Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults

Tina Akhavan, Bohdan L Luhovyy, Peter H Brown, Clara E Cho, and G Harvey Anderson

ABSTRACT

Background: Dairy protein ingestion before a meal reduces food intake and, when consumed with carbohydrate, reduces blood glucose.

Objective: The objective was to describe the effect of whey protein (WP) or its hydrolysate (WPH) when consumed before a meal on food intake, pre- and postmeal satiety, and concentrations of blood glucose and insulin in healthy young adults.

Design: Two randomized crossover studies were conducted. WP (10–40 g) in 300 mL water was provided in experiment 1, and WP (5–40 g) and WPH (10 g) in 300 mL water were provided in experiment 2. At 30 min after consumption, the subjects were fed an ad libitum pizza meal (experiment 1) or a preset pizza meal (12 kcal/kg, experiment 2). Satiety, blood glucose, and insulin were measured at baseline and at intervals both before and after the meals.

Results: In experiment 1, 20–40 g WP suppressed food intake (P < 0.0001) and 10–40 g WP reduced postmeal blood glucose concentrations and the area under the curve (AUC) (P < 0.05). In experiment 2, 10–40 g WP, but not WPH, reduced postmeal blood glucose AUC and insulin AUC in a dose-dependent manner (P < 0.05). The ratio of cumulative blood glucose to insulin AUCs (0–170 min) was reduced by 10 g WP but not by 10 g WPH.

Conclusions: WP consumed before a meal reduces food intake, postmeal blood glucose and insulin, and the ratio of cumulative blood glucose to insulin AUCs in a dose-dependent manner. Intact WP, but not WPH, contributes to blood glucose control by both insulin-dependent and insulin-independent mechanisms. This trial was registered at clinicaltrials.gov as NCT00988377 and NCT00988182.

INTRODUCTION

A role of milk protein consumption and its physiologic functionality beyond the provision of nutrients in the management of obesity and the metabolic syndrome is of interest because of strong associations between high dairy consumption and low body weight (1, 2). In addition, experimental studies have shown that milk proteins reduce short-term appetite, food intake (3–6), and blood glucose responses when consumed with carbohydrate (7–9).

Whey protein accounts for 20% of cow milk protein and is of specific interest because it is a readily available byproduct of cheese making. It enhances satiety and suppresses food intake in humans (3) and reduces food intake more than does casein (10), egg albumin, or soy protein (4). When whey is consumed with carbohydrate, it reduces the subsequent glycemic response (11–13), as do other proteins (9). The reduction in blood glucose is suggested to occur because of whey protein’s rapid digestion (14, 15) and high content of branched-chain amino acids (11), resulting in rapid insulin release (5, 6, 16). Support for the importance of the branched-chain amino acids in whey protein as the mediator of its effect has arisen by the similarity of the effect of intact whey, hydrolyzed whey protein (17) and branched-chain amino acids (13) on plasma insulin and glucose concentrations. However, mechanisms other than insulin may be of importance because a branched-chain amino acid mixture does not reproduce the effect of the intact whey protein on gut peptides involved in the control of glycemia and stomach emptying (13).

There are many reports that large amounts of whey protein (40–60 g) when consumed alone in beverage form reduce subsequent food intake (4, 10, 18) or when consumed with carbohydrate reduce the glycemic response (11–13). However, there is only one recent report suggesting that consumption of whey protein alone before a meal may be beneficial to glycemic control (19). Consumption of 55 g whey protein in a soup before a serving of mashed potato with added glucose (total of 59 g carbohydrate) reduced glycemic excursions in patients with type 2 diabetes (19). However, the relation between the dose of whey protein consumed before a meal of usual quantities and carbohydrate content on efficacy for reducing food intake and postmeal glycemic and insulin response in either healthy individuals or patients with type 2 diabetes has not been reported.

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Therefore, the objective of these 2 experiments was to determine the relations between whey protein dose, or its hydrolysate, on satiety, food intake, and pre- and postmeal glycemic and insulin responses in healthy subject when consumed in beverages 30 min before an ad libitum pizza meal (experiment 1) or before a preset meal of a fixed quantity (experiment 2).

SUBJECTS AND METHODS

Participants

Healthy individuals participated in the experiments (experiment 1: 16 men; experiment 2: 12 men and 10 women). Participants were of normal weight, characterized by a body mass index (in kg/m²) between 18 and 24.9 and aged 20–27 y. They were recruited through advertisements posted on the campus of the University of Toronto. Breakfast skippers, smokers, dieters, or individuals with diabetes (fasting blood glucose ≥ 7.0 mmol/L) or other metabolic diseases were excluded. Restrained eaters identified by a score of ≥11 on the Eating Habits Questionnaire (20) and those taking medication were also excluded. On completion of the study and analysis of the blood samples, one female participant was found to be hyperinsulinemic, and her data were excluded from the study. The sample size required was based on a previous short-term food intake study on protein (4). Participants were financially compensated for completing the studies. The procedures of the studies were approved by the Human Subject Review Committee, University of Toronto Ethics Review Office.

Preloads

Premeal treatments were as follows: control and 10, 20, 30, and 40 g whey protein (NZMP Whey Protein Concentrate 392, Fonterra Co-operative Group Limited, New Zealand) in experiment 1, and control and 5, 10, 20, and 40 g whey protein and 10 g whey protein hydrolysate (WPH) (Hilmar 8350; Hilmar Ingredients, CA) in experiment 2. The whey protein (both experiments) and WPH (experiment 2) powders contained approx 80% protein, 5% lactose, 6% fat, 4% ash, and 4% moisture (see Supplementary Table 1 under “Supplemental data” in the online issue). Therefore, for example, to provide the equivalent of 10 g protein, 12.5 g either whey protein or WPH was added to the treatment, which provided approx 50 kcal energy. On the basis of the observation that a branched-chain amino acid mixture did not result in similar effects on the release of gut peptides as intact protein (13), a WPH was included in experiment 2 to further explore the role of intact protein, compared with hydrolyzed protein, on pre- and postmeal glycemic control. The WPH was prepared by enzymatic hydrolysis and, as reported by the manufacturer, resulted in a distribution profile of approx 40% free amino acids and short peptides of up to 10 amino acids, 27% of peptides of 10–200 amino acids, 16% of 50–200 amino acids, and 17% of amino acid chains larger than 200.

All preloads were isovolumetric (300 mL) and served chilled in beverage form. Isovolumetric flavored water was used as a control in both experiments. The addition of orange-flavored energy-free sweetener (1 g Kool-Aid; Kraft Canada Inc, North York, Canada) equalized the palatability of the preloads, as judged by a test panel of 12 subjects. An additional 100 mL water was provided to participants after consumption of the preload to reduce the aftertaste of the protein preloads.

Protocol

The experiment protocol was similar to previously published procedures (21). A standard breakfast (300 kcal) consisted of a single serving of a ready-to-eat breakfast cereal (Honey Nut Cheerios; General Mills, Mississauga, Canada), a 250-mL box of 2% milk (Sealtest Skim Milk, Markham, Canada) and a 250-mL box of orange juice (Tropicana Products Inc, Bradenton, FL).

Breakfasts were given to subjects to be consumed at their preferred time in the morning (0600–0900) after a 10-h overnight fast and were asked not to consume anything between the breakfast and the study session 4 h later (1000 to 1300), except water, until 1 h before the session. Each participant was scheduled to arrive at the same time for each treatment in the Department of Nutritional Sciences at University of Toronto and instructed to refrain from alcohol consumption and any unusual exercise and activity the night before the study sessions. Because impaired insulin sensitivity has been observed after an oral-glucose-tolerance test in the luteal phase of the menstrual cycle in healthy women (22), women were scheduled for the sessions during the follicular phase.

On their arrival, participants completed visual analog scale (VAS) questionnaires concerning “Sleep Habits,” “Stress Factors,” “Food Intake and Activity Level,” “Feelings of Fatigue,” and “Motivation to Eat.” A composite score of the 4 appetite questions in the “Motivation to Eat” VAS was calculated to obtain the average appetite score (21, 23) for statistical analysis.

A baseline capillary blood sample was taken by finger prick to measure glucose and insulin. Blood samples were obtained with a Monojector Lancet Devices (Sherwood Medical, St Louis, MO). Concentrations of blood glucose that corresponded to the plasma concentration were measured with a glucose meter (Accu-Chek Compact; Roche Diagnostics Canada, Laval, Canada). In experiment 2, after measurement of blood glucose, 300 μL capillary blood was collected into Microvette 300 blood collection tubes (Sarstedt, Nümbrecht, Germany). Insulin was measured by enzyme immunoassay (Insulin EIA, Alpco Diagnostics, Salem, NH).

Men were provided the preloads in random order once per week in the first experiment and twice weekly in the second experiment. The women in the second experiment were studied twice a week in the first 2 wk of their menstrual cycle. They were instructed to drink the preload within 5 min at a constant pace. After consumption, the palatability, taste, and texture of the preloads were measured by VAS. Subjective appetite and blood glucose were measured 15 and 30 min from the time when subjects started drinking the treatments. Participants were asked to remain seated throughout the experimental session and were allowed to read or listen to music.

At 30 min, participants were fed either an ad libitum pizza meal (McCain Foods Ltd, Florenceville, NB) in experiment 1 (23) or a fixed quantity of pizza (based on 12 kcal/kg of body weight for subjects in experiment 2) with a bottle of spring water (500 mL Crystal Springs; Aquaterra Corp, Crystal Springs Division, Mississauga, Canada). In experiment 1, subjects were provided 3 varieties of pizza (Deluxe, Pepperoni, and Three Cheese) based on their preferences and were asked to eat until...
they were “comfortably full” and were allowed 20 min to eat. In experiment 2, they were provided only the Deluxe variety and were asked to consume all of the food in 20 min. Immediately after the meal at 50 min and again at 65, 80, and 95 min (in both experiments) and 110, 140, and 170 min (in experiment 2), subjective appetite and blood glucose were measured. In experiment 2, insulin was also measured at 0, 30, 50, 95, 110, 140, and 170 min.

Detailed information of the nutrient content of the pizza and method of cooking was reported previously (21, 23). Test meal consumption was calculated from the weight of the consumed pizza based on the compositional information provided by the manufacturer. Water intake was measured by weight (g).

Cumulative energy intake was calculated by adding the energy consumed from the whey preload to the energy consumed at the test meal (21). Caloric compensation, expressed as a percentage, was calculated by subtracting the calories consumed after the whey preload from those after the water control divided by the calories in the whey preload and multiplied by 100. Caloric compensations of <100% indicated that the subject had low compensation for the whey preload energy at the test meal, whereas scores >100% indicated overcompensation for the whey preload energy at the test meal.

The ratios of blood glucose to insulin concentration (mmol·L⁻¹/µIU·mL⁻¹) and cumulative incremental areas under the curve (AUCs) (mmol·min/L to µIU·min/mL) were calculated to provide an evaluation of the efficacy of insulin action as previously used to identify the relation between postmeal glucose and insulin ratios (24). The lower the ratio, the higher the efficacy of insulin action (25).

### Statistical analysis

All analyses were conducted by using SAS version 9.1 (SAS Institute Inc, Cary, NC). Two and 3-factor repeated-measures analysis of variance (ANOVA; via PROC MIXED procedure) were performed to analyze the effects of time, sex, preload, and their interaction on outcome variables measured over the study period, including average appetite scores and blood glucose and insulin responses. When a preload and time interaction was statistically significant, one-factor ANOVA using the PROC MIXED procedure was followed by Tukey’s post hoc test to investigate the effect of preload on absolute and changes from baseline for blood glucose and insulin at each time of measurement. Premeal changes from baseline were calculated from 0 min (immediately before preload consumption) and postmeal changes from 30 min (before meal consumption).

The effect of preload on food intake, cumulative energy intake, and caloric compensation in experiment 1 and on premeal, postmeal, and AUC (26) for appetite and blood glucose (in both experiments) and insulin (in experiment 2) were tested by one-factor ANOVA (Proc Mixed procedure) followed by Tukey’s post hoc test to identify differences between preloads.

Pearson’s correlation coefficients were used to detect associations between dependent measures. Significance was set at P < 0.05. Data are presented as means ± SEMs.

### RESULTS

#### Participant characteristics

In experiment 1, men (n = 16) had a mean (±SEM) age of 22.3 ± 0.6 y, body weight of 69.5 ± 1.6 kg, height of 1.8 ± 0.0 m, and body mass index of 22.6 ± 0.4. In experiment 2, men (n = 12) and women (n = 9) with a mean age of 21.8 ± 0.6 and 21.8 ± 0.9 y, body weight of 69.7 ± 2.1 and 59.9 ± 2.2 kg, height of 1.8 ± 0.0 and 1.67 ± 0.0 m, and body mass index of 22.1 ± 0.5 and 21.4 ± 0.5, respectively, completed the study.

#### Food and water intake

In experiment 1, treatment affected food intake (P < 0.0001), and all doses of whey protein, except 10 g, significantly suppressed food intake at 30 min compared with control, with the lowest food intake after 40 g whey protein (Table 1). Cumulative energy intake from the preload and pizza meal, caloric compensation, and water intake were not significantly different between the treatments.

### Table 1

<table>
<thead>
<tr>
<th>Whey protein preload</th>
<th>Energy intake</th>
<th>Caloric compensation</th>
<th>Water intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test meal²</td>
<td>Cumulative³</td>
<td>%</td>
</tr>
<tr>
<td>Control⁵</td>
<td>1142 ± 59*</td>
<td>1142 ± 59</td>
<td>1142 ± 59</td>
</tr>
<tr>
<td>10 g</td>
<td>1064 ± 55*</td>
<td>1115 ± 55</td>
<td>153 ± 87</td>
</tr>
<tr>
<td>20 g</td>
<td>989 ± 71*</td>
<td>1091 ± 71</td>
<td>150 ± 59</td>
</tr>
<tr>
<td>30 g</td>
<td>983 ± 50*</td>
<td>1136 ± 50</td>
<td>104 ± 30</td>
</tr>
<tr>
<td>40 g</td>
<td>837 ± 41*</td>
<td>1041 ± 41</td>
<td>150 ± 23</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1. All values are means ± SEMs; n = 16. Values in the same column with different superscript letters are significantly different, P < 0.0001 (one-factor ANOVA for preload effect followed by Tukey’s post hoc test).
2. Energy consumed in an ad libitum meal 30 min after the preloads.
3. Energy in preloads + energy from the meal.
4. Caloric compensation = [(kcal consumed at the meal after the water control – kcal consumed at the meal after the whey protein preload)/kcal in the whey protein preload] × 100.
5. Water (300 mL).
Subjective average appetite score

In experiment 1, average appetite was affected by time \((P < 0.0001)\), being suppressed more at 15 min after the preloads than at 30 min and more at 50 min (immediately after the meal) than at 80 and 95 min. However, average appetite was not affected by preload or time and preload interaction. The mean average appetite scores were 69.6 ± 2.0 and 63.6 ± 1.9 mm at 0 and 30 min, respectively (see Supplementary Figure 1 under “Supplemental data” in the online issue).

In experiment 2, average appetite was affected by preload \((P < 0.01)\) and sex \((P < 0.0001)\). When expressed as a mean concentration over all measured times, men had higher subjective average appetite scores than did women \((40.2 ± 1.0\) compared with 22.9 ± 1.0 mm; 2-factor ANOVA, \(P < 0.0001\)). However, average appetite was not affected by a preload-by-sex interaction. Thus, the data are shown as pooled for the sexes.

Mean average appetite scores were 64.5 ± 2.1 and 63.8 ± 1.8 mm at 0 and 30 min, respectively. The average appetite response was affected by time \((P < 0.0001)\), being lower 15 min after all preloads than at 0 or 30 min and markedly reduced at 50 min (immediately after the meal) (see Supplementary Figure 1 under “Supplemental data” in the online issue). Preload was a factor \((P < 0.0001)\), with the 20-g and 40-g whey protein preload suppressing average appetite more than the control. However, there was no time-by-preload interaction.

Subjective average appetite AUC

In experiment 1, there was no difference between preloads in premeal average appetite AUC \((0–30\) min) (see Supplementary Figure 2 under “Supplemental data” in the online issue). However, postmeal average appetite AUC \((30–95\) min) was suppressed less after 30 and 40 g whey protein than after the control, likely because of the lower food intake after these preloads \((P < 0.01)\).

In experiment 2, no differences were found in the premeal AUC due to treatments. As expected, because of consumption of the preset meal, postmeal subjective average appetite AUCs were not affected by the premeal treatments (see Supplementary Figure 3 under “Supplemental data” in the online issue).

Blood glucose concentration

In both experiments, the baseline data are reported followed by an analysis of the change from baselines \((0\) min for premeal and \(30\) min for postmeal responses) because, in experiment 2, differences in baseline blood glucose concentrations between treatments were observed \((P < 0.05)\). In experiment 1, the blood glucose response was affected by time \((P < 0.0001)\), preload \((P < 0.0001)\), and the interaction between time and preload \((P < 0.0001)\).

At 0 and 30 min, overall mean blood glucose concentrations in both sexes combined were \(5.0 ± 0.0\) and \(5.2 ± 0.0\) mmol/L, respectively (Table 2). At 30 min, 20, 30, and 40 g whey resulted in lower blood glucose concentrations than did the control \((P < 0.01)\), but no difference was detected between the doses. Posttest meal blood glucose was lower at 50 and 65 min after all whey protein doses than after the control. This effect was sustained at 80 and 95 min for all except the 10-g doses \((P < 0.0001)\).

In experiment 2, the blood glucose response was affected by time \((P < 0.0001)\), preload \((P < 0.0001)\), and an interaction between time and preload \((P < 0.0001)\). There was no effect of sex or sex with preload interaction on blood glucose response \((2\)-factor ANOVA\). Therefore, the results shown for blood glucose are the pooled data for the sexes.

At 0 and 30 min, overall mean blood glucose concentrations in both sexes combined were \(4.9 ± 0.0\) and \(4.9 ± 0.0\) mmol/L, respectively (Table 3). At 15 min, 10 g WPH and at 30 min 10 g WPH and 40 g whey protein resulted in higher blood glucose responses than did the control \((P < 0.0001)\).

### Table 2
Experiment 1: effect of premeal whey protein on pre- and postmeal blood glucose responses

<table>
<thead>
<tr>
<th>Time</th>
<th>Control(^2)</th>
<th>10 g</th>
<th>20 g</th>
<th>30 g</th>
<th>40 g</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min(^3)</td>
<td>5.0 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Change from 0 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>30 min</td>
<td>0.0 ± 0.1(^b)</td>
<td>0.1 ± 0.1(^b)</td>
<td>0.3 ± 0.1(^a)</td>
<td>0.3 ± 0.1(^a)</td>
<td>0.2 ± 0.1(^a)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Absolute concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min(^3)</td>
<td>5.0 ± 0.1(^b)</td>
<td>5.2 ± 0.1(^b,c)</td>
<td>5.5 ± 0.1(^a)</td>
<td>5.3 ± 0.1(^b)</td>
<td>5.3 ± 0.1(^b,c)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change from 30 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 min</td>
<td>2.0 ± 0.2(^c)</td>
<td>0.9 ± 0.2(^b)</td>
<td>0.3 ± 0.2(^c)</td>
<td>0.2 ± 0.2(^c)</td>
<td>-0.2 ± 0.1(^c)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>65 min</td>
<td>2.5 ± 0.2(^c)</td>
<td>1.5 ± 0.2(^b)</td>
<td>0.7 ± 0.2(^c)</td>
<td>0.7 ± 0.2(^c)</td>
<td>0.1 ± 0.1(^c)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>80 min</td>
<td>1.9 ± 0.3(^c)</td>
<td>1.6 ± 0.2(^a)</td>
<td>0.8 ± 0.2(^c)</td>
<td>0.8 ± 0.2(^c)</td>
<td>0.3 ± 0.1(^b)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>95 min</td>
<td>1.6 ± 0.2(^c)</td>
<td>1.3 ± 0.2(^b)</td>
<td>0.8 ± 0.2(^c)</td>
<td>1.0 ± 0.1(^b,c)</td>
<td>0.6 ± 0.2(^c)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^{1}\) All values are means ± SEMs; \(n = 16\). Values at each time of measurement with different superscript letters are significantly different \(2\)-factor ANOVA, time-by-treatment interaction \((P < 0.0001)\), followed by one-factor ANOVA for preload effect and Tukey’s post hoc test \((P < 0.05)\).

\(^{2}\) Water control \((300\) mL)\).

\(^{3}\) Prior consumption of preloads \((baseline)\).
Postmeal blood glucose, expressed as the change from 30 min, was reduced by the whey protein preloads in a dose-dependent manner (Table 3). From 50 to 95 min, 20 and 40 g resulted in lower postmeal blood glucose concentrations, as did 40 g whey protein at 110 min \((P < 0.001)\). Compared with the control, 10 g whey protein reduced postmeal blood glucose at 65, 80, and 170 min, but 10 g WPH reduced the postmeal blood glucose increase only at 65 min \((P < 0.05)\). There was no difference in blood glucose between the preloads at 140 min.

**TABLE 3** Experiment 2: effect of premeal whey protein on pre- and postmeal blood glucose response

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (^1)</th>
<th>5 g</th>
<th>10 g (^2)</th>
<th>10 g</th>
<th>20 g</th>
<th>40 g</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mmol/L</td>
<td></td>
<td>mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute concentration 0 min (^3)</td>
<td>4.8 ± 0.1(^{a,b})</td>
<td>4.8 ± 0.1(^{a,b})</td>
<td>4.7 ± 0.1(^{b})</td>
<td>5.0 ± 0.1(^{a})</td>
<td>4.9 ± 0.1(^{b})</td>
<td>4.9 ± 0.1(^{a,b})</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Change from 0 min 15 min</td>
<td>−0.0 ± 0.1(^{b})</td>
<td>0.1 ± 0.1(^{b})</td>
<td>0.3 ± 0.1(^{a})</td>
<td>−0.1 ± 0.1(^{b})</td>
<td>0.2 ± 0.1(^{b})</td>
<td>0.2 ± 0.1(^{a,b})</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>30 min</td>
<td>−0.1 ± 0.1(^{c})</td>
<td>0.0 ± 0.1(^{c})</td>
<td>0.2 ± 0.1(^{a})</td>
<td>−0.1 ± 0.1(^{b,c})</td>
<td>0.2 ± 0.1(^{b,c})</td>
<td>0.2 ± 0.1(^{a,b})</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Absolute concentration 30 min (^2)</td>
<td>4.7 ± 0.1(^{c})</td>
<td>4.8 ± 0.1(^{b,c})</td>
<td>5.0 ± 0.1(^{a,b})</td>
<td>4.9 ± 0.1(^{a})</td>
<td>5.0 ± 0.1(^{b})</td>
<td>5.1 ± 0.1(^{a})</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Change from 30 min 50 min</td>
<td>0.6 ± 0.1(^{a})</td>
<td>0.5 ± 0.1(^{a})</td>
<td>0.4 ± 0.1(^{a,b})</td>
<td>0.2 ± 0.1(^{a,b})</td>
<td>0.0 ± 0.1(^{b,c})</td>
<td>−0.3 ± 0.1(^{c})</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>65 min</td>
<td>2.6 ± 0.2(^{a})</td>
<td>2.4 ± 0.2(^{a})</td>
<td>2.0 ± 0.1(^{b,c})</td>
<td>1.6 ± 0.2(^{e})</td>
<td>0.7 ± 0.1(^{d})</td>
<td>0.1 ± 0.1(^{c})</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>80 min</td>
<td>27 ± 0.3(^{a})</td>
<td>24 ± 0.3(^{a})</td>
<td>2.2 ± 0.2(^{a,b})</td>
<td>1.8 ± 0.2(^{b})</td>
<td>1.0 ± 0.1(^{c})</td>
<td>0.3 ± 0.1(^{d})</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>95 min</td>
<td>2.3 ± 0.3(^{a})</td>
<td>1.9 ± 0.3(^{a})</td>
<td>2.0 ± 0.3(^{a})</td>
<td>1.6 ± 0.2(^{a,b})</td>
<td>1.0 ± 0.1(^{b,c})</td>
<td>0.5 ± 0.2(^{c})</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>110 min</td>
<td>2.0 ± 0.3(^{a})</td>
<td>1.6 ± 0.2(^{a})</td>
<td>1.7 ± 0.3(^{a,b})</td>
<td>1.4 ± 0.2(^{a,c})</td>
<td>1.2 ± 0.1(^{b,c})</td>
<td>0.8 ± 0.2(^{c})</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>140 min</td>
<td>1.5 ± 0.2(^{a})</td>
<td>1.6 ± 0.2(^{a})</td>
<td>1.4 ± 0.2(^{a})</td>
<td>1.3 ± 0.1(^{a})</td>
<td>1.3 ± 0.1(^{b})</td>
<td>1.1 ± 0.2(^{a})</td>
<td>NS</td>
</tr>
<tr>
<td>170 min</td>
<td>1.4 ± 0.1(^{a})</td>
<td>1.0 ± 0.1(^{a,b})</td>
<td>1.3 ± 0.1(^{a,b})</td>
<td>1.0 ± 0.1(^{a,b})</td>
<td>1.0 ± 0.1(^{a,b})</td>
<td>1.3 ± 0.2(^{b})</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^1\)All values are means ± SEMs; \(n=21\). Values at each time of measurement with different superscript letters are significantly different [2-factor ANOVA, time-by-treatment interaction \((P < 0.0001)\), followed by one-factor ANOVA for preload effect and Tukey’s post hoc test \((P < 0.05)\)].

\(^2\)Water control (300 mL).

\(^3\)Whey protein hydrolysate (10 g).

\(^4\)Prior consumption of preloads (baseline).

\(^5\)Prior consumption of preset fixed pizza meal (30 min).

**FIGURE 1.** Mean (±SEM) premeal (0–30 min) and postmeal (30–95 min) (A) and cumulative (0–95 min) (B) blood glucose areas under the curve (AUC) after consumption of the whey protein preload \((n=16)\). Values with different lowercase letters are significantly different, \(P < 0.0001\) (one-factor repeated-measures ANOVA followed by Tukey’s post hoc test).
Blood glucose AUC

In experiment 1, 20 and 30 g whey protein, with no difference between the whey doses, led to a small but significantly higher premeal blood glucose AUC (0–30 min) compared with the control \((P = 0.01)\) (Figure 1A). The postmeal blood glucose AUC (30–95 min) was reduced by all whey protein preloads (Figure 1). Of the whey protein preloads, 10 and 40 g resulted in the highest and lowest postmeal blood glucose AUCs \((P < 0.0001)\), respectively, and the 20- and 30-g whey protein preloads resulted in intermediate AUCs. Compared with the control, the cumulative blood glucose AUC (0–95 min) was reduced by all whey protein doses, the lowest value being after 40 g \((P < 0.0001)\) (Figure 1B).

In experiment 2, 20 and 40 g whey protein and 10 g WPH resulted in higher premeal blood glucose AUCs than did 10 g whey or the control \((P < 0.01)\) (Figure 2A). The premeal blood glucose AUC after the 5-g whey protein preload did not differ from any other preloads. Postmeal blood glucose AUCs decreased after 10, 20, and 40 g whey protein \((P < 0.0001)\), but not after 5 g whey protein or 10 g WPH, compared with the control. 

FIGURE 2. Mean (±SEM) premeal (0–30 min) and postmeal (30–170 min) blood glucose (A) and insulin (B) areas under the curve (AUC) after consumption of the whey protein and whey protein hydrolysate (WPH) preload \((n = 21)\). Values with different lowercase letters are significantly different, \(P < 0.05\) (one-factor repeated-measures ANOVA followed by Tukey’s post hoc test).

FIGURE 3. Mean (±SEM) cumulative (0–170 min) blood glucose (A) and insulin (B) areas under the curve (AUC) after consumption of the whey protein and whey protein hydrolysate (WPH) preload \((n = 21)\). Values with different lowercase letters are significantly different, \(P < 0.001\) (one-factor repeated-measures ANOVA followed by Tukey’s post hoc test).
control (Figure 2). The cumulative blood glucose AUC (0–170 min) was lower after the 10-, 20-, and 40-g whey protein preloads than after 5 g whey protein, 10 g WPH, and the control (%  < 0.0001), with no differences between the latter (Figure 3A).

Insulin

In experiment 2, insulin was affected by time (%  < 0.0001), by preload (%  < 0.01), and by the interaction between time and preload (%  < 0.01). Insulin was affected by sex (%  < 0.0002). When expressed as a mean concentration over all measured times, capillary insulin concentrations were higher in women than in men (29.5 compared with 17.4 μIU/mL; 2-factor ANOVA, P < 0.002). However, there was no interaction between the effect of sex and preload. Therefore, the pooled data are presented for men and women.

There was a trend for a significant difference in insulin concentrations between the preloads at baseline (%  = 0.07). Therefore, the baseline data are reported followed by an analysis of the change from baselines.

At 0 and 30 min, overall mean insulin concentrations in both sexes combined were 4.5 ± 0.2 and 11.8 ± 0.8 μIU/mL, respectively (Table 4). All whey doses, except 5 g, increased the insulin response to 30 min, immediately before the meal, compared with the control (%  < 0.0001); the highest response was after 20 and 40 g whey protein, followed by 10 g whey protein and 10 g WPH. At 50 min, right after the meal, 40 g whey protein resulted in less of an increase in insulin than did the control (%  < 0.05). From 80 to 170 min, all whey doses, except 5 g, reduced postmeal insulin responses (%  < 0.0001).

Insulin AUC

The premeal insulin AUC (0–30 min) was higher after all whey protein doses, except 5 g, than after the control (%  < 0.0001) (Figure 2B). The 20- and 40-g whey protein preloads resulted in the highest premeal insulin AUC, followed by 10 g WPH and 10 g whey protein (%  < 0.0001). Conversely, the postmeal insulin AUC (30–170 min) was lower after all doses of whey protein, except 5 g, than after the control (%  < 0.001) (Figure 2). There was no statistically significant difference in the cumulative insulin AUC (0–170 min) between the protein preloads and the control (Figure 3B).

Relations between dependent measures

In experiment 1, food intake was not correlated with pre- or postmeal blood glucose AUC or subjective average appetite AUC. The premeal blood glucose AUC was inversely associated with the postmeal blood glucose AUC (r = −0.35, P = 0.002). Larger premeal and postmeal blood glucose AUCs were associated with greater suppression of premeal (r = −0.33, P = 0.003) and postmeal (r = −0.22, P = 0.05) average appetite AUCs. The greater the suppression of premeal average appetite AUC, the lower the suppression of postmeal average appetite AUC (r = −0.30, P = 0.006).

In experiment 2, positive associations were found between premeal (r = 0.31, P < 0.001), postmeal (r = 0.56, P < 0.0001), and cumulative (r = 0.35, P < 0.0001) blood glucose with insulin AUCs. The premeal insulin AUC was inversely correlated with the postmeal blood glucose AUC (r = −0.42, P < 0.0001), but the premeal blood glucose AUC was not associated with the postmeal insulin AUC (r = −0.1, P = 0.1) or the postmeal insulin AUC (r = −0.7, P = 0.4). The premeal insulin AUC (0–30 min) was inversely associated with the postmeal insulin AUC (30–170 min) (r = −0.22, P < 0.05). The greater the suppression of postmeal and cumulative average appetite AUCs, the greater the postmeal blood glucose AUC (r = −0.22, P > 0.05) and cumulative blood glucose AUC (r = −0.20, P < 0.05). Postmeal and cumulative average appetite AUCs and insulin AUCs were not associated.

### TABLE 4
Experiment 2: effect of premeal whey protein on pre- and postmeal insulin responses

<table>
<thead>
<tr>
<th>Time</th>
<th>Control²</th>
<th>5 g</th>
<th>10 g²³</th>
<th>10 g</th>
<th>20 g</th>
<th>40 g</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute concentration</td>
<td>μIU/mL</td>
<td>μIU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>3.6 ± 0.5</td>
<td>4.7 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>4.5 ± 0.5</td>
<td>4.9 ± 0.5</td>
<td>5.2 ± 0.6</td>
<td>0.07</td>
</tr>
<tr>
<td>Change from 0 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>0.0 ± 0.3</td>
<td>3.2 ± 0.7</td>
<td>6.3 ± 0.8</td>
<td>7.2 ± 0.9</td>
<td>12.4 ± 1.7</td>
<td>14.5 ± 2.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Absolute concentration</td>
<td>μIU/mL</td>
<td>μIU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 min²</td>
<td>3.6 ± 0.5</td>
<td>7.9 ± 0.8</td>
<td>10.7 ± 1.0</td>
<td>11.7 ± 1.2</td>
<td>17.3 ± 2.0</td>
<td>19.7 ± 2.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Change from 30 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 min</td>
<td>19.0 ± 3.7</td>
<td>12.4 ± 3.1</td>
<td>13.5 ± 3.0</td>
<td>13.7 ± 3.1</td>
<td>12.7 ± 2.2</td>
<td>8.5 ± 2.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>80 min</td>
<td>38.8 ± 5.6</td>
<td>30.2 ± 3.8</td>
<td>25.9 ± 3.8</td>
<td>23.2 ± 3.3</td>
<td>18.8 ± 3.7</td>
<td>17.0 ± 2.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>110 min</td>
<td>31.3 ± 5.3</td>
<td>23.9 ± 3.9</td>
<td>18.7 ± 3.2</td>
<td>16.0 ± 2.9</td>
<td>11.1 ± 2.6</td>
<td>13.3 ± 3.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>140 min</td>
<td>23.5 ± 3.4</td>
<td>19.6 ± 3.0</td>
<td>12.1 ± 1.9</td>
<td>11.5 ± 1.7</td>
<td>8.3 ± 2.4</td>
<td>11.9 ± 2.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>170 min</td>
<td>20.7 ± 3.6</td>
<td>13.3 ± 2.1</td>
<td>8.9 ± 1.4</td>
<td>8.8 ± 1.3</td>
<td>2.7 ± 1.9</td>
<td>6.7 ± 2.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

¹ All values are means ± SEMs; n = 21. Values at each time of measurement with different superscript letters are significantly different [2-factor ANOVA, time-by-treatment interaction (%  < 0.01), followed by one-factor ANOVA for preload effect and Tukey’s post hoc test (%  < 0.05)].

² Water control (300 mL).

³ Whey protein hydrolysate (10 g).

⁴ Prior consumption of preloads (baseline).

⁵ Prior consumption of preset fixed pizza meal (30 min).
An inverse association was found between the dose of whey protein and the ratios of cumulative blood glucose/insulin AUC ($r = -0.33, P < 0.001$) (Figure 4) and the change from 30-min blood glucose/insulin responses at 80 min ($r = -0.41, P < 0.001$) (Figure 5).

**DISCUSSION**

These studies are the first to evaluate the effect of consuming whey protein alone before a meal on blood glucose and insulin responses in healthy young adults. The results of these studies showed that whey protein, in relatively small amounts and when consumed before a meal, reduces food intake and postmeal blood glucose while reducing postmeal insulin response. Thus, the effects of premeal intact whey protein, but not WPH, on postmeal blood glucose control are not explained in full by its insulinotropic action. Furthermore, because WPH did not reduce the postmeal blood glucose response, it may be suggested that noninsulintropic mechanisms require stimulation arising from the digestion of intact proteins.

The preload doses were given 30 min before the meal, based on the peak insulin response time after protein and carbohydrate loads (10, 14). Thus, it was anticipated that responses in food intake and in blood glucose and insulin would be observed at lower doses than used previously. In experiment 2, a fixed-size meal was fed with the objective of isolating the effect of whey protein and WPH on blood glucose control by insulin, independent of variations in food intake. However, it is clear that the benefit of premeal consumption of whey protein in ad libitum eating patterns resides in its effect on reducing both food intake and postmeal glycemic and insulin responses.

Because plasma insulin increased similarly after 10 g of both whey protein and WPH preloads (Table 3), the role of branched-chain amino acids in stimulating insulin release and secretion is supported (11). However, 4 lines of evidence suggest that insulin alone, as indicated by plasma insulin concentrations, cannot be the only cause of the lower postmeal blood glucose after premeal consumption of intact whey protein. First, the lower postmeal blood glucose with increasing doses of whey protein in both experiments (Figures 1A and 2A) was achieved in the presence of a lower, not higher, postmeal insulin AUC (Figure 2B) and a similar cumulative (0–170 min) insulin AUC (Figures 3B) in experiment 2. Second, when the cumulative AUC for blood glucose was divided by the cumulative AUC for insulin to evaluate the efficacy of insulin action (24, 25), the ratio was decreased, in a dose-dependent manner, to 50% of the control after a premeal consumption of intact whey protein of 40 g (Figure 4). Thus, it is clear that lower blood glucose concentrations occurred without an overall increase in insulin requirement. Third, the ratio of blood glucose to insulin response at 80 min was also decreased in a dose-dependent manner to <25% of the control (Figure 5). Fourth, in contrast with 10 g intact protein, 10 g WPH did not result in a lower cumulative blood glucose concentration than did the control, even though it increased the postmeal (Figure 2B) and cumulative insulin (Figure 3B) AUC similarly.

Although the mechanism by which premeal whey protein brings about improved postmeal glucose control is unclear, the most probable explanation for the insulin-independent actions of
premeal consumption of whey protein on blood glucose control resides in the effect of protein on gastric emptying. Even a modest change in the gastric emptying rate affects the magnitude and timing of postprandial blood glucose and insulin increases (27, 28) and is decreased by protein ingestion consumed either with carbohydrate (29) or alone (10). A reduction in stomach emptying was suggested in the present study by the lower peak blood glucose concentration at 80 min (30 min after the meal), after all protein preloads, but the peak rise in blood glucose was much more attenuated after 20, 30, and 40 g whey protein and continued to rise until 140 min (90 min postmeal), at which time the blood glucose concentrations were not different between the treatments (Table 3). Furthermore, slower stomach emptying would be expected because whey protein and other proteins release cholecystokinin, glucagon-like peptide 1 (10), glucose-dependent insulinotropic polypeptide (13), and peptide tyrosine tyrosine from the intestinal enteroendocrine cells (19, 29, 30). The release of these hormones may be an explanation for the lack of effect of 10 g WPH on blood glucose, but not intact whey protein, because a branched-chain amino acid mixture produces the effect of the intact whey protein on insulin but not on gut hormones, including cholecystokinin and glucagon-like peptide 1 (13).

The dose of whey protein required to suppress food intake when consumed 30 min before the meal was shown to range from 20 to 40 g in experiment 1, based on a sample size of 16 individuals. However, based on a recalculation of sample size using the variability found in this study, the 78-kcal reduction after 10 g whey protein may have been found to be statistically significant with a sample size of 40 subjects.

The efficacious dose of premeal whey protein required to affect the insulin and postmeal glycemic response in healthy young adults was found to be as low as 10 g and possibly 5 g. Power calculations suggest that an increased sample size of 40 subjects would detect an effect of 5 g whey with a power of 0.8. The efficacy of consumption of 55 g whey protein before a meal in controlled type 2 diabetic patients has been confirmed (19) and suggested to be comparable with the effect of pharmacologic therapy, such as the reduction in postprandial glycemia with sulfonylureas, but the subjects were provided only 59 g carbohydrate at the meal. In our study, the men and women averaged 103 and 82 g carbohydrate intake at the fixed-size pizza meal, which suggests that premeal administration of whey protein in relatively small amounts is very efficacious in contributing to glycemic control in healthy subjects. It remains to be determined, however, whether these small doses have a benefit in patients with type 2 diabetes.

In both experiments, an inverse association was found between postmeal blood glucose and average appetite AUCs (experiment 1: $r = -0.22$; experiment 2: $r = -0.21$, $P < 0.05$), which suggests that, independent of either premeal treatment or the amount of carbohydrate consumed (experiment 2), the higher the postmeal blood glucose concentration, the greater the suppression of average appetite, as observed in previous studies (21). In experiment 2, there was no significant association between insulin and average appetite AUCs, which suggests that blood glucose was a better predictor of satiety, even though insulin acts as a satiety hormone in the short term (31).

In conclusion, whey protein consumed before a meal reduces food intake and postmeal blood glucose and insulin and the ratio of the cumulative blood glucose/insulin AUC in a dose-dependent manner. Intact whey protein, but not WPH, contributes to blood glucose control by both insulin-dependent and insulin-independent mechanisms. Thus, the premeal ingestion of whey protein...
protein may be an effective strategy for achieving blood glucose control in healthy and insulin-resistant humans.

The authors’ responsibilities were as follows—GHA: conceived the hypothesis, designed the experiment, and wrote the manuscript; CEC: conducted experiment 1 as part of her summer student project; TA: contributed to the design and writing of the manuscript, conducted the experiments, and collected and analyzed the data; BLL and PHB: collaborated in the design of the study and reviewed the manuscript; and PHB: arranged the financial donation, provided the test materials, participated in the discussion of the design, and reviewed the manuscript. PHB is an employee of Kraft Foods Global LLC (Chicago, IL). None of the other authors declared a conflict of interest.

REFERENCES