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Effects of randomized whey-protein loads on energy intake, appetite, gastric emptying, and plasma gut-hormone concentrations in older men and women

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ABSTRACT

Background: Protein- and energy-rich supplements are used widely for the management of malnutrition in the elderly. Information about the effects of protein on energy intake and related gastrointestinal mechanisms and whether these differ between men and women is limited.

Objective: We determined the effects of whey protein on energy intake, appetite, gastric emptying, and gut hormones in healthy older men and women.

Design: Eight older women and 8 older men [mean ± SEM age: 72 ± 1 y; body mass index (in kg/m^2): 25 ± 1] were studied on 3 occasions in which they received protein loads of 30 g (120 kcal) or 70 g (280 kcal) or a flavored water control drink (0 kcal). At regular intervals over 180 min, appetite (visual analog scales), gastric emptying (3-dimensional ultrasonography), and blood glucose and plasma gut-hormone concentrations [insulin, glucagon, ghrelin, cholecystokinin, gastric inhibitory polypeptide (GIP), glucagon-like peptide 1 (GLP-1), and peptide tyrosine tyrosine (PYY)] were measured, and ad libitum energy intake was quantified from a buffet meal (180–210 min; energy intake, appetite, and gastric emptying in the men have been published previously).

Results: Energy intake at the buffet meal was ∼80% higher in older men than in older women ($P < 0.001$). Energy intake was not suppressed by protein compared with the control in men or women ($P > 0.05$). There was no effect of sex on gastric emptying, appetite, gastrointestinal symptoms, glucose, or gut hormones ($P > 0.05$). There was a protein load–dependent slowing of gastric emptying, an increase in concentrations of insulin, glucagon, cholecystokinin, GIP, GLP-1, and PYY, and an increase in total energy intake (drink plus meal: 12% increase with 30 g and 32% increase with 70 g; $P < 0.001$). Energy intake at the buffet meal was inversely related to the stomach volume and area under the curve of hormone concentrations ($P < 0.05$).

Conclusion: In older men and women, whey-protein drinks load-dependently slow gastric emptying and alter gut hormone secretion compared with a control but have no suppressive effect on subsequent ad libitum energy intake. This trial was registered at www.anzctr.org.au as ACTRN12612000941864. *Am J Clin Nutr* 2017;106:865–77.

Keywords: aging, appetite and energy intake, gastrointestinal function, gastrointestinal mechanisms, sex, whey protein

INTRODUCTION

Over recent decades, the prevalence of malnutrition, both as undernutrition and obesity, has increased in older men and women (1, 2). A growing awareness of the prevalence and adverse effects of the major muscle loss that occurs during aging, irrespective of BMI (e.g., reduced functional capacity and decreased quality of life) (1, 3, 4), has led to the development of nutritional strategies that were designed specifically to preserve or restore skeletal muscle mass and function. A common strategy is the use of supplements, which are usually high-energy drinks that are rich in whey protein (5–9).

Despite the increasing use of protein-rich drinks, information about their effects on energy intake and underlying gastrointestinal

Supported by an Adelaide Geriatrics Training and Research with Aged Care Resthaven grant. S Soenen was supported by a Royal Adelaide Hospital Florey Fellowship (2129), NDL-M was supported by a National Health and Medical Research Council (NHMRC) New Investigator project grant (627118), KLJ was supported by an NHMRC Senior Clinical Career Development award (627011), and LGT was supported by an Australian postgraduate award and a Dawes scholarship from the Royal Adelaide Hospital. Whey protein was donated by the Fonterra Research and Development Center, Palmerston North, New Zealand, and antisera was donated by B Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Munich, Germany.

Supplemental Figure 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Abbreviations used: GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; PYY, peptide tyrosine tyrosine; T50, 50% gastric-emptying time; 3D, 3-dimensional.

Received February 5, 2017. Accepted for publication June 27, 2017.

First published online July 26, 2017; doi: https://doi.org/10.3945/ajcn.117.154377.

mechanisms in older men and, particularly, older women is lacking. In young adults, protein is the most satiating of the 3 macronutrients (10). After a mixed macronutrient preload, young women, compared with young men, appear to compensate less in the subsequent meal, which results in higher total (i.e., meal plus preload) energy intake (11, 12). Variations in gut-hormone secretion or action [e.g., ghrelin, cholecystokinin, glucose-dependent insulinotropic polypeptide/gastric inhibiting polypeptide (GIP), glucagon-like polypeptide-1 (GLP-1), and peptide tyrosine tyrosine (PYY)] as well as the rate of gastric emptying and gastric distension are likely to play a role in the regulation of appetite and energy intake in younger adults particularly in the short-term after nutrient ingestion (13–19).

Compared with young adults, healthy older people exhibit decreased taste and food palatability, are less hungry and fuller during fasting and postprandial states, and consume less food and energy including protein (20). These factors have been termed "the physiologic anorexia of aging" (3, 4). Healthy aging is associated with a reduced responsiveness to the suppressive effects of nutrients on appetite and energy intake (21–24). We have recently shown that the acute administration of 30-g (120-kcal) and 70-g (280-kcal) oral whey-protein loads suppressed subsequent energy intake by 12–17% in young people and without suppression in healthy older men (24). Accordingly, compared with the control, protein ingestion increased total energy intake (protein plus subsequent ad libitum energy intake) more in older men than in younger men.

In this study, we aimed to further characterize the feeding and gut (hormone) responses to orally ingested whey-protein loads in older people by studying older women as well as older men. We hypothesized that orally administered whey protein would have load-related effects on gastric emptying and plasma gut-hormone concentrations (insulin, glucagon, ghrelin, cholecystokinin, GIP, GLP-1, and PYY) in healthy older men and women, suppress subsequent ad libitum energy intake less, and, therefore, result in a greater increase in overall energy intake (protein drink plus meal intake) than would a control in older women compared with older men (previously published data of energy intake, appetite, and gastric emptying).

METHODS

Subjects

The study included 8 older men [mean ± SEM age: 73 ± 1 y; body weight: 77 ± 4 kg; height: 1.73 ± 0.02 m; BMI (in kg/m^2): 26 ± 1; the men were included in our previous study comparing energy intake, appetite, and gastric emptying between young and older men (24)] and 8 older women [age: 70 ± 1 y; body weight: 63 ± 2 kg; height: 1.58 ± 0.02 m; BMI: 25 ± 1] who were recruited through an advertisement (a participant flowchart is shown in **Supplemental Figure 1**). There were no differences in either age ($P = 0.14$) or BMI ($P = 0.70$) between the men and women. On the basis of our previous work (23), with an observed within-subjects SD of 181 kcal and upper 60% confidence limit of 234 kcal, we calculated that 8 subjects/group would allow detection of a within-groups difference between treatments of 271 kcal ($n = 8$ older women) and a between-sexes difference of 353 kcal ($n = 8$ older women compared with $n = 8$ older men), with power equal to 0.8 and $\alpha = 0.05$.

Exclusion criteria were as follows: smoking; alcohol abuse; use of illicit substances; diabetes; gallbladder or pancreatic disease; gastrointestinal surgery (apart from an uncomplicated appendectomy); gastrointestinal symptoms (abdominal pain, gastroesophageal reflux, diarrhea, or constipation); use of medications that are known to potentially affect energy intake, appetite, or gastrointestinal motor function; impaired cognitive function [score <25 on the Mini-Mental State (25)], depression [score ≥11 on the Geriatric Depression Questionnaire (26)], and undernutrition [score <24 on the Mini Nutritional Assessment (27)]; being lactose intolerant or having food allergies; low ferritin concentrations or blood donation in the 12 wk before the study days; and failing to comprehend the study protocol. The Royal Adelaide Hospital Human Research Ethics Committee approved the study protocol, and the study was conducted in accordance with the Declaration of Helsinki. The study was registered as a clinical trial with the Australian New Zealand Clinical Trial Registry (www.anzctr.org.au; ACTRN12612000941864). All subjects provided written informed consent before their inclusion.

Protocol

The protocol was identical to that of our previous study that compared young and older men, and the results (i.e., energy intake, appetite, and gastric emptying) in the older men have been published previously (24). Each subject was studied on 3 occasions that were separated by 3–14 d to determine the effects of 2 oral whey-protein loads [30 g (120 kcal) and 70 g (280 kcal)] and a flavored-water control drink (~0 g protein) on energy intake, gastric emptying, perceptions of appetite, and gastrointestinal symptoms in a randomized [via the method of randomly permuted blocks; www.randomization.com (16 subjects were randomly assigned in 1 block with random permutations)], double-blind, crossover design.

Protein drinks were served in a covered cup and prepared by dissolving whey protein isolate (Fonterra Co-Operative Group Ltd.) in varying volumes of demineralized water and diet lime cordial (Bickford's Australia Pty Ltd.) to achieve the desired loads [i.e., 30 g whey protein (volume of the powder: 19 mL) in 335 mL distilled water and 85 mL cordial (2.5 kcal/100 mL) and 70 g whey protein (volume of the powder: 45 mL) in 280 mL H_2O and 100 mL cordial; the control drink consisted of 359 mL distilled water and 90 mL cordial]. Sodium chloride in the amounts of 0.3 and 1.2 g was added to the 30-g and control drinks, respectively, to match the osmolarity (88 mOsm/L) with the 70-g drink. To ensure that all ingredients were dissolved evenly throughout and to minimize the layer of foam on top of the solution, the drinks were stirred continuously at low speed on a stirring plate. The volume of each drink was measured before serving, and the recorded volumes differed modestly (i.e., control: 450 mL; 30 g protein: 439 mL; and 70 g protein: 425 mL). All drinks were provided to participants in a covered cup so that both the investigator who was conducting the data collection and the participants were blinded to the test drinks. The drinks were prepared by a research assistant who was not involved in the data analysis of the study results.

Subjects were provided a standardized evening meal [beef lasagna (McCain Foods Pty Ltd.) (~591 kcal) to consume on the night before each study day at ~1900. They were instructed to fast overnight from solids and liquids and to refrain from strenuous physical activity until they attended the laboratory at the Discipline of Medicine, The University of Adelaide, Royal Adelaide Hospital, at ~0830.

On arrival, subjects were seated in an upright position on a wooden chair where they remained for the duration of the study,

and an intravenous cannula for blood taking was inserted. In each subject, measurements of total gastric volume and perceptions of appetite and gastrointestinal symptoms were performed immediately before (during fasting; 0 min) and immediately after ingestion of the drink and at 15-min intervals until 180 min. Subjects were instructed to consume the drink within 2 min. Gastric volume was measured with the use of 3-dimensional (3D) ultrasonography (24). Perceptions of appetite and gastrointestinal symptoms were assessed with the use of validated visual analog scales, and blood samples were collected for the measurements of gut hormones. At 180 min, each subject was presented with a standard, cold, buffet-style meal in excess of what they were expected to consume (total energy content of 2457 kcal; 19% protein, 50% carbohydrates, and 31% fat) for 30 min (180–210 min) until they were comfortably full in a room by themselves to limit external distractions (28). The buffet-style meal consisted of palatable food items including sliced bread, cheese, ham and chicken, fruits, yogurt, custard, margarine, mayonnaise, iced coffee, orange juice, fruit salad, and water (24).

Measurements

Energy intake

The amount that was eaten at the buffet meal (grams) was quantified by weighing the food before and after consumption. Energy intake (kilocalories) at the buffet meal and proportions of protein, carbohydrate, and fat were calculated with the use of commercially available software (Foodworks version 8; Xyris Software Pty Ltd.). Energy intake was calculated both as intake at the buffet meal and as cumulative energy intake, which was defined as the sum of energy intake at the buffet meal and the energy content of the preload drink. Absolute (kilocalories) and percentage suppression per change of energy intakes at the buffet meal (the percentage of energy intake of the control per day) by a given protein load compared with the control were calculated.

Perceptions of appetite and gastrointestinal symptoms

Perceptions of hunger, desire to eat, prospective consumption, fullness, nausea, and bloating were rated with the use of a visual analog scale questionnaire at 0, 5, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180, and 210 min (29). The questionnaire consisted of 100-mm horizontal lines whereby 0 represented that the sensation was "not felt at all," and 100 represented that the sensation was "felt the greatest." Subjects placed a vertical mark on each horizontal line to indicate the strength of each sensation at the specified time points.

Gastric emptying

Total gastric volume was measured with the use of a Logiq 9 ultrasound system (GE Healthcare Technologies) with TruScan Architecture (built-in magnetically sensored 3D positioning and orientation measurement) including a 3D sensor, which was attached to a 3.5C broad spectrum 2.5–4-MHz convex transducer, and a transmitter, which was placed at the level of the stomach immediately behind the subject at 0, 5, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min (30). Because the transmitter produces a spatially varying magnetic field that is distorted by conductive metals, all metal objects were removed from the subject to minimize interference during image acquisition. The stomach was scanned by a continuous translational movement along its long axis (~10 s). During each scan, the subject was instructed to sit still and hold their breath at the end of inspiration. If gastric contractions were observed, the acquisition was paused until the contraction wave had passed. The raw data (original scan planes) were transferred for 3D reconstructions and a volume estimation with the use of EchoPAC–3D purpose-built software (GE Vingmed Sound). Gastric retention was calculated as total gastric volume minus baseline fasting gastric volume at each time point and expressed as the percentage of the maximal gastric volume (100%) (i.e., volume of the ingested drink). When ultrasound images lacked sufficient clarity to determine the volume of the stomach, data were imputed by linear interpolation. In one male subject, the quality of ultrasound stomach images was insufficient to determine gastric emptying in all 3 conditions, and therefore, data of gastric emptying of this subject were excluded from the analysis. The time at which 50% of the preload drink had emptied from the stomach [50% gastric-emptying time (T50); minutes] was calculated for all conditions. The rate of gastric emptying was calculated as the mean of the rates of emptying (kilocalories per minute) during each 15-min interval, respectively, of the early phase (0–60-min), late phase (60–180-min), and total (0–180-min) time periods.

Blood glucose and plasma insulin, glucagon, ghrelin, cholecystokinin, GIP, GLP-1, and PYY

Blood samples were collected, at 0, 5, 15, 30, 45, 60, 90, 120, 150, and 180 min into ice-chilled, EDTA-coated tubes. No inhibitors were added (31). Plasma was obtained by centrifugation for 15 min at 3200 × *g* at 4°C, and samples were stored at −80°C for further analysis of hormone concentrations.

Blood glucose (millimoles per liter) was determined immediately after collection via the glucose oxidase method with the use of a portable glucometer (Optium Xceed; Abbott Laboratories). Intra-assay and interassay CVs were 3.2% and 10.8%, respectively.

Total plasma insulin (milliunits per liter) was measured with an ELISA immunoassay (10-1113; Mercodia). The minimum detectable limit was 1.0 mU/L. Intra-assay and interassay CVs were 3.0% and 8.7%, respectively.

Total plasma glucagon (picograms per milliliter) was measured with a radioimmunoassay (GL-32K; Millipore). The minimum detectable limit was 20 pg/mL. The intra-assay and interassay CVs were 4.3% and 7.1%, respectively. The ratio of insulin to glucagon was calculated for each time point in each subject. A homeostatic model assessment index at baseline was calculated according to the following formula (32):

$$\text{Insulin concentration}(\mu\text{U/L}) \times \text{glucose concentration}(\text{nM/L}) \div 22.5 \quad (1)$$

Total plasma ghrelin (picograms per milliliter) was measured with the use of a radioimmunoassay with some modifications to a published method (33). The radiolabel (NEX388) was supplied by Perkin Elmer. The standard and samples were incubated with the antibody and radiolabel for 3–4 d at 4°C. The detection limit was 40 pg/mL. Intra-assay and interassay CVs were 6.7% and 12.1%, respectively.

Plasma cholecystokinin-8 (picomoles per liter) was measured via a radioimmunoassay with the use of an adaption of a previous method (34). Samples were extracted in 66% ethanol; extracts

were dried down and resuspended in assay buffer (50 mmol phosphate/L, 10 mmol EDTA/L, and 2 g gelatin/L; pH = 7.4). Standards were prepared with synthetic sulfated cholecystokinin-8 (Sigma Chemical), antibody (C2581, lot 105H4852; Sigma Chemical) was added at a working dilution of 1:17,500, and sulfated cholecystokinin-8 ^{125}I-labeled with Bolton and Hunter reagent (Perkin Elmer) was used as a tracer. Incubation was for 7 d at 4°C. The antibody-bound fraction was separated by the addition of dextran-coated charcoal-containing gelatin (0.015 g gelatin, 0.09 g dextran, and 0.15 g charcoal in 30 mL assay buffer), and the radioactivity was determined in the supernatant fluid after centrifugation. The detection limit was 1 pmol/L. The Intra-assay and interassay CVs were 5.4% and 13.9%, respectively.

Total plasma GIP (picomoles per liter) was measured via a radioimmunoassay (35). The standard curve was prepared in buffer rather than extracted charcoal stripped serum, and the radioiodinated label was supplied by Perkin Elmer. The minimum detectable limit was 2 pmol/L. The intra-assay and interassay CVs were 3.9% and 9%, respectively.

Total plasma GLP-1 (picomoles per liter) was measured via a radioimmunoassay (GLPIT-36HK; Millipore). The detection limit was 3 pmol/L. Intra-assay and interassay CVs were 6.3% and 10.3%, respectively.

Total plasma PYY (picomoles per liter) was measured via a radioimmunoassay with the use of antisera (B Otto, Medizinische Klinik, Klinikum Innenstadt, University of Munich) against human peptide YY (1–36) (Sigma-Aldrich) and raised in rabbits. This antisera showed <0.001% cross-reactivity with human pancreatic polypeptide or sulfated cholecystokinin-8 and 0.0025% cross-reactivity with human neuropeptide Y. Standards (1.6–50 fmol/tube) or samples (200 μL plasma) were incubated in 200 μL assay buffer (50 mmol $NaPO_4$/L, 10 mmol EDTA/L, 2 g gelatin/L, and 0.1 g Na azide/L; pH = 7.4) and a 1:12,000 dilution of antisera for 24 h. The standards and samples were further incubated with 10,000 counts/min tracer (NEX3410; Perkin Elmer) for 24 h. Separation of the antibody-bound tracer from the free tracer was performed by second antibody precipitation [i.e., 500 μL 1:100 dilution of sheep antirabbit immunoglobulin in wash buffer comprising 50 mmol Tris base/L, 150 mmol NaCl/L, and 8% polyethylene glycol 6000 (pH = 8.0) and 50 μL normal rabbit serum diluted 1:50 in wash buffer], incubated for 2 h at room temperature, centrifuged at 4000 × g for ≥20 min at 4°C, supernatant fluid was poured off, and pellets were counted in a γ counter. The detection limit was 1.5 pmol/L. Intra-assay and interassay CVs were 8.7% and 18.2%, respectively.

Data and statistical analyses

Statistical analyses were performed with the use of SPSS software (version 21; IBM). Main effects of sex and protein load and their interaction effects on energy intake and gastric emptying were determined with the use of a repeated-measures ANOVA with the protein load as the within-subject factor and sex as the between-subject factor. Main effects of sex and protein load and their interaction effects on perceptions of appetite and gastrointestinal symptoms, blood glucose, and plasma hormone concentrations were determined with the use of a repeated-measures mixed-effect model with the protein load as the within-subject factor and sex as the between-subject factor, including baseline values at each treatment visit as a covariate. Post hoc comparisons, which were adjusted for multiple comparisons with the use of Bonferroni correction, were performed when there were significant main or interaction effects.

Within-subject correlations were determined with the use of a general linear model with a fixed slope and random intercept (36). The AUC was calculated from baseline to 180 min with the use of the trapezoidal rule. Assumptions of normality were verified for all outcomes before the statistical analysis. Statistical significance was accepted at $P < 0.05$. All data are presented as means ± SEMs.

RESULTS

The study protocol was well tolerated by all subjects.

Energy intake

Energy intake at the buffet meal (**Figure 1**) was ~80% higher in older men than in older women (mean energy intake of 3 study days in men: 1042 ± 69 kcal; in women: 584 ± 61 kcal; main effect of sex: $P < 0.001$; main effect of protein load: $P = 0.34$; interaction effect of sex by protein load: $P = 0.81$). Energy intake at the buffet meal did not differ between study days [preload drink containing 0 (control), 30, or 70 g protein] with no significant suppression of energy intake compared with that on the control day by either protein load [mean suppression of 45 ± 23 kcal or 5% ± 3% after the 30-g (120-kcal) or 70-g

FIGURE 1 Mean ± SEM energy intake at the buffet meal (kilocalories) in older men (energy intake at the buffet meal I shown by gray shading; $n = 8$) and women (energy intake at the buffet meal is shown by black shading; $n = 8$) after drinks (energy intake of the drink as the white part of each bar) containing flavored water (control) and whey-protein loads of 30 g (120 kcal) or 70 g (280 kcal). Main sex and protein-load effects and interaction effects were determined with the use of a repeated-measures ANOVA and post hoc Bonferroni correction. $^{\#}P < 0.001$ (main effect of sex: energy intake at the buffet meal was higher in older men than in older women; main effect of protein load: $P = 0.34$; interaction effect of sex by protein load: $P = 0.81$). $^{*}P < 0.001$ [main effects of sex and protein load: total energy intake (preload drink plus meal) was higher in older men than in older women, and total energy intake was higher after the 30-g protein load (9.5% increase) and 70-g protein load (27% increase) than after the control (interaction effect of sex by protein load: $P = 0.81$)]. The suppression of energy intake by protein (30 or 70 g) compared with the control (main effect of sex: $P = 0.62$; main effect of protein load: $P = 0.83$; interaction effect of sex by protein load: $P = 0.67$).

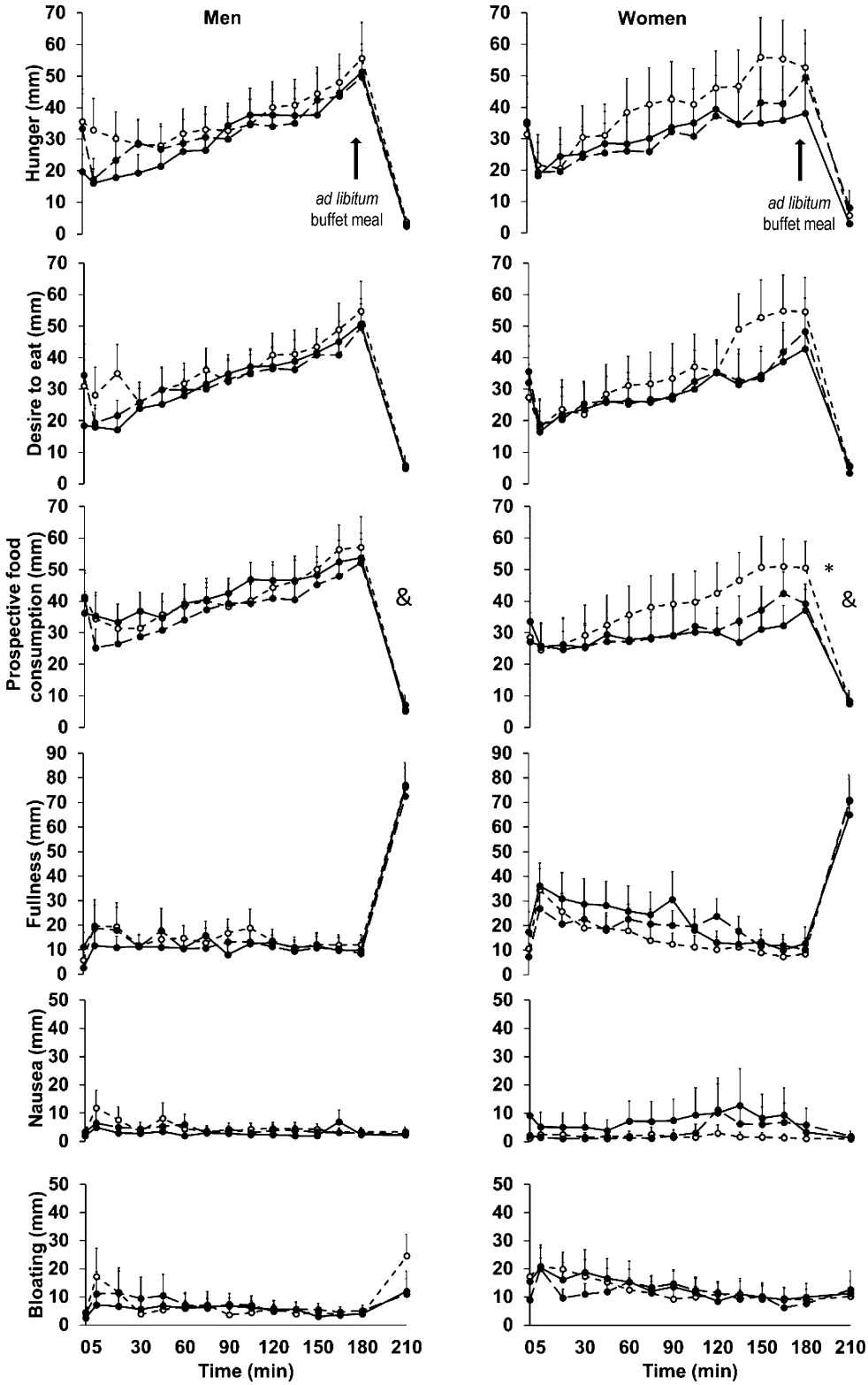

FIGURE 2 Mean ± SEM visual analog scores of hunger, desire to eat, prospective food consumption, fullness, nausea, and bloating in older men ($n = 8$) and women ($n = 8$) after intakes of drinks containing flavored water (control; dotted lines with open circles) and whey-protein loads of 30 g (dashed lines with closed circles) or 70 g (solid lines with closed circles). The main effect of sex and protein load and interaction effects were determined with the use of a mixed-effects model with baseline concentrations as a covariate and post hoc Bonferroni correction. $P > 0.05$: main effect of sex and protein load and interaction effect of sex by protein load for visual analogue scores of hunger, desire to eat, prospective food consumption, fullness, nausea, and bloating (AUC from 0 to 180 min). [&]Interaction effect of sex by protein load, $P < 0.05$. *For 70 g protein compared with the control (in women, perceptions of prospective food consumption were higher after the 70-g protein drink than after the control), $P < 0.05$.

(280-kcal) protein drinks compared with the control; main effect of sex: $P = 0.62$; main effect of protein load: $P = 0.83$; interaction effect of sex by protein load: $P = 0.67$]. There was a dose-dependent effect of the protein load on total (preload drink plus meal) energy intake [mean total energy intake of men and women: control: 843 ± 77 kcal; 30 g protein: 923 ± 75 kcal (12% increase); and 70 g protein: 1073 ± 78 kcal (32% increase); mean total energy intake of 3 study days in men: 1175 ± 69 kcal; and in women: 717 ± 61 kcal; main effect of sex: $P < 0.001$; main effect of protein load: $P < 0.001$; interaction effect of sex by protein load: $P = 0.81$]. Macronutrient preferences during the buffet meal did not differ between either men or women or study visits (mean macronutrient composition of the buffet meal: protein: 20% ± 1%; fat: 28% ± 1%; and carbohydrates 53% ± 1%; $P > 0.05$).

Perceptions of appetite and gastrointestinal symptoms

Baseline perceptions of hunger, desire to eat, prospective food consumption, fullness, nausea, and bloating were not different between men and women or study days ($P > 0.05$) (**Figure 2**). There was an interaction effect of sex by protein load for prospective food consumption (effect of sex: $P = 0.50$; effect of protein load: $P = 0.10$; interaction effect of sex by protein load: $P = 0.015$); the post hoc analysis revealed that women had higher perceptions of prospective food consumption during the control condition than during the 70-g protein condition ($P = 0.018$). The main effects of sex and protein load and the interaction effect of sex by protein load for hunger, desire to eat, fullness, nausea, and bloating (AUC from 0 to 180 min) as well as the sex and treatment effect of prospective food consumption were NS ($P > 0.05$).

Gastric emptying

Gastric-emptying variables are detailed in **Table 1**. Baseline gastric volumes were not different between men and women (39 ± 3 and 34 ± 5 mL, respectively; $P = 0.45$) or study days ($P = 0.76$). The control drink (water) as well as the 30-g protein drink emptied in an overall nonlinear pattern, whereas the pattern of emptying of the 70-g protein drink was linear (**Figure 3**).

There was a dose-dependent effect of the whey-protein load to slow gastric emptying [T50 for the mean of men and women: control: 23 ± 2 min; 30 g: 65 ± 7 min; and 70 g: 130 ± 10 min; effect of sex T50: $P = 0.41$; effect of protein load T50: $P < 0.001$; interaction effect of sex by protein load T50: $P = 0.77$; effect of sex AUC from 0 to 180 min: $P = 0.22$, AUC from 0 to 60 min (early phase): $P = 0.27$, AUC from 60 to 180 min (late phase): $P = 0.24$; effect of protein load AUC from 0 to 180 min: $P < 0.001$, AUC from 0 to 60 min: $P < 0.001$, AUC from 60 to 180 min: $P < 0.001$; interaction effect of sex by protein load AUC from 0 to 180 min: $P = 0.58$, AUC from 0 to 60 min: $P = 0.43$, AUC from 60 to 180 min: $P = 0.46$] with no difference in the rate of gastric emptying between men and women [mean rate of gastric emptying of men and women total phase from 0 to 180 min: 30 g: 0.6 ± 0.02 kcal/min (range: 0.4–0.7 kcal/min); and 70 g: 1.0 ± 0.07 kcal/min (range: 0.5–1.4 kcal/min); early phase from 0 to 60 min (when drinks were still emptying): 30 g: 1.0 ± 0.09 kcal/min (range: 0.2–1.5 kcal/min); and 70 g: 1.2 ± 0.1 kcal/min (range: 0.2–2.2 kcal/min); late phase from 60 to 180 min: 30 g: 0.4 ± 0.03 kcal/min (range: 0.3–0.8 kcal/min); and 70 g: 0.9 ± 0.07 kcal/min (range: 0.3–1.3 kcal/min); effect of sex: $P = 0.29$; effect of protein load: $P < 0.001$; interaction effect of sex by protein load: $P = 0.25$]. By 180 min, the 30-g protein drink had complete gastric emptying (≥90%) in 7 subjects, an additional 7 subjects showed emptying of ∼85%, and one subject showed emptying of ∼60%, whereas the 70-g protein drink was emptied from the stomach by approximately ≥85% in only 3 subjects.

Glucose and gut hormones

Baseline concentrations of blood glucose and plasma insulin, glucagon, ghrelin, cholecystokinin, GIP, GLP-1, and PYY as well as the HOMA-IR and the ratio of insulin to glucagon were not significantly different between men and women or study visits ($P > 0.05$, **Figures 4** and **5**).

The main effect of sex was NS for concentrations (AUC from 0 to 180 min) of glucose ($P = 0.70$), glucagon ($P = 0.94$), ghrelin ($P = 0.35$), cholecystokinin ($P = 0.16$), GIP ($P = 0.18$), or GLP-1 ($P = 0.55$). Older women had higher plasma concentrations of insulin (main effect of sex: $P = 0.040$) and PYY ($P = 0.037$) and an increased ratio of insulin to glucagon ($P = 0.008$) compared with those of older men.

The main effect of protein load was significant for concentrations (AUC from 0 to 180 min) of insulin, glucagon, ghrelin, cholecystokinin, GIP, GLP-1, and PYY and ratio of insulin to glucagon (all $P < 0.001$) but was NS for glucose ($P = 0.36$).

The interaction effect of sex by protein load was significant for concentrations (AUC from 0 to 180 min) of the ratio of insulin to glucagon ($P = 0.018$) but was NS for glucose ($P = 0.44$), insulin ($P = 0.081$), glucagon ($P = 0.45$), ghrelin ($P = 0.26$), cholecystokinin ($P = 0.18$), GLP-1 ($P = 0.60$), and PYY ($P = 0.45$). Post hoc analyses revealed that the ratio of insulin to glucagon was higher in women than in men after the 30-g protein drink ($P = 0.034$) and 70-g protein drink ($P = 0.006$).

In women, the ratio of insulin to glucagon was higher after both the 70- and 30-g protein drinks than after the control (all $P < 0.001$). In men, the ratio of insulin to glucagon was higher after the 70-g protein drink than after the control ($P = 0.002$).

In many cases, the protein-load effects were time dependent. The difference (earlier return to baseline after 30 compared with 70 g protein intake) in late-phase responses (greater than ∼90–120 min) of ghrelin in men, cholecystokinin in women, and glucagon and GIP in men and women between both protein loads (Figures 4 and 5) may have been related to the gastric emptying being completed earlier after the 30-g protein load than after the 70-g protein load (Table 1, Figure 3).

Relations between energy intake, appetite, gastric emptying, and gut hormones

Energy intake (kilocalories) at the buffet meal was, within subjects, inversely related to gastric emptying (T50 and gastric retention AUC from 0 to 180 min, early phase AUC from 0 to 60 min, and late phase AUC from 60 to 180 min) and gastric volume at 180 min in women (**Table 2**) [i.e., the slower the drink emptied from the stomach within a subject (70 < 30 < 0 g), the lower the subsequent energy intake (180–210 min)].

TABLE 1
Gastric emptying of water (control) and protein drinks in older men and women[1]

	Older men (n = 7)			Older women (n = 8)			P		
	0 g	30 g	70 g	0 g	30 g	70 g	Protein load	Sex	Interaction
50% gastric-emptying time, min	23 ± 3	59 ± 5	123 ± 13	23 ± 3	70 ± 13	136 ± 16	<0.001	0.41	0.77
Rate of gastric emptying,[2] kcal/min	—	0.6 ± 0.0	1.1 ± 0.1	—	0.6 ± 0.0	0.9 ± 0.1	<0.001	0.29	0.25
Early phase (from 0 to 60 min)	—	1.1 ± 0.1	1.3 ± 0.1	—	0.9 ± 0.2	1.1 ± 0.2	0.08	0.25	0.86
Late phase (from 60 to 180 min)	—	0.4 ± 0.0	1.0 ± 0.1	—	0.5 ± 0.1	0.8 ± 0.1	<0.001	0.67	0.12
Amount emptied, %									
At 60 min	84 ± 2	54 ± 3	28 ± 3	82 ± 5	43 ± 8	23 ± 5	<0.001	0.19	0.67
At 180 min	99 ± 0	89 ± 2	70 ± 5	99 ± 1	87 ± 4	59 ± 6	<0.001	0.29	0.29

[1] All values are means ± SEMs. Main sex and protein-load effects and interaction effects were determined with the use of a repeated-measures ANOVA.
[2] Calculated as the mean of rates of emptying during each 15-min interval, respectively, of the early phase (0–60-min), late phase (60–180-min), and total (0–180-min) time periods.

Energy intake (kilocalories) at the buffet meal was, within subjects, positively related to plasma concentrations (AUC from 0 to 180 min) of ghrelin and inversely related to plasma concentrations of PYY in older men and women combined and to insulin, glucagon, cholecystokinin, GIP, and PYY in older women [e.g., the greater the increase in plasma concentrations of insulin, glucagon, cholecystokinin, GIP, and PYY within a subject (70 > 30 > 0 g), the lower the subsequent energy intake]. Energy intake was also, within subjects, related to plasma hormone concentrations before the meal (180 min) in older women (insulin: $r = -0.50$, $P = 0.041$; ghrelin: $r = 0.65$, $P = 0.005$; cholecystokinin: $r = -0.54$, $P = 0.025$; GIP: $r = -0.49$, $P = 0.048$; and PYY: $r = -0.73$, $P = 0.001$).

Gastric emptying (gastric retention AUC from 0 to 180 min) was, within subjects, related to plasma insulin, glucagon, cholecystokinin, GIP, GLP-1, and PPY concentrations (AUC) and inversely related to ghrelin concentrations as well as perceptions of appetite and gastrointestinal symptoms (**Table 3**) (i.e., the higher the plasma hormone concentrations of insulin, glucagon, cholecystokinin, GIP, GLP-1, and PPY, the slower the rate of gastric emptying within a subject and the lower the concentrations of ghrelin and feelings of hunger).

Hunger and prospective food consumption were, within subjects, inversely related to cholecystokinin concentrations, and the desire to eat was inversely related to cholecystokinin and GIP concentrations. Fullness correlated positively with cholecystokinin concentrations and nausea correlated positively with glucose concentrations and negatively with ghrelin concentrations (**Table 4**).

GIP was, within subjects, related to GLP-1 ($r = 0.52$, $P = 0.002$) (i.e., the greater the increase in plasma GIP concentrations, the greater the increase in GLP-1). Ghrelin was, within subjects, inversely related to insulin ($r = -0.59$, $P < 0.001$) (i.e., the greater the increase in plasma insulin concentrations, the greater the inhibition of ghrelin production).

DISCUSSION

This study examined the acute effects of oral whey protein consumption on energy intake, appetite, gastric emptying, and plasma gut-hormone concentrations in older women as well as men. The protein drinks did not suppress subsequent ad libitum food intake in either sex. Consequently, there was a dose-dependent effect of the whey-protein drinks (30 and 70 g protein) to increase total energy intake (preload drink plus meal) compared with that of the control drink in both men and women. In both sexes, protein caused a load-dependent slowing of gastric emptying and an increase in plasma concentrations of insulin, glucagon, ghrelin, cholecystokinin, GIP, GLP-1, and PYY. Because the protein doses that were used were in the range that have been reported to have favorable effects on muscle mass (13), our observations of comparable effects of protein on appetite and underlying gastrointestinal mechanisms in men and women support the use of protein supplements in older people to preserve or increase skeletal muscle mass and function without suppressing energy intake.

Total energy intake was increased most by the highest protein dose [70 g (280 kcal)] compared with the control with a substantial increase of 32% or 230 kcal compared with an increase of 12% or 80 kcal after the 30-g (120-kcal) protein load. These observations are consistent with evidence of a reduced suppression of energy intake by nutrient ingestion in older men than

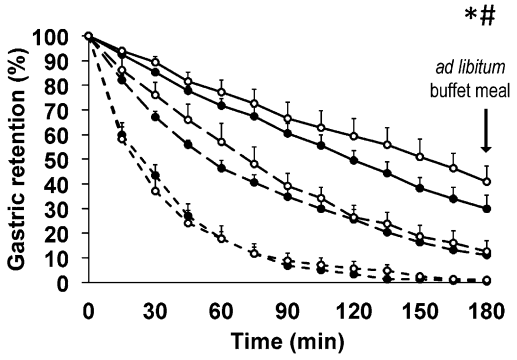

FIGURE 3 Mean ± SEM percentage of gastric retention in older men (n = 7; closed circles) and women (n = 8; open circles) after intakes of drinks containing flavored water (control; dotted line) and whey-protein loads of 30 g (dashed line) or 70 g (solid line). Main sex and protein-load effects and interaction effects were determined with the use of a repeated-measures ANOVA. *$P < 0.001$: main effect of protein load for 50% gastric-emptying time (main effect of sex: $P = 0.41$; interaction effect of sex by protein load: $P = 0.77$). #$P < 0.001$: main effect of protein load AUC from 0 to 180 min, from 0 to 60 min (early phase), and from 60 to 180 min (late phase) (main effect of sex AUC from 0 to 180 min: $P = 0.22$, AUC from 0 to 60 min: $P = 0.27$, AUC from 60 to 180 min: $P = 0.24$; interaction effect of sex by protein load AUC from 0 to 180 min: $P = 0.58$, AUC from 0 to 60 min: $P = 0.43$, AUC from 60 to 180 min: $P = 0.46$).

in young men (21–24) and extend these findings to older women. These observations indicate that doses of protein that have been shown to be sufficient to cause dietary protein muscle deposition can be ingested by both older women and men without suppressing appetite or overall energy intake. In older adults compared with younger adults, the sensitivity of muscle-protein synthesis to the ingestion of small amounts (≤20 g) of whey protein may be reduced (37). However, these postprandial differences between the young and old are not evident after consumption of ample amounts of dietary protein (greater than ~35 g). Moreover, the administration of protein supplements in older people may increase total energy intake (supplement plus subsequent meal) as was observed in this study. This result contrasts with the effects of protein in younger adults whereby identical whey drinks that were given according to the same study protocol produced a significant ~15% suppression of ad libitum food intake compared with that of a control in our previous study (24). To our knowledge, the long-term effects of protein supplements on energy intake in older adults are unknown, and reported effects on muscle mass and function have been inconsistent (38–41).

Appetite and energy intake are dependent on the precise co-ordination of interrelated gastric and small intestinal mechanisms that are triggered by the interaction with the nutrients ingested. We, and other authors, have shown that healthy aging is associated with a modest slowing of gastric emptying of both solids and liquids although the rate of emptying generally remains within the normal range for young subjects

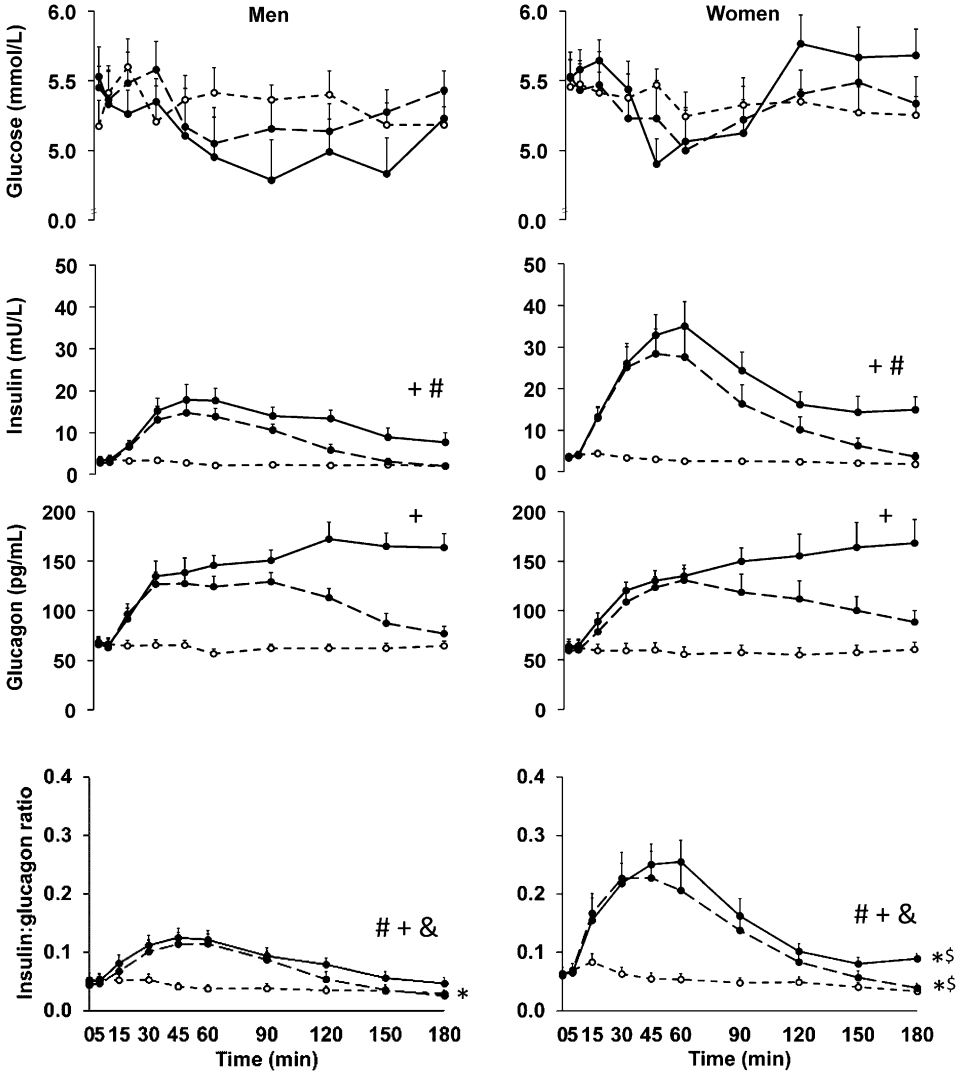

FIGURE 4 Mean ± SEM concentrations (AUC from 0 to 180 min) of blood glucose and plasma insulin and glucagon and the ratio of insulin to glucagon in older men (n = 8) and women (n = 8) after intakes of drinks containing flavored water (control; dotted lines with open circles) and whey-protein loads of 30 g (dashed lines with closed circles) or 70 g (solid lines with closed circles). The main effect of sex and protein load and interaction effects were determined with the use of a mixed-effect model with baseline concentrations as a covariate and post hoc Bonferroni correction. $^{\#}$Main effect of sex (plasma insulin and the ratio of insulin to glucagon concentrations were higher in older women than in older men), $P = 0.034$. $^{+}$Main effect of protein load (plasma insulin, glucagon, and the ratio of insulin to glucagon concentrations were protein-load dependent), $P < 0.001$. $^{\&}P < 0.05$: interaction effect of sex by protein load (post hoc tests: $^{\$}P < 0.05$: plasma insulin and the ratio of insulin to glucagon concentrations were higher in women than in men after both the 30 and 70 g protein load; $^{*}P < 0.05$: in women the ratio of insulin to glucagon was higher after both the 30- and 70-g protein loads than after the control; in men, the ratio of insulin to glucagon was higher after the 30-g protein drink than after the control).

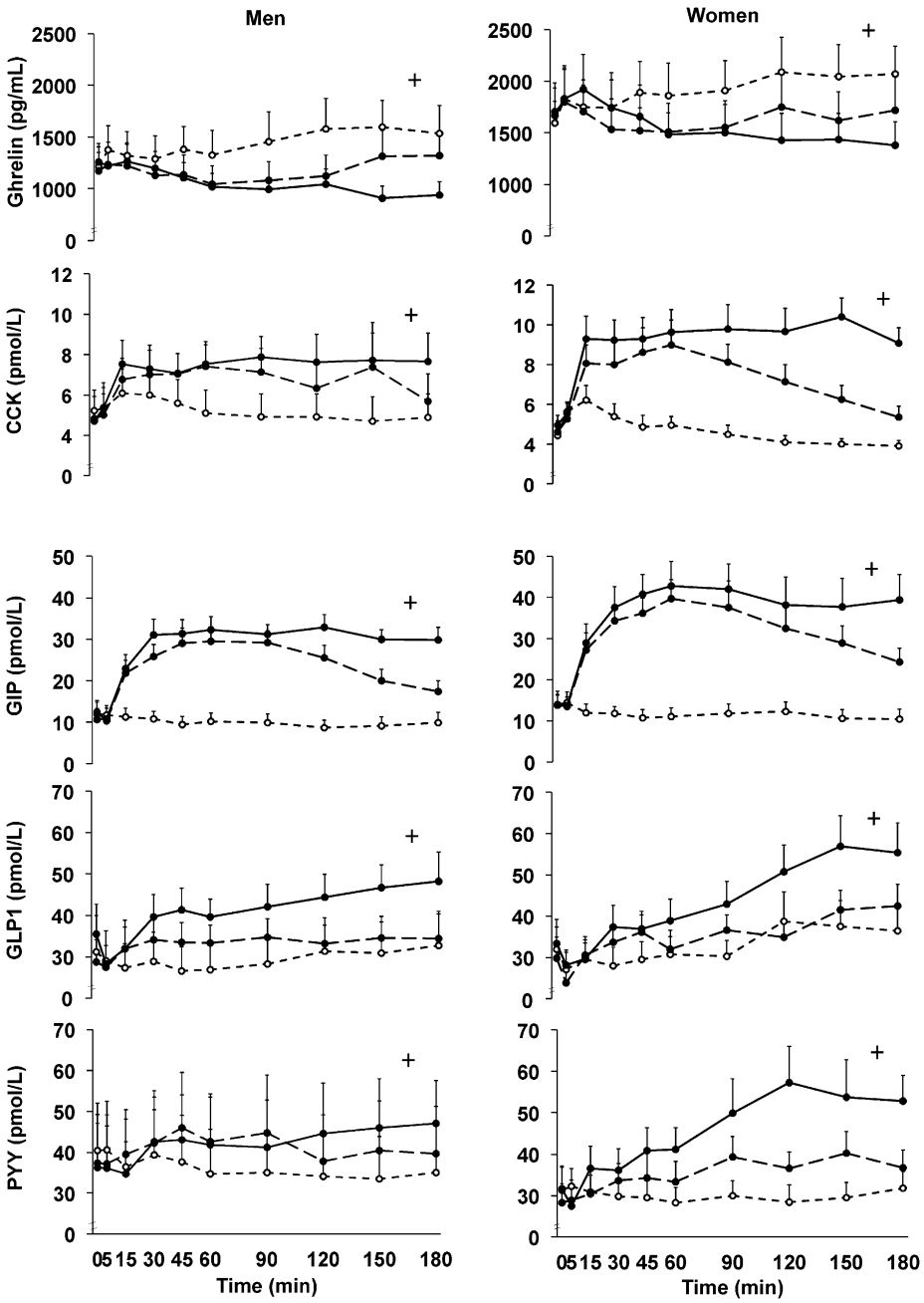

FIGURE 5 Mean ± SEM plasma concentrations (AUC from 0 to 180 min) of ghrelin, CCK, GIP, GLP1, and PYY in older men ($n = 8$) and women ($n = 8$) after intakes of drinks containing flavored water (control; dotted lines with open circles) and whey-protein loads of 30 g (dashed lines with closed circles) or 70 g (solid lines with closed circles). Main effects of sex and protein load and interaction effects were determined with the use of a mixed-effect model with baseline concentrations as a covariate. Main effects of sex and interaction effects of sex by protein load of the gut hormones were NS. $^{+}$Main effect of protein load, $P < 0.001$. CCK, cholecystokinin; GIP, gastric inhibitory polypeptide; GLP1, glucagon-like peptide 1; PYY, peptide tyrosine tyrosine.

(i.e., ~1–4 kcal/min) (24, 42–44). Slower gastric emptying results in greater distension of the stomach at any given time after ingestion of a meal. This effect can, in turn, lead to greater fullness and, at least in young adults, a consequent reduction in subsequent energy intake. In the present study, there was a marked, dose-dependent, slowing of gastric emptying by intake of the protein drinks with the T50 more than doubling from the control to 30-g protein days and again from the 30- to 70-g protein days. The rate of gastric emptying of the protein drinks in both men and women (~1 kcal/min) was apparently at the lower end of the normal range. Gastric emptying of the 30-g protein drink slowed further after the early phase (~0.5 kcal/min) when, on average, ~48% of the drink was emptied after 60 min, whereas the 70-g protein drink continued being emptied at ~1 kcal/min after ~25% had emptied, on average, after 60 min.

There was no significant difference between emptying rates in older men and women. Controversy exists regarding the sex-related difference in gastric emptying. Previously gastric emptying, as determined via scintigraphy, has been shown to be modestly slower in young and middle-aged, lean, but not obese

(45), women than in men (46–48), but not in all studies (49). Bennink et al. (50) also observed slower gastric emptying of a solid, but not liquid, test meal in lean, healthy, young women than in men. We have reported that energy intake at a buffet meal is associated negatively ($r = -0.90$, $P < 0.001$) with the antral area (distal stomach) immediately before the meal in young and older subjects who received mixed macronutrient drinks (0, 250, and 750 kcal) (51). Although the negative association between gastric retention and energy intake in the female (but not male) subjects in this study is consistent with some suppression of energy intake by gastric distension, the finding that the marked protein-induced slowing of gastric emptying after ingestion of the protein drinks was not associated with the suppression of subsequent energy intake suggests that this effect, if present, is probably minor in elderly people. This possibility would be consistent with the finding of Rayner et al. (52) that the perception of gastric distension is diminished in healthy older people.

Gastric emptying was, within subjects, related to plasma gut-hormone concentrations, whereby higher plasma concentrations of insulin, glucagon, cholecystokinin, GIP, GLP-1, and PYY were correlated with lower plasma ghrelin concentrations and perceptions of hunger and slower gastric emptying of the protein drink (70 < 30 < 0 g). There was an immediate load-dependent increase in the plasma hormone concentrations of cholecystokinin and GIP (both mainly produced in the duodenum and proximal jejunum) that reached a plateau from 15 to 30 min onwards, whereas the hormones that are produced more distally in the gut [i.e., GLP-1 and PYY (GLP-1 is mainly produced in the ileum, and PYY is mainly produced in the ileum and colon)] showed a more constant increase. Gastric emptying was completed earlier after the 30-g than the after the 70-g protein load, which resulted in a time-dependent response (earlier return to baseline after intake of 30 compared with 70 g protein) in plasma concentrations of cholecystokinin in women and glucagon and GIP in men and women after the 30- compared with 70-g protein loads. Healthy older people, compared with young people, have higher postprandial concentrations of cholecystokinin, GIP, GLP-1, and PYY, which may contribute to the slowing of gastric emptying (51, 53–55). The latter effect may in part be related to an impairment of clearance including GIP and GLP-1 inactivation by dipeptidyl peptidase IV and renal processes (55).

Healthy aging is characterized by impaired glucose tolerance or insulin resistance (55, 56). The latter effect may reflect

TABLE 2
Within-subject correlations between energy intake at the buffet meal and perceptions of appetite and gastrointestinal symptoms, gastric emptying, and concentrations of blood glucose and plasma gut hormones in older men and women[1]

	Within-subject correlations					
	Older men ($n = 8$)[2]		Older women ($n = 8$)		Combined	
	r	P	r	P	r	P
Hunger	0.43	0.11	0.29	0.30	0.35	0.06
Desire to eat	0.23	0.42	−0.43	0.11	−0.07	0.71
Prospective food consumption	0.11	0.70	0.22	0.43	0.14	0.47
Fullness	0.10	0.72	0.29	0.29	0.16	0.42
Nausea	0.14	0.62	−0.40	0.14	−0.09	0.66
Bloating	−0.05	0.85	0.00	1.00	−0.01	0.94
50% gastric-emptying time	−0.10	0.71	−0.66	0.004	−0.29	0.11
Gastric retention AUC						
From 0 to 180 min	−0.15	0.59	−0.61	0.01	−0.30	0.10
From 0 to 60 min	−0.16	0.57	−0.59	0.01	−0.30	0.10
From 60 to 180 min	−0.15	0.60	−0.59	0.01	−0.29	0.11
Gastric retention at 180 min (before buffet meal)	−0.36	0.19	−0.62	0.008	−0.41	0.021
Glucose	−0.10	0.71	0.26	0.31	0.04	0.84
Insulin	−0.10	0.70	−0.64	0.006	−0.29	0.10
Glucagon	−0.10	0.70	−0.57	0.016	−0.24	0.17
Ghrelin	0.33	0.19	0.43	0.08	0.35	0.045
Cholecystokinin	−0.11	0.66	−0.52	0.031	−0.25	0.16
GIP	−0.18	0.49	−0.54	0.027	−0.28	0.11
GLP-1	−0.42	0.10	0.13	0.63	−0.05	0.77
PYY	−0.43	0.08	−0.69	0.002	−0.45	0.009

[1] r and P values are for within-subject correlations between energy intake at the buffet meal (kilocalories) and visual analog scale (expressed as mm; AUC from 0 to 180 min) perceptions of hunger, desire to eat, prospective consumption, fullness, nausea, and bloating (expressed as mm; AUC from 0 to 180 min), 50% gastric-emptying time, gastric retention (expressed as %; AUC from 0 to 180 min), gastric retention at 180 min, and concentrations (AUC from 0 to 180 min) of blood glucose (expressed as mmol/L) and plasma insulin (expressed as mU/L), glucagon (expressed as pg/mL), ghrelin (expressed as pg/mL), cholecystokinin (expressed as pmol/L), GIP (expressed as pmol/L), GLP-1 (expressed as pmol/L), and PYY (expressed as pmol/L) in older men and women. Within-subject correlations were determined with the use of a general linear model with a fixed slope and random intercept. GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; PYY, peptide tyrosine tyrosine.

[2] $n = 7$ for gastric retention in the older men.

TABLE 3
Within-subject correlations between gastric retention and perceptions of appetite and gastrointestinal symptoms and concentrations of blood glucose and plasma gut hormones in older men and women[1]

	Older men (n = 7)		Older women (n = 8)		Combined	
	r	P	r	P	r	P
Hunger	0.00	0.99	−0.44	0.08	−0.63	0.12
Desire to eat	−0.09	0.76	−0.52	0.031	−0.36	0.044
Prospective food consumption	0.14	0.62	−0.65	0.005	−0.34	0.06
Fullness	−0.45	0.09	0.51	0.004	0.20	0.29
Nausea	0.11	0.70	0.14	0.58	0.12	0.54
Bloating	0.21	0.46	0.07	0.79	0.09	0.63
Glucose	−0.55	0.033	−0.23	0.38	−0.35	0.053
Insulin	0.90	<0.001	0.89	<0.001	0.87	<0.001
Glucagon	0.94	<0.001	0.96	<0.001	0.94	<0.001
Ghrelin	−0.73	0.020	−0.57	0.017	−0.64	<0.001
Cholecystokinin	0.78	0.001	0.92	<0.001	0.87	<0.001
GIP	0.92	<0.001	0.85	<0.001	0.87	<0.001
GLP-1	0.76	0.001	0.75	0.001	0.75	<0.001
PYY	0.72	0.002	0.85	<0.001	0.80	<0.001

[1] r and P values are for within-subject correlations between gastric retention (expressed as %; AUC from 0 to 180 min) and visual analog scale (expressed as mm, AUC from 0 to 180 min) perceptions of hunger, desire to eat, prospective consumption, fullness, nausea and bloating, and concentrations (AUC 0–180) of blood glucose (expressed as mmol/L), plasma insulin (expressed as mU/L), glucagon (expressed as pg/mL) ghrelin (expressed as pg/mL), cholecystokinin (expressed as pmol/L), GIP (expressed as pmol/L), GLP-1 (expressed as pmol/L), and PYY (expressed as pmol/L) in older men and women. Within-subject correlations were determined with the use of a general linear model with a fixed slope and random intercept. GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; PYY, peptide tyrosine tyrosine.

increased adiposity and a reduction in the secretion of, or pancreatic β cell sensitivity to (57), the incretins GLP-1 and GIP (55, 58). Insulin peaked between 30 and 60 min and returned to baseline after 180 min, and this effect was greater in older women than in older men. Glucagon increased almost concurrently in older men and women, before decreasing again after 90 min after the 30-g protein load, whereas it stayed elevated after the 70-g protein load.

Our study has several limitations that should be recognized. The number of subjects was relatively small, and therefore, the study may have been underpowered for secondary outcomes including perceptions of appetite and gastrointestinal symptoms and the change in gastric emptying after the 70-g whey-protein load in older women than in older men. Energy intake was assessed 3 h after protein intakes at a buffet meal and not during the remainder of the day; accordingly, potential compensating changes in energy intake after lunch were not evaluated. Although the drinks were matched for taste and osmolality, we did not assess the subject's perceptions of taste, pleasantness, or palatability of the drinks. As a consequence of the study design, the protein-preload drinks were isocaloric for both older men and women. Older women are expected to have lower energy requirements than are older men, and therefore, the drinks that were given to the female group in this study could be judged to be larger than those that were given to the male group when considered in relation to energy requirements. Blood glucose was measured with a glucometer, and blood samples of glucagon and GLP-1 were collected without protease inhibitors, which could be considered to be less than optimal; however, the results appeared clear cut with significant changes in both glucagon and GLP-1 in response to the protein loads in the direction expected.

In conclusion, ingestion of protein drinks at doses that have been previously shown to suppress energy intake in young men has no effect on ad libitum energy intake in either older women or men 3 h after consumption. Consequently, the consumption of protein drinks leads to an increase in total energy intake.

We thank Rachael Rigda, Seva Hatzinikolas, and Judith Wishart, National Health and Medical Research Council of Australia Center of Clinical Research Excellence in Translating Nutritional Research to Good Health, Discipline of Medicine, Royal Adelaide Hospital, The University of Adelaide, for assistance with studies (Rachael Rigda and Seva Hatzinikolas) and the analysis of the hormones (Judith Wishart).

The authors' responsibilities were as follows—S Soenen, IC, MH, and NDL-M: designed the research; CG, LGT, and S Soenen: conducted the

TABLE 4
Within-subject correlations between perceptions of hunger, desire to eat, prospective food consumption, fullness, nausea, and bloating and concentrations of blood glucose and plasma gut hormones in older men and women (n = 16)[1]

	Hunger		Desire to eat		Prospective food consumption		Fullness		Nausea		Bloating	
	r	P	r	P	r	P	r	P	r	P	r	P
Glucose	0.09	0.63	0.01	0.97	−0.09	0.63	0.14	0.44	0.37	0.047	−0.22	0.23
Insulin	−0.31	0.09	−0.35	0.06	−0.36	0.05	0.29	0.12	0.09	0.63	0.09	0.63
Glucagon	−0.27	0.14	−0.32	0.09	−0.30	0.10	−0.06	0.75	0.10	0.58	0.10	0.59
Ghrelin	0.18	0.33	0.12	0.50	0.10	0.57	−0.14	0.44	−0.37	0.048	−0.02	0.90
Cholecystokinin	−0.41	0.029	−0.50	0.009	−0.56	0.004	0.38	0.043	0.18	0.32	0.11	0.56
GIP	−0.35	0.06	−0.37	0.047	−0.34	0.07	0.21	0.25	0.13	0.46	0.08	0.68
GLP-1	−0.22	0.23	−0.27	0.149	−0.25	0.17	0.04	0.83	0.05	0.77	0.06	0.75
PYY	−0.12	0.53	−0.19	0.30	−0.30	0.10	0.15	0.40	0.04	0.83	0.16	0.39

[1] r and P values are for within-subject correlations between perceptions of appetite and gastrointestinal symptoms (AUC from 0 to 180 min) and concentrations (AUC from 0 to 180 min) of blood glucose (expressed as mmol/L), plasma insulin (expressed as mU/L), glucagon (expressed as pg/mL), ghrelin (expressed as pg/mL), cholecystokinin (expressed as pmol/L), GIP (expressed as pmol/L), GLP-1 (expressed as pmol/L), and PYY (expressed as pmol/L) in older men and women. Within-subject correlations were determined with the use of a general linear model with a fixed slope and random intercept. GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; PYY, peptide tyrosine tyrosine.

research; S Soenen: generated the random-allocation sequence and had primary responsibility for the final content of the manuscript; CG: enrolled and assigned the participants to the interventions; S Soenen, CG, and KL: performed the statistical analyses; S Soenen, CG, IC, TH, KLJ, MH, and KL: contributed to the data interpretation; and all authors: contributed to the writing of the manuscript and read and approved the final manuscript. Fonterra, Geriatrics Training and Research with Aged Care Resthaven, and the Royal Adelaide Hospital Research Foundation did not have any input in the design, implementation, analysis, or interpretation of the data. None of the authors reported a conflict of interest related to the study.

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