

# The metabolic response to ingested glycine<sup>1-3</sup>

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## ABSTRACT

**Background:** The metabolic effects of dietary protein are complex. In persons with type 2 diabetes, protein ingestion results in little or no increase in plasma glucose concentrations but a stimulation of insulin and glucagon secretion. Furthermore, when protein is ingested with glucose, a synergistic effect on insulin secretion is observed. The most potent protein is gelatin, which consists of 30% glycine residues.

**Objective:** The objective of the present study was to determine whether glycine per se stimulates insulin secretion or reduces the glucose response when ingested with glucose.

**Design:** Nine healthy subjects were tested on 4 separate occasions. Plasma glucose, insulin, glucagon, and glycine concentrations were measured at various times during a 2-h period after the ingestion of 1 mmol glycine/kg lean body mass, 25 g glucose, 1 mmol glycine/kg lean body mass + 25 g glucose, or water only, given in random order.

**Results:** Plasma concentrations of glycine and glucagon were elevated after the ingestion of glycine, as expected. The serum insulin concentration also was slightly elevated after the ingestion of glycine alone. When glycine was ingested with glucose, the plasma glucose area response was attenuated by >50% compared with the response after the ingestion of glucose alone. The dynamics of the insulin response after the ingestion of glycine plus glucose were modestly different from those after the ingestion of glucose alone, but the area response was not significantly different.

**Conclusion:** The data are compatible with the hypothesis that oral glycine stimulates the secretion of a gut hormone that potentiates the effect of insulin on glucose removal from the circulation. *Am J Clin Nutr* 2002;76:1302-7.

**KEY WORDS** Glycine, insulin, glucose, glucagon, gut hormones, incretin, amino acids

## INTRODUCTION

Our laboratory is interested in the metabolic response to ingested proteins, particularly in persons with type 2 diabetes. The reason for this interest is that ingested protein either results in no increase in peripheral blood glucose concentrations or increases them only modestly (1, 2). However, ingested protein stimulates both insulin and glucagon secretion. In addition, in persons with type 2 diabetes, protein ingested with glucose reduces the integrated, single-meal glucose area response when compared with that after the ingestion of only glucose by the same subjects. This is due to a synergistic stimulation of insulin secretion when protein is ingested simultaneously with glucose (3).

Of 7 different protein sources tested previously, one of the most potent was gelatin (4). This was somewhat surprising because gelatin is an atypical protein. On a molar basis, glycine makes up ≈30% of the total amino acids present (5). Therefore, we were interested in determining whether glycine itself stimulates insulin secretion or reduces the integrated glucose response when ingested with a standardized amount of glucose. We first determined the response in normal young subjects.

## SUBJECTS AND METHODS

Nine healthy subjects were studied (4 women, 5 men). The subjects' ages ranged from 21 to 52 y. Their mean body mass was 75 kg (range: 58.5-88.7), and their mean lean body mass was 61 kg (range: 48-72 kg). The subjects' mean body mass index (in kg/m<sup>2</sup>) was 25.9 ± 0.5 (range: 24.1-28.2). The results of thyroid, renal, and liver function tests were normal. The subjects were nondiabetic on the basis of the National Diabetes Data Group criteria, which were in effect at the time this study was begun (6). At that time, a fasting glucose concentration on more than one occasion of ≥7.8 mmol/L (140 mg/dL) for venous plasma or ≥6.7 mmol/L (120 mg/dL) for venous whole blood or capillary whole blood was considered compatible with a diagnosis of diabetes. 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 for 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 on separate occasions 1-14 d apart 1 mmol glycine/kg lean body mass,

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1 mmol glycine/kg lean body mass + 25 g glucose, 25 g glucose, or water only. 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 glycine.

The plasma glucose concentration was measured 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 measured by a standard double-antibody radioimmunoassay method with kits produced by Endotech (Louisville). Glucagon was measured by radioimmunoassay with 30K antiserum purchased from Health Science Center (Dallas). Individual amino acids were measured by HPLC in the laboratory of KS Nair (Rochester, MN). Lean body mass was determined by bioelectrical impedance analysis (with an instrument from RJL Systems, Clinton Town, MI).

The areas under the curves were calculated by using the trapezoidal rule (7). The initial fasting value of the respective hormone or metabolite was used as a baseline, and the area was measured over the 2-h period after the ingestion of the test substance. Statistics were determined by repeated-measures analysis of variance (ANOVA) with the MINITAB computer program (version 10.5; Minitab, Inc, State College, PA), followed by post hoc matched, paired *t* tests, which were Bonferroni-corrected for multiple comparisons. Data are presented as means  $\pm$  SEMs.

## RESULTS

### Glycine concentration

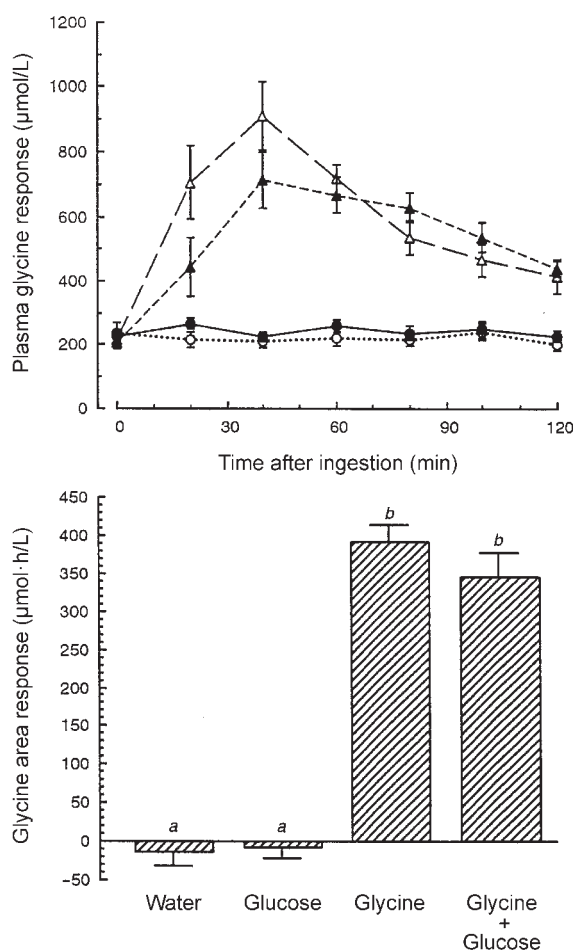
The mean amount of glycine given was 4.6 g (range: 3.6–5.4 g). The mean fasting glycine concentration for all studies was  $220 \pm 22$   $\mu\text{mol/L}$  (range: 205–236  $\mu\text{mol/L}$ ; **Figure 1**, top). After the ingestion of water or glucose only, the plasma glycine concentration remained constant. After the ingestion of glycine, the plasma glycine concentration increased from a baseline of  $217 \pm 21$   $\mu\text{mol/L}$  to a peak of  $909 \pm 106$   $\mu\text{mol/L}$  at 40 min, after which it decreased toward the fasting baseline. At the end of the study, however, the plasma glycine concentration was still elevated (414  $\mu\text{mol/L}$ ). When glucose was ingested with glycine, the response was attenuated and modestly prolonged compared with that after glycine ingestion alone. The area response to glycine + glucose was slightly but not significantly less than that after the ingestion of glycine alone (**Figure 1**, bottom).

### Other amino acid concentrations

Data for the other amino acids that yielded a significant area response are presented in **Table 1**. The net alanine, glutamine, and serine area responses after the ingestion of glycine were significantly greater than after the ingestion of water only. Plasma arginine, glutamate, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine, and valine were also measured but did not change significantly (data not shown).

### Glucose concentration

The mean fasting plasma glucose concentration was  $4.3 \pm 0.17$  mmol/L ( $77 \pm 3$  mg/dL) (**Figure 2**, top). After the ingestion of water only, the plasma glucose concentration remained stable. After the ingestion of glycine, the glucose concentration also was stable. The glucose concentration after glucose ingestion increased, reached a maximum of 6.9 mmol/L (124 mg/dL) at 30 min, and returned to baseline at 70 min. When



**FIGURE 1.** Top: Mean ( $\pm$  SEM) plasma glycine response in 9 healthy subjects after the ingestion of water only ( $\circ$ ... $\circ$ ), 25 g glucose ( $\bullet$ — $\bullet$ ), 1 mmol glycine/kg lean body mass ( $\Delta$ — $\Delta$ ), or 25 g glucose + 1 mmol glycine/kg lean body mass ( $\blacktriangle$ — $\blacktriangle$ ). Bottom: The 120-min net integrated glycine area response with the use of the concentration at time zero as baseline.  $n = 9$ . Bars with different letters are significantly different,  $P \leq 0.01$ .

glycine was ingested with glucose, the rise in the plasma glucose concentration was attenuated by  $\approx 15\%$ . The plasma glucose concentration then rapidly decreased to below the fasting baseline at 70 min. By 100 min, the glucose concentration was not significantly different whether or not glycine was ingested with glucose. The 2-h integrated glucose area response to glycine + glucose was  $< 50\%$  of the response to glucose alone (**Figure 2**, bottom).

### Insulin concentration

The mean fasting serum insulin concentration was  $48 \pm 6$  pmol/L ( $8.1 \pm 1$   $\mu\text{U/mL}$ ) (**Figure 3**, top). Glycine ingestion stimulated a modest increase in insulin concentration. After glucose ingestion, there was a rapid rise in insulin concentration, which corresponded with the rise in glucose concentration. When glycine was ingested with glucose, the insulin peak occurred later and was slightly less than when glucose was ingested alone.

The mean insulin area response to glycine alone was greater than that for the ingestion of water and this difference was highly significant ( $P < 0.01$ ). The mean insulin area response to

**TABLE 1**  
Plasma amino acid concentrations<sup>1</sup>

	0 min	60 min	120 min
	$\mu\text{mol/L}$		
<b>Alanine</b>			
Water	330 ± 58	315 ± 53	264 ± 32
Glycine	279 ± 28	311 ± 40	272 ± 35
Glucose	357 ± 53	360 ± 45	311 ± 45
Glycine + Glucose	277 ± 47	314 ± 44	300 ± 33
<b>Glutamine</b>			
Water	257 ± 37	238 ± 34	224 ± 34
Glycine	224 ± 38	271 ± 43	228 ± 38
Glucose	276 ± 54	247 ± 45	213 ± 33
Glycine + Glucose	220 ± 42	271 ± 47	246 ± 42
<b>Serine</b>			
Water	138 ± 18	126 ± 14	111 ± 10
Glycine	116 ± 15	144 ± 7	124 ± 10
Glucose	156 ± 23	141 ± 16	115 ± 13
Glycine + Glucose	123 ± 12	141 ± 8	139 ± 13

<sup>1</sup> $\bar{x} \pm \text{SEM}$ ;  $n = 9$ . The net area responses for alanine, glutamine, and serine were significantly greater after glycine ingestion than after the ingestion of water alone by repeated-measures ANOVA followed by post hoc matched-paired  $t$  tests, which were Bonferroni-corrected for multiple comparisons (data not shown).

glycine + glucose was not significantly different from the response to glucose alone (Figure 3, bottom). This was despite the fact that the glucose area response was much less after glycine plus glucose ingestion.

### Glucagon concentration

Glycine strongly stimulated an increase in glucagon concentration when ingested alone, and this increase persisted for the duration of the study (Figure 4, top). After the ingestion of water alone, glucose alone, or glycine plus glucose, the glucagon concentration decreased modestly; the decrease was greatest after glucose ingestion.

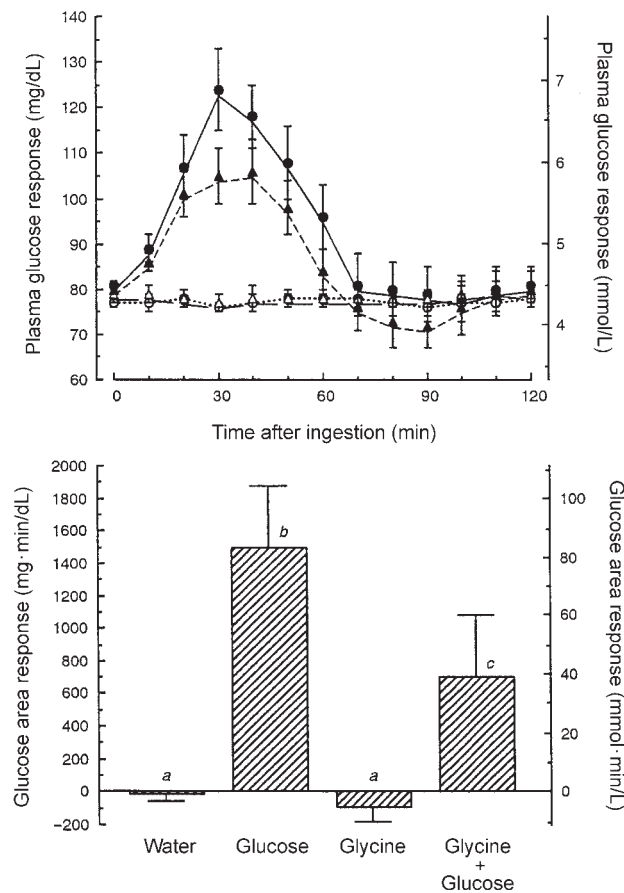
The glucagon area increase in response to the ingestion of glycine alone was significantly different from that resulting from the ingestion of water, glucose, or the combination of glycine + glucose, as expected (Figure 4, bottom). None of the last 3 were significantly different from each other.

### DISCUSSION

Glycine is the smallest amino acid. It is readily synthesized from serine, which also can be synthesized endogenously. Thus, neither glycine nor serine is a nutritionally required amino acid. Glycine is utilized in the synthesis of several biologically important compounds, including glucose. It also is utilized in detoxification reactions and itself is a neurotransmitter.

Glycine is present in nearly all proteins, and its presence in an amino acid chain allows for an acute angulation of the chain during the folding process. This often is required for the assumption of the proper three-dimensional structure required for that protein to fulfill its functional role (8, 9).

The concentration of glycine is particularly high in structural collagen, in which 1 of every 3–4 amino acids is glycine. When these structural proteins are subjected to alkaline or acid hydrolysis and heated, they denature and form gelatin, an ingredient commonly

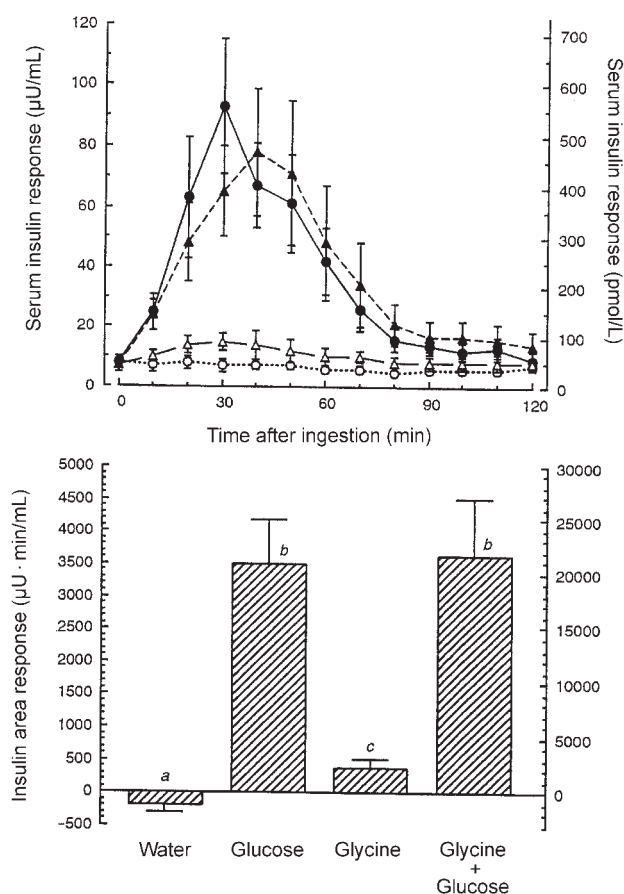


**FIGURE 2.** Top: Mean ( $\pm \text{SEM}$ ) plasma glucose response in 9 healthy subjects after the ingestion of water only ( $\circ \dots \circ$ ), 25 g glucose ( $\bullet \dots \bullet$ ), 1 mmol glycine/kg lean body mass ( $\triangle \dots \triangle$ ), or 25 g glucose + 1 mmol glycine/kg lean body mass ( $\blacktriangle \dots \blacktriangle$ ). Bottom: The 120-min net integrated glucose area response with the use of the concentration at time zero as baseline.  $n = 9$ . Bars with different letters are significantly different,  $P \leq 0.01$ .

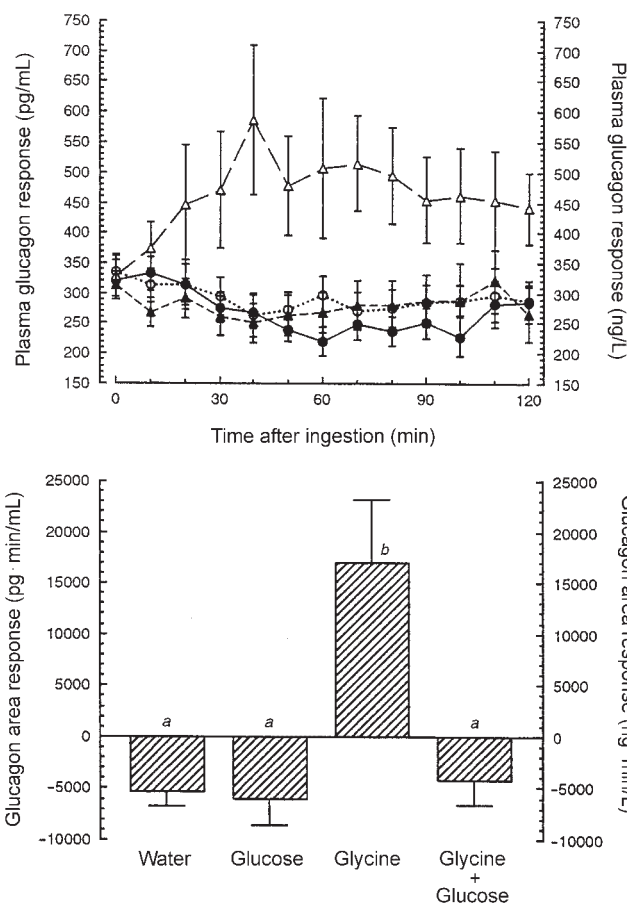
used in foods. As indicated in the Introduction, gelatin, when ingested with glucose, strongly potentiates the glucose-stimulated increase in insulin in persons with type 2 diabetes (4).

Some individual amino acids, when given intravenously in large amounts, are known to be insulin secretagogues. The most potent of these has been reported to be arginine (10). However, a mixture of 10 nutritionally required or provisionally nutritionally required amino acids was most potent in stimulating insulin secretion. Nonnutritionally required amino acids have not been systematically studied in this regard, but are generally considered to have no effect on insulin secretion (11). Much less is known about the ability of ingested single amino acids, whether nutritionally required or not, to stimulate insulin secretion. This is particularly the case when they are ingested in amounts likely to be present in a meal.

We have begun a systematic study of the metabolic effects of individual amino acids ingested in amounts likely to be present in a high-protein meal. We initiated these studies by studying the response to arginine (12) and to glycine. Arginine was selected because it is considered to be the prototypical amino acid secretagogue. Glycine was selected because it is the major amino acid



**FIGURE 3.** Top: Mean ( $\pm$ SEM) serum insulin response in 9 healthy subjects after the ingestion of water only ( $\circ$ ... $\circ$ ), 25 g glucose ( $\bullet$ ... $\bullet$ ), 1 mmol glycine/kg lean body mass ( $\triangle$ ... $\triangle$ ), or 25 g glucose + 1 mmol glycine/kg lean body mass ( $\blacktriangle$ ... $\blacktriangle$ ). Bottom: The 120-min net integrated insulin area response with the use of the concentration at time zero as baseline.  $n = 9$ . Bars with different letters are significantly different,  $P \leq 0.01$ .



**FIGURE 4.** Top: Mean ( $\pm$ SEM) plasma glucagon response in 9 healthy subjects after the ingestion of water only ( $\circ$ ... $\circ$ ), 25 g glucose ( $\bullet$ ... $\bullet$ ), 1 mmol glycine/kg lean body mass ( $\triangle$ ... $\triangle$ ), or 25 g glucose + 1 mmol glycine/kg lean body mass ( $\blacktriangle$ ... $\blacktriangle$ ). Bottom: The 120-min net integrated glucagon area response with the use of the concentration at time zero as baseline.  $n = 9$ . Bars with different letters are significantly different,  $P \leq 0.01$ .

in gelatin. As indicated earlier, gelatin strongly stimulates insulin secretion in persons with type 2 diabetes. This occurs even though glycine, when infused intravenously in a large amount, has been reported to have no effect on the glucose, insulin, or glucagon concentration in obese subjects who fasted overnight (13).

Many years ago it was reported that glycine given orally in amounts of 40–50 g results in a moderate reduction in blood glucose concentrations in healthy and diabetic adults [from 5.7 to 4 mmol/L (102 to 72 mg/dL) in healthy subjects and from 14.2 to 8.9 mmol/L (256 to 161 mg/dL) in diabetic subjects] (14, as quoted in 15). Others reported no effect on the blood glucose concentration in fasting subjects (16, 17, as quoted in 15). At that time, it was not possible to measure serum insulin concentrations.

Subsequently, glycine (0.3 mol) administered orally or intraduodenally was reported to have no effect on the blood glucose or insulin concentration over 180 min in overnight-fasted healthy subjects or in subjects with a partial gastrectomy. Serum glycine concentrations were increased  $\approx$ 3-fold. In subjects with mild untreated diabetes, the authors also stated that intraduodenally administered glycine did not affect either the glucose or the insulin concentration. Interestingly, glycine administration

stimulated a rise in growth hormone in the nondiabetic but not in the diabetic subjects (11).


In the present study, ingestion of 1 mmol glycine/kg lean body mass ( $\approx$ 4.6 g) also did not significantly affect the plasma glucose concentration. It clearly stimulated an increase in insulin, although the increase was modest (Figures 2 and 3). The glycine was rapidly absorbed and the dose used increased the circulating glycine concentration by  $\approx$ 4-fold.

After this manuscript was submitted for publication, an article reporting the effect of glycine on insulin secretion and action in healthy first-degree relatives of patients with type 2 diabetes was published by Gonzalez-Ortiz et al (18). These authors reported an increase in peripheral insulin concentrations in 6 volunteers who received 5 g glycine orally 30 min before a hyperglycemic, hyperinsulinemic clamp. A small increase in insulin concentrations in 6 other volunteers who received a placebo (magnesium oxide) also was reported, but was only approximately one-quarter of that after glycine. Insulin action was not significantly different between the 2 groups. Thus, although both the experimental design and the patient population differed between our study and that of Gonzalez-Ortiz et al, the increase in peripheral insulin concentration after oral glycine administration was observed in both.

In the present study, glycine also resulted in a large increase in glucagon concentrations (Figure 4). Intravenously administered glycine potently stimulates glucagon secretion in dogs (19). However, even supraphysiologic concentrations of glycine, obtained by intravenous administration of the amino acid, do not increase the glucagon concentration in humans (13). Because intravenously administered glycine does not stimulate a rise in glucagon concentration, but oral glycine was rather potent in this regard, the current data suggest that the rise in glucagon was due to the release of a glucagon-stimulating hormone in response to glycine in the lumen of the gastrointestinal tract. However, to our knowledge, such a gut hormone has not been identified. In the same study, intravenously infused glycine also did not stimulate an increase in insulin concentration (13). However, when given orally in the present study, glycine did stimulate a modest increase in insulin. Thus, the same putative gut hormone may stimulate insulin secretion as well.

The dramatic reduction in the glucose area response when glycine was ingested with glucose is of considerable interest from a physiologic point of view. However, the mechanism remains to be determined. The integrated insulin area response after the ingestion of glycine + glucose was essentially the same as when only glucose was ingested, but it occurred at a greatly attenuated increase in glucose concentration and without a delay in the return of the glucose concentration to the initial fasting value. This result is similar to that we and others observed in healthy subjects when fat was ingested with glucose (20, 21). It suggests that the glucose clearance rate was accelerated. The clearance rate of ingested glucose was not determined in any of these studies. Therefore, whether the attenuated increase in glucose and rapid return to the fasting value was due to 1) an increased removal rate either directly or indirectly as a result of the rise in insulin, 2) an independent mechanism, or 3) a decrease in the endogenous glucose production rate cannot be determined.

A decrease in the glucose production rate would not be expected in the presence of a persistent large increase in the glucagon concentration. In fact, one would anticipate an increase in glucose production. A reduction in the glucose absorption rate also is unlikely but cannot be ruled out. Glycine has been reported to compete with glucose for absorption (22). Cholecystokinin and glucagon-like peptide 1 (GLP-1, 7-36 amide) strongly reduce the rate of gastric emptying. The latter hormone also may reduce duodenal motility (23). Intraduodenal instillation of a mixture of nutritionally required amino acids stimulates cholecystokinin secretion; individually, phenylalanine, valine, and methionine were most potent. Nonnutritionally required amino acids including glycine did not stimulate cholecystokinin secretion (24). GLP-1 also stimulates insulin secretion and reduces glucagon secretion. Because glycine stimulated an increase in both insulin and glucagon, a glycine-stimulated increase in GLP-1 is not likely to explain the results (23). Overall, the data suggest that oral glycine stimulates the secretion, either directly or indirectly, of a gut hormone that potentiates or is additive with the effect of insulin in stimulating the removal of glucose from the circulation. It also inhibits the effect of glucagon on endogenous glucose production. Nevertheless, this remains only a speculation at present.

In any regard, the ingested glycine effect on the postprandial glucose concentration may be important therapeutically if a similar effect can be shown in persons with type 2 diabetes. It will also be of interest to determine whether glycine synergistically facilitates insulin secretion by other amino acids that are quantitatively prominent in gelatin protein. 

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