Effects of protein on glycemic and incretin responses and gastric emptying after oral glucose in healthy subjects1–3

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ABSTRACT

Background: Dietary interventions represent a promising therapeutic strategy to optimize postprandial glycemia. The addition of protein to oral glucose has been reported to improve the glycemic profile.

Objective: The aim of the current study was to evaluate the mechanisms by which protein supplementation lowers the blood glucose response to oral glucose.

Design: Nine healthy men were studied on 3 d each in a random order. Subjects consumed 300-mL drinks containing either 50 g glucose (Glucose), 30 g gelatin (Protein), or 50 g glucose with 30 g gelatin (Glucose + Protein) in water labeled with 150 mg [13C]lactate. Blood and breath samples were subsequently collected for 3 h to measure blood glucose and plasma insulin, glucagon-like peptide 1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP) concentrations and gastric half-emptying time, which was calculated from 13CO2 excretion.

Results: The blood glucose response was less after Glucose + Protein than after Glucose (P < 0.005); GIP was lower (P < 0.005), and there were no significant differences in plasma insulin or GLP-1. Protein alone stimulated insulin, GLP-1, and GIP (P < 0.05 for each) without elevating blood glucose. The gastric half-emptying time was greater after Glucose + Protein than after Glucose (P < 0.05) and tended to be greater for Glucose than for Protein (P = 0.06).

Conclusions: In healthy humans, the addition of protein to oral glucose lowers postprandial blood glucose concentrations acutely, predominantly by slowing gastric emptying, although protein also stimulates incretin hormones and non-glucose-dependent insulin release. Am J Clin Nutr 2007;86:1364–8.

KEY WORDS  Glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1, breath test

INTRODUCTION

Glycemic control is a major determinant of the development and progression of the complications associated with diabetes (1–3), and recent studies have highlighted the particular importance of postprandial hyperglycemia (4, 5). High-protein diets are reported to decrease both postprandial blood glucose and glycated hemoglobin in type 2 diabetes (6), but the mechanisms responsible for these effects are poorly defined.

The capacity of protein to enhance insulin secretion has been recognized for many years. Gannon et al (7) reported that the addition of 25 g protein (from sources such as meat, fish, or gelatin) to a 50-g oral glucose load reduced the subsequent blood glucose response in type 2 diabetic patients (by =30% with gelatin), whereas it increased plasma insulin (by ≈2.5 times with gelatin) when compared with glucose alone.

It is now established that the rate of gastric emptying is a major determinant of postprandial glycemia, so that even modest changes may have a substantial effect on the magnitude and timing of postprandial increases in blood glucose and insulin (8, 9). Gastric emptying is normally regulated at a relatively constant rate of 2–3 kcal/min, predominantly by feedback arising from the small intestine (10, 11). The addition of energy in the form of protein to an oral glucose load would be expected, therefore, to slow gastric emptying of glucose and thereby reduce the glycemic response. In this circumstance, it may be expected that there would be a reduction, rather than an increase, in the plasma insulin response. Previous studies that evaluated the effects of protein supplementation on oral glucose tolerance (7, 12, 13) did not measure the rate of gastric emptying.

Oral glucose stimulates insulin secretion to a much greater degree than does an equivalent intravenous glucose load (14). This “incretin effect” is mediated by the small intestinal peptides glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Both carbohydrate and fat are potent stimuli for GLP-1 and GIP secretions (15), but the response to protein is more variable; amino acids (16–18) and casein hydrolysates (19) tend to stimulate GIP and GLP-1 more potently than do intact proteins, at least in healthy humans (15, 20). Strategies to enhance the incretin response are of particular interest in the management of patients with type 2 diabetes, in whom both the secretion of GLP-1 (21) and the β cell response to GIP (22)
appear to be impaired. We have now examined the potential contributions of gastric emptying, incretin peptides, and insulin in mediating the reduction in the glycemic response to oral glucose by the addition of protein, in healthy humans.

SUBJECTS AND METHODS

Subjects

Nine healthy men [median age: 24 y (range: 19–35 y); median body mass index (in kg/m²): 24.2 (range: 21.6–27.5)] were studied. In each subject, measurements were performed on 3 d after an overnight fast, and each measurement was separated by ≥3 d.

Each subject gave written informed consent in accordance with the guidelines of the Declaration of Helsinki. The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital.

Protocol

The order of the studies was randomized and single-blinded. An intravenous cannula was placed in a forearm vein 30 min before each study to allow repeated blood samples to be drawn.

On each day, a 300-mL drink was consumed, which contained 50 g glucose (“Glucose” drink; 200 kcal), 30 g powdered gelatin (incorporating 25 g protein; Davis Gelatin; Gelita NZ Ltd, Christchurch, New Zealand; “Protein” drink; 100 kcal), or both glucose and gelatin (“Glucose + Protein” drink; 300 kcal), dissolved in water. Each drink contained 150 mg [13C]acetate and nonnutritive fruit flavoring, was served at 37 °C, and was consumed within 3 min.

Blood samples were collected at −5, 0, 15, 30, 45, 60, 90, 120, 150, and 180 min for measurements of blood glucose and plasma insulin, GLP-1, and GIP concentrations. To calculate the rate of gastric emptying, breath samples were obtained at 5-min intervals from 0 to 60 min and then at 15-min intervals from 60 to 180 min.

Measurements

Blood glucose, plasma insulin, GLP-1, and GIP concentrations

Blood samples for the determinations of plasma insulin, GLP-1, and GIP were collected in ice-chilled tubes containing EDTA and 400 kIU apronin (Trasylol; Bayer Australia Ltd, Pymble, Australia) per liter of blood. Plasma was separated by centrifugation and stored at −70 °C for subsequent analysis. Blood glucose concentrations were determined immediately by using a portable glucometer (Medisense Precision QID; Abbott Laboratories, Bedford, MA). The accuracy of this method was confirmed by our laboratory with the use of the hexokinase technique (8). Plasma insulin was measured by enzyme-linked immunosorbent assay (Diagnostics Systems Laboratories Inc, Webster, TX); the intrasassay CV was 2.6%, and the interassay CV was 6.2% (23). Plasma GLP-1 concentrations were measured by radioimmunoassay; the intrasassay CV was 17%, and the interassay CV was 18% (24). Plasma GIP was also measured by radioimmunoassay; intra- and interassay CVs were both 15% (24).

Gastric emptying

[13C] CO2 enrichment in the breath samples was measured by mass spectroscopy (ABCA 20–20 mass spectrometer; Europa Scientific, Crewe, United Kingdom) to determine the percentage [13C] CO2 recovery per hour and the cumulative percentage [13C] CO2 recovery over 3 h (25). The method of Ghoos et al (26) was applied to calculate the gastric half-emptying time and gastric emptying coefficient. Breath tests that use the [13C] acetate label were validated against the gold standard of scintigraphy for the measurement of liquid gastric emptying (27).

Statistical analyses

Data were evaluated by analysis of variance compared with the guidelines of the Declaration of Helsinki. The protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital.

RESULTS

All subjects tolerated the study well. Subjects could distinguish the drinks containing gelatin because of their viscous texture.

Blood glucose, plasma insulin, GLP-1, and GIP concentrations

Blood glucose, plasma insulin, GLP-1, and GIP concentrations are shown in Figure 1. Blood glucose concentrations increased from baseline after the Glucose and Glucose + Protein drinks (P < 0.05 for each), but not after the Protein drink, and the blood glucose profile differed significantly between the 3 study days (P < 0.005 for both treatment effect and treatment × time interaction). Blood glucose concentrations were lower after the Glucose + Protein drink than after the Glucose drink (P < 0.05), with a lower peak blood glucose value for Glucose + Protein (8.0 ± 0.4 mmol/L) than for Glucose (9.4 ± 0.3 mmol/L) (P < 0.05). For the drinks containing glucose, blood glucose concentrations fell below baseline values late in the study, so that blood glucose was lower for Glucose + Protein than for Protein at 120 and 150 min and was also lower for Glucose than for Protein at 150 min (P < 0.05 for all).

Plasma insulin concentrations increased from baseline after all 3 drinks (P < 0.005 for treatment effect; P < 0.001 for treatment × time interaction). Insulin concentrations were lower after Protein than after the other drinks (P < 0.05) but did not differ between Glucose and Glucose + Protein. Insulin remained elevated above baseline concentrations after drinks containing glucose, even after blood glucose had returned to, or fallen below, baseline values.

Plasma GLP-1 concentrations increased on all 3 d (P < 0.05), with a relatively early peak followed by a steady decline. There was no significant difference between the 3 drinks.

Plasma GIP concentrations are shown in Figure 1D. Plasma GIP increased on all 3 d (P < 0.05 for each), and GIP concentrations differed between study days (P < 0.005 for treatment effect; P < 0.001 for treatment × time interaction). Plasma GIP concentrations were greater after Glucose than after each of the
other drinks ($P < 0.05$ for each) and were greater after Glucose + Protein than after Protein ($P < 0.05$). As for insulin, GIP remained elevated above baseline beyond the return of blood glucose to baseline.

**Gastric emptying**

Gastric half-emptying time differed between the 3 study days ($P < 0.005$) and was greater for Glucose + Protein (51.2 ± 1.4 min) than for either the Glucose (45.6 ± 1.3 min; $P < 0.05$) or the Protein (42.1 ± 1.3 min; $P < 0.005$) drinks (Figure 2). Half-emptying time tended to be greater for Glucose than for Protein ($P = 0.06$). The gastric emptying coefficient tended to differ between the 3 study days ($P = 0.05$) and on direct comparison was greater for Glucose (3.82 ± 0.07) than for Glucose + Protein (3.61 ± 0.06) ($P < 0.05$), but it did not differ significantly between Protein (3.98 ± 0.16) and Glucose ($P = 0.29$) or between Protein and Glucose + Protein ($P = 0.07$).

**Relation between blood glucose and gastric emptying**

When the Glucose and Glucose + Protein drinks were compared (Figure 3), there was an inverse correlation between the change in blood glucose increment at 15 min and the change in gastric half-emptying time ($r = -0.77$, $P < 0.05$).

**DISCUSSION**

The present study is the first to evaluate both gastric emptying and incretin responses to the addition of protein to an oral glucose load in humans. We showed that the addition of protein reduces the glycemic response in healthy subjects and is associated with a slowing of gastric emptying. The latter was predictable given that the stomach empties at a relatively constant caloric rate (11); it is possible that cholecystokinin contributed to this effect, because protein is a potent stimulus for its release (28), although we did not measure plasma cholecystokinin.

![Figure 1](image1.png)

**FIGURE 1.** Mean (±SE) concentrations of blood glucose, plasma insulin, plasma glucagon-like peptide 1 (GLP-1), and plasma glucose-dependent insulinotropic polypeptide (GIP) after 50 g oral glucose (Glucose drink; ○), 25 g oral protein (Protein drink; □), and 50 g oral glucose with 25 g oral protein (Glucose + Protein drink; ●). $n = 9$ subjects. Blood glucose, plasma insulin, and plasma GIP differed significantly between study days ($P < 0.005$ for all, repeated-measures ANOVA treatment effects and treatment × time interactions), but there was no significant difference in GLP-1 between treatments.

- **Significant difference between Glucose + Protein and Glucose drinks, $P < 0.05$.**
- **Significant difference between Glucose and Protein drinks, $P < 0.05$.**
- **Significant difference between Glucose + Protein and Protein drinks, $P < 0.05$.**

![Figure 2](image2.png)

**FIGURE 2.** Gastric half-emptying time ($t_{1/2}$) of the 3 drinks. $n = 9$ subjects. A significant treatment effect was observed ($P < 0.0005$, ANOVA).

- **Significant difference between Glucose + Protein and Glucose drinks, $P < 0.05$.**
- **Significant difference between Glucose and Protein drinks, $P < 0.05$.**
- **Significant difference between Glucose + Protein and Protein drinks, $P < 0.005$.**
secretion to oral glucose and a diminished glucose-sensing ability. Type 2 diabetes is associated with an impaired early-phase insulin response. These observations are consistent with the evidence that reduction in blood glucose but no increment in the insulin release, and, indeed, plasma GIP concentrations were lower after Glucose + Protein than after the Glucose drink alone. This occurred despite the fact that protein, when consumed alone, stimulated both incretin and insulin release. The increase in plasma insulin after the Protein drink was not associated with any elevation in blood glucose, which suggests that it was mediated by a mechanism other than GLP-1 and GIP, because these peptides stimulate insulin secretion in a glucose-dependent manner. A possible alternative mechanism for this effect was the stimulation of β cells by amino acids. The fact that the blood glucose did not decrease in response to the release of insulin could be explained by concurrent glucagon release, although we did not measure this.

The loads of glucose and protein administered in this study were chosen to match those evaluated previously in patients with type 2 diabetes. The observed lowering of glycemia after the addition of protein to the glucose drink is in accordance with the report of Gannon et al. In contrast with our findings, these authors observed a 2.5-fold stimulation of insulin release. However, the insulin response to the glucose drink alone in their type 2 diabetic patients was markedly less than in our healthy subjects. The same investigators then studied the effects of adding glycine or proline, both of which are found in gelatin, to oral glucose in healthy volunteers and reported a decrease in blood glucose but no increment in the insulin response. These observations are consistent with the evidence that type 2 diabetes is associated with an impaired early-phase insulin secretion to oral glucose and a diminished glucose-sensing ability of the β cell, whereas the insulin responses to other stimuli may be relatively intact, at least in the early stages of the disease.

Several groups have investigated the insulin response to ingested protein and found that not only the presence of diabetes, but also the relative quantities of protein and carbohydrate and the presence of free amino acids (as opposed to intact protein) are important variables. When ingested in isocaloric quantities, glucose and protein from lean beef have additive effects on insulin secretion in healthy subjects, whereas if the amount of protein is less than that of glucose, the increase in insulin is substantially less than additive. Furthermore, the insulin response correlates with plasma amino acid concentrations; the insulin response to intact protein is less than that observed with protein hydrolysates, which presumably reflects the more rapid increase in plasma amino acid concentrations with the latter.

Little information is available on the effects of supplementing carbohydrate with protein on the incretin response. Frid et al reported that the addition of whey protein to carbohydrate in type 2 diabetic patients increased the insulin response and decreased the glycemic profile more than did the addition of lean ham. The GIP response was greater with whey (area under the curve increased by 30% over 120 min), but GLP-1 concentrations did not significantly differ. Gunnarsson et al recently reported that in gastric gavage-fed mice, the insulin response to glucose (ie, area under the curve) was trebled by the addition of an equal weight of whey protein, and this was associated with a substantial decrease in plasma glucose. The total plasma GLP-1 concentration increased, but GIP did not. However, analysis of intact (or active) incretins indicated an increase in intact GIP, and the authors suggested that fragments of whey protein could inhibit dipeptidyl peptidase IV, the enzyme that degrades the incretin peptides, in the small intestine. As in our study, gastric emptying was slowed by the addition of whey protein to glucose. The fact that the addition of protein in the present study did not increase plasma GLP-1 and decreased GIP is likely to reflect the relatively smaller load and the nature of the protein used. Although we did not measure intact, as opposed to total, GIP, gelatin may be less easily digested to fragments that block dipeptidyl peptidase IV than is whey protein.

We evaluated the acute effects of modest supplementation with protein in healthy individuals. Acute and longer-term studies would be of interest in patients with type 2 diabetes to evaluate the effect of protein supplementation on glycemic control, and the load and type of protein could be optimized for a greater incretin response. An alternative approach, eg, the ingestion of a protein “preload” at a given interval before meals, could also be evaluated to determine whether the stimulation of incretins and insulin could improve glycemia after the subsequent meal.

In summary, we confirmed the capacity of protein supplementation to improve the glycemic response to glucose in healthy humans, and we showed that the slowing of gastric emptying makes an important contribution to this effect. Further studies aimed at optimizing this phenomenon are indicated in patients with type 2 diabetes, who may derive additional benefit, in terms of postprandial glycemia, from the stimulation of non–glucose-dependent insulin secretion.

The authors’ responsibilities were as follows—AK: contributed to the study design, conducted the study, and drafted the manuscript; RC: conducted the study, analyzed the data, and contributed to the writing of the manuscript; SD: contributed to the study design and helped conduct the study; MB: analyzed the breath test data and revised the manuscript; FDB: helped plan the study design with regard to breath testing and reviewed the manuscript; JMW: undertook the peptide assays and analysis of the data; KLL: contributed to the data analysis and revision of the manuscript; MH: contributed to the conception and design, data analysis, and revision of the manuscript; CKR: conceived the study and oversaw the conduct and analysis of the study and the writing of the manuscript. None of the authors had a conflict of interest to declare.
REFERENCES


