kg lean body mass⁻¹ · d⁻¹ or placebo for 10 wk. The doses were 4–5 g/d of each amino acid. The pilot studies suggested that higher doses resulted in significant abdominal cramps and diarrhea. Lysine plus arginine supplementation did not significantly affect the glucose or insulin response. However, there was a significant increase in glucagon concentration (13).

In a different study, ingestion of a single dose of 1200 mg of lysine plus 1200 mg of arginine by healthy individuals resulted in an increase in insulin concentration (65% increase from baseline at 30 min) and an increase in growth hormone concentration. This effect was not observed when either of the amino acids was ingested alone (14).

We are not aware of any previous studies in humans in which the effect of oral lysine with or without glucose on glucose metabolism in healthy individuals has been studied. As mentioned above, lysine is a potent insulin secretagogue when administered intravenously in very large amounts. In our study, ingestion of an amount of lysine likely to be ingested with a very large protein meal resulted in a small insulin response that was not statistically significantly different from water ingestion only ($P = 0.08$). When lysine was ingested with glucose, the insulin response was similar to that resulting from ingestion of glucose alone. However, the rate of insulin increase in the first 20 min was greater after lysine with glucose ingestion compared with glucose alone. This implies that lysine may have an effect on first-phase insulin secretion.

Lysine ingestion alone did have a significant lowering effect on the glucose area response compared with water ingestion ($P = 0.02$). Similarly, when lysine was given with glucose, it dramatically attenuated the glucose area response compared with glucose ingestion alone. This suggests that lysine is either facilitating the beta cell insulin response to a rise in glucose concentration or it independently facilitates the removal of glucose.

Lysine in this study was given at a high dose (mean 11 g). Others have reported that lysine, when given at more physiologic doses (between 1 and 5 g/d), did not have an effect on insulin secretion (13, 14). These studies did not evaluate the effect of the combination of lysine with glucose. Thus, it will be of interest to examine whether lysine attenuates the glucose response when ingested at lower doses using the same protocol.

We have reported the effect of arginine on insulin and glucose response when ingested with or without glucose using the same

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**FIGURE 4.** A: Mean (±SEM) time course of serum insulin concentrations. B: Net integrated areas under the curve after ingestion of test meals. Areas with different letters are statistically significantly different by Student’s $t$ test ($P < 0.05$).
protocol. Even though it was a potent insulin secretagogue when administered intravenously, as was lysine, arginine did not stimulate an increase in insulin concentration when ingested alone nor did it synergize in stimulating insulin secretion when ingested with glucose (5). Our preliminary data also indicate that oral histidine, another basic amino acid, does not stimulate insulin secretion or lower the glucose concentration (FQN and MCG, unpublished data, 2009).

Lysine, as did other amino acids, stimulated a glucagon response (3–5, 7, 15). Lysine has been used extensively at high doses both in short- and long-term studies, as indicated above, and no serious side effects have been described. It was reported to be well tolerated in a study of 3 individuals who ingested 10–20 g/d of lysine in divided doses for >12 d (16). However, diarrhea was reported in patients with heart disease who ingested 40 g/d in divided doses for 2–5 d. Symptoms were mild and self-limiting and patients completed the study (17). In agreement with these reports, the majority of the participants in our study described mild gastrointestinal upset, mainly diarrhea. The symptoms started after the completion of the study and did not last for >3 h. This could be explained by the observation that the lysine concentration was still rising at the end of the study. Symptoms were not related to the total amount of lysine ingested, sex of the subject, or body weight. All of the participants ingested ≥8 g of lysine.

The participants reported less desire to eat when they consumed lysine with or without glucose compared with water. The participants did not complain of any gastrointestinal symptoms when poststudy meals were served. Therefore, we can assume that lysine had a mild satiety effect. Food energy was the lowest after lysine ingestion, but this was not statistically significant. The subjects consumed more protein after lysine compared with water ingestion and fewer carbohydrates when lysine was added to glucose. Even though the major focus of the study was not to examine the effect of lysine ingestion on satiety and food intake, these remain interesting observations that deserve further investigation. Previous studies have shown that either intravenous infusion of amino acids or oral amino acid ingestion results in higher satiety scores but not necessarily lower energy intakes (18, 19). It would be interesting to extend the study period to study

![FIGURE 5. A: Mean (±SEM) time course of the change in serum glucagon concentrations. Fasting glucagon concentrations were 64 ± 5 pg/mL for water, 63 ± 4 pg/mL for glucose, 64 ± 4 pg/mL for glucose plus lysine, and 59 ± 4 pg/mL for lysine, respectively. B: Net integrated areas under the curve after ingestion of test meals. Areas with different letters are statistically significant by Student’s t test (P < 0.05).](image-url)
effect on lysine concentration on satiety, gastrointestinal symptoms, and insulin concentrations.

In conclusion, lysine, when ingested in relatively high physiological doses, results in a small decrease in the glucose concentration and an increase in glucagon and insulin concentrations. However, when lysine was ingested with glucose, it resulted in a dramatic attenuation of the glucose response without an accompanying increase in insulin concentration. Whether similar effects on glucose will be observed in smaller, more physiologic doses needs to be determined.

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The authors’ responsibilities were as follows—DK, LL, and KS: involved in subject recruitment and data collection; DK: performed the data analysis; FQN, MCG, and DK: participated in the discussion of the results, wrote the manuscript, and approved the final version of the manuscript; and FQN and MCG: conceived the hypothesis for the study and were responsible for the overall study design. None of the authors had a personal or financial conflict of interest.

REFERENCES