Co-ingestion of a protein hydrolysate and amino acid mixture with carbohydrate improves plasma glucose disposal in patients with type 2 diabetes[1–3]

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ABSTRACT

Background: Although insulin secretion after carbohydrate ingestion is severely impaired in patients with type 2 diabetes, amino acid and protein co-ingestion can substantially increase plasma insulin responses.

Objective: We investigated insulin responses and the subsequent plasma glucose disposal rates after the ingestion of carbohydrate alone (CHO) or with a protein hydrolysate and amino acid mixture (CHO+PRO) in patients with a long-term diagnosis of type 2 diabetes.

Design: Ten type 2 diabetic patients [mean (±SEM) age: 62 ± 2 y; body mass index (kg/m^2): 27 ± 1] and 9 healthy control subjects (age: 58 ± 1 y; body mass index: 27 ± 1) participated in 2 trials in which the plasma insulin response was measured after the ingestion of 0.7 g carbohydrate $\cdot$ kg^{-1} $\cdot$ h^{-1} with or without 0.35 g $\cdot$ kg^{-1} $\cdot$ h^{-1} of a mixture that contained a protein hydrolysate, leucine, and phenylalanine. Continuous infusions with [6,6-^{2}H$_2$]glucose were then given to investigate plasma glucose disposal.

Results: Plasma insulin responses were higher by 299 ± 64% and 132 ± 63% in the CHO+PRO trial than in the CHO trial in the diabetic patients and the matched control subjects, respectively ($P <$ 0.001). The subsequent plasma glucose responses were reduced by 28 ± 6% and 33 ± 3% in the CHO+PRO trial than in the CHO trial in the diabetic patients and the matched control subjects, respectively ($P < 0.001$). The reduced plasma glucose response in the diabetic patients was attributed to a 13 ± 3% increase in glucose disposal ($P < 0.01$).

Conclusions: The combined ingestion of carbohydrate with a protein hydrolysate and amino acid mixture significantly increases de novo insulin production in patients with a long-term diagnosis of type 2 diabetes. The increased insulin response stimulates plasma glucose disposal and reduces postprandial glucose concentrations. *Am J Clin Nutr* 2005;82:76–83.

KEY WORDS Glucose disposal, protein hydrolysate, leucine, phenylalanine, metabolism, type 2 diabetes

INTRODUCTION

The stimulating effect of the combined intake of carbohydrate and protein on plasma insulin release was reported in the 1960s (1, 2) and has since been confirmed in healthy subjects (3) and in patients with type 2 diabetes (4–6). Furthermore, intravenous infusion of free amino acids was reported to increase insulin secretion (7–9). In agreement with these findings, various in vitro studies with incubated β cells have attributed strong insulinotropic properties to arginine, leucine, and phenylalanine (10–17). We have performed various in vivo studies in which we defined an optimal insulinotropic amino acid and protein mixture containing leucine, phenylalanine, and a protein hydrolysate that has repeatedly been shown to augment the insulin response by an additional 100% in healthy subjects (18, 19). Nutritional interventions that effectively stimulate endogenous insulin secretion could be of particular significance in patients with type 2 diabetes. An increase in endogenous insulin secretion could increase blood glucose disposal and thus improve glucose homeostasis. Moreover, preventing or reducing the postprandial rise in blood glucose concentration that follows carbohydrate intake could reduce the risk of developing diabetic and cardiovascular complications (20, 21). Furthermore, the combined administration of amino acids and protein with carbohydrate, which leads to a state of hyperinsulinemia and hyperaminoacidemia, may represent an effective strategy to inhibit proteolysis and to stimulate protein synthesis (22, 23). This outcome would be of particular interest, because muscle protein breakdown rates are markedly elevated in uncontrolled diabetes (24).

In patients with a long-term diagnosis of type 2 diabetes, hyperglycemia is not accompanied by a compensatory hyperinsulinemia. As such, it is generally assumed that the capacity of the β cell to secrete insulin is severely impaired as the result of several defects (25). These defects, which are all indicative of a progressive insensitivity of the β cell to glucose, include a reduced early-insulin secretory response to oral glucose, a reduced ability of the β cell to compensate for the degree of insulin resistance, a decline in the glucose-sensing ability of the β cell, and a shift to the right in the dose-response curve relating glucose

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and insulin secretion (26). All of these defects involve glucose-sensing and -signaling pathways in the β cell. Although insulin secretion in response to carbohydrate intake is impaired in patients with type 2 diabetes, we recently showed that co-ingestion of a protein and amino acid mixture can increase the plasma insulin response 2–3-fold (27). Although such nutritional interventions can effectively stimulate endogenous insulin secretion in patients with a long-term diagnosis of type 2 diabetes, the clinical significance of these interventions in regard to blood glucose homeostasis remains to be established.

In the present study, we investigated the insulinotropic properties of a combination of a mixture of protein hydrolysate, leucine, and phenylalanine with carbohydrate and the glucose disposal rate after its ingestion in patients with a long-term diagnosis of type 2 diabetes and in healthy, matched control subjects.

SUBJECTS AND METHODS

Subjects

Ten male patients with a long-term diagnosis of type 2 diabetes and 10 healthy, matched control subjects were selected to participate in the present study. The baseline characteristics of the subjects are shown in **Table 1**. Exclusion criteria were impaired renal or liver function, obesity [body mass index (in kg/m^2) >35], cardiac disease, hypertension, diabetic complications, and exogenous insulin therapy. All except one of the type 2 diabetic patients ($n = 9$) were using oral antidiabetic agents (metformin alone or in combination with sulfonylureas). One control subject withdrew from the experiment for personal reasons. In the type 2 diabetic patients, any medication being used was withheld for 2 d before the screening process. The subjects were screened for glucose intolerance and type 2 diabetes by use of a standard oral-glucose-tolerance test according to the World Health Organization criteria of 1999 (28). All subjects were informed about the nature and the risks of the experimental procedures before their written informed consent was obtained. All clinical trials were approved by the local medical ethical committee.

Screening

Before selection into the study, all subjects were given an oral-glucose-tolerance test. The subjects arrived at the laboratory at 0800 by car or public transportation after having fasted overnight. A blood sample was collected from the fasting subjects, after which a bolus of 75 g glucose (dissolved in 250 mL water) was ingested ($t = 0$ min). After 120 min, a second blood sample was obtained. Plasma glucose concentrations were measured to determine glucose intolerance and type 2 diabetes according to the World Health Organization criteria of 1999 (28). In addition, basal fasting plasma glucose and insulin concentrations were used to assess whole-body insulin resistance with the homeostasis model assessment insulin resistance index (29), which was calculated as the product of basal fasting plasma glucose (mmol/L) and insulin (mU/L) concentrations divided by 22.5.

Medication, diet, and activity before testing

Medication that stimulates insulin production or secretion (sulfonylurea derivatives) was withheld for 2 d before each test to prevent confounding effects on amino acid–induced insulin secretion. The use of insulin sensitizers (metformin) was continued to support the benefits of increasing endogenous insulin secretion on glucose homeostasis. All subjects maintained normal dietary and physical activity patterns throughout the entire experimental period. In addition, the subjects refrained from heavy physical labor and exercise for ≥3 d before each trial and filled out a food-intake diary for 2 d before the first trial to keep their dietary intake as identical as possible before the second trial. The evening before each trial, the subjects received a standardized meal (43.80 kJ/kg body wt that consisted of 60% of energy as carbohydrate, 28% of energy as fat, and 12% of energy as protein).

TABLE 1
Subject characteristics[1]

	Control group ($n = 9$)	Type 2 diabetic group ($n = 10$)
Age (y)	58.2 ± 1.0	61.5 ± 2.3
Body weight (kg)	84.89 ± 2.86	81.8 ± 3.89
Height (m)	1.76 ± 0.02	1.73 ± 0.02
BMI (kg/m^2)	27.49 ± 1.07	27.19 ± 0.97
Basal plasma glucose (mmol/L)	5.31 ± 0.12	10.71 ± 0.56[2]
Plasma glucose$_{\text{OGTT120}}$ (mmol/L)[3]	4.98 ± 0.41	20.01 ± 1.14[2,4]
Basal plasma insulin (mU/L)	6.44 ± 0.90	10.30 ± 1.59
Hb A$_{1c}$ (%)	5.10 ± 0.13	7.49 ± 0.38[2]
HOMA-IR	1.52 ± 0.22	5.02 ± 0.96[2]
Diagnosed with type 2 diabetes (y)	NA	11 ± 2
Medication	NA	Metformin or SU derivatives

[1] All values are $\bar{x}$ ± SEM. OGTT, oral-glucose-tolerance test; Hb A$_{1c}$, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance (29); NA, not applicable; SU, sulfonylureas.

[2] Significantly different from control group, $P < 0.01$ (t test comparing patient and control group).

[3] Plasma glucose concentration 2 h after ingestion of 75 g glucose.

[4] Significantly different from basal values, $P < 0.01$ (t test comparing pre- and post-OGTT values).

Design

Each subject participated in 2 trials, separated by a 2-wk period, in which the plasma insulin response and subsequent plasma glucose disposal rate were measured after the ingestion of 2 different beverage compositions (CHO, carbohydrate only; or CHO+PRO, carbohydrate and a mixture that contained a protein hydrolysate and the free amino acids leucine and phenylalanine). The subjects were placed in a supine position and remained inactive for 3 h. Drinks were provided in a randomized order and a double-blind fashion. The beverages were flavored to make the taste comparable in both trials (*see* below).

Protocol

The subjects reported to the laboratory at 0800 after an overnight fast. A catheter (Baxter BV, Utrecht, Netherlands) was inserted into an antecubital vein for the isotope infusion. Another catheter was inserted into a dorsal vein on the contralateral hand and was placed in a hot-box (60 °C) for arterialized blood sampling. After 10 min, a blood sample was collected from the resting subjects ($t = 0$ min). After the administration of an intravenous bolus of 13.5 μmol $[6,6\text{-}^2H_2]$glucose/kg, a continuous infusion of 277 ± 3 nmol $\cdot$ kg^{-1} $\cdot$ min^{-1} of $[6,6\text{-}^2H_2]$glucose was started via a calibrated IVAC 560 pump (IVAC Corp, San Diego, CA) and continued until $t = 180$ min. At $t = 0$ min, the subjects drank an initial bolus (2 mL/kg) of the test drink (CHO or CHO+PRO). Repeated boluses (2 mL/kg) were ingested every 15 min until $t = 165$ min. Blood samples were drawn every 15 min during the first hour and then every 30 min until $t = 180$ min for the measurement of plasma glucose, glucose enrichment, and insulin. In addition, proinsulin and C-peptide concentrations were measured in the blood samples that were collected at $t = 0$, 60, 120, and 180 min.

Beverages

The subjects received repeated boluses of 2 mL/kg to ensure a given dose of 0.7 g carbohydrate $\cdot$ kg^{-1} $\cdot$ h^{-1} (50% as glucose and 50% as maltodextrin) with or without 0.35 g $\cdot$ kg^{-1} $\cdot$ h^{-1} of a protein hydrolysate and amino acid mixture (50% as casein hydrolysate, 25% as free leucine, and 25% as free phenylalanine) every 15 min until $t = 165$ min. Glucose and maltodextrin were obtained from AVEBE (Veendam, Netherlands), crystalline amino acids were from BUFA (Uitgeest, Netherlands), and the casein protein hydrolysate was prepared by DSM Food Specialties (Delft, Netherlands). The casein hydrolysate (Insuvital; DSM Food Specialties) was obtained by enzymatic hydrolysis of sodium caseinate with the use of a neutral protease and a prolyl-specific endoproteinase. Both drinks were uniformly flavored by the addition of 0.2 g sodiumsaccharinate, 1.8 g citric acid, and 5 g of a cream vanilla flavor (Quest International, Naarden, Netherlands) for each 1 L of beverage.

Isotope tracer calculations

The glucose tracer (99% enriched; Cambridge Isotope laboratories, Andover, MA) was first dissolved in 0.9% saline. The glucose tracer concentration in the infusates averaged 22 ± 0.4 mmol/L. The $[6,6\text{-}^2H_2]$glucose infusion rate averaged 277 ± 3 nmol $\cdot$ kg^{-1} $\cdot$ min^{-1}. Plasma glucose enrichments are expressed as tracer/tracee ratios. The rate of appearance (Ra) and rate of disappearance (Rd) of glucose were calculated with the use of the single-pool non–steady state Steele equations (30) adapted for stable-isotope studies as described elsewhere (31).

$$\mathrm{Ra} = \{F - V[(C_2 + C_1)/2][(E_2 - E_1)/(t_2 - t_1)]\}/[(E_2 + E_1)/2] \quad (1)$$

$$\mathrm{Rd} = \mathrm{Ra} - V \cdot [(C_2 - C_1)/(t_2 - t_1)] \quad (2)$$

where F is the infusion rate (in μmol $\cdot$ kg^{-1} $\cdot$ min^{-1}); V is the distribution volume for glucose (160 mL/kg); $C1$ and $C2$ are the glucose concentrations (in mmol/L) at time 1 (t_1) and 2 (t_2), respectively; and $E1$ and $E2$ are the plasma glucose enrichments (tracer/tracee ratios) at time 1 and 2, respectively.

Blood sample analysis

Blood (10 mL) was collected in EDTA-containing tubes and centrifuged at 1000 × *g* for 10 min at 4 °C. Aliquots of plasma were immediately frozen in liquid nitrogen and stored at −80 °C until analyzed. Glucose concentrations (Uni Kit III; Roche, Basel, Switzerland) were analyzed with the COBAS FARA semiautomatic analyzer (Roche). Plasma insulin, proinsulin, and C-peptide concentrations were assayed with a modified, solid-phase, 2-site fluoroimmunometric assay based on a direct sandwich technique (DELFIA method; Perkin Elmer, Turku, Finland). To measure the glycated hemoglobin content, a 3-mL blood sample was collected in EDTA-containing tubes and was analyzed by HPLC (Bio-Rad Diamat, Munich, Germany). After derivatization of the plasma samples, plasma $[6,6\text{-}^2H_2]$glucose enrichment was measured by electron ionization gas chromatography–mass spectrometry (Finnigan INCOS-XL; Finnigan Mat, Ilemel, Hemstead, United Kingdom).

Statistics

Data are expressed as means ± SEMs. The plasma responses were calculated as the area under the curve minus baseline values. To compare plasma metabolite concentrations and tracer kinetics over time between trials, a two-way repeated-measures analysis of variance (ANOVA) was performed. Subgroups were analyzed further whenever significant time-by-treatment interactions were observed. Changes in time within each group were checked for statistical significance with the use of a one-way repeated-measures ANOVA. When an F ratio was significant, a Scheffe's post hoc test was performed to locate specific differences. For non-time-dependent variables, a multiway ANOVA alone or with a Student's t test for unpaired observations was used. Significance was set at $P < 0.05$. All calculations were performed with STATVIEW 5.0 (SAS Institute Inc, Cary, NC).

RESULTS

Insulin

Plasma insulin concentrations in subjects that had fasted overnight were similar in both groups and in both trials. Insulin concentrations increased significantly in both groups after the ingestion of carbohydrate alone and carbohydrate with the protein and amino acid mixture ($P < 0.001$; **Figure 1**A). A repeated-measures ANOVA showed a significant time-by-treatment interaction for plasma insulin concentrations ($P < 0.01$). After $t = 60$ min, plasma insulin concentrations in the diabetes group were higher in the CHO+PRO trial than in the CHO trial ($P < 0.05$). No significant differences were found between trials in the control group. After the insulin response was expressed as the area under the curve (minus baseline values), significantly greater plasma insulin responses were observed in the CHO+PRO trial than in the CHO trial in both groups ($P < 0.01$, Figure 1B). Plasma insulin responses were 299 ± 64% and 132 ± 63% greater in the CHO+PRO trial than in the CHO trial in the diabetes and control groups, respectively ($P < 0.01$).

C-peptide and proinsulin

Plasma C-peptide concentrations in fasting subjects were similar in both groups. A repeated-measures ANOVA showed a significant time-by-treatment interaction for plasma C-peptide concentrations ($P < 0.01$). In both trials, C-peptide concentrations increased significantly over time ($P < 0.05$; **Figure 2**). After $t = 60$ min, plasma C-peptide concentrations in the diabetes group were significantly higher in the CHO+PRO trial than in the CHO trial ($P < 0.05$). When expressed as the area under the curve, significantly greater C-peptide responses were observed in the CHO+PRO trial than in the CHO trial in both groups ($P < 0.01$). Plasma C-peptide responses were 98 ± 18% and 56 ± 26%

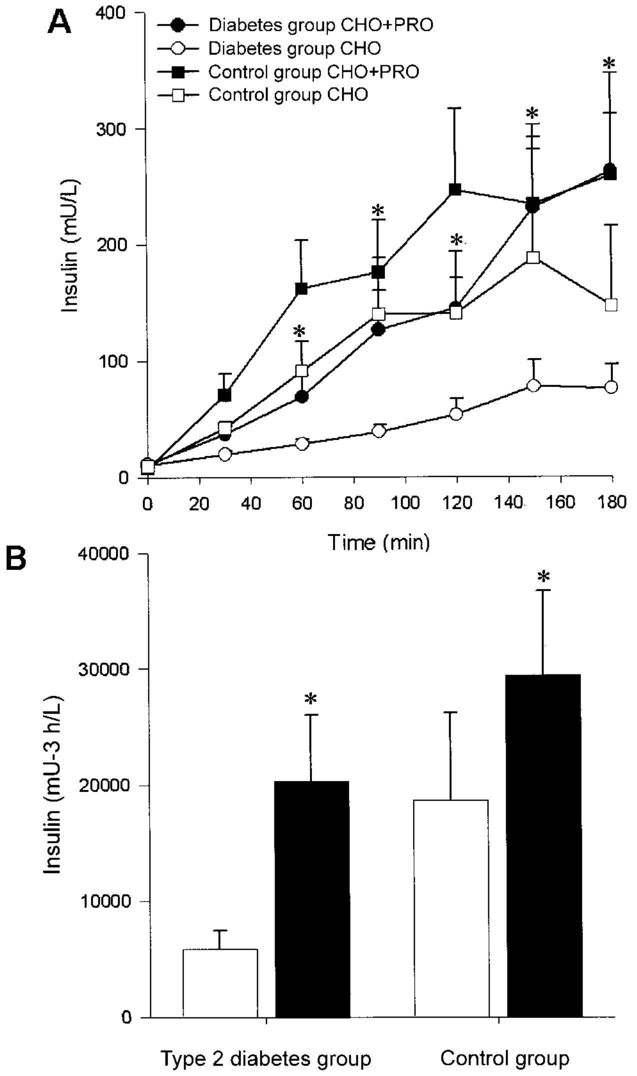

FIGURE 1. Mean (±SEM) plasma insulin concentrations (A) and responses (B) over a 3-h period after the ingestion of carbohydrate (CHO; open symbols) or carbohydrate and a protein hydrolysate and amino acid mixture (CHO+PRO; filled symbols) in patients with type 2 diabetes ($n = 10$) and in healthy, matched control subjects ($n = 9$). (A) Insulin concentrations increased significantly over time in both groups and trials, $P < 0.001$. No significant differences were found between trials in the control group. There was a significant time × treatment interaction for insulin ($P < 0.01$). *Significantly different from the CHO trial in the diabetes group, $P < 0.05$ (Scheffe's adjustment). (B) *Significantly different from the CHO trial, $P < 0.05$ (ANOVA).

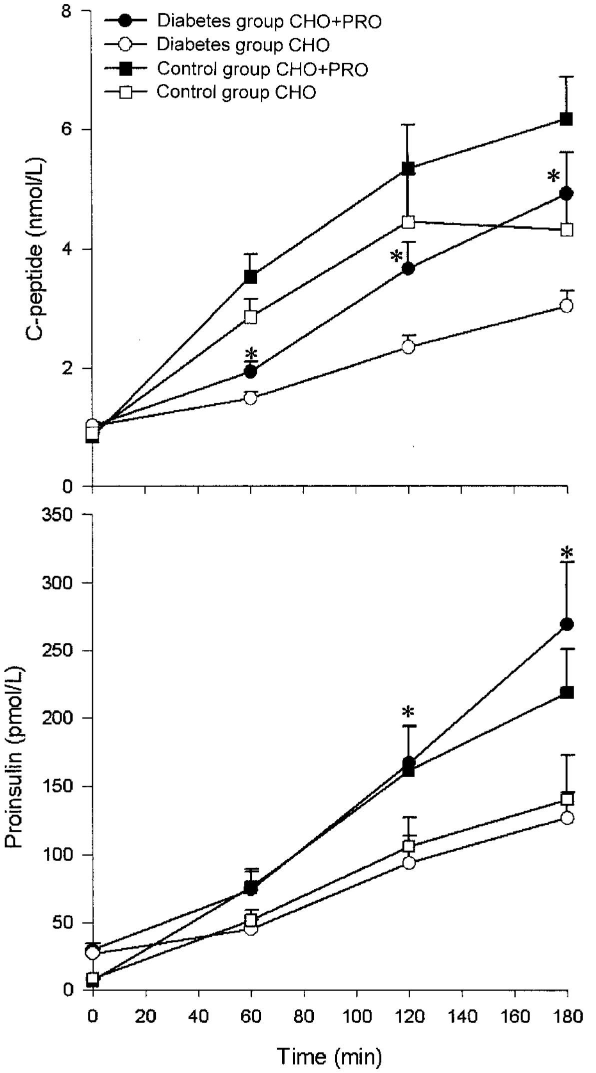

FIGURE 2. Mean (±SEM) plasma C-peptide and proinsulin concentrations over a 3-h period after the ingestion of carbohydrate (CHO) or carbohydrate and a protein hydrolysate and amino acid mixture (CHO+PRO) in patients with type 2 diabetes ($n = 10$) and in healthy, matched control subjects ($n = 9$). We found a significant time × treatment interaction for C-peptide and proinsulin ($P < 0.01$ for all). C-peptide concentrations increased significantly over time in both trials and groups, $P < 0.05$. After $t = 60$ min, C-peptide concentrations were significantly different between trials in the diabetes group (Scheffe's adjustment); no significant differences were found between trials in the control group. Proinsulin concentrations increased significantly over time in both trials and groups, $P < 0.01$. After $t = 120$ min, proinsulin concentrations were significantly different between trials in the diabetes group (Scheffe's adjustment); no significant differences were found between trials in the control group. *Significantly different from the CHO trial, $P < 0.05$.

greater in the CHO+PRO trial than in the CHO trial in the diabetes and control groups, respectively ($P < 0.01$). Plasma C-peptide concentrations correlated well with plasma insulin concentrations ($r = 0.89$, $P < 0.001$).

Plasma proinsulin concentrations in fasting subjects were higher in the type 2 diabetes group than in the normoglycemic control subjects (28.3 ± 2.9 mmol/L compared with 7.5 ± 0.5 mmol/L, respectively $P < 0.01$). In both trials, proinsulin concentrations increased significantly over time ($P < 0.01$; Figure 2) and showed a significant time-by-treatment interaction ($P < 0.01$). After $t = 120$ min, plasma proinsulin concentrations in the diabetes group were higher in the CHO+PRO trial than in the CHO trial ($P < 0.05$). No significant differences were observed between trials in the control group. When expressed as the area under the curve, significantly greater proinsulin responses were observed in the CHO+PRO trial than in the CHO trial in both groups ($P < 0.05$). The plasma proinsulin responses were 151 ± 28% and 84 ± 37% greater in the CHO+PRO trial than in the CHO trial in the diabetes and the control groups, respectively ($P < 0.05$). Plasma proinsulin concentrations correlated with both plasma insulin and plasma C-peptide concentrations ($r = 0.79$ and $r = 0.85$, respectively; $P < 0.001$)

Glucose

Plasma glucose concentrations in fasting subjects were higher in the type 2 diabetic patients than in the normoglycemic control subjects (9.7 ± 0.3 mmol/L compared with 5.7 ± 0.1 mmol/L, respectively; $P < 0.01$). A repeated-measures ANOVA showed a significant time-by-treatment interaction for plasma glucose concentrations ($P < 0.01$). In the type 2 diabetic patients, plasma glucose concentrations in the CHO trial increased after carbohydrate ingestion until $t = 150$ min, after which values reached a plateau. In the CHO+PRO trial, glucose concentrations increased significantly during the first 90 min ($P < 0.01$), after which they either reached a plateau or tended to decline (**Figure 3**A). At $t = 180$ min, the plasma glucose concentration was significantly lower in the CHO+PRO trial than in the CHO trial ($P < 0.05$) for the type 2 diabetes group. In the control group, plasma glucose concentrations slightly increased during the first 60 min in both trials and then returned to baseline levels over the next 2 h (Figure 3A). Plasma glucose concentrations were significantly higher in the type 2 diabetic patients than in the matched control subjects ($P < 0.05$). After expressing the plasma glucose response as the area under the curve, we observed a significantly higher plasma glucose response in the type 2 diabetic patients than in the matched normoglycemic control subjects ($P < 0.001$; Figure 3B). In both groups, significantly lower plasma glucose responses were observed in the CHO+PRO trial than in the CHO trial ($P < 0.001$; Figure 3B). The plasma glucose response was 28 ± 6% and 33 ± 3% lower in the CHO+PRO trial than in the CHO trial in the diabetes and matched control groups, respectively ($P < 0.001$).

Glucose tracer kinetics

In the type 2 diabetes group, the plasma glucose Ra was stable over the entire testing period and averaged 42.4 ± 0.8 and 41.2 ± 1.1 μmol · kg^{-1} · min^{-1} in the CHO and CHO+PRO trials, respectively. In the control group, the plasma glucose Ra was also stable and averaged 39.8 ± 0.7 and 37.9 ± 0.8 μmol · kg^{-1} · min^{-1} in the CHO and CHO+PRO trials, respectively (**Table 2** and **Figure 4**A and B). No significant differences in the plasma glucose Ra were observed between trials or groups.

The glucose Rd increased over time in both trials and in both groups ($P < 0.05$; Figure 4C and D). In the diabetes group, the Rd averaged 19.7 ± 2.4 and 20.4 ± 2.8 μmol · kg^{-1} · min^{-1} at $t = 30$ min and increased over time to reach 45.1 ± 1.8 and 45.4 ± 3.6 μmol · kg^{-1} · min^{-1} in the CHO and CHO+PRO trials, respectively (Figure 4C). In the control group, the Rd averaged 14.7 ± 1.4 and 19.4 ± 1.7 μmol · kg^{-1} · min^{-1} at $t = 30$ min and increased to 45.4 ± 2.4 and 44.8 ± 2.2 μmol · kg^{-1} · min^{-1} in the CHO and CHO+PRO trials, respectively (Figure 4D). The increase in the Rd over time was significantly different between groups ($P < 0.05$).

Plasma glucose disposal, expressed as the percentage of the appearing glucose that disappears from the circulation, was significantly lower in the diabetic patients than in the matched control subjects ($P < 0.001$; Table 2). In the diabetes group, plasma glucose disposal was 12.5 ± 3.1% higher in the CHO+PRO trial than in the CHO trial ($P < 0.01$). In the control group, plasma glucose disposal was not significantly improved in the CHO+PRO trial (3.4 ± 2.2%; $P = 0.2$; Table 2).

In the diabetes group, the glucose disposal rate was significantly improved by 15.8 g (≈88 mmol) over the 150-min period in the CHO+PRO trial compared with the CHO trial ($P < 0.01$). In the control group, an additional 11.7 g (≈65 mmol) glucose was disposed of during the 150-min period in the CHO+PRO trial compared with the CHO trial ($P = 0.2$).

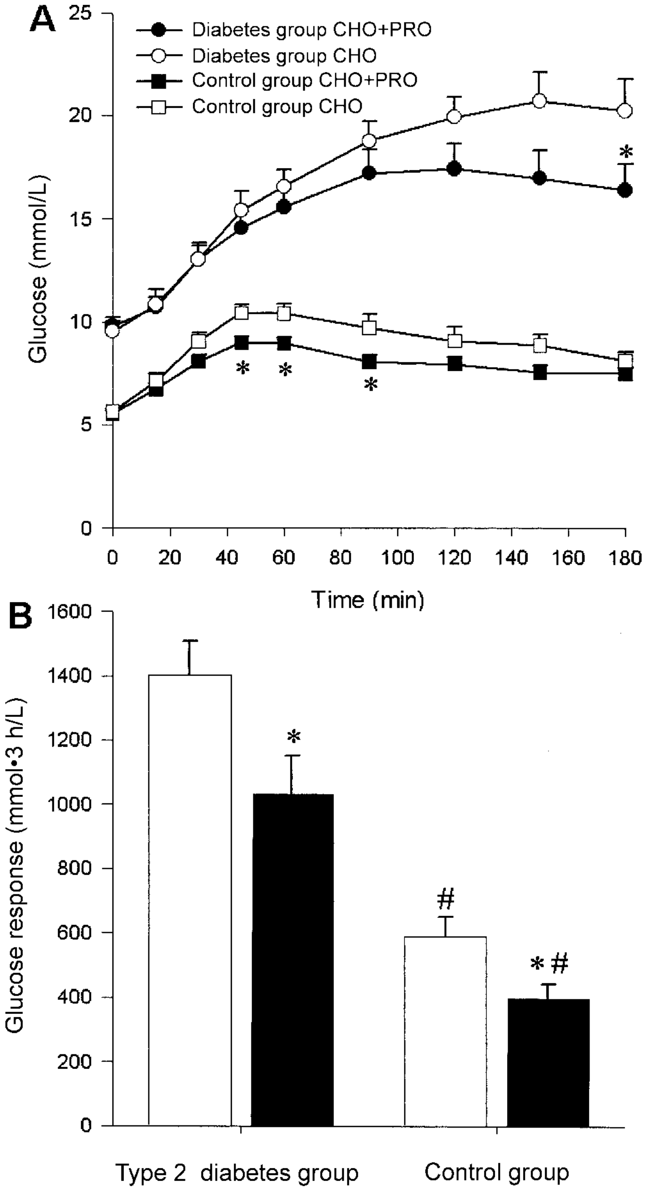

FIGURE 3. Mean (±SEM) plasma glucose concentrations (A) and responses (B) over a 3-h period after the ingestion of carbohydrate (CHO; open symbols) or carbohydrate and a protein hydrolysate and amino acid mixture (CHO+PRO; filled symbols) in patients with type 2 diabetes ($n = 10$) and in healthy, matched control subjects ($n = 9$). (A) Glucose concentrations increased significantly over time in both trials for the diabetic patients, $P < 0.01$. There was a significant time × treatment interaction for glucose, $P < 0.01$. *Significantly different from the CHO trial for both groups, $P < 0.05$ (Scheffe's adjustment). (B)$^{*,\#}$Significantly different from the CHO trial (ANOVA): $^{*}P < 0.05$, $^{\#}P < 0.001$.

DISCUSSION

The present study showed that co-ingestion of carbohydrate with a mixture containing casein hydrolysate, leucine, and phenylalanine substantially increased insulin secretion when compared with the ingestion of carbohydrate alone. The substantial

TABLE 2
Plasma glucose kinetics[1]

	CHO	CHO + PRO
Control group ($n = 9$)		
Ra (μmol · kg^{-1} · min^{-1})	39.8 ± 0.7	37.9 ± 0.8
Rd (μmol · kg^{-1} · min^{-1})	36.2 ± 1.7	35.7 ± 1.3
Glucose disposal (Rd as % of Ra)	91 ± 4	94 ± 3
Time for Rd to match Ra (min)	90 ± 8	75 ± 6[2]
Type 2 diabetic group ($n = 10$)		
Ra (μmol · kg^{-1} · min^{-1})	42.4 ± 0.8	41.2 ± 1.1
Rd (μmol · kg^{-1} · min^{-1})	30.3 ± 1.3	33.2 ± 1.5
Glucose disposal (Rd as % of Ra)	72 ± 3[3]	81 ± 3[2,3]
Time for Rd to match Ra (min)	179 ± 8[4]	135 ± 9[2,4]

[1] All values are $\bar{x}$ ± SEM of [6.6-^{2} H$_2$]glucose tracer rate of appearance (Ra) and disappearance (Rd) and Rd expressed as percentage of Ra over the entire 150-min period. CHO, carbohydrate; CHO + PRO, carbohydrate and protein mixture.

[2] Significantly different from CHO trial, $P < 0.01$ (t test comparing trials within each group).

[3,4] Significantly different from control group (t test comparing trials between groups): $^3P < 0.001$, $^4P < 0.01$.

3–4-fold greater insulin response significantly improved postprandial glucose disposal and resulted in lower plasma glucose concentrations in type 2 diabetic patients. This study indicates that nutritional interventions that improve endogenous insulin secretion can be practical and effective tools in the treatment of type 2 diabetes.

The synergistically stimulating effect of the combined ingestion of carbohydrate and intact protein on plasma insulin release was first reported in the late 1960s (1, 2) and was later confirmed in both healthy subjects (3) and type 2 diabetic patients (4–6). Floyd et al (7–9, 32) investigated the effects of intravenous infusions of various amino acids on plasma insulin secretion and reported that arginine, leucine, and phenylalanine were the most insulinotropic amino acids. We have confirmed many of these findings after testing the oral administration of these amino acids in combination with carbohydrate (18, 19). Consequently, we defined a practical and optimal insulinotropic amino acid and protein mixture composed of a protein hydrolysate, free leucine, and phenylalanine (18, 19). Recently, we investigated the insulinotropic properties of this mixture in patients with a long-term diagnosis of type 2 diabetes and reported a 189% greater plasma insulin response in these patients when the mixture was coingested with carbohydrate than when carbohydrate was ingested alone (27). Although that study clearly showed that endogenous insulin secretion can be substantially increased in patients with a long-term diagnosis of type 2 diabetes, the clinical relevance of these findings had not yet been established. Therefore, in the present study, we investigated plasma glucose disposal after the ingestion of carbohydrate with or without the addition of such an insulinotropic protein hydrolysate and amino acid mixture in healthy subjects and in type 2 diabetic patients.

The patients with type 2 diabetes who were selected for this study had been diagnosed with type 2 diabetes for ≥10 y. Basal fasting glucose concentrations, oral-glucose-tolerance test values, glycated hemoglobin content, and the homeostasis model assessment insulin resistance index values confirmed their type 2 diabetic state (Table 1). Hyperinsulinemia, a compensatory response to the prevailing hyperglycemia, was no longer present in these patients (Table 1 and Figure 1). After ingestion of only carbohydrate in the CHO trial, insulin responses were substantially lower in the diabetic patients than in the control subjects (Figure 1B). This finding clearly illustrates the reduced sensitivity of the β cell to glucose in the type 2 diabetic state (26). Interestingly, co-ingestion of carbohydrate with the protein hydrolysate and amino acid mixture in the CHO+PRO trial significantly increased the plasma insulin response by 299 ± 64% and 132 ± 63% in the diabetic patients and the normoglycemic control subjects, respectively ($P < 0.01$; Figure 1B). The insulin response in the CHO+PRO trial in the type 2 diabetic patients was similar to the insulin response reported in the CHO trial in the healthy subjects (Figure 1B). In other words, although the sensitivity of the pancreas to carbohydrate intake is significantly reduced in patients with a long-term diagnosis of type 2 diabetes, the capacity to secrete insulin in response to other stimuli (such as amino acids) remains intact. Therefore, the defects in the insulin response after the ingestion of a meal in these patients are mainly attributed to the reduced sensitivity of the β cell to glucose and not to an overall defect in the capacity to produce or to secrete insulin.

To confirm that the elevated plasma insulin concentrations in the CHO+PRO trial are indeed secondary to increased insulin production, we measured plasma C-peptide and proinsulin concentrations according to the method of Hovorka and Jones (33). In the process of insulin production, the precursor proinsulin is cleaved into insulin and the 31-kD residue connecting peptide (C-peptide). Insulin, C-peptide, and a small amount of residual proinsulin are stored in the secretory granules of the β cell until secretion (34). In the present study, we observed a significant increase in plasma C-peptide and proinsulin concentrations over time in all trials (Figure 2). Significantly greater plasma C-peptide responses were observed in the CHO+PRO than in the CHO trial (98 ± 18% and 56 ± 26% in the diabetic patients and healthy control subjects, respectively; $P < 0.01$). Similarly, plasma proinsulin responses were also 151 ± 28% and 84 ± 37% greater in the CHO+PRO trial than in the CHO trial in the diabetes and control groups, respectively ($P < 0.05$). Both C-peptide and proinsulin concentrations correlated well with plasma insulin concentrations ($r = 0.89$ and $r = 0.79$, respectively: $P < 0.001$). Thus, these data further support the observation that co-ingestion of carbohydrate with the protein and amino acid mixture in the CHO+PRO trial effectively stimulates de novo insulin production.

In response to the increased insulin production and secretion rate in the CHO+PRO trial, plasma glucose concentrations were significantly decreased when compared with values observed in the CHO trial (Figure 3A). In the CHO+PRO trial, plasma glucose responses were decreased by as much as 28 ± 6% and 33 ± 3% in the diabetic patients and normoglycemic control subjects, respectively, compared with responses in the CHO trial ($P < 0.001$). This decrease in the plasma glucose response is much more prominent than in our earlier observations (27), which can be explained by the longer trial duration in the present study. Interventions that effectively reduce the postprandial rise in plasma glucose concentrations after carbohydrate intake are of clinical significance and have been associated with a reduced risk of developing diabetic and cardiovascular complications (20, 21). Many food components or pharmacologic agents that effectively lower postprandial glucose concentration after meal ingestion inhibit gastric emptying or intestinal uptake of glucose or both (35–37). In the present study, we applied a continuous

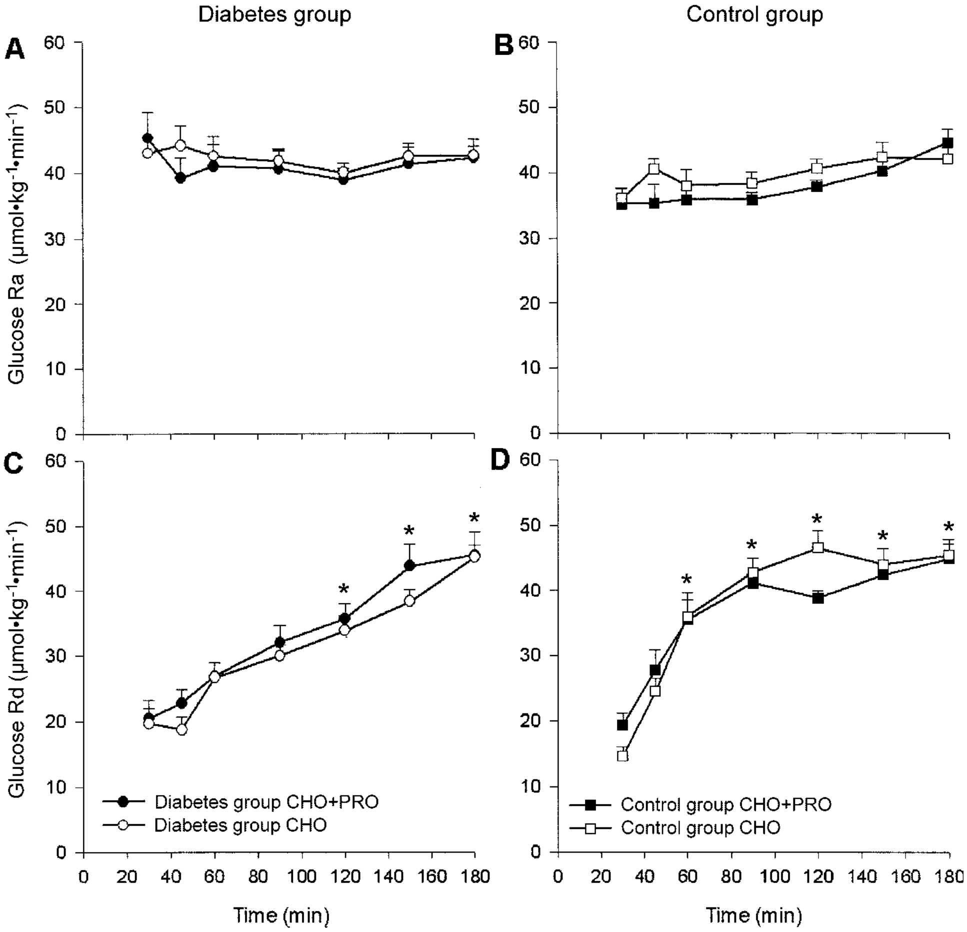

FIGURE 4. Mean (±SEM) plasma glucose rate of appearance (Ra: A and B) and disappearance (Rd: C and D) over time in patients with type 2 diabetes (A and C) and in healthy, matched control subjects (B and D) after the ingestion of carbohydrate (CHO) or carbohydrate and a protein hydrolysate and amino acid mixture (CHO+PRO) in patients with type 2 diabetes ($n = 10$) and in healthy, matched control subjects ($n = 9$). No significant differences in Ra were observed between trials or groups. Glucose Rd increased over time in both trials in both groups, $P < 0.05$. The increase in Rd over time was significantly different between groups ($P < 0.05$) but not between trials. *Significantly different from $t = 30$ min, $P < 0.05$.

infusion of a [6,6-$^2H_2$]glucose tracer to measure the Ra of glucose in the circulation. Plasma glucose Ras were similar in both groups and trials and remained constant throughout the trials (Table 2, Figure 4A and B). This finding indicates that inhibition of gastrointestinal uptake of glucose is not responsible for the observed decline in the postprandial blood glucose response after co-ingestion of carbohydrate with the protein and amino acid mixture.

Whereas the plasma glucose Ra remained stable throughout the trials, the plasma glucose Rd from the circulation significantly increased over time in both trials (Figure 4; $P < 0.01$). In contrast with the Ra values, the plasma glucose Rd was strikingly different between the diabetic patients and the healthy, matched control subjects (Figure 4C and D). Whereas Rd values increased exponentially in the control subjects, a more gradual rise in the glucose Rd was observed in the diabetic patients ($P < 0.01$). It took about twice as long in the diabetic patients as in the healthy subjects for the plasma glucose Ra to be matched by its Rd ($P < 0.01$). Consequently, plasma glucose disposal (calculated as Rd expressed as a percentage of Ra) was significantly lower in the diabetic patients than in the normoglycemic control subjects (Table 2; $P < 0.01$). In both groups, the time for the Rd to match the Ra was significantly reduced in the CHO+PRO trial (Table 2; $P < 0.01$). Accordingly, plasma glucose disposal after co-ingestion of carbohydrate with the protein and amino acid mixture improved plasma glucose disposal by 13 ± 3% ($P < 0.01$) and 3 ± 2% ($P = 0.2$) in diabetic patients and healthy control subjects, respectively.

In conclusion, ingestion of a protein hydrolysate, leucine, and phenylalanine mixture can substantially augment insulin responses after carbohydrate intake. In patients with a long-term diagnosis of type 2 diabetes, co-ingestion of carbohydrate with such a mixture can induce a 3–4-fold greater plasma insulin response than ingestion of carbohydrate alone. This response effectively improves plasma glucose disposal and thereby reduces the postprandial plasma glucose concentration. The combined ingestion of an amino acid and protein mixture with carbohydrate represents an effective interventional strategy in the treatment of type 2 diabetes.

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RJFM, AJMW, and LJCvL designed the study. RJFM organized and carried out the clinical trials with the assistance of RK and AHGZ. RJFM performed the statistical analysis and wrote the manuscript together with LJCvL. PPCAM performed the plasma proinsulin, insulin, and C-peptide

analyses. NCS and WHMS provided medical assistance. None of the authors had a personal or financial conflict of interest.

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