The Metabolic Response to Ingestion of Proline With and Without Glucose

Frank Q. Nuttall, Mary C. Gannon, and Kelly Jordan

Ingested protein results in an increase in circulating insulin and glucagon concentrations and no change, or a slight decrease, in circulating glucose. In subjects with type 2 diabetes, when protein is ingested with glucose, insulin is further increased and the glucose rise is less than when glucose is ingested alone. Presumably these effects are due to the amino acids present in the proteins. The effects of individual amino acids, ingested in physiologic amounts, with or without glucose, have not been determined. Therefore, we have begun a systematic study of the response to ingested amino acids. Eight young, non-obese, subjects (4 men, 4 women) ingested 1 mmol proline/kg lean body mass, 25 g glucose, 25 g glucose + 1 mmol proline/kg lean body mass or water only on 4 separate occasions at 8 AM. Blood was obtained before and after ingestion of the test meal over the following 150 minutes. Proline ingestion resulted in a 13-fold increase in the plasma proline concentration. This was decreased by 50% when glucose was ingested with proline. Proline alone had little effect on glucose, insulin, or glucagon concentrations. However, ingestion of proline with glucose resulted in a 23% attenuation of the glucose area response and no change in insulin response compared with the response to that of glucose alone. A glucose-stimulated decrease in glucagon was further facilitated by proline. Ingested proline is readily absorbed. It reduces the glucose-induced increase in glucose concentration in the presence of an unchanged insulin and a decreased glucagon response.

DIETARY PROTEIN ingestion clearly results in an increase in circulating insulin and glucagon in people with and without type 2 diabetes.[1] However, it does not result in an increase in glucose concentration even though the amino acids resulting from digestion of the protein can be converted into glucose through gluconeogenesis.[2] Indeed, protein ingested with glucose may actually diminish the glucose response to the ingested glucose.[3,4] Nevertheless, the insulin and glucose response to individual protein sources varied considerably.[3]

Because proteins are composed of 20 different amino acids and the composition of each protein is different, we have begun a systematic study of the effect of ingested individual amino acids on the circulating glucose, insulin, and glucagon concentrations when ingested with and without 25 g glucose. The amount of amino acid ingested in each case is 1 mmol/kg lean body mass. The subjects are normal young adults.

We realize that the metabolic response to intact proteins may be different from that resulting from ingestion of the individual constituent amino acids. Nevertheless, we think evaluating the response to individual amino acids should be the first step in trying to understand how proteins stimulate insulin secretion and lower the blood glucose concentration after glucose ingestion and why the response varies among different protein sources.

The results from the ingestion of arginine[5] and glycine[6] have been published previously. Others have reported that alanine administered orally also increases plasma insulin,[7] with little change in plasma glucose. The effect of ingestion of alanine with glucose was not studied. The response to ingestion of proline is presented in this report.

MATERIALS AND METHODS

Four men and 4 women were studied. All subjects were nondiabetic, based on the National Diabetes Data Group criteria for the diagnosis of diabetes. The mean age of the subjects was 28 (range, 22 to 38), and mean body mass index (BMI) was 23 (range, 21 to 34). Mean body weight was 80 kg (range, 58 to 107 kg).

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 Committee on Human Subjects. All subjects had fasted 12 hours overnight before testing.

Each subject was admitted to the Special Diagnostic and Treatment Unit (SDTU) on the morning of the study. Body composition was determined using bioelectrical impedance (RJL Systems, Detroit, MI).

An indwelling catheter was inserted into a forearm vein and kept patent with intravenous saline. Baseline blood samples were drawn at 7:30 AM, 7:40 AM, and 7:50 AM. At 8 AM, subjects ingested 1 of 4 test solutions in random order. The test solutions consisted of: (1) 1 mmol proline/kg lean body mass, (mean 6.0 g [53 mmol] with a range of 5.9 g [41 mmol] to 8.2 g [71 mmol]); (2) 25 g glucose (45 mL Glutol); (3) 1 mmol proline/kg lean body mass plus 25 g glucose; or (4) water only. The pH of the amino acid containing solutions was adjusted to 7.0. All subjects ingested all 4-test solutions. The volume of the solutions ingested was 150 mL and the time for ingestion was less than 1 minute. Blood was obtained every 10 minutes after ingestion of the test solution for 120 minutes and then again at 150 minutes.

There is some evidence that protein has an effect on satiety.[8] Therefore, we were interested in whether individual amino acids also could affect satiety. To assess this, the subjects were asked to complete a satiety index after the final blood draw (155 minutes after ingestion of test solution). The satiety index consisted of the following 5 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 could eat? (5) How pleasant have you found the test substances? The subjects provided a numerical response using a linear scale of 1 to 100 with 1 being the least and 100 being the most. They then were served a regular mixed meal with more food energy than the subjects could eat and the amount of food energy (kcal) ingested was calculated by the study

From the Section of Endocrinology, Metabolism, and Nutrition, Veterans Affairs Medical Center, Minneapolis; and the Departments of Medicine, and Food Science and Nutrition, University of Minnesota, Minneapolis, MN.

Submitted June 20, 2003; accepted September 9, 2003.

Supported in part by a grant from the Minnesota Medical Foundation, by funds from the Minnesota Obesity Center, and by Merit Review funds from the Medical Research Service, Department of Veterans Affairs.

Address reprint requests to Frank Q. Nuttall, MD, PhD, Chief, Section of Endocrinology, Metabolism, and Nutrition, VA Medical Center (111G), One Veterans Dr, Minneapolis, MN 55417.

0026-0495/04/5302-0016$30.00/0

doi:10.1016/j.metabol.2003.09.013

dietician using Nutritionist V as a reference. The 4-day study was conducted over a 2-week period.

Plasma glucose was determined by a glucose oxidase method using a Beckman glucose analyzer with an O_2 electrode (Beckman Instruments, Fullerton, CA). Serum immunoreactive insulin was measured using an automated chemiluminescent assay on DPC's IMMULITE machine (Diagnostic Products, Los Angeles, CA). Glucagon was determined by radioimmunoassay (RIA) using kits purchased from Linco (St Louis, MO). Alpha amino nitrogen (AAN) was determined by the method of Goodwin.[9] Individual amino acid concentrations were determined by high-performance liquid chromatography (HPLC) in the laboratory of Dr K.S. Nair at the Mayo Medical School, Rochester, MN.

The net 150-minute area responses, ie, areas above baseline, were calculated using a computer program based on the trapezoid rule.[10] Food energy consumption was calculated using the computer software Nutritionist V (Hearst, San Bruno, CA). Statistics were determined using Student's *t* test for paired variates, analysis of variance (ANOVA), or Wilcoxon's signed-rank with the StatView 512+ program (Abacus Concepts, Calabasas, CA) for the Macintosh computer (Apple Computer, Cupertino, CA), as appropriate. A P value of $\leq .05$ was the criterion for significance. Data are presented as means ± SEM.

RESULTS

After the subjects ingested proline alone the proline concentration increased rapidly and reached a concentration approximately 13-fold over the initial concentration. Ingested glucose had little effect on the proline concentration. However, when glucose was ingested with proline, it reduced the proline area response by 50% (Fig 1).

The mean fasting glucose concentration was 4.6 ± 0.13 mmol/L (82 ± 2.3 mg/dL). After ingestion of glucose alone, the glucose concentration increased steadily to 7 mmol/L (126 mg/dL) at 40 minutes. It remained elevated for 30 minutes before gradually returning to the fasting concentration at approximately 150 minutes. After ingestion of glucose plus praline, the glucose concentration increased at the same rate, but reached a peak earlier (30 minutes) when compared with glucose alone. The maximum concentration also was lower (6.2 mmol/L or 111 mg/dL). After the ingestion of proline alone, the glucose concentration was essentially unchanged from that when only water was ingested (Fig 2A).

When proline was ingested with glucose, the glucose area response above baseline was attenuated by 23% when compared with the ingestion of glucose alone. This was statistically significant ($P < .05$). The area response to proline alone was not different from the area response to water ingestion (Fig 2B).

The mean fasting serum insulin concentration was 8.5 ± 1.7 μU/mL (51 ± 10 pmol/L). When subjects ingested glucose alone, the serum insulin concentration increased to a maximum of 33 μU/mL (199 pmol/L) at 30 minutes. It remained elevated for 30 minutes before gradually decreasing to a final concentration of 12 ± 4.6 μU/mL (72 ± 28 pmol/L) at 150 minutes. After the ingestion of glucose with proline, the serum insulin concentration reached a peak of 41 ± 13 μU/mL (246 ± 76) pmol/L). The peak occurred earlier (20 minutes) and remained elevated at this concentration for 20 minutes over that observed when only glucose was ingested. It then returned to the fasting concentration at 150 minutes. When proline was ingested alone, there was a slight, but persistent, increase in serum insulin concentration (Fig 3A).

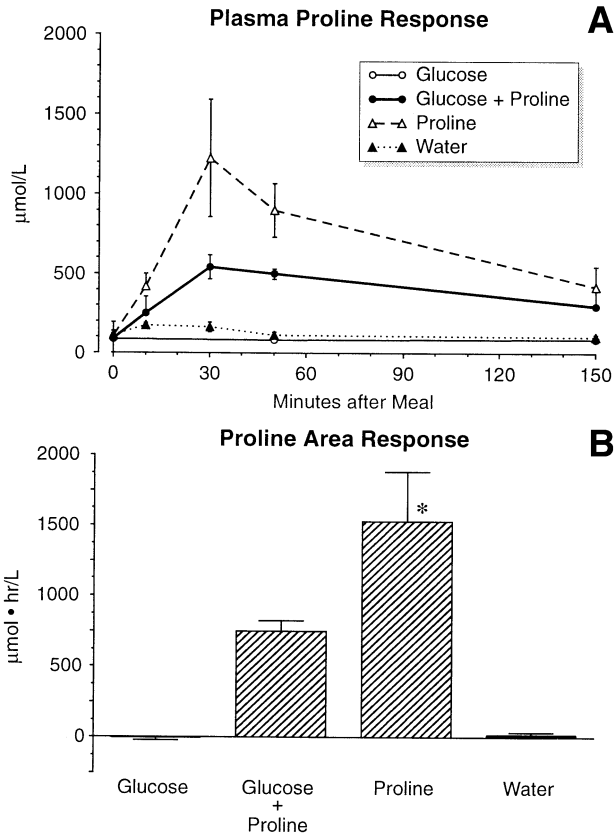

Fig 1. (A) Mean (± SEM) plasma proline concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated areas under the curve (AUC) using the fasting values as baseline. *Significantly different from glucose + proline using Student's *t* test ($P < .008$).

The mean 150-minute integrated serum insulin area response was 11% greater after subjects ingested glucose plus proline compared with when glucose was ingested alone. This was not significant ($P = .29$). When analyzed over 50 minutes as an index of the more rapid increase in insulin, the difference was 20% greater than when subjects ingested glucose alone. This was statistically significant ($P = .047$). In addition, the rate of increase in insulin concentration determined by slope analysis was statistically significantly greater after glucose + proline compared with proline alone ($P < .04$). This increased rate occurred even though the proline increase was greatly diminished (Fig 1A). The insulin area response to ingested proline alone compared with water integrated over 150 minutes, although small, was statistically significant ($P < .02$) (Fig 3B). In fact, it was greater in every individual.

The initial mean fasting glucagon concentration varied modestly. However, the overall mean was 62 ± 9 pg/mL. After the subjects ingested glucose, the glucagon concentration decreased as expected. A similar result was seen after ingestion of glucose plus proline. The mean glucagon concentration remained below the fasting concentration throughout the period of study. After the ingestion of proline alone, the mean gluca-

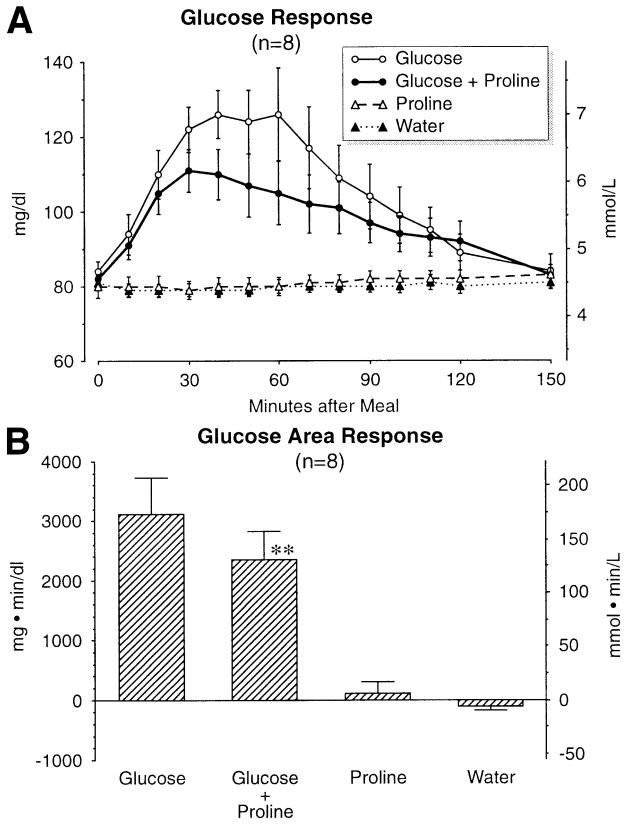

Fig 2. (A) Mean (± SEM) plasma glucose concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated AUC using the fasting values as baseline. **Significantly different from glucose alone using Student's *t* test ($P < .05$).

gon concentration gradually decreased and generally the decrease was greater than after water ingestion (Fig 4A).

The mean integrated glucagon area response was negative after glucose ingestion. The mean glucose area response after the ingestion of proline with glucose also was negative. This was more negative than with glucose alone, and the difference was statistically significant using Wilcoxon's signed-rank analysis ($P = .03$). Proline ingested independently also resulted in a decrease in glucagon area response, but this did not quite reach statistical significance using Wilcoxon's signed-rank analysis ($P = .08$) (Fig 4B).

The mean fasting AAN concentration was 3.7 ± 0.1 mg/dL. The AAN concentration decreased after the ingestion of glucose alone, whereas, it remained unchanged after water ingestion. The AAN concentration increased promptly after proline ingestion, reached a maximum at 30 minutes, and then decreased, but was still elevated at the end of the study. When glucose was ingested with the proline, the increase was attenuated by approximately 50%, but the elevation in concentration remained for the duration of the study (Fig 5A).

The mean integrated negative AAN area response after glucose ingestion was statistically significant when compared with water ingestion alone ($P < .02$). The smaller increase in AAN area after ingestion of proline with glucose, compared with ingestion of only glucose, was highly significant ($P < .001$) (Fig 5B).

The response of individual amino acids to ingestion of water, glucose, praline, and proline with glucose is shown in Table 1. The concentrations of leucine, isoleucine, and valine decreased after ingestion of glucose alone or after ingestion of glucose with proline, but not when proline alone was ingested. The concentration of the other amino acids (serine, tyrosine, alanine, glycine, phenylalanine, methionine, threonine, cysteine, glutamine, lysine, arginine, histidine, and glutamate) did not change significantly after ingestion of proline, glucose, or proline with glucose (data not shown). Because blood was drawn from a forearm vein, rather than from an arterialized hand vein, it is possible that part of the effect noted could have been due to local extraction of glucose and/or amino acids by forearm tissues. However, we doubt that this was significant because the venous catheter generally was inserted just above the wrist.

The subjects' sensory response to the ingested substances is shown in Table 2. The subjects' desire to eat was significantly lower by rank sum analysis ($P = .05$) after subjects ingested proline alone and after glucose plus proline ($P = .002$), but not after glucose alone (rank sum $P = 0.1$) when compared with water only. The subjects also reported a greater degree of fullness after ingestion of proline alone, proline plus glucose,

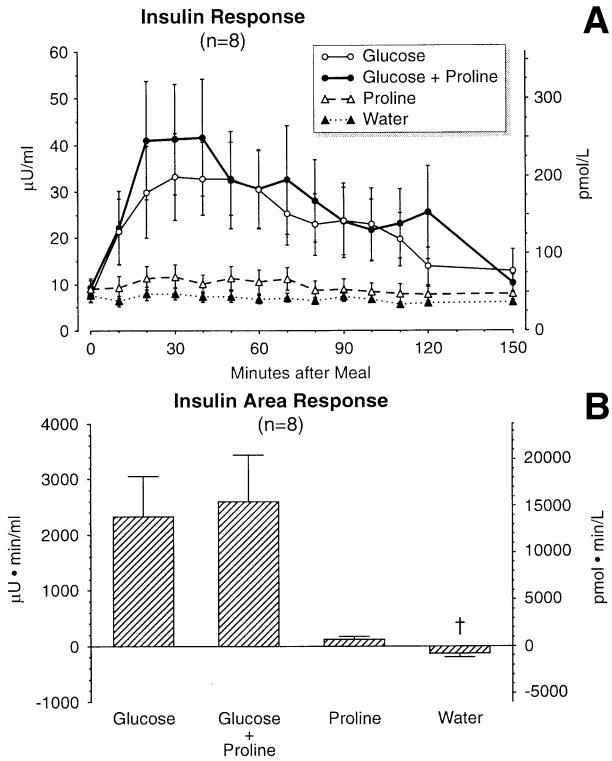

Fig 3. (A) Mean (± SEM) serum insulin concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated AUC using the fasting values as baseline. †Significantly different from proline alone using Student's *t* test ($P < .02$).

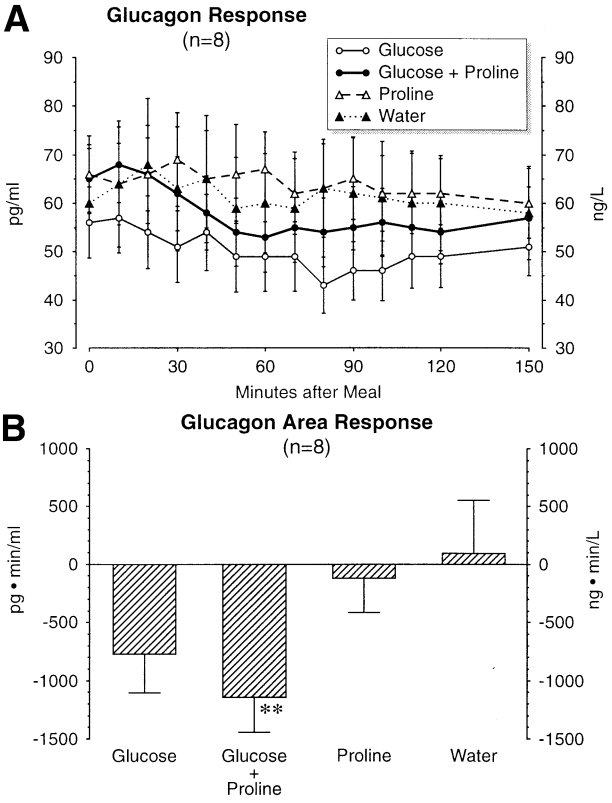

Fig 4. (A) Mean (± SEM) plasma glucagon concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated AUC using the fasting values as baseline. **Significantly different from glucose alone using Wilcoxon's sign rank test.

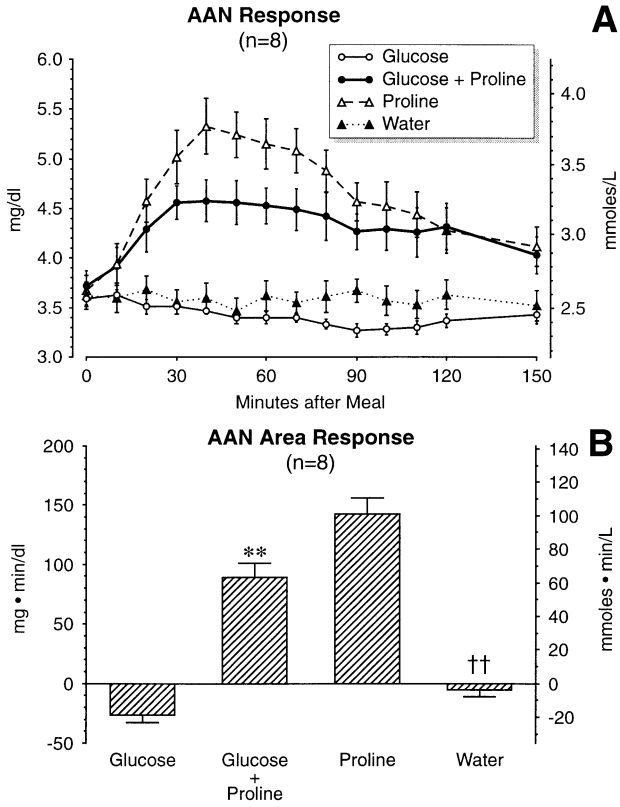

Fig 5. Mean (± SEM) plasma AAN concentration in 8 healthy subjects after ingestion of water only (▲), 25 g glucose (○), 1 mmol proline/kg lean body mass (△), or 25 g glucose + 1 mmol proline/kg lean body mass (●). (B) Net integrated AUC using the fasting values as baseline. **Significantly different from glucose alone using Student's *t* test ($P < .001$); ††significantly different from glucose alone using Student's *t* test ($P < .02$).

and glucose alone compared with when they ingested water only ($P < .05$). However, the difference in fullness was not significantly different when the subjects ingested glucose with or without proline. Nevertheless, on average, they ate more of the mixed meal after proline was ingested (797 ± 80 kcal *v* 642 ± 81 kcal) than after water alone, but this was not statistically significant. After ingestion of glucose alone and glucose plus proline, subjects ingested 555 ± 68 kcal and 635 ± 53 kcal, respectively. Again, the subjects ingested more after proline ingestion. These differences also were not significant ($P = .23$).

The subjects found the sandy, grainy texture of the proline unpleasant, and a few described a bitter or sour taste. The subjects did not experience any unpleasant sensations, an unpleasant after-taste, or constitutional or gastrointestinal symptoms after the test meals.

DISCUSSION

Proline in proteins is important because its structure does not allow it to be present in the formation of an a-helix in the amino acid chain.[11] Proline is quantitatively absorbed after digestion of proteins.[12] Indeed, in the present study the peripheral circulating proline concentration had increased by 10 minutes after its ingestion, and the concentration had increased approximately 13-fold by 30 minutes. Absorbed proline is metabolized to glutamine, which can enter the tricarboxylic cycle and ultimately be converted to glucose.[11]

Table 1. Plasma Amino Acid Concentrations

Time	0	50 Minutes	150 Minutes
Isoleucine			
Water	61 ± 7	53 ± 4	57 ± 5
Glucose	65 ± 4	43 ± 5*	39 ± 2*
Proline	56 ± 4	50 ± 3	51 ± 4
Pro + gluc	62 ± 5	49 ± 7	34 ± 5*
Leucine			
Water	118 ± 6	117 ± 10	112 ± 4
Glucose	127 ± 5	95 ± 5*	89 ± 4*
Proline	114 ± 8	105 ± 8	104 ± 4
Pro + gluc	132 ± 10	100 ± 12	74 ± 11*
Valine			
Water	211 ± 12	183 ± 7	199 ± 16
Glucose	162 ± 39	128 ± 33	129 ± 31
Proline	184 ± 15	168 ± 6	179 ± 13
Pro + gluc	219 ± 22	198 ± 22	154 ± 20

*Statistically significant compared with 0 time (ANOVA).

Table 2. Satiety Index and Caloric Consumption

	Glucose	Glucose + Proline	Proline	Water	Significance
Desire to eat	64 ± 6	58 ± 8	62 ± 8	71 ± 7	¥¥∧
Degree of hunger	59 ± 8	57 ± 8	59 ± 7	70 ± 6	†† ¥¥∧
Fullness	33 ± 7	42 ± 9	36 ± 8	24 ± 5	†¥∧
Proposed intake	62 ± 6	63 ± 8	68 ± 7	70 ± 7	†¥
Test meal taste	58 ± 8	47 ± 11	46 ± 11	59 ± 6	∧∧
Caloric intake (kcal)	563 ± 84	635 ± 53	797 ± 80	642 ± 81	
Protein (g)	24 ± 5	24 ± 4	28 ± 6	24 ± 3	
CHO (g)	67 ± 15	96 ± 11	117 ± 10	85 ± 19	ΔΔ
Fat (g)	23 ± 6	19 ± 4	27 ± 4	25 ± 5	

Statisical Significance Denoted by the Following Symbols		
	Rank Sum	P Value and Rank Sum
Glucose *v* glucose + proline	*	**
Glucose *v* proline	Δ	ΔΔ
Glucose *v* water	†	††
Glucose + Pro *v* proline	√	√√
Glucose + Proline *v* water	¥	¥¥
Proline *v* water	∧	∧∧

NOTE. Average response on a scale of 1-100 with 1 being the least and 100 the most. See Materials and Methods for more details.
Desire to eat: Glucose + proline is significantly less than glucose. Proline is significantly less than water (rank sum only).
Degree of hunger: Glucose, glucose and proline are significantly less than water. Proline is significantly less than water (rank sum only).
Fullness: Glucose, glucose + proline and proline are significantly higher than water (rank sum only).
Proposed intake: Glucose, glucose + proline are significantly less than water (rank sum only).
Test meal taste: Proline is significantly less than water.
CHO intake: Glucose is significantly less than proline.

The proline content of meats is approximately 4% to 5% on a molar basis. In cottage cheese, it is approximately 10%.[12] Thus, the amount of proline ingested by the subjects in this study was the equivalent of that found in 0.38 pounds (6 ounces) of beef or 0.15 pounds (2.4 ounces) of cottage cheese. These represent readily attainable amounts of proline ingested in a single meal. In the collagen family of proteins, another dietary source of protein, proline and its hydroxylated derivative, hydroxyproline, make up approximately 20% to 24% of the total number of amino acids present.

We were particularly interested in the effect of proline on circulating insulin and glucagon concentrations, because we previously had shown that ingested gelatin, the hydrolyzed product of collagen, strongly potentiated a glucose-stimulated increase in insulin (~270%) and reduced the plasma glucose response by approximately 30% in people with type 2 diabetes.[3] We were surprised by these results because the amino acid composition was considerably different from that of the other proteins studied.

Glycine, proline, and hydroxyproline make up approximately 55% to 65% of the total amino acids present in collagen, thus, we assumed that these amino acids contributed to the greatly increased insulin and smaller glucose response in people with type 2 diabetes when gelatin was ingested with glucose compared with when glucose was ingested alone.[3] However, neither glycine nor proline had been reported to stimulate insulin secretion.

Using the same protocol as used here, we previously have reported that glycine, the other major amino acid present in collagen, reduced the plasma glucose area response to ingested glucose by greater than 50% without a difference in insulin area response in normal subjects. This suggests that glycine stimulated insulin secretion in the presence of an increased glucose concentration. When glycine was ingested alone, it also stimulated a small increase in insulin concentration. Glycine ingestion also strongly stimulated an increase in glucagon concentration.[6]

The present data indicate that proline derived from the digestion of gelatin also is likely to have contributed to the smaller glucose response noted previously in the diabetic subjects when they ingested gelatin with glucose compared with when they ingested only glucose.[3] As with glycine, a proline-facilitated insulin response to glucose likely was playing a role. However, on a molar basis, glycine was more effective[6] than proline in reducing the glucose response. This occurred even though the quantitative insulin responses were similar.[6] A kinetic difference in response also was present. Proline stimulated an early increase in insulin, whereas, with glycine, the increase was slower than when glucose was ingested alone. An early increase in insulin, ie, a first phase insulin response is clearly important in maintaining a normal glucose concentration after a meal.[13,14]

To our knowledge, this is the first report of an effect on glucose and insulin metabolism by proline. In addition, based on the amount ingested, the effect is likely to be physiologically relevant.

The glucagon responses to glycine and proline clearly were different. Glycine stimulated an increase in concentration; proline did not. In fact, it resulted in a further decrease in glucagon concentration when it was ingested with glucose (Fig 3). Thus, glycine, but not proline, could have contributed to the robust

increase in glucagon concentration observed after gelatin ingestion.[3]

Of interest from a mechanistic perspective, the attenuation in glucose response was greater after glycine ingestion with glucose than after proline ingestion with glucose even though glycine and not proline stimulated an increase in glucagon concentration. This is contrary to the traditional concept of glucagon and insulin having opposing effects on glucose production in the liver.[15]

Another unexpected finding was that when glucose was ingested with proline, it greatly reduced the increase in proline concentration observed when proline was ingested alone (Fig 1). This must have been due to a decreased absorption rate or increased removal rate or both. The present data do not allow us to choose between these possibilities. Because glucose, when ingested alone, did not affect the proline concentration, most likely the simultaneous ingestion of glucose with the proline affected the absorption rate. Nevertheless, this remains to be documented. The decrease in leucine, isoleucine, and valine concentrations, which occurred after glucose or after glucose with proline ingestion, was as expected with an increase in insulin concentration.[16]

It also is of interest that proline, when ingested alone, decreased the subjects desire to eat and increased their sense of fullness (Table 2). However, on average, they ate more after the study than when they ingested water, glucose, or glucose with proline. The explanation for this is uncertain.

In the future, it will be of interest to determine if the hydroxylated prolines affect the circulating glucose, insulin, or glucagon concentrations. It also will be of interest to determine if there is an additive or even a synergistic effect on blood glucose and insulin concentrations when proline and glycine are ingested simultaneously in the same molar amounts. In addition, whether proline ingestion will reduce the glycine-stimulated increase in glucagon just as glucose did,[6] will be of interest. Whether the effect of the 2 amino acids can explain the additional stimulation of insulin secretion when gelatin was ingested with glucose by people with untreated type 2 diabetes[3] also will be of interest to determine. Finally, it will be of interest to determine if proline stimulates food ingestion significantly when a larger number of subjects are studied and when the amount of proline ingested is varied.

ACKNOWLEDGMENT

We thank the subjects for participating in the study, the staff of the Special Diagnostic and Treatment Unit, the staff of the Clinical Chemistry Laboratory, Dr S.K. Nair, and Dawn Morse for the amino acid determinations, Terry Masai for his interest in our work and help in obtaining the amino acids, and Dr Michael Kuskowski for advice on the statistical presentation of the data. Proline was a gift from Terry Masai and the Ajinomoto Company.

REFERENCES

1. Gannon MC, Nuttall FQ: Protein and diabetes. in Franz MJ, Bantle JP (eds): American Diabetes Association Guide to Medical Nutrition Therapy for Diabetes. Alexandria, VA, American Diabetes Association, 1999, pp 107-125

2. Nuttall FQ, Gannon MC: Metabolic response to dietary protein in persons with and without diabetes mellitus. Diabetes Nutr Metab 4:71-88, 1991

3. Gannon MC, Nuttall FQ, Neil BJ, et al: The insulin and glucose responses to meals of glucose plus various proteins in type 2 diabetic subjects. Metabolism 37:1081-1088, 1988

4. Nuttall FQ, Mooradian AD, Gannon MC, et al: Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. Diabetes Care 7:465-470, 1984

5. Gannon MC, Nuttall JA, Nuttall FQ: Oral arginine does not stimulate an increase in insulin concentration but delays glucose disposal. Am J Clin Nutr 76:1016-1022, 2002

6. Gannon MC, Nuttall JA, Nuttall FQ: The metabolic response to ingested glycine. Am J Clin Nutr 76:1302-1307, 2002

7. Genuth SM, Castro J: Effect of oral alanine on blood beta-hydroxybutyrate and plasma glucose, insulin, free fatty acids, and growth hormone in normal and diabetic subjects. Metabolism 23:375-386, 1974

8. Eisenstein J, Roberts SB, Dallal G, et al: High-protein weight-loss diets: Are they safe and do they work? A review of the experimental and epidemiologic data. Nutr Rev 60:189-200, 2002

9. Goodwin JF: The colorimetric estimation of plasma amino nitrogen with DFNB. Clin Chem 14:1080-1090, 1968

10. Fuller G, Parker RM: Approximate Integration. Applications 13-16, in Analytical Geometry and Calculus. Princeton NJ, Van Nostrand, 1964, pp 367-368

11. McGilvery RW, Goldstein GW: Biochemistry. A Functional Approach. Philadelphia, PA, Saunders, 1983

12. Cheftel JC, Cuq J-L, Lorient D: Amino acids, peptides, and proteins, in Fennema OH (ed): Food Chemistry. New York, NY, Marcel Dekker, 1985, pp 245-369

13. Weyer C, Bogardus C, Mott DM, et al: The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest 104:787-794, 1999

14. Gerich JE: Is reduced first-phase insulin release the earliest detectable abnormality in individuals destined to develop type 2 diabetes? Diabetes 51:S117-S121, 2002 (suppl 1)

15. Cherrington AD, Stevenson RW, Steiner KE, et al: Insulin, glucagon, and glucose, as regulators of hepatic glucose uptake and production in-vivo. Diabetes Metab Rev 3:307-332, 1987

16. Zinneman HH, Nuttall FQ, Goetz FC: Effect of endogenous insulin on human amino acid metabolism. Diabetes 15:5-8, 1966