

See corresponding editorial on page 267.

Mechanisms of action of a carbohydrate-reduced, high-protein diet in reducing the risk of postprandial hypoglycemia after Roux-en-Y gastric bypass surgery

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ABSTRACT

Background: Postprandial hypoglycemia is a risk after Roux-en-Y gastric bypass (RYGB).

Objectives: We speculated that a carbohydrate-reduced, high-protein (CRHP) diet might reduce the risk of hypoglycemia and therefore compared the acute effects of a conventionally recommended (CR) diet and CRHP diet [55/30 energy percent (E%) carbohydrate and 15/30 E% protein, respectively] in RYGB patients.

Methods: Ten individuals (2 males, 8 females, mean \pm SD age 47 ± 7 y; stable body mass index 31 ± 6 kg/m²; 6 ± 3 y post-RYGB) with recurrent postprandial hypoglycemia documented by plasma glucose (PG) ≤ 3.4 mmol/L were examined on 2 d with isoenergetic CRHP or CR diets comprising a breakfast and subsequent lunch meal.

Results: Peak PG was significantly reduced on the CRHP diet after breakfast and lunch by 11% and 31% compared with the CR diet. Nadir PG increased significantly on CRHP (by 13% and 9%). Insulin secretion was reduced, and glucagon secretion increased on the CRHP diet after both meals. Glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide secretion were lower after lunch but unaltered after breakfast on CRHP; β -cell function and insulin clearance were unchanged.

Conclusions: The CRHP diet lowered glucose excursions and reduced insulin secretion and incretin hormone responses, but enhanced glucagon responses compared with the CR diet. Taken together, the results may explain the decreased glucose variability and lower risk of postprandial hypoglycemia. This study was registered at clinicaltrials.gov as NCT02665715. *Am J Clin Nutr* 2019;110:296–304.

Keywords: low carbohydrate diet, postprandial hypoglycemia, Roux-en-Y gastric bypass, obesity, insulin secretion

Introduction

Roux-en-Y gastric bypass (RYGB) surgery induces a long-term sustainable weight loss of ~ 15 BMI units and is widely considered an effective treatment of obesity and its multiple comorbidities, including type 2 diabetes (and is hence sometimes referred to as metabolic surgery) (1). The operation potentially has a number of complications, one of which is postprandial hypoglycemia (2–4). Following the surgically changed gastrointestinal anatomy after RYGB, nutrient delivery to the small intestine and absorption are accelerated, resulting in an earlier and higher peak of blood glucose concentration after meal intake, but in the subsequent period (1.5–3 h after meal intake) the nadir blood glucose concentration is lower compared with before surgery (5). In some patients, overt postprandial hypoglycemia develops, which may be severe with neuroglycopenic symptoms,

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Supplemental Figure 1 is 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: carb, carbohydrates; CGM, continuous glucose monitoring; CR diet, conventional recommended diet; CRHP diet, carbohydrate-reduced, high-protein diet; E%, energy percent; GI, glycemic index; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; IGI, insulinogenic index; ISR, insulin secretion rate; PAL, physical activity level; PG, plasma glucose; REE, resting energy expenditure; RYGB, Roux-en-Y gastric bypass; TEE, total energy expenditure; β -GS, β -cell glucose sensitivity.

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cognitive impairment, and risk of loss of consciousness (2, 3). Symptomatic postprandial hypoglycemia is typically reported as a complication 1–5 y post-RYGB when maximal weight loss has been obtained (2, 6–8). It is also reported that after RYGB, many patients have frequent asymptomatic hypoglycemic episodes with reduced neurohumoral and glucagon responses to hypoglycemia (9–13). Up to 75% of patients with prior gastric bypass surgery may have asymptomatic hypoglycemia [<3.0 mmol/L (55 mg/dL)] as measured by continuous glucose monitoring (CGM) (4).

The precise pathophysiology of postprandial hypoglycemia after RYGB remains incompletely understood and is probably multifactorial (14,15). An inappropriate hypersecretion of insulin attributed to an exaggerated secretion of glucagon-like peptide 1 (GLP-1) in combination with an earlier and higher peak of plasma glucose, as well as a reduced insulin clearance, have been suggested to be involved (16–20). This overshoot of insulin can in some patients lead to a mismatch between the rate of glucose absorption from the intestine, insulin secretion and whole body glucose disposal, and insulin secretion resulting in postprandial hyperinsulinemic hypoglycemia (8, 16–18). Thus, postprandial hypoglycemia can be prevented by blocking the insulinotropic effect of GLP-1 and thereby suppressing insulin secretion (17, 18). Defective counter-regulation by adrenalin and glucagon may also be involved (12, 14).

Therapeutic options for postprandial hypoglycemia primarily include dietary changes with restriction of carbohydrate intake and reduction of high-glycemic-index carbohydrates in order to lower the postprandial glucose surges and insulin responses. The efficacy of such dietary changes has been demonstrated in some small-scale studies (21–24). The precise mechanism of action of a carbohydrate-restricted diet has never been investigated in patients with postprandial hypoglycemia after RYGB surgery and was the aim of the present study. Here, we related the postprandial glucose excursion to the responses of incretin hormones, insulin, glucagon and free fatty acids, and evaluated whether a CRHP diet could reduce the risk of postprandial hypoglycemia compared with a CR diet.

Methods

Participants

The study participants were 10 RYGB-operated patients (2 males, 8 females, mean \pm SD age 47 ± 7 y; BMI 31 ± 6 kg/m²; 6 ± 3 y post-RYGB) from Hvidovre Hospital (Copenhagen, Denmark). All patients were recruited from the outpatient clinic to which they had been referred because of classic hypoglycemic symptoms which were documented by glucose concentrations ≤ 3.4 mmol/L during screening (see **Supplemental Figure 1** for flow chart of enrollment procedure).

Six participants (2 males) were included based on the in-house screen test and 4 were included based on CGM data. At the in-house screen test, patients were served a liquid mixed-meal [Fresubin 300 kcal, carbohydrate 50%, protein (P) 15%, fat (F) 35%], which was consumed over 10 min followed by frequent blood sampling for 3 h. For CGM, a Medtronic iPro2 was inserted according to the manufacturer's directions and participants were instructed to measure capillary blood glucose at least 4 times/d for CGM calibration. Patients wore the CGM monitor for 5 consecutive days while maintaining everyday living. CGM data

were analyzed with iPro2 software. Patients were blinded to the CGM data.

Body composition was assessed by dual-energy X-ray absorptiometry (Discovery A, S/N 83487; Hologic Inc., with the Apex 2.3 software package) to allow calculation of individual resting energy expenditure (REE). Physical activity level (PAL) was set to be 1.4 for all participants during intervention days, and total energy expenditure (TEE) was calculated as REE \times PAL. All meals were prepared individually to match each participant's estimated TEE.

Written, informed consent was obtained from all patients. The study was approved by the Municipal Ethics Committee of Copenhagen; it was in accordance with the Helsinki-II Declaration and was registered at www.clinicaltrials.gov as NCT02665715.

Study design

Patients were studied in a randomized crossover design consisting of 2 test days, with a washout period of 2 full days up to 6 weeks between the 2 diets. Each test day consisted of 2 consecutive mixed-meal tests (semisolid breakfast and solid lunch) comparing 2 isoenergy diets, a CRHP and CR diet [30/55 energy percent (E%) carbohydrate, 30/15 E% protein, 40/30 E% fat, respectively]. All meals were weighed out individually to match each participant's estimated TEE, with 15% of TEE being allocated for breakfast and 20% for lunch. The ingredients used in the CRHP diet for breakfast were eggs, olive oil, rye bread, tomato, mozzarella (45 E% fat in dry matter cheese), ham, yogurt (0.2 E% fat, high protein), and for lunch were chicken, olive oil, tomato, feta cheese (40 E% fat in dry matter cheese), spring onions, rye bread, chickpeas, bell peppers, parsley, milk (0.5% F). The CR diet was in accordance with the recommended diet for gastric bypass patients. The ingredients used for the CR breakfast were strawberry jam, apples, almonds, wheat bread, yogurt (3.5 E% fat), egg, cheese (45 E% fat in dry matter cheese), and for lunch were tomato, spring onions, bell peppers, butter, chicken, pasta, pesto, wheat bread, milk (0.5 E% fat). Fiber content was higher in the CR diet, whereas added sugar content and glycemic index (GI) were comparable (with GI >55) in both diets. Vitamin D and calcium contents were higher in the CRHP diet. Patients were randomly allocated consecutively to start with either the CRHP or CR diet by each participant drawing a folded note out of a concealed envelope to avoid prediction of the last assignment. The randomization was performed by a third-party study nurse who was unrelated to the study. Six subjects were tested with CRHP and 4 subjects with CR diets on the first test day (Supplemental Figure 1). On the evening before each test day, a standardized dinner meal (25% of estimated TEE; macronutrient composition: carbohydrate 55 E%, protein 15 E%, fat 30 E%) was provided to all at home. The standardized meal consisted of smoked salmon, eggs, tomatoes, rye bread, potatoes, mayonnaise, roasted onions, chives, pears, and honey.

On each study day, patients arrived after a 10-h overnight fast. An intravenous cannula was inserted into an antecubital vein. Patients were placed in a reclined position on a bed throughout the test except during the actual ingestion, where they were seated in an upright position in the bed with a table in front of them. Blood was sampled 3 times at fasting and for 8 h postprandially,

at time points -10, -5, 0, 15, 30, 35, 40, 45, 50, 60, 75, 90, 105, 120, 150, 180, 240, 255, 270, 275, 280, 285, 290, 300, 315, 330, 345, 360, 390, 420, and 480 min. Breakfast was ingested at $t = 0-30$ min and lunch at $t = 240-270$ min. To estimate intestinal nutrient entry, 1 g of crushed paracetamol (Pamol; Nycomed) was added to the breakfast meal and ingested with yogurt at $t = 20-30$.

Analyses

Plasma glucose was analyzed immediately bedside with the use of the glucose oxidase technique (YSI model 2300 STAT Plus; Yellow State Instruments). Serum C-peptide was analyzed with the IMMULITE 2000 Immunoassay System (Siemens Healthcare). Plasma was acquired by collecting blood in prechilled EDTA tubes that were immediately centrifuged at 4°C for 10 min at 2000 g, and serum was acquired by collecting blood in clot activator tubes that were left for 30 min before centrifugation. Plasma was used for analysis of total GLP-1, total glucose-dependent insulinotropic polypeptide (GIP), and glucagon. For analysis of GLP-1, GIP, and glucagon, plasma samples were extracted with 70% ethanol (by volume, final concentration) to remove unspecific, cross-reacting substances. The plasma concentrations of GLP-1 were measured against standards of synthetic GLP-1 7-36amide through the use of antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore does not react with GLP-1-containing peptides from the pancreas. The results of the assay accurately reflect the rate of secretion of GLP-1 because the assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9-36amide, into which GLP-1 is rapidly converted. For both assays, the sensitivity was <1 pmol/L, the intra-assay coefficient of variation was <6% at 20 pmol/L, and the recovery of standard, added to plasma before extraction, was ~100% when corrected for losses inherent in the plasma extraction procedure. For the GIP radioimmunoassay, we used the C-terminally directed antiserum code no. 867, which was raised against a synthetic peptide corresponding to the C-terminus of human GIP. It does not cross-react with the so-called GIP 8000, whose chemical nature and relationship to GIP secretion is uncertain. It reacts fully with the primary metabolite, GIP 3-42. Human GIP and [¹²⁵I]-human GIP (70 MBq/nmol) were used as standards and tracer. Plasma glucagon concentrations were measured with the antibody employed (code no. 4305) directed against the C-terminus of the glucagon molecule and therefore mainly measured glucagon of pancreatic origin. Standards were human glucagon and the tracer was mono-iodinated human glucagon (NovoNordisk). The sensitivity and detection limit was <1 pmol/L, the intra-assay coefficient of variation was <6% at 20-30 pmol/L, and the recovery of standard, added to plasma before extraction, was ~100% when corrected for losses inherent in the plasma extraction procedure. The assay has been validated against sandwich ELISA and mass spectrometry.

Outcomes

The primary outcome was nadir plasma glucose. Secondary outcomes included: peak plasma glucose; time to nadir glucose;

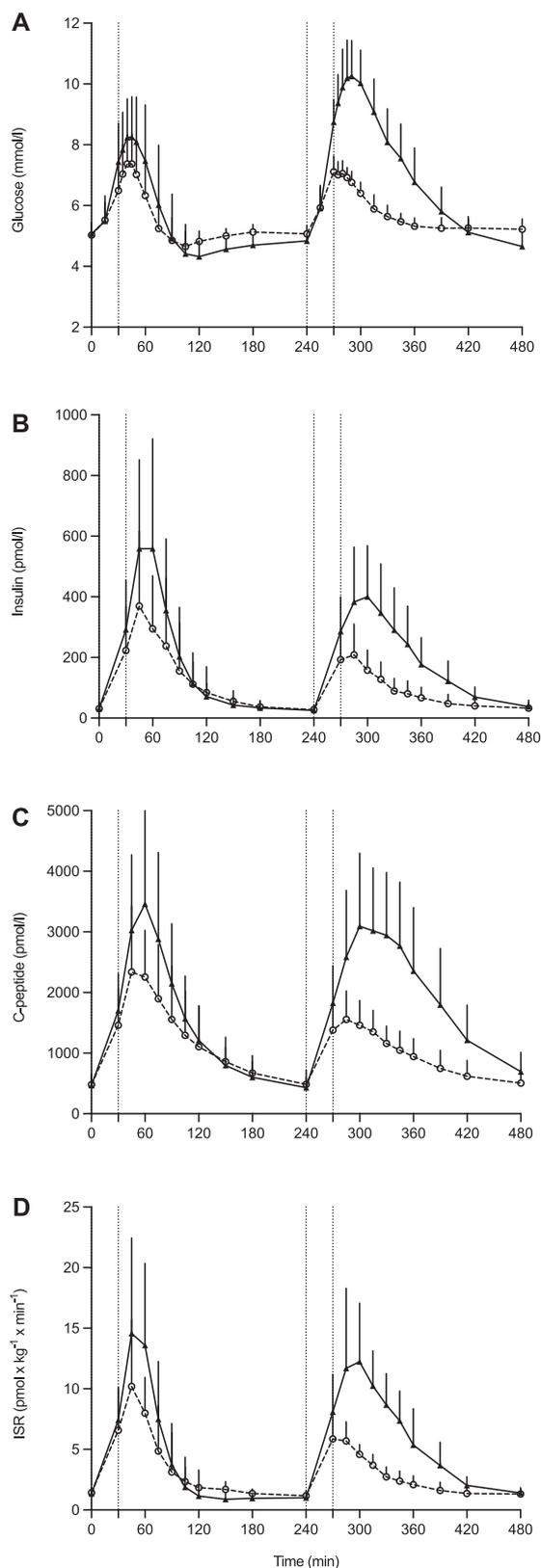


FIGURE 1 Concentrations of plasma glucose (A), serum insulin (B), and serum C-peptide (C), and insulin secretion rate (D) in response to a CRHP diet (dotted line, white circles) and a CR diet (solid line, black triangles). Values are mean \pm SD. CR, conventional recommended; CRHP, carbohydrate-reduced high-protein; ISR, insulin secretion rate.

TABLE 1 Responses to diets¹

	Breakfast			Lunch		
	CRHP diet	CR diet	<i>P</i> value	CRHP diet	CR diet	<i>P</i> value
Glucose						
Fasting, mmol/L	5.0 ± 0.4 ²	5.1 ± 0.4	0.5571	NA	NA	NA
tAUC, mmol/L × min	1289 ± 95	1291 ± 144	0.9410	1359 ± 53	1629 ± 120	4.23 × 10 ⁻⁵
iAUC, mmol/L × min	112 ± 29	155 ± 70	0.5251 ³	148 ± 48	480 ± 100	9.75 × 10 ⁻⁵³
Peak, mmol/L	7.6 ± 0.9	8.6 ± 1.3	0.0146 ³	7.3 ± 0.4	10.5 ± 1.6	2.75 × 10 ⁻⁵³
Nadir, mmol/L	4.5 ± 0.5	4.0 ± 0.7	0.0075	5.0 ± 0.2	4.6 ± 0.4	0.0039 ⁴
Insulin						
Fasting, pmol/L	31 ± 18	31 ± 19	1	NA	NA	NA
tAUC, nmol/L × min	29 ± 16	39 ± 19	0.0079	20 ± 9	44 ± 17	1.91 × 10 ⁻⁴
iAUC, nmol/L × min	22 ± 13	32 ± 17	0.0428 ³	14 ± 7	38 ± 15	0.0026 ³
Peak, pmol/L	432 ± 268	626 ± 348	0.0172	222 ± 101	466 ± 167	0.0012
C-peptide						
Fasting, pmol/L	479 ± 159	470 ± 185	0.7195	NA	NA	NA
tAUC, nmol/L × min	276 ± 95	333 ± 116	0.0052	224 ± 66	446 ± 162	0.0020 ⁴
iAUC, nmol/L × min	161 ± 68	221 ± 86	0.0006	108 ± 31	342 ± 134	0.0020 ⁴
Peak, pmol/L	2653 ± 1128	3646 ± 1622	0.0144	1617 ± 432	3346 ± 1106	0.0020 ⁴
Insulin secretion rate						
Fasting, pmol · kg ⁻¹ · min ⁻¹	1.4 ± 0.4	1.3 ± 0.4	0.4453 ⁴	NA	NA	NA
tAUC, pmol/L · kg ⁻¹	787 ± 225	934 ± 263	0.0087	633 ± 120	1315 ± 339	2.17 × 10 ⁻⁵
iAUC, pmol/L · kg ⁻¹	475 ± 187	671 ± 256	0.0039	358 ± 98	1071 ± 314	4.61 × 10 ⁻⁵
Peak, pmol · kg ⁻¹ · min ⁻¹	10.9 ± 5.4	15.7 ± 7.9	0.0082	6.3 ± 1.7	13.8 ± 6.1	0.0024

¹*n* = 10. *P* values were assessed with Student's paired *t* test if not otherwise stated. CR diet, conventional recommended diet; CRHP diet, carbohydrate-reduced, high-protein diet; iAUC, incremental AUC; NA, not available; tAUC, total AUC.

²Mean ± SD (all such values).

³Bonferroni corrected *P* value, for secondary outcomes.

⁴*P* value was calculated with the Wilcoxon signed-rank test.

time to peak glucose; incremental glucose area; decremental glucose area; incremental insulin area; incremental GLP-1 area; time below baseline glucose concentrations; and glycemic excursions. All other analyses were exploratory.

Statistics and calculations

To test the effect of randomization sequence on the primary outcome, a 2-way ANOVA was performed with sequence as fixed effect and treatment as repeated measures.

Time to peak and time to nadir were calculated as the mean of the observations. The total area under the curve (tAUC) was calculated through the use of the trapezoid rule, the incremental AUC (iAUC) was calculated as the area above the fasting values, and the decremental AUC (dAUC) was calculated as the area below the fasting values. All results are expressed as mean ± SD. For all results, *P* values <0.05 were considered significant. Comparisons between secondary outcomes were adjusted with the use of the Bonferroni corrections, taking multiplicity into account. For exploratory outcomes, no adjustments of *P* values for multiplicity were performed. Differences between AUCs, peaks, etc., were assessed with Student's paired *t* tests when normal distribution was achieved with the use of the Shapiro-Wilk test. When normality was not achieved, significance was assessed through the use of the Wilcoxon signed-rank test on the difference scores as specified in the tables. Statistical analyses were performed with GraphPad Prism version 7.0d (GraphPad Software).

Insulin secretion rates (ISRs) were calculated for each patient by deconvolution of C-peptide concentrations, utilizing a 2-compartment model of C-peptide kinetics and population-based C-peptide parameters (25) as employed in the ISEC software program (26). ISRs are expressed in pmol · kg⁻¹ · min⁻¹.

β -cell function was evaluated by calculating several meal-related indices: 1) the incremental iAUC(ISR)/iAUC(PG), a β -cell index including both the early and late insulin responses to meals; 2) early insulin secretory responses during breakfast and lunch were normalized to the glycemic stimulus by calculating the insulinogenic index IGI = Δ ISR/ Δ PG, with Δ PG from fasting to peak PG, and Δ ISR from fasting to ISR at time for peak PG; and lastly, 3) β -cell glucose sensitivity (β -GS), which expresses the efficacy with which changes in plasma glucose concentrations stimulate insulin secretion. For this, the relationship between plasma glucose concentrations and ISR was evaluated by cross-correlation analysis, and β -GS calculated as the slope of the regression line, indicating the change in insulin secretion per unit change in glucose concentration. β -GS was calculated separately during each meal both for increasing (upslope) glucose and declining (downslope) glucose concentrations. Insulin clearance during the meals was calculated as iAUC(insulin)/iAUC(ISR).

Results

Glucose, insulin, and C-peptide

No differences in fasting glucose, insulin, or C-peptide were found on the 2 days.

Glucose

The tAUC of glucose and the iAUC were similar after breakfast on the 2 days ($P = 0.9410$ and $P = 0.5251$ respectively). At lunch, the CRHP diet reduced glucose tAUC by 17% ($P = 4.23 \times 10^{-5}$) and glucose iAUC by 69% ($P = 9.75 \times 10^{-5}$) compared with CR diet (Figure 1A; Table 1). Peak plasma glucose was reduced on the CRHP diet by 11% ($P = 0.0146$) after breakfast and by 31% ($P = 2.75 \times 10^{-5}$) after lunch. The CRHP diet raised the nadir plasma glucose by 13% ($P = 0.0075$) after breakfast and by 9% ($P = 0.0039$) after lunch. The randomization sequence had no effect on the primary outcome (nadir plasma glucose) at breakfast ($P = 0.9956$) or at lunch ($P = 0.4147$). During breakfast, glucose peak concentrations were reached at 45 ± 8 min during the CRHP diet and 46 ± 9 min during the CR diet ($P = 1$) and at 275 ± 10 and 290 ± 10 min ($P = 0.0011$), respectively, after lunch. After breakfast glucose nadirs were observed at 98 ± 18 min during the CRHP diet and at 120 ± 23 min ($P = 0.0418$) on the CR day and after 428 ± 61 and 462 ± 29 min ($P = 1$), respectively, after lunch. No hypoglycemic episodes (plasma glucose < 3.4 mmol/L) were seen after the CRHP diet, whereas 2 individuals experienced plasma glucose levels < 2.9 mmol/L and 3 individuals between 3.4 and 3.9 mmol/L following breakfast on the CR diet, and one single individual experienced plasma glucose < 3.9 mmol/L after lunch (no episodes < 3.4 mmol/L).

Insulin

During breakfast, the CRHP diet reduced insulin tAUC by 26% ($P = 0.0079$) and iAUC by 32% ($P = 0.0428$) compared with the CR diet (Figure 1B; Table 1). The corresponding values during lunch were reductions in the tAUC by 53% ($P = 1.91 \times 10^{-4}$) and the iAUC by 63% ($P = 0.0026$). The CRHP diet reduced peak serum insulin concentration by 31% ($P = 0.0172$) and 52% ($P = 0.0012$) after ingestion of breakfast and lunch, respectively (Figure 1B; Table 1).

C-peptide

At breakfast, the CRHP diet reduced C-peptide tAUC by 17% ($P = 0.0052$) and iAUC by 27% ($P = 0.0006$), compared with the CR diet and at lunch by 50% (tAUC, $P = 0.0020$) and 68% (iAUC, $P = 0.0020$). The CRHP diet reduced peak serum C-peptide concentration by 27% ($P = 0.0144$) and 52%

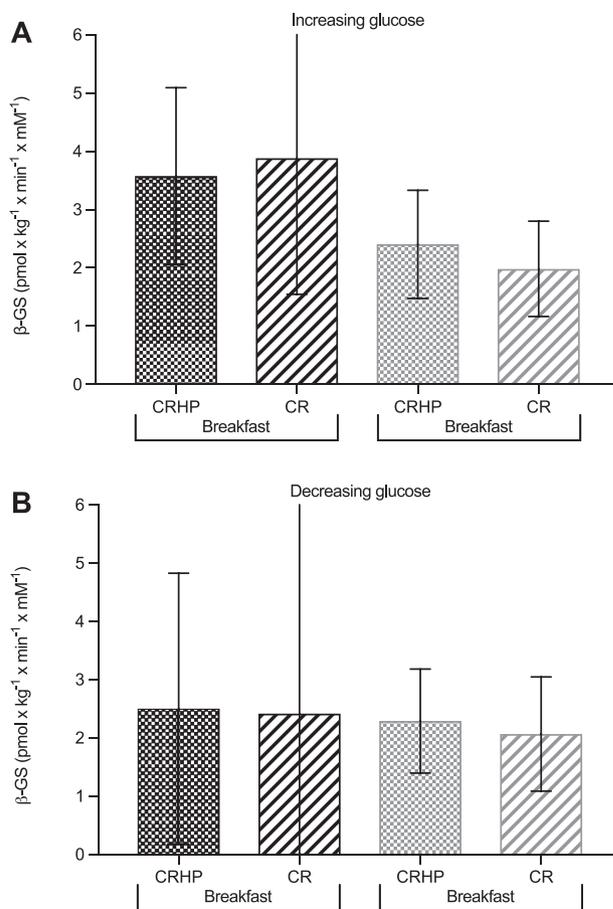


FIGURE 2 β -cell glucose sensitivity: increasing glucose (A); decreasing glucose (B). Squared boxes show CRHP diet breakfast (black) and lunch (gray). Crossed boxes show CR diet breakfast (black) and lunch (gray). Values are mean \pm SD. CR, conventional recommended; CRHP, carbohydrate-reduced high-protein; β -GS, β -cell glucose sensitivity.

($P = 0.0020$) after breakfast and lunch, compared with the CR diet.

ISRs

During breakfast, the CRHP diet reduced the ISR tAUC by 16% ($P < 0.0087$) and iAUC by 53% ($P = 0.0039$), compared

TABLE 2 β -cell function in relation to changes in glucose¹

	Breakfast			Lunch		
	CRHP diet	CR diet	<i>P</i> value	CRHP diet	CR diet	<i>P</i> value
iAUC(ISR)/iAUC(PG)	4.3 \pm 1.4 ²	4.9 \pm 2.1	0.5002	2.6 \pm 0.8	2.3 \pm 0.7	0.4549
IGI	3.6 \pm 1.5	4.1 \pm 2.6	0.3454	2.4 \pm 0.9	2.0 \pm 0.8	0.1431
β -cell sensitivity upslope	3.6 \pm 1.5	3.9 \pm 2.3	1 ³	2.4 \pm 0.9	2.0 \pm 0.8	0.0979
β -cell sensitivity downslope	2.5 \pm 2.3	2.4 \pm 3.6	0.9170	2.3 \pm 0.9	2.1 \pm 1.0	0.4078
Insulin clearance	44 \pm 16	47 \pm 17	0.1807	39 \pm 16	35 \pm 11	0.2370

¹ $n = 10$. *P* values were assessed with Student's paired *t* test if not otherwise stated. CR diet, conventional recommended diet; CRHP diet, carbohydrate-reduced, high-protein diet; iAUC, incremental AUC; IGI, insulinogenic index; ISR, insulin secretion rate; PG, plasma glucose.

²Mean \pm SD (all such values).

³*P* value was calculated with the Wilcoxon signed-rank test.

with CR diet, and during lunch by 52% (tAUC, $P = 2.17 \times 10^{-5}$) and 67% (iAUC, $P = 4.61 \times 10^{-5}$). The CRHP diet reduced the peak ISR by 30% ($P = 0.0082$) and 54% ($P = 0.0024$) after ingestion of breakfast and lunch compared with the CR diet (Figure 1D; Table 1). The peak insulin secretion on the CRHP and CR diets was reached 50 ± 12 and 54 ± 10 min after ingestion of breakfast ($P = 0.50$) and at 284 ± 18 and at 300 ± 20 min ($P = 0.0078$), respectively, during lunch. Furthermore, the CRHP diet reduced insulin secretion in relation to glucose concentrations throughout the entire 480-min postprandial period (tAUC ISR₄₈₀/tAUC glucose₄₈₀) by 30% (0.53 ± 0.10 compared with 0.77 ± 0.16 pmol · kg⁻¹ · mmol/L · min⁻¹, $P < 0.0001$), compared with the CR diet.

β-cell function in relation to changes in glucose

iAUC(ISR)/iAUC(PG) did not differ between the 2 diets at breakfast ($P = 0.5002$) or lunch ($P = 0.4549$). In addition, the IGI and β -GS for the upslope and downslope did not differ between the 2 diets, either at breakfast or at lunch (Figure 2; Table 2).

Insulin clearance

Insulin clearance was similar on the CRHP and CR diets during both breakfast ($P = 0.1807$) and lunch ($P = 0.2370$) (Table 2).

Gut hormones and glucagon

Fasting concentrations of incretin hormones and glucagon were similar on the 2 experimental days.

Total GLP-1

The GLP-1 tAUC and peak did not differ between the 2 diets after the breakfast test ($P = 0.3957$ and $P = 0.3113$, respectively), but GLP-1 tAUC was reduced on the CRHP diet after lunch by 18% ($P = 0.0339$) compared with the CR diet (Figure 3A; Table 3).

Total GIP

At breakfast, the tAUCs and peaks of GIP were similar ($P = 0.5566$ and $P = 0.4258$, respectively). During lunch, the GIP tAUC and peak GIP were reduced on the CRHP diet by 25% ($P = 3.09 \times 10^{-4}$) and 33% ($P = 6.72 \times 10^{-5}$), respectively (Figure 3B; Table 3).

Glucagon

Total glucagon tAUC was increased on the CRHP diet after ingestion of both breakfast (33%, $P = 0.0246$) and lunch (52%, $P = 0.0059$) compared with the CR diet (Table 3). Indeed, although glucagon secretion was slightly stimulated during CRHP, a suppression was observed on the CR diet. The difference in glucagon responses on the 2 diets was most marked during lunch (Figure 3C; Table 3).

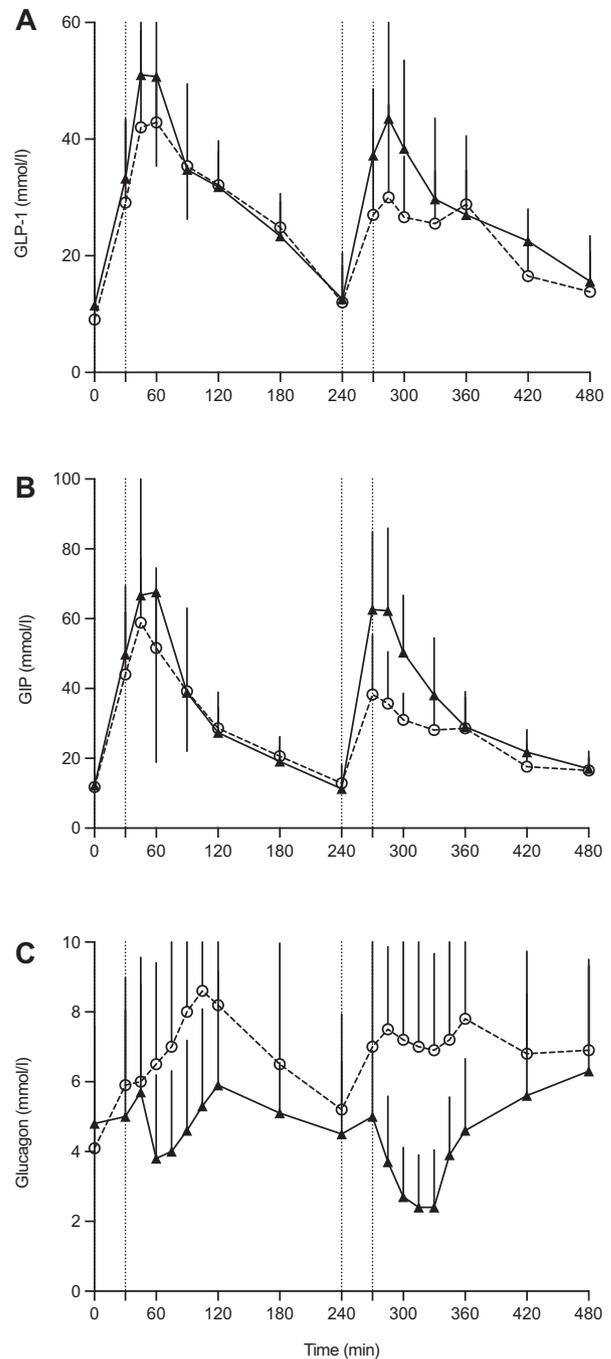


FIGURE 3 Plasmatotal GLP-1 (A), plasma total GIP (B), and plasma glucagon (C) concentrations in response to CRHP diet (dotted line, white circles) and a CR diet (solid line, black triangles). Values are mean \pm SD. CR, conventional recommended; CRHP, carbohydrate-reduced high-protein; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1.

Fatty acids and cortisol

Fatty acids

Suppression of plasma fatty acids did not differ between the 2 diets when adjusted for fasting values. Nadir plasma concentrations of FAs were similar between the diets (Figure 4A; Table 4).

TABLE 3 Incretins and glucagon responses to diets¹

	Breakfast			Lunch		
	CRHP diet	CR diet	<i>P</i> value	CRHP diet	CR diet	<i>P</i> value
GLP-1						
Fasting, pmol/L	9 ± 5 ²	12 ± 5	0.2406	NA	NA	NA
tAUC, pmol/L · min	5640 ± 1703	6005 ± 1161	0.3957	5301 ± 1436	6467 ± 1777	0.0339
Peak, pmol/L	48 ± 16	54 ± 14	0.3113	38 ± 14	46 ± 17	0.1531
GIP						
Fasting, pmol/L	12 ± 3	12 ± 4	0.5806	NA	NA	NA
tAUC, pmol/L · min	7298 ± 2222	7712 ± 2837	0.5566 ³	5969 ± 1618	7913 ± 2257	3.09 × 10 ⁻⁴
Peak, pmol/L	62 ± 21	75 ± 45	0.4258 ³	45 ± 17	67 ± 23	6.72 × 10 ⁻⁵
Glucagon						
Fasting, pmol/L	4 ± 2	5 ± 3	0.2091	NA	NA	NA
tAUC, pmol/L · min	1589 ± 649	1198 ± 520	0.0246	1680 ± 590	1104 ± 454	0.0059 ³

¹*n* = 10. *P* values were assessed with Student's paired *t*-test if not otherwise stated. CR diet, conventional recommended diet; CRHP diet, carbohydrate-reduced, high-protein diet; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; NA, not available; tAUC, total AUC.

²Mean ± SD (all such values).

³*P* value was calculated with the Wilcoxon signed-rank test.

Cortisol

Secretion of cortisol was largely similar between the 2 diets (Figure 4B; Table 4).

Gastric emptying rate

The time to peak of plasma concentrations of paracetamol did not differ between the 2 diets (CRHP: 41 ± 13 min; CR: 36 ± 5 min; *P* = 0.225).

Discussion

In the present study we examined the acute changes in glucose metabolism following a reduction in dietary carbohydrate energy content from 55% to 30% with no difference in GI, while fat and protein contents were isoenergetically increased. Glucose fluctuations were reduced during CRHP with 11% and 31% lower glucose peak concentrations, and 13% and 10% higher nadir concentrations after breakfast and lunch, respectively. The amount of insulin secreted was also less on the CRHP diet. The secretion of GLP-1 and GIP were significantly lower after lunch but insignificantly reduced after breakfast on the CRHP diet. The glucagon responses were substantially higher on the CRHP diet than on the CR diet. Notably, indices of β -cell function did not differ between the 2 diets.

Gastric bypass surgery results in an accelerated delivery of nutrients into the distal small intestine and accelerated entry into the systemic circulation, inducing an excessive increase in blood glucose and postprandial GLP-1 as well as in insulin secretion (16–20). Against this pathophysiologic background, treatment of postprandial hypoglycemia should include a reduction in intake of carbohydrates, especially high-GI carbohydrates. The efficacy of a carbohydrate-reduced diet to treat mild postprandial hypoglycemia has been demonstrated in various studies (21–24). A low-carbohydrate and low-GI diet is also a treatment for dumping syndrome, i.e., abdominal discomfort experienced early after intake in some patients.

The present study is the first to compare the acute effects of a 30 E% carbohydrate-reduced, high-protein diet with a conventionally recommended diet of 55 E% carbohydrate and 15 E% protein. The major determinant of the postprandial

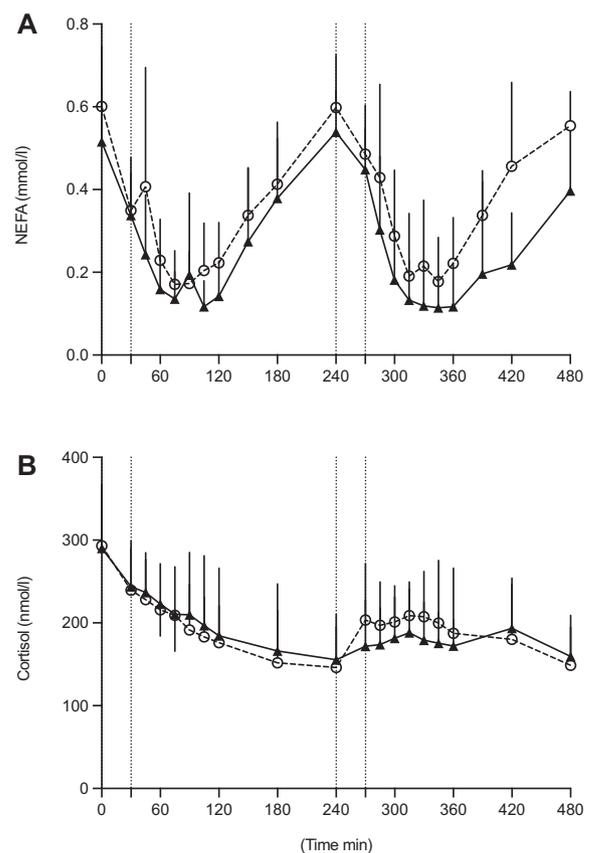


FIGURE 4 Concentrations of nonesterified fatty acids (A) and cortisol (B) in response to a CRHP diet (dotted line, white circles) and a CR diet (solid line, black triangles). Values are mean ± SD. CR, conventional recommended; CRHP, carbohydrate-reduced high-protein; NEFA, nonesterified fatty acids.

TABLE 4 Fatty acids and cortisol responses to diets¹

	Breakfast			Lunch		
	CRHP diet	CR diet	<i>P</i> value	CRHP diet	CR diet	<i>P</i> value
Fatty acids						
Fasting, mmol/L	0.60 ± 0.15 ^{2,3}	0.52 ± 0.13	0.1367	NA	NA	NA
tAUC, mmol/L · min	86 ± 18	73 ± 24	0.0334	92 ± 22	61 ± 26	0.0039 ³
dAUC, mmol/L · min	61 ± 31	53 ± 18	0.4287	55 ± 22	69 ± 27	0.2715
Cortisol						
Fasting, nmol/L	293 ± 72	290 ± 77	0.9090	NA	NA	NA
tAUC, μmol/L · min	45.3 ± 11.7	47.5 ± 12.6	0.4774	44.3 ± 11.1	42.5 ± 6.7	0.6019
Peak, nmol/L	293 ± 72	313 ± 79	0.4082	265 ± 63	238 ± 45	0.0929

¹*n* = 10. *P* values were assessed with Student's paired *t* test if not otherwise stated. CR diet, conventional recommended diet; CRHP diet, carbohydrate-reduced, high-protein diet; dAUC, decremental AUC; NA, not available; tAUC, total AUC.

²Mean ± SD (all such values).

³*P* value was calculated with the Wilcoxon signed-rank test.

glucose excursion is the amount of carbohydrate in the meal, the GI and the gastric emptying rate (27, 28). Glucose is, compared with protein and fat, a strong stimulator of insulin secretion and, compared with fat and protein, is also the strongest stimulator of GLP-1 secretion from the intestinal L-cells after RYGB surgery (29). GLP-1 and GIP in nonoperated subjects are responsible for up to two-thirds of the meal-induced insulin secretion. After gastric bypass surgery, the exaggerated GLP-1 response during a meal is a very strong stimulator of insulin secretion (17, 30). Accordingly, it has been reported that patients with symptomatic hypoglycemia have greater postprandial GLP-1 and insulin responses than patients without hypoglycemia after RYGB (31), which could be corrected by administration of a GLP-1 receptor agonist (16, 18).

The present study reveals that the CRHP diet resulted in a significant reduction in insulin secretion at breakfast and lunch, probably primarily explained by a combination of the lower carbohydrate intake, which resulted in smaller glucose excursions, and an attenuated response of the incretin hormones compared with a conventional diet. GLP-1 as well as GIP responses after breakfast did not differ significantly between the CRHP and CR diet, whereas the responses were significantly reduced after lunch with the CRHP diet. The lower GLP-1 and GIP responses might have influenced the insulin secretion during lunch. Notably, the β -cell function evaluated by several indices of early as well as late insulin secretion to changes in glucose did not differ between the 2 diets.

Differences in nutrient entry into the small intestine do not seem to be able to explain the effects, because the time to peak concentration of paracetamol and the time to peak plasma glucose and insulin secretion did not differ at breakfast, whereas peak glucose and insulin concentrations occurred earlier after lunch on the CRHP diet. Other suggested explanations of postprandial hypoglycemia after gastric bypass have included reduced hepatic insulin extraction resulting in peripheral hyperinsulinemia and a defective response of counter-regulatory hormones (32). In the present acute study, insulin clearance did not differ between the 2 diets. The glucagon responses to the 2 diets were very different, with the highest glucagon responses occurring with the CRHP diet. Glucagon is an important counter-regulatory hormone in defense against hypoglycemia (33–35). It is also known that

amino acids are powerful stimulants of glucagon secretion (36), and therefore the high protein content can explain the increased secretion of glucagon on the CRHP diet. The increased glucagon secretion could in part account for the reduction in risk of hypoglycemia after meal ingestion on the CRHP diet compared with the CR diet. Cortisol responses did not differ between the diets.

Two individuals experienced hypoglycemia of <2.9 mmol/L and 5 participants had a plasma glucose <3.9 mmol/L at breakfast on the CR diet. One single subject had a plasma glucose <3.9 mmol/L at lunch during the CR diet. It should be noted that breakfast was semisolid whereas lunch was solid, which might explain some of the differences in the risk of hypoglycemia during breakfast and lunch. Peak plasma glucose concentration was higher after lunch than after breakfast on the CR diet and was substantially lower after lunch and similar to after breakfast on the CRHP diet. These findings may be explained by the “second meal effect”, i.e., a meal with low GI can lower the glycemic response of the second meal (37).

In conclusion, the present study demonstrates that a carbohydrate-reduced high-protein diet lowers glucose excursion and reduces insulin secretion and incretin hormone responses but enhances glucagon responses compared with a conventionally recommended diet. Taken together, these results explain the observations of decreased glucose variability and lower risk of postprandial hypoglycemia.

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