Effects of a protein ‘preload’ on gastric emptying, glycemia, and gut hormones after a carbohydrate meal in diet-controlled type 2 diabetes

Short running title: Protein preload and glycemic response

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**Objective:** We evaluated whether a whey ‘preload’ could slow gastric emptying, stimulate incretin hormones, and attenuate postprandial glycemia in type 2 diabetes.

**Research Design and Methods:** 8 type 2 patients ingested 350mL beef soup, 30min before a potato meal; 55g whey was added either to the soup (‘whey preload’), or potato (‘whey in meal’), or ‘no whey’ was given.

**Results:** Gastric emptying was slowest after the ‘whey preload’ (P<0.0005). The incremental area under the blood glucose curve was less after the ‘whey preload’ and ‘whey in meal’ than ‘no whey’ (P<0.005). Plasma glucose-dependent insulinotropic polypeptide, insulin and cholecystokinin concentrations were higher on both whey days than after ‘no whey’, whereas glucagon-like peptide-1 was greatest after the ‘whey preload’ (P<0.05).

**Conclusions:** Whey protein consumed before a carbohydrate meal can stimulate insulin and incretin hormone secretion, and slow gastric emptying, leading to marked reduction in postprandial glycemia in type 2 diabetes.
The rate of gastric emptying and the incretin, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulino tropic polypeptide (GIP), response to a meal are known to be major determinants of postprandial blood glucose excursions (1; 2). One strategy to minimise postprandial glycemia could be to administer a small load of protein or fat before a meal, so that the presence of nutrients in the small intestine induces the release of peptides such as GLP-1, GIP, and cholecystokinin (CCK), to slow gastric emptying, and stimulate insulin secretion in advance of the main nutrient load (3; 4). We hypothesized that a protein ‘preload’ would reduce the postprandial glycemic excursion in type 2 patients by these mechanisms.

RESEARCH DESIGN AND METHODS

Protocol: Eight diet-controlled type 2 patients (7 male; age 58 ± 3 yr; body mass index 28.6 ± 1.3 kg/m²; duration of known diabetes 5.4 ± 1.1 yr; glycated hemoglobin 6.5 ± 0.2%) attended the laboratory after an overnight fast (14h for solids, 12h for liquids) on three separate occasions. Each patient consumed beef flavored soup (3.8g non-caloric beef flavoring dissolved in 350mL water), 30 min before a mashed potato meal, containing 65g powdered potato (Deb, Epping, Australia) with 20g glucose (total of 59.1g carbohydrate, 4.3g fat, 5.2g protein; 1276.5kJ), labeled with 20MBq 99mTc-sulfur colloid (4). On one day, 55g whey protein (876.7kJ) was added to the soup. On another day, 55g whey was mixed into the potato meal. On a third day, neither the preload nor the meal contained whey. Blood was sampled frequently for blood glucose and plasma hormone measurements.

Measurements: Gastric emptying was assessed by scintigraphy. Data were corrected for radionuclide decay, subject movement, and gamma-ray attenuation, and the gastric half-emptying time (T50) was calculated (4).

Blood glucose concentrations were measured using a glucometer (Medisense Precision QID, Abbott Laboratories, Bedford, MA, USA), which we have validated against the hexokinase technique (5). Plasma insulin was measured by chemiluminescent immunometric assay (Immulite 2000 Insulin, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Total GLP-1 (GLP1-36HK, Linco Research, St. Charles, Missouri, MO, USA), total GIP, and CCK-8 were measured by radioimmunoassay (RIA) (6).

Cardiovascular autonomic function was assessed by the variation in R-R interval during deep breathing, and the systolic blood pressure changes in response to standing (7).

Data were evaluated using repeated measures ANOVA, with treatment and time as factors (StatView 5.0, Abacus Concepts, Inc., Berkeley, CA, USA). Data are shown as mean values ± standard error; P<0.05 was considered significant.

RESULTS (Figure 1)

Two of the eight subjects had definite autonomic dysfunction. The study was well tolerated.

On the ‘no whey’ and ‘whey in meal’ days, emptying was rapid initially, and subsequently slower, whereas emptying after the ‘whey preload’ approximated a linear pattern. Gastric emptying was slowest on the ‘whey preload’ day (T50: 87.3 ± 5.4 min; P=0.0001) and was slower with ‘whey in the meal’ (53.0 ± 8.3 min; P<0.01) than ‘no whey’ (39.0 ± 6.2 min).

There were no differences in baseline blood glucose, plasma insulin, GLP-1, GIP or CCK concentrations. The incremental area under the curve (iAUC) for blood glucose was less after the ‘whey preload’ (363.7 ± 64.5 mmol.min/L) and ‘whey in meal’ (406.3 ± 85.9 mmol.min/L), compared to ‘no whey’ (734.9 ± 98.9 mmol.min/L; P<0.005 for both).
The iAUCs for insulin, GLP-1, GIP, and CCK were greater when whey was given as a preload (P < 0.05 for all) or in the meal (P<0.005 for all) compared to ‘no whey’. Despite an earlier response, the iAUC for insulin did not differ between ‘whey preload’ and ‘whey in meal’ (P=0.50). GLP-1 was greater between -15 min and 90 min with the ‘whey preload’ when compared with ‘whey in meal’ (P=0.0001), but the overall iAUC did not differ significantly.

DISCUSSION

We demonstrated that whey protein, when given before or with a high carbohydrate meal, resulted in a substantial reduction in postprandial glycemia in diet-controlled type 2 patients. Given that the magnitude of the reduction was comparable to what would be hoped for with pharmacological therapy, such as sulphonylureas, these data have considerable implications for nutritional strategies in the management of diabetes.

The pivotal role of the gastrointestinal tract in determining postprandial glycemia has often been overlooked, but is assuming increasing prominence, partly because of the development of gut peptide-based therapies for diabetes, such as the GLP-1 analog, exenatide (8), and the amylin analog, pramlintide (9), which may act predominantly by slowing gastric emptying. Similar to what we reported after an oil preload (4), whey slowed gastric emptying substantially, particular when given before the meal, associated with stimulation of GLP-1 and CCK. However, in contrast to the delayed insulin response observed after oil, whey augmented insulin secretion markedly, possibly by a combination of the incretin effect and direct stimulation of the beta cells by absorbed amino acids (10). It is likely that the stimulation of insulin by whey was responsible for the much greater reduction in glycemia after whey than oil, given that effects on gastric emptying were comparable.

While our study involved a small number of subjects who had well-controlled, predominantly uncomplicated, type 2 diabetes, the improvement in postprandial glycemia was marked, and highly consistent. Further evaluation is now required in poorly controlled patients and those taking oral hypoglycemic agents, and to determine whether the acute effects are sustained in the longer term. It would also be important to confirm whether the effects are evident with a smaller load of protein, in order to minimise additional energy intake. Although concerns have been raised about hyperinsulinemia as a risk factor for vascular disease (11), more likely it represents a marker for other risk factors (12), and in the UKPDS study, stimulation of insulin by sulphonylureas was not associated with increased cardiovascular events (13).

The concept of using dietary manipulations to treat type 2 diabetes, based on our knowledge of the contribution of gastric emptying and gut peptides to postprandial glycemic responses, appears to hold much promise.

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Disclosures: None of the authors has any conflict of interest to disclose.
REFERENCES
Figure 1 Gastric emptying (A), and concentrations of blood glucose (B), plasma insulin (C), plasma GLP-1 (D), plasma GIP (E) and plasma CCK (F) in response to a mashed potato meal in 8 type 2 diabetic patients. On each of study day, subjects ingested 350mL beef flavored soup 30min before a radiolabeled mashed potato meal; either 55g whey protein was added to the soup (‘whey preload’), or the whey was added to the potato (‘whey in meal’), or no whey was given (‘no whey’). Data are mean ± standard error. *P<0.05, ‘whey preload’ vs. ‘whey in meal’; #P<0.05, ‘whey in meal’ vs. ‘no whey’; §P<0.05, ‘whey preload’ vs. ‘no whey’.