A small dose of whey protein co-ingested with mixed-macronutrient breakfast and lunch meals improves postprandial glycemia and suppresses appetite in men with type 2 diabetes: a randomized controlled trial

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ABSTRACT

Background: Large doses of whey protein consumed as a preload before single high–glycemic load meals has been shown to improve postprandial glycemia in type 2 diabetes. It is unclear if this effect remains with smaller doses of whey co-ingested at consecutive mixed-macronutrient meals. Moreover, whether hydrolyzed whey offers further benefit under these conditions is unclear.

Objective: The aim of this study was to investigate postprandial glycemic and appetite responses after small doses of intact and hydrolyzed whey protein co-ingested with mixed-nutrient breakfast and lunch meals in men with type 2 diabetes.

Design: In a randomized, single-blind crossover design, 11 men with type 2 diabetes [mean ± SD age: 54.9 ± 2.3 y; glycated hemoglobin: 6.8% ± 0.3% (51.3 ± 3.4 mmol/mol)] attended the laboratory on 3 mornings and consumed *1*) intact whey protein (15 g), *2*) hydrolyzed whey protein (15 g), or *3*) placebo (control) immediately before mixed-macronutrient breakfast and lunch meals, separated by 3 h. Blood samples were collected periodically and were processed for insulin, intact glucagon-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP), leptin, peptide tyrosine tyrosine ($PYY_{3–36}$), and amino acid concentrations. Interstitial glucose was measured during and for 24 h after each trial. Subjective appetite was assessed with the use of visual analog scales.

Results: Total postprandial glycemia area under the curve was reduced by 13% ± 3% after breakfast following the intact whey protein when compared with control ($P < 0.05$). Hydrolyzed whey attenuated early glucose after breakfast when compared with control ($P < 0.05$). Glycemia was improved postlunch after the intact whey protein only when compared with control ($P < 0.05$). Greater satiety was observed after the intact whey protein only after both meals when compared with control ($P < 0.05$). Insulin concentrations increased after both the intact and hydrolyzed whey protein, showing strong positive correlations with increases in valine and isoleucine ($P < 0.05$). Incretin and appetite regulatory hormone responses were similar across trials ($P > 0.05$).

Conclusions: The consumption of a small 15-g dose of intact whey protein immediately before consecutive mixed-macronutrient meals improves postprandial glycemia, stimulates insulin release, and increases satiety in men with type 2 diabetes. This trial was registered at www.clinicialtrials.gov as NCT02903199. *Am J Clin Nutr* 2018;107:550–557.

Keywords: type 2 diabetes, whey protein, postprandial, glucose, insulin, incretin, appetite, hydrolyzed

INTRODUCTION

Reducing postprandial glucose excursions is important in the management of type 2 diabetes due to their predictive relation with glycated hemoglobin (HbA1c) (1) and future cardiovascular disease events (2). Moreover, postprandial glycemia has been shown to be an independent risk factor for cardiovascular disease (3, 4) due to the high glucose excursions driving increased glucose variability, oxidative stress, inflammation, and vascular dysfunction (3–6), and thus promotes diabetes complications

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Supplemental Figures 1–3 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CGM, continuous glucose monitoring; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; HbA1c, glycated hemoglobin; $PYY_{3–36}$, peptide tyrosine tyrosine.

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(7, 8). A substantial economic cost is associated with poorly controlled postprandial hyperglycemia in type 2 diabetes (9), underlining the need for more refined and cost-effective strategies for improvement in postmeal glycemic control.

Current interventional studies have sought to improve postprandial glycemia through premeal supplementation of whey protein, including intact and hydrolyzed (more rapidly digested) forms (10–12). Whey protein contains an abundant source of amino acids and bioactive peptides that are rapidly absorbed into the circulation after digestive breakdown (13). These properties of whey protein are potent insulin secretagogues that directly stimulate pancreatic β cells (14) and augment the incretin effect through glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) secretion (10, 11), thereby creating a postprandial glycemia-reducing milieu (15, 16). In addition to increased insulinotropic activity (17), incretin peptide secretion exerts positive influences on gastric emptying, reduced hepatic glucose production, and increased satiety (18, 19). An increase in satiety has also been reported after whey protein ingestion in nondiabetic individuals (20), mediated by a suppression of orexigenic drive and stimulation of episodic satiety signals (21); however, this has yet to be assessed in type 2 diabetes.

There are practical limitations associated with implementing premeal whey protein supplementation as a therapeutic option in type 2 diabetes. First, studies have investigated the glycemic response to a single test meal of primarily high–glycemic index carbohydrate content, such as powdered potatoes and glucose syrup (10, 22), without investigations at subsequent meals. Second, dosages of whey protein administered are generally unrealistically large (45–55 g) (10, 23, 24), providing a significant caloric burden (~220 kcal). Finally, whey protein has shown benefit in type 2 diabetes when supplemented ~30 min before the main meal (10, 22), thus restricting its ecologic validity when applied in free-living conditions.

Therefore, the objective of this study was to assess the glycemic and appetite effects of whey protein, in intact and hydrolyzed fractions, within the parameters of small, realistic doses ingested immediately before the initiation of mixed-nutrient and habitually consumed breakfast and lunch meals. Second, we investigated the relative contribution of putative mechanisms of incretin peptide secretion and amino acid appearance on the insulinotropic effect of whey protein.

METHODS

Participants

The CONSORT (Consolidated Standards of Reporting Trials) flow diagram is shown in **Supplemental Figure 1**. Eleven male patients with type 2 diabetes, managed by metformin monotherapy (500–2000 mg/d; $n = 8$) or diet and lifestyle modification ($n = 3$), were studied after providing written informed consent. Their mean ± SEM age was 54.9 ± 2.3 y, with a BMI (kg/m^2) of 31.8 ± 2.6, HbA1c of 6.8% ± 0.3% (51.3 ± 3.4 mmol/mol), and a duration of known diabetes of 4 ± 1 y. Exclusion criteria included smokers and those with prescribed medications affecting appetite and gastrointestinal function, those receiving insulin therapy, and those with food intolerances or allergies. The study was approved by the local National Health Service Research Ethics Committee with procedures in accordance with the revised Helsinki Declaration of 1983. All medication doses were kept the same throughout the trial period. This trial was registered at www.clinicialtrials.gov as NCT02903199.

Prelaboratory phase

For standardization of appetite perceptions and gut hormone variables (25), patients were provided with a meal to be consumed the evening before each trial (635 kcal; beef lasagna; Tesco). Patients also received dietary recording sheets, food scales (kitchen scale; Salter), and a self-monitoring glucose analyzer (Accu-Chek Mobile; Roche Diagnosis Ltd.). Continuous glucose-monitoring (CGM) systems (Dexcom G4; Dexcom) and pedometers (Digital Daffodil) were fitted to patients ~36 h before each trial initiation. CGM sensors were fitted as previously described by Campbell et al. (26) and removed 24 h after leaving the laboratory. For CGM calibration purposes, self-reported capillary blood glucose concentrations were performed ≥4 times/d with the use of a finger-prick glucose analyzer (Accu-Chek Mobile; Roche Diagnostics Ltd.). Sensor data were retrospectively stored and analyzed with the use of Dexcom software (Dexcom Studio; Dexcom). Patients were requested to record and replicate diet and activity patterns (steps per day) for the 24 h preceding each trial and to avoid strenuous activity and alcohol for the previous 48 h. Stature, mass, and waist circumference were recorded ~36 h before the first trial.

Laboratory protocol

Each patient was studied on 3 separate occasions, separated by 7 d, in a randomized, single-blind, crossover design. Trial sequences were randomly assigned with the use of a computerized random-number generator (www.randomization.com). After an overnight fast, patients reported to the Newcastle National Institute for Health Research Clinical Research Facility of the Royal Victoria Infirmary in Newcastle upon Tyne, United Kingdom, at 0800. On arrival, patients were seated and an intravenous cannula was inserted into the antecubital vein for repeated blood sampling. After fasted blood sampling, patients consumed *1*) intact whey protein concentrate (68 kcal; Lacprodan DI-8790; Arla Foods), *2*) hydrolyzed whey protein (68 kcal; PSNU 28600; Arla Foods), or *3*) a placebo beverage (<1 kcal; flavored water) immediately followed by mixed-nutrient breakfast or lunch meals. Both whey beverages contained 15 g protein. For breakfast and lunch, patients ate 60 g whole-grain cereal (Nestlè) with 250 mL whole milk (Tesco) (387 kcal, 56 g carbohydrate, 11 g fat, and 13 g protein) and 4 slices of wheat bread (Warburtons), 100 g chicken sandwich filler (Tesco), and 5 g butter (Arla) (879 kcal, 117 g carbohydrate, 27 g fat, and 37 g protein), respectively. Treatments were masked by standardization of supplement taste, smell, and visual cues and served with calorie-free citrus flavoring (Fun One; <1 kcal) in 150-mL opaque bottles. Patients were permitted ad libitum water intake, for which timing and quantity were recorded during the initial trial and replicated at subsequent trials.

Venous blood plasma samples were collected at 5, 10, 15, 30, 45, 60, 90, 120, 150, and 180 min post-breakfast and -lunch meals to capture time-course changes in plasma amino acids, insulin, leptin, GIP, active GLP-1, and peptide tyrosine tyrosine ($PYY_{3–36}$). The Vacutainers (Becton Dickinson, Sweden) were

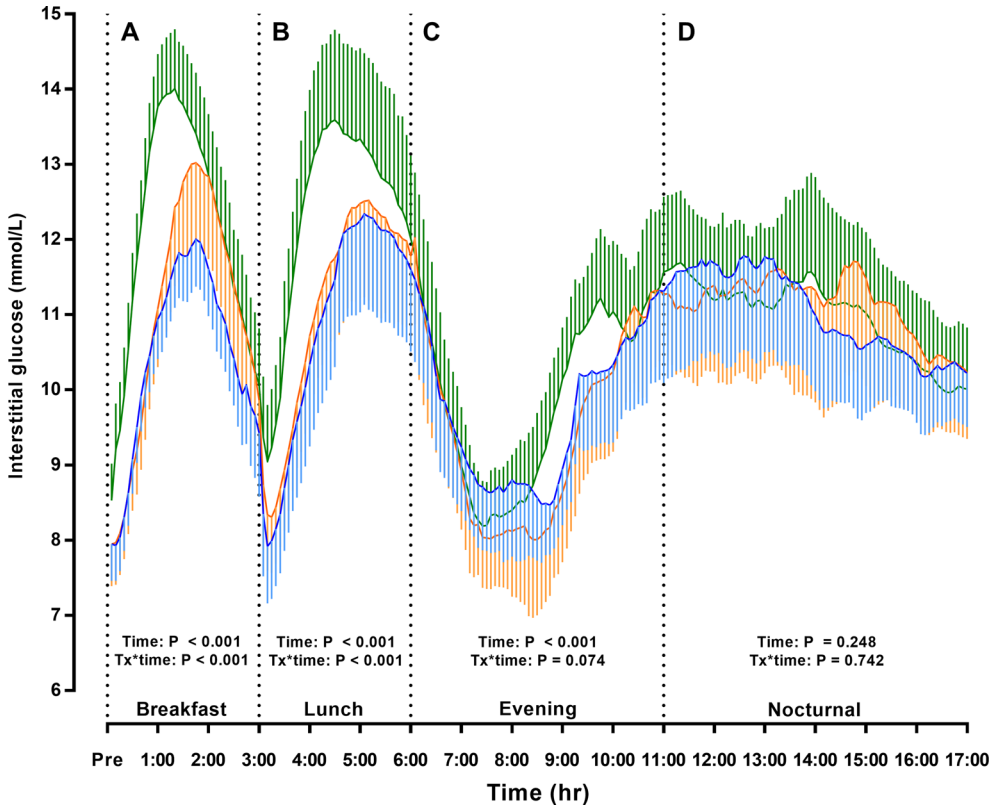

FIGURE 1 Time-course changes in interstitial glucose during breakfast (A), lunch (B), and evening (C) and nocturnal (D) phases ($n = 11$). The vertical dashed lines indicate the start of each phase. The blue-shaded area indicates intact whey protein; the orange-shaded area indicates hydrolyzed whey protein; and the green shaded area indicates control. Values are means ± SEMs and time and interaction (Tx*time) effects. Data were analyzed by 2-factor (Tx*time) repeated-measures ANOVA, with Bonferroni-adjusted post hoc comparisons where significant time and time × treatment effects were found. Pre, before treatment; Tx*time, treatment × time.

pretreated with protease inhibitors, di-peptidyl peptidase-4 inhibitor (DPP-IV; 30 μL) and aprotinin (~500 Kallikrein inhibitor unit), to preserve GIP and intact GLP-1 without affecting concentrations of other measured hormones (27) before centrifugation at 3000 rev/min for 15 min at 4°C. Plasma was separated and stored at −80°C for subsequent analysis. An ELISA was performed to measure plasma concentrations of human insulin, active GLP-1, and leptin (Multi-spot Assay; Meso Scale Discovery), GIP (Human Total GIP Kit; Meso Scale Discovery), and $PYY_{3–36}$ ($PYY_{3–36}$ EIA Kit; Phoenix Pharmaceuticals, Inc.). Plasma amino acids were analyzed via HPLC (Waters 474 scanning fluorescence detector; Milford) via o-phthaladehyde derivitization. Detection was performed fluorometrically, as described by Frank and Powers (28).

Postlaboratory phase

Before leaving the laboratory, patients were given an evening meal, consisting of 450 g chicken biryani (Tesco) and a mini naan bread (Tesco) (1007 kcal, 145 g carbohydrate, 28 g fat, and 37 g protein) to be consumed at ~1900. Patients were instructed to report water intake immediately upon leaving the laboratory at the first trial and replicate this at subsequent visits. No further supplementation of whey protein was administered at the evening meal. Dietary, activity, and CGM measures were recorded until 24 h posttrial.

Outcomes and measurements

The primary outcomes were mean and AUC concentrations for postprandial glucose. Secondary outcome measures were mean and AUC concentrations of plasma insulin, amino acids, incretin and appetite hormones, and subjective measures of appetite. AUC was calculated with the use of the trapezoidal rule over 0–30 min, 0–60 min, 0–90 min, and 0–180 min to capture early and total postprandial concentrations of glycemic and appetite variables. Measures of subjective appetite (hunger, fullness, satisfaction, and prospective food intake) were assessed with the use of previously validated visual analog scales (29).

Sample-size calculation

Sample-size calculations were based on protocol pilot data. To detect a difference of ≥10% in glucose AUC, a sample of 11 participants would be required to test the null hypothesis that the population means are similar across trials with a probability of 0.8 and associated type 1 error of 0.05.

Statistical analysis

The variables were assessed with the use of 2-factor repeated-measures ANOVA, with treatment and time as factors. Post hoc analyses, adjusted for multiple comparisons by Bonferroni correction, were performed if ANOVAs showed significant treatment effects. AUC values were compared with the use of 1-factor

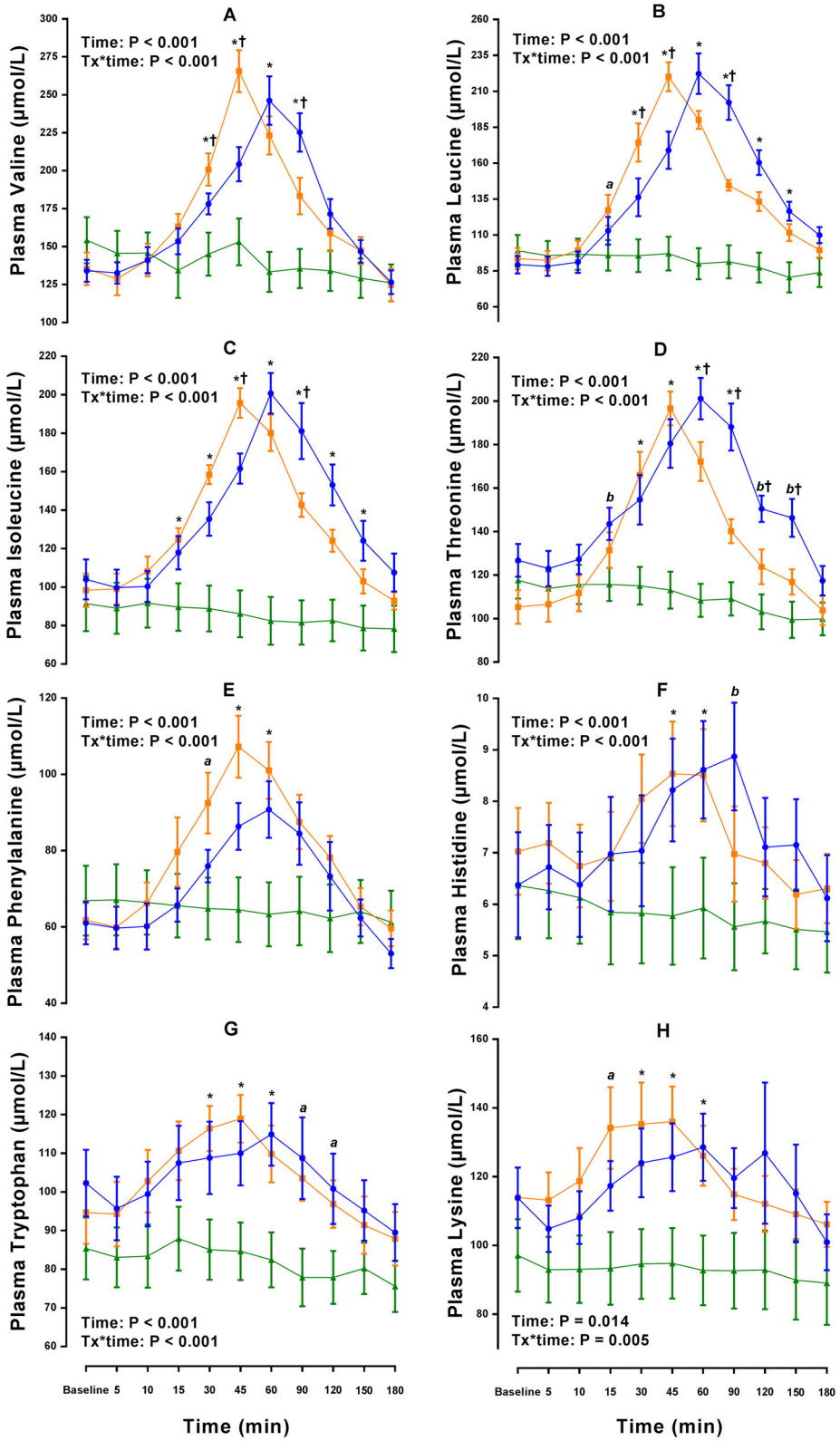

FIGURE 2 Time-course changes in the plasma amino acids valine (A), leucine (B), isoleucine (C), threonine (D), phenylalanine (E), histidine (F), tryptophan (G), and lysine (H) after breakfast ($n = 11$). Blue lines indicate intact whey protein; orange lines indicate hydrolyzed whey protein; and green lines indicate control. *Control different from intact and hydrolyzed whey protein, [a]control different from hydrolyzed whey protein, [b]control different from intact whey protein, and [†]intact whey protein different from hydrolyzed whey protein ($P < 0.05$). Values are means ± SEMs and time and interaction (Tx*time) effects. Data were analyzed by 2-factor (time × treatment) repeated-measures ANOVA, with Bonferroni-adjusted post hoc comparisons where significant time and time × treatment effects were found. Tx*time, treatment × time.

TABLE 1
Postprandial AUCs for plasma insulin after breakfast and lunch meals in men with type 2 diabetes[1]

	Plasma insulin AUC, μU/mL (%)		
	Control	Intact whey protein	Hydrolyzed whey protein
Breakfast			
0–30 min	1656.0 ± 209.5	2178.9 ± 215.6 (31.5)*	1913.9 ± 211.3 (15.5)
0–60 min	3999.9 ± 490.1	5011.0 ± 480.7 (25.3)*	4745.9 ± 469.6 (18.7)
0–90 min	6507.9 ± 766.9	8236.1 ± 791.7 (26.6)*	7608.6 ± 712.1 (16.9)
0–180 min	12,779.1 ± 1767.5	15,371.4 ± 1693.5 (20.3)*	14,586.2 ± 1588.7 (14.1)*
Lunch			
0–30 min	1973.6 ± 307.9	2585.9 ± 410.6 (31.1)	2455.8 ± 412.5 (24.5)
0–60 min	4853.6 ± 758.1	5659.0 ± 1013.9 (16.6)	5581.7 ± 888.4 (15)
0–90 min	7806.2 ± 1341.7	8691.7 ± 1572.9 (11.3)	8630.1 ± 1503.8 (10.6)
0–180 min	17,233.4 ± 3115.3	18,366.9 ± 3133.8 (9.6)	18,444.6 ± 3409.0 (7)

[1] Values are means ± SEMs unless otherwise indicated; $n = 11$. Percentages (in parentheses) represent the change in postprandial response as a percentage of the control trial. *Different from control, $P < 0.05$. Data were analyzed by 1-factor (treatment) repeated-measures ANOVA, with Bonferroni-adjusted post hoc pairwise comparisons where significant treatment effects were found.

repeated-measures ANOVA. To study whether the plasma insulin concentrations correlated with the postprandial concentrations of any amino acids, the AUC for insulin was divided by the AUC for blood glucose to obtain the insulinogenic index (30). Relations between variables were assessed with the use of univariate linear regression analysis. All of the analyses were performed with SPSS (version 22; IBM). Data are presented as means ± SEMs. $P < 0.05$ was considered significant.

RESULTS

Prelaboratory phase

All of the participants showed full compliance with the study methodology. Prelaboratory dietary (kilocalories per day), physical activity (steps per day), and interstitial glycemia were similar during the 24 h before arriving at the laboratory ($P > 0.05$).

Laboratory phase

Baseline concentrations of interstitial glucose, plasma insulin, GLP-1, GIP, leptin, $PYY_{3–36}$, valine, leucine, isoleucine, threonine, phenylalanine, histidine, tryptophan, and lysine were considered highly comparable. Capillary glucose $AUC_{0–60}$ values are presented in **Supplemental Figure 2**, and time-course changes in interstitial glucose postbreakfast and postlunch are presented in **Figure 1**A, B. There was a significant time effect and condition × time interaction for absolute interstitial glucose concentrations after breakfast [$P < 0.001$ (partial $\eta^2 = 0.842$); $P < 0.001$ (partial $\eta^2 = 0.321$)] and after lunch [$P < 0.001$ (partial $\eta^2 = 0.731$); $P < 0.001$ (partial $\eta^2 = 0.201$)], respectively. Reductions in peak postprandial hyperglycemia and reduced postprandial $AUC_{0–180}$ were observed after breakfast and lunch after the intact whey protein compared with the control (Supplemental Figure 2; $P < 0.05$). Similar glycemic responses were observed between the intact and hydrolyzed trials. There were no differences in postprandial glycemic variability across trials at breakfast (percentage of CV—intact whey protein: 13.9% ± 1.2%; hydrolyzed whey protein: 15.7% ± 1.6%; control: 15.5% ± 1.4%; $P = 0.650$) or lunch (percentage of CV—intact whey protein: 14.4% ± 2.1%; hydrolyzed whey protein: 14.2% ± 1.4%; control: 11.4% ± 1.3%; $P = 0.347$).

The postbreakfast responses of amino acids valine, leucine, isoleucine, threonine, phenylalanine, histidine, tryptophan, and lysine are presented in **Figure 2**. There were significant increases in each amino acid after the intact and hydrolyzed whey protein trials when compared with placebo ($P < 0.05$). There was a more rapid release of the amino acids valine, isoleucine, leucine, and threonine within 30–45 min after the hydrolyzed trial than after the intact whey protein trial ($P < 0.05$). There were strong positive correlations between 0–30-min insulinogenic index scores and incremental AUCs for valine ($r_s = 0.680$, $P = 0.021$) and isoleucine ($r_s = 0.751$, $P = 0.008$) concentrations and for valine concentrations only for 0–90 min ($r_s = 0.671$, $P = 0.024$) and 0–180 min ($r_s = 0.669$, $P = 0.024$).

Plasma insulin AUC responses after breakfast and lunch are presented in **Table 1**. Significantly greater concentrations of plasma insulin were observed after both intact and hydrolyzed trials at breakfast when compared with the control ($P < 0.05$; Table 1). Absolute plasma active GLP-1 and total GIP responses after breakfast and lunch are presented in **Figure 3**. There were similar responses observed for $PYY_{3–36}$ and leptin after the breakfast and lunch meals (see **Supplemental Figure 3**).

Subjective ratings of fullness ($AUC_{0–180\ min}$) were significantly greater after breakfast when intact whey protein was ingested when compared with control (973 ± 46.2 compared with 801 ± 40.6 cm/min; $P = 0.043$). After lunch, increased satiety was reported after intact whey compared with after placebo, with reduced ratings of hunger $AUC_{0–180\ min}$ (595.4 ± 62.3 compared with 718.1 ± 67.0 cm/min; $P = 0.041$) and prospective food intake $AUC_{0–180\ min}$ (662.7 ± 68.9 compared with 891.0 ± 75.7 cm/min; $P < 0.001$).

Postlaboratory phase

Postlaboratory evening and nocturnal interstitial glucose responses are presented in Figure 1C, D. At the evening meal (1800–2100), observations of interstitial glucose concentrations showed a significant main effect for time ($P < 0.001$, partial $\eta^2 = 0.381$) but no differences were observed between conditions ($P = 0.889$, partial $\eta^2 = 0.074$). Dietary intake (kilocalories per day) and physical activity (steps per day) in the following 24 h were also similar between conditions [$P = 0.505$ (partial $\eta^2 = 0.046$) and $P = 0.883$ (partial $\eta^2 = 0.012$), respectively].

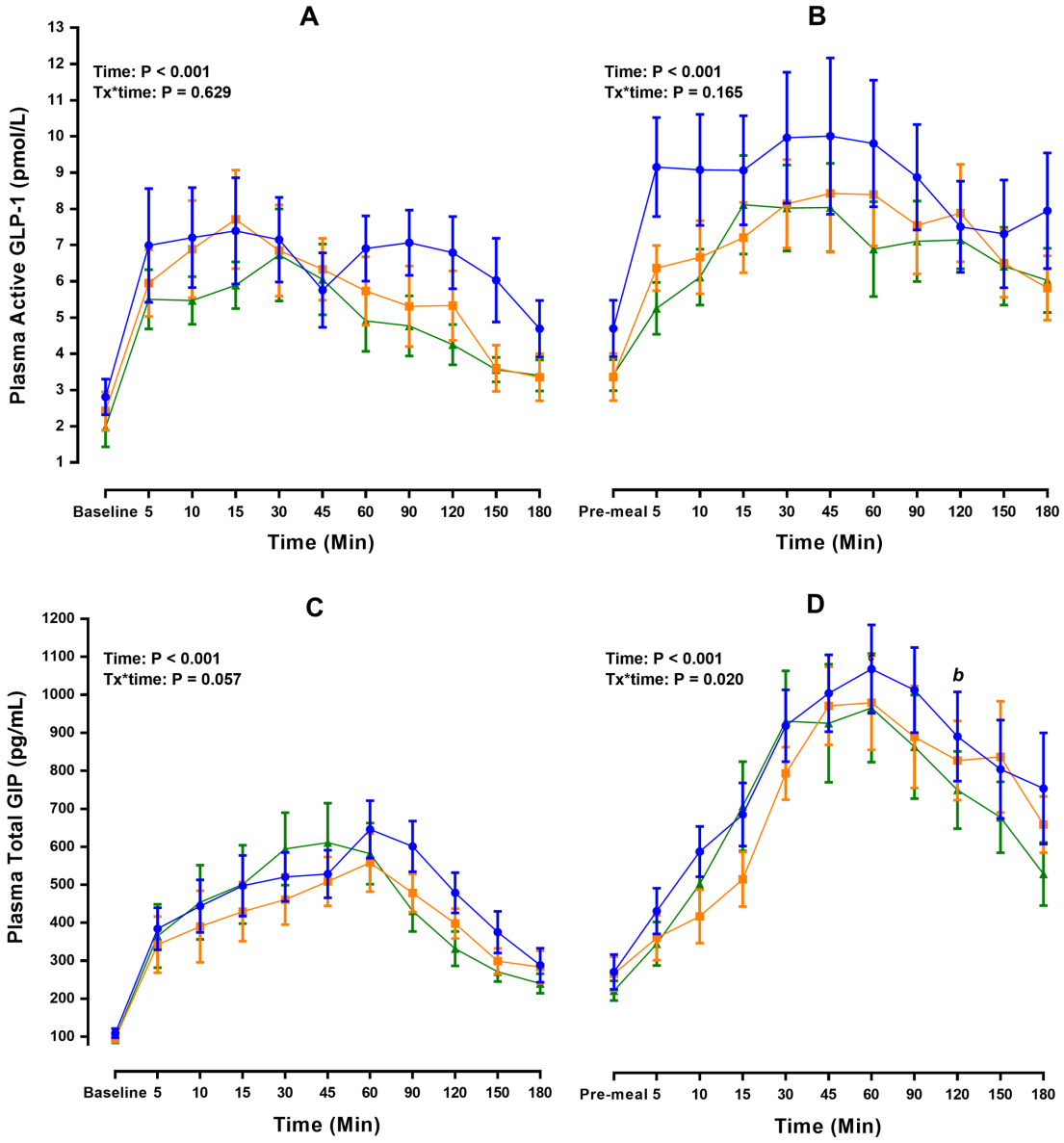

FIGURE 3 Time-course changes in plasma active GLP-1 and GIP after breakfast (A, C) and lunch (B, D) ($n = 11$). Blue lines indicate intact whey protein; orange lines indicate hydrolyzed whey protein; and green lines indicate control. Values are means ± SEMs and time, and interaction (Tx*time) effects. Data were analyzed by 2-factor (Tx*time) repeated-measures ANOVA, with Bonferroni-adjusted post hoc comparisons where significant time and Tx*time effects were found. GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; Tx*time, treatment × time.

DISCUSSION

The aim of this study was to investigate postprandial glycemia and appetite responses after breakfast and lunch meals co-ingested with either small doses of intact or hydrolyzed whey protein in men with type 2 diabetes. We show a reduction in postprandial glycemia after both the intact and hydrolyzed whey protein after breakfast and an increase in satiety after breakfast and lunch after the intact whey protein only. Moreover, despite a more rapid release of amino acids into the circulation, hydrolyzed whey did not provide any further benefit toward glycemic control or appetite hormone response.

An attenuation of postprandial glucose concentrations after whey protein ingestion was observed alongside elevations in plasma insulin despite similar plasma GIP and GLP-1 responses. Previous research has identified an important role for incretin peptides in mediating the insulinotropic activity of whey protein (10). Our study is in contrast to these data; studies that administered larger doses of whey protein, from 27 to 55 g (10, 11, 24), observed a significantly increased incretin response, whereas administering smaller doses, including 25 g (22) and 15 g in the current study, showed no difference in GLP-1 and GIP responses. Furthermore, the lack of change in the incretin hormones is also likely due to whey protein co-ingestion with the meal, as opposed to being ingested as a preload 30 min before the meal (10). A reduced gastric-emptying rate after whey protein ingestion has been suggested to influence the attenuation of glucose concentrations when ingested as a 30-min preload (31). However, gastric-emptying rates may still be slowed but to a much lesser extent

when whey is co-ingested with a meal (10), compared with as a preload; therefore, the observed glycemic responses in our study are potentially due to a minor slowing of gastric emptying combined with increased postprandial insulin concentrations.

Whether the glycemic-lowering effect of the whey protein would still be as effective if it was incorporated into each meal, rather than as a preload, is unknown. Previous research has shown that there is a loss of the glycemic-lowering effect when the whey is consumed within the meal, as opposed to as a 30-min preload (10). However, with our participants consuming the whey immediately before each meal, and with a far lower dose than the study of Ma et al. (10) (55 compared with 15 g), there is potential that the same effect would have been elicited whether the whey was a preload bolus or incorporated into the meal. However, from a clinical and real-world viewpoint, the use of a preload is likely a more practical option for patients, rather than having to incorporate whey into each meal.

The amino acids leucine, isoleucine, phenylalanine, and lysine are also reported to increase insulin through several amino acid–mediated pathways in pancreatic β cells (17, 32, 33). Our results showed marked elevations in each of these amino acids after whey protein ingestion, with plasma concentrations of valine and isoleucine strongly correlated to insulinogenic index values, which suggests that branched-chain amino acids may have been important determinants of the insulinotropic effect of whey we observed. Despite a more rapid absorption of valine, leucine, and isoleucine after the hydrolyzed whey trial, when compared with intact whey protein, our data showed no discernible glycemic, insulinemic, or hormonal differences between whey fractions. With intact whey also being a quickly digested protein form, it is likely that any further benefit of hydrolysis would be minimal.

To the best of our knowledge, this is the first study to investigate self-rated appetite responses in patients with type 2 diabetes after whey protein ingestion. Our findings show increased satiety (fullness, hunger, and prospective food intake) after co-ingestion of intact whey protein with breakfast and lunch when compared with a control. Despite a caloric surplus of 68 kcal with whey meals, our findings are supported by Doyon et al. (34), who documented a similar impact on satiety when yogurt was enriched with whey in isocaloric testing conditions.

The subjective appetite responses were observed without any conditional differences in the postprandial appetite control hormones GLP-1, GIP, $PYY_{3–36}$, or leptin. However, insulin has a potent anorectic effect (35) and has been shown to be positively associated with fullness sensations after the ingestion of whey protein (36). In addition, increases in plasma amino acids are also potential mediators of the increased satiety response (37).

In summary, a small dose (15 g) of whey protein, when co-ingested with mixed-nutrient meals, improves postprandial glycemia and increases satiety in men with type 2 diabetes. Further research is required to explore the clinical efficacy of mealtime whey protein ingestion on long-term glycemic control (HbA1c, glucose variability), food intake, and weight management in type 2 diabetes.

The authors' responsibilities were as follows—DGK, EJS, and DJW: designed the research; DGK, MDC, and MW: conducted the research; DGK and LB: analyzed the data; DGK, MW, LB, EJS, and DJW: wrote the manuscript; DJW: had primary responsibility for the final content; and all authors: read and approved the final manuscript. The authors had no conflicts of interest relevant to this article.

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