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ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 502 – 511
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Effects of meal size and composition on incretin, α-cell, and β-cell responses

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Received 26 December 2008; accepted 28 July 2009

Abstract

The incretins glucagon-like peptide–1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) regulate postprandial insulin release from the β-cells. We investigated the effects of 3 standardized meals with different caloric and nutritional content in terms of postprandial glucose, insulin, glucagon, and incretin responses. In a randomized crossover study, 18 subjects with type 2 diabetes mellitus and 6 healthy volunteers underwent three 4-hour meal tolerance tests (small carbohydrate [CH]-rich meal, large CH-rich meal, and fat-rich meal). Non–model-based and model-based estimates of β-cell function and incremental areas under the curve of glucose, insulin, C-peptide, glucagon, GLP-1, and GIP were calculated. Mixed models and Friedman tests were used to test for differences in meal responses. The large CH-rich meal and fat-rich meal resulted in a slightly larger insulin response as compared with the small CH-rich meal and led to a slightly shorter period of hyperglycemia, but only in healthy subjects. Model-based insulin secretion estimates did not show pronounced differences between meals. Both in healthy individuals and in those with diabetes, more CH resulted in higher GLP-1 release. In contrast with the other meals, GIP release was still rising 2 hours after the fat-rich meal. The initial glucagon response was stimulated by the large CH-rich meal, whereas the fat-rich meal induced a late glucagon response. Fat preferentially stimulates GIP secretion, whereas CH stimulates GLP-1 secretion. Differences in meal size and composition led to differences in insulin and incretin responses but not to differences in postprandial glucose levels of the well-controlled patients with diabetes.

1. Introduction

The incretins glucagon-like peptide–1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are hormones released from endocrine cells in the intestinal mucosa after a meal to regulate postprandial insulin release from the pancreatic β-cell. Because incretin function is known to be reduced or absent in patients with diabetes [1], whereas the insulinotropic effect of GLP-1 is preserved, there is substantial interest in dipeptidyl peptidase-4 (DPP-IV) inhibitors and GLP-1 analogs for controlling postprandial blood glucose levels in type 2 diabetes mellitus. Augmentation of the secretion of endogenous GLP-1 could be another approach; the effect of the composition of a meal on incretin levels and subsequent insulin, glucagon, and glucose concentrations is therefore of interest.

A mixed-meal test offers the potential for assessing insulin secretion during physiologically relevant situations and for evaluating the physiologic effects of incretins. The few studies that have examined incretin responses after one or more meals are not consistent about the contributions of meal composition and size in stimulating incretin and/or insulin secretion in patients with diabetes. A recent study showed that, in both healthy subjects and type 2 diabetes

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0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.07.039

mellitus patients, GIP responses were higher after mixed-meal ingestion than after an oral glucose load, but GLP-1 levels were similar during both tests [2]. In a study with 5 healthy volunteers, the time course of plasma GIP concentrations paralleled the gastric emptying of fat and protein [3]. On the other hand, it has been reported that ingestion of fat before a carbohydrate (CH) meal attenuated the postprandial rises in GIP but stimulated GLP-1 in type 2 diabetes mellitus [4]. In a study comparing 50-g vs 100-g glucose loads in healthy individuals, GLP-1 and GIP were dose-dependently increased [5]. As for meal size, one study has demonstrated higher incretin and C-peptide responses after a large as compared with a small meal [6]. β-Cell glucose sensitivity, an estimate of β-cell function, tended to increase in obese individuals during a large meal, perhaps reflecting increased incretin response. However, the meal composition of the large and small meal was similar; therefore, the influence of the macronutrients could not be derived.

It is unknown how meals with different caloric content and CH/fat load compare with one another in subjects with and without type 2 diabetes mellitus. In particular, the degree to which size of a meal (caloric load) and varying nutrient composition may influence the incretin, insulin, and glucagon responses has not been well elucidated. Furthermore, little is known about postprandial glucagon responses, which might also be influenced by meal composition.

Our objective was to investigate the effects of 3 standardized meals with different caloric and nutritional content given in randomized order to subjects with and without type 2 diabetes mellitus in terms of glucose, insulin, glucagon, and incretin postprandial response and to evaluate the potential roles of those meals for enhancing incretin function in patients with diabetes and healthy individuals.

2. Subjects and methods

2.1. Study procedure

The study had a randomized, 3-period crossover design and included both subjects with type 2 diabetes mellitus (n = 18) and healthy volunteers (n = 6). Subjects were randomized to 1 of 6 sequences (*abc*, *acb*, *bac*, *bca*, *cab*, or *cba*) of 3 meals to be administered as meal tolerance test. The 3 test meals included (*a*) a small (low-calorie) CH-rich meal, (*b*) a large (high-calorie) CH-rich meal, and (*c*) a (high-calorie) fat-rich meal. Meal compositions are shown in Table 1. Participants were instructed to be consistent in their diet the day preceding each test and to avoid strenuous exercise the day before each test. The study was approved by the ethical committee of the VU University Medical Center, and all subjects signed informed consent.

On the days of the visit to the research center, the subjects arrived in fasting state (no food or drink except water for at least 12 hours); patients with diabetes did not take any of their diabetes medication that morning. If subjects used statins, they were instructed not to take the statin from the night before each meal test. Subjects needed to consume the entire meal within 10 minutes. Blood was obtained before and 10, 20, 30, 60, 90, 120, 180, and 240 minutes after meal ingestion for determination of plasma glucose, insulin, C-peptide, and glucagon. Because of financial restrictions, GLP-1 and GIP were assessed on less time points: before and 30, 60, and 120 minutes after meal ingestion. Physical (weight, height, and waist and hip circumference) and blood pressure (Collin Press-mate BP-8800; Colin, Komaki City, Japan) measurements were completed during the first test.

2.2. Inclusion and exclusion criteria

Inclusion criteria were age between 50 and 70 years, and body mass index (BMI) between 25 and 38 kg/m^2 for patients with diabetes and between 23 and 38 kg/m^2 for healthy subjects. Eighteen persons diagnosed with type 2 diabetes mellitus who had been on a glucose-lowering agent for at least 3 months were included. In addition, 6 healthy volunteers with fasting plasma glucose less than 5.5 mmol/L were included. Subjects on insulin, thiazolidinediones, and/or glucocorticoids as well as subjects with end-stage renal disease and subjects with hypersensitivity or dietary restrictions to the contents of the nutrition drink or bars were excluded.

Table 1
Composition of the meals

	Small CH-rich meal	Large CH-rich meal	Fat-rich meal
Approximate total nutrient content (kcal)	460	680	833
CH	75 g (66.2 En%)	109 g (65.5 En%)	74.8 g (36.4 En%)
Fat	9 g (17.9 En%)	14 g (18.9 En%)	48.9 g (51.8 En%)
Protein	18 g (15.89 En%)	26 g (15.62 En%)	24.2 g (11.8 En%)
Content	1 Nutrition bar	2 Nutrition bars	96 g croissant
	1 Nutrition drink/Boost	1 Nutrition drink/	10 g butter
	(Nestlé HealthCare Nutrition, Minnetonka, MN)	Boost	40 g cheese
			150 g fatty milk
			100 g yogurt drink (flavor red fruits)
			10 g Fantomalt powder
			(Nutricia, Zoetermeer, The Netherlands)
			(added to yogurt)

2.3. Laboratory analysis

Serum total cholesterol and high-density lipoprotein cholesterol were measured by enzymatic colorimetric assays (Roche, Mannheim, Germany). Low-density lipoprotein cholesterol was calculated according to the Friedewald formula [7] except when fasting triglyceride levels exceeded 5.0 mmol/L. Plasma glucose levels were determined with a glucose hexokinase method (Gluco-quant, Roche Diagnostics); C-peptide and insulin, with immunometric assays (ACS Centaur; Bayer Diagnostics, Mijdrecht, the Netherlands). Glucagon was determined in EDTA samples with added aprotinin with a radioimmunoassay (Linco Research, St Charles, MO). Total GIP and GLP-1 concentrations in plasma were measured after extraction of plasma with 70% ethanol (vol/vol, final concentration). For the GIP radioimmunoassay [8], the C terminally directed antiserum R 65 was used, which cross-reacts fully with human GIP but not with the so-called GIP 8000, whose chemical nature and relationship to GIP secretion are uncertain. It reacts fully with the primary metabolite, GIP 3-42. Human GIP and 125-I human GIP (70 MBq/nmol) were used for standards and tracer. The plasma concentrations of GLP-1 were measured [9] against standards of synthetic GLP-1 7-36amide using antiserum code no. 89390, which is specific for the amidated C-terminus of GLP-1 and therefore mainly reacts with GLP-1 of intestinal origin.

2.4. Non–model-based β-cell function parameters

The insulinogenic index (as an estimate of early insulin secretion) was calculated by dividing the increment in insulin during the first 30 minutes by the increment in glucose over the same period ($\Delta I_{30}/\Delta G_{30}$) [10]. Negative or infinite insulinogenic index results (n = 4) were excluded from the analyses. Area under the curve (AUC) of insulin and glucose was calculated by the trapezoid rule [11]. Overall glucose-stimulated insulin secretion was calculated as $AUC_{insulin}/AUC_{glucose}$ ratio.

2.5. Model-based β-cell function parameters

Model-based β-cell function parameters were estimated using a mathematical model developed by Mari et al [12,13], with insulin secretion rate calculated by means of deconvolution of C-peptide levels [14]. In summary, glucose-mediated insulin secretion ($S[t]$) in this mathematical model is the sum of 2 components: $S(t) = P(t)f(G) + S_d(t)$. $P(t)f(G)$ represents the product of the dose-response relation between insulin secretion and glucose concentration ($f[G]$) and the potentiation factor ($P[t]$), which modulates this dose-response relation. The slope of the dose-response is denoted as β-cell glucose sensitivity. Potentiation may be induced by several factors, for example, glucose potentiation (higher insulin secretion due to repeated glucose stimulation), incretin potentiation, or neural modulation [12,15]. The potentiation factor ratio is the ratio between potentiation at the end of the test (220-240 minutes) and the initial value (0-20 minutes). $S_d(t)$ represents the enhancement of insulin secretion proportional to the rate of change of the plasma glucose concentration and is denoted as rate sensitivity.

2.6. Statistical analysis

Descriptive statistics are presented as means ± SD or median and interquartile range as appropriate. A mixed model with subject as a random factor and with meal and period as fixed factors was used to test for differences in β-cell function parameters between meals. A test for the interaction of meal-by-period was performed to assess potential carryover effects. Because the interaction between glucose status (diabetes vs healthy) and meal was significant ($P < .05$), the analyses were performed separately for both groups. Natural log-transformed variables were used in case of nonnormal distributions. If a significant difference was found, pairwise comparisons with Bonferroni correction for multiple testing were performed to determine differences between meals. Fasting incretin concentrations; incretin concentrations at $t = 30$; and incremental AUC (ΔAUC) of glucose, insulin, C-peptide, glucagon, GIP, and GLP-1 were tested nonparametrically with a Friedman test because a natural log-transformation did not improve their distributions. If an overall significant difference was present, a Sign test was used to determine pairwise differences between meals. Pearson's r was used to determine correlations. P values < .05 were considered statistically significant.

3. Results

3.1. Study population

Characteristics of the study population are shown in Table 2. All patients with diabetes were treated with oral glucose-lowering medication: 7 patients used metformin,

Table 2
Characteristics of the population

	Healthy subjects (n = 6)	Diabetes patients (n = 18)
Age (y)	61.4 (4.5)	63.3 (3.8)
Height (cm)	170.7 (9.2)	169.7 (9.3)
BMI (kg/m^2)	26.9 (1.8)	28.9 (2.1)
Waist circumference (cm)	90.3 (8.7)	100.5 (5.3)
Hip circumference (cm)	102.3 (3.8)	103.9 (5.9)
Waist-hip ratio	0.88 (0.08)	0.97 (0.05)
Systolic blood pressure (mm Hg)	138.2 (11.8)	146.3 (17.5)
Diastolic blood pressure (mm Hg)	80.3 (5.0)	82.4 (7.8)
Total cholesterol (mmol/L)	5.9 (0.6)	5.1 (1.0)
HDL cholesterol (mmol/L)	1.55 (0.35)	1.19 (0.24)
LDL cholesterol (mmol/L)	3.6 (0.7)	3.0 (0.9)
HbA_{1c} (%)	5.5 (0.1)	6.8 (0.8)

Means (SD). HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; HbA_{1c}, hemoglobin A_{1c}.

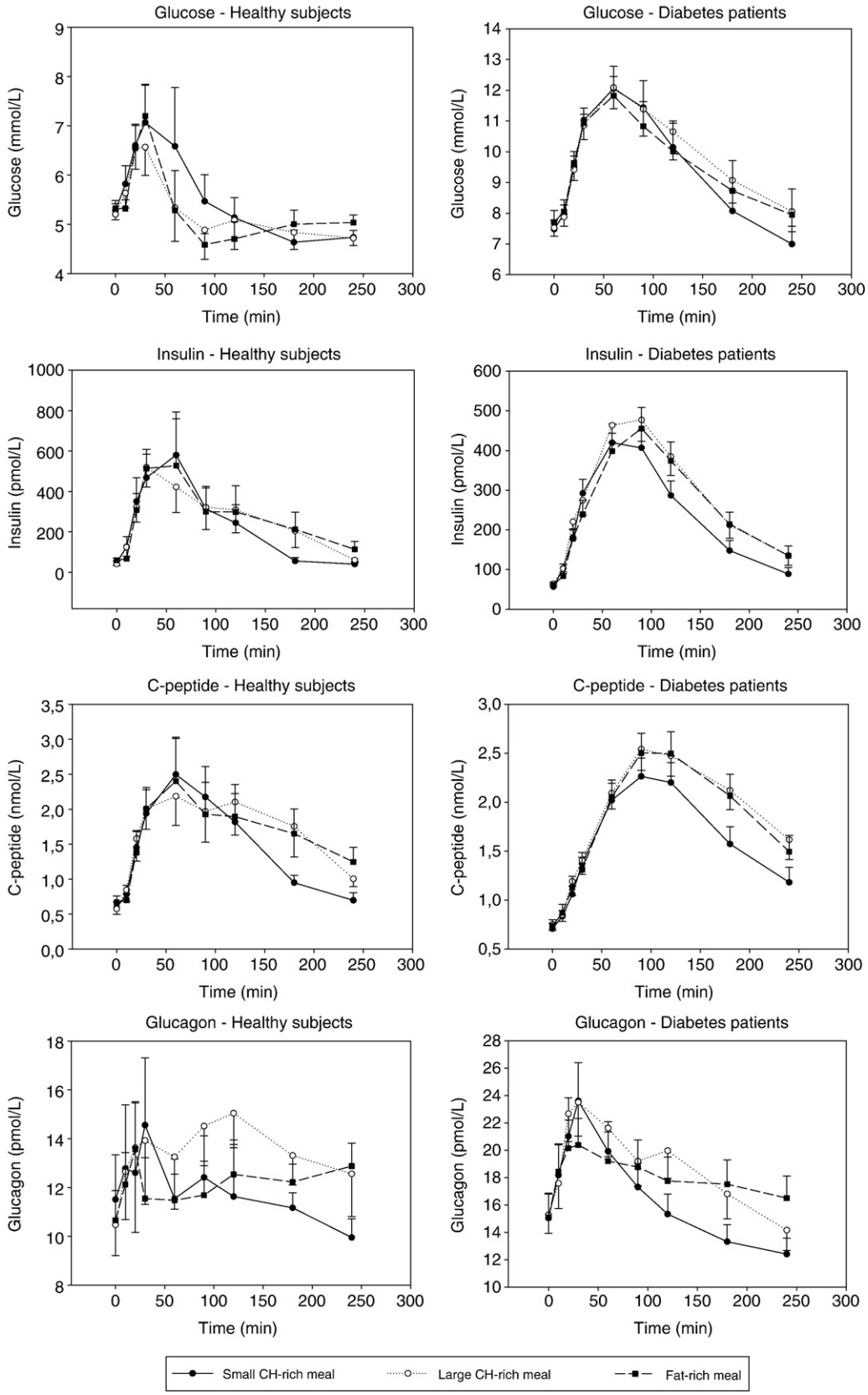

Fig. 1. Glucose, insulin, C-peptide, and glucagon (mean ± SE) after ingestion of the small CH-rich meal (closed circles), large CH-rich meal (open circles), and the fat-rich meal (closed squares) in healthy individuals and diabetes patients (left and right hand panels, respectively). Note that the scaling of the y-axis is different for healthy subjects vs diabetes patients.

Table 3
Incremental AUCs during meals

	Small CH-rich meal	Large CH-rich meal	Fat-rich meal
Healthy subjects			
$\Delta AUC_{glucose}$ (mmol·h/L)	1.4 (−2.6, 3.3)	−0.9 (−3.1, 3.4)	−0.8 (−2.9, 2.1)
$\Delta AUC_{insulin}$ (pmol·h/L)	549.6 (422.1, 1084.6)	714.8 (527.6, 1244.0)	653.7 (283.9, 1374.4)
$\Delta AUC_{C\text{-}peptide}$ (nmol·h/L)	3.5 (2.3, 5.1)	4.0 (3.1, 6.0)*	3.2 (2.3, 6.3)
$\Delta AUC_{glucagon}$ (pmol·h/L)	2.1 (−7.3, 8.2)	14.4 (5.2, 17.6)	5.5 (1.5, 10.0)
$\Delta AUC_{GLP\text{-}1}$ (pmol·h/L)	13.5 (10.5, 15.8)	16.8 (11.5, 28.6)	19.1 (7.8, 26.9)
ΔAUC_{GIP} (pmol·h/L)	97.1 (88.3, 110.3)	98.4 (84.5, 128.6)	117.3 (110.2, 143.4)
Diabetes patients			
$\Delta AUC_{glucose}$ (mmol·h/L)	8.4 (1.7, 13.7)	9.2 (1.9, 17.0)	4.9 (1.6, 13.6)
$\Delta AUC_{insulin}$ (pmol·h/L)	586.2 (397.1, 1018.5)	904.0 (537.0, 1259.6)*	707.9 (537.1, 1315.8)*
$\Delta AUC_{C\text{-}peptide}$ (nmol·h/L)	3.3 (2.6, 5.4)	4.6 (3.7, 6.6)*	4.6 (3.2, 6.7)*
$\Delta AUC_{glucagon}$ (pmol·h/L)	6.4 (3.3, 9.1)	13.0 (6.8, 22.2)*	11.0 (2.7, 21.0)
$\Delta AUC_{GLP\text{-}1}$ (pmol·h/L)	18.3 (9.5, 29.0)	23.8 (18.8, 29.6)	13.6 (10.5, 20.0)†
ΔAUC_{GIP} (pmol·h/L)	79.7 (63.0, 106.0)	87.9 (70.3, 130.7)	99.2 (74.1, 158.1)

Values are median (interquartile range).
* $P < .05$ compared with small CH-rich meal.
† $P < .05$ compared with large CH-rich meal.

8 used sulfonylureas, and 3 patients used both metformin and sulfonylureas. Antihypertensives were used by 11 diabetes patients and 1 healthy subject; lipid-lowering medication by 9 patients and 1 healthy individual.

3.2. Glucose, C-peptide, insulin, and glucagon responses

Postprandial profiles of all parameters are shown in Fig. 1. Fasting concentrations were not different before each of the meals except for a minor difference in fasting insulin and C-peptide between the large CH-rich meal and the fat-rich meal in healthy subjects.

In healthy subjects, the $\Delta AUC_{glucose}$ did not differ between meals (Table 3), although the glucose level remained elevated for a longer period after the small CH-rich meal than after the other 2 meals (Fig. 1). The 3 different meals induced relatively similar glucose responses (Fig. 1) in subjects with diabetes. Although the $\Delta AUC_{glucose}$ was slightly smaller after the fat-rich meal in individuals with diabetes, there was no significant difference between meals (Table 3).

The small CH-rich meal resulted in a significantly lower C-peptide response ($\Delta AUC_{C\text{-}peptide}$) than the large CH-rich meal in healthy subjects (Table 3). In individuals with diabetes, $\Delta AUC_{insulin}$ and $\Delta AUC_{C\text{-}peptide}$ were significantly lower for the small CH-rich meal than the other meals because of a more rapid decrease.

Patients with diabetes had higher glucagon secretion than healthy subjects (Fig. 1). The large CH-rich meal resulted in (nonsignificantly) higher glucagon response compared with the small CH-rich meal and the fat-rich meal in healthy subjects. In patients with diabetes, glucagon secretion ($\Delta AUC_{glucagon}$) was significantly higher after the large CH-rich meal than after the small CH-rich meal (Table 3). Whereas levels were decreasing after both CH-rich meals during the last 2 hours, after the fat-rich meal, there was an increase (healthy subjects) or stabilization (diabetes patients) in the last 2 hours. After the small CH-rich meal, values had dropped to even less than fasting values after 240 minutes in both groups of participants.

3.3. Incretin responses

Fasting incretin levels were similar before all study meals within both groups. The large CH-rich meal induced the highest GLP-1 responses both in healthy individuals as well as in those with diabetes (Fig. 2). $\Delta AUC_{GLP\text{-}1}$ was significantly higher for the large CH-rich meal vs the fat-rich meal but only in diabetes patients (Table 3). The other 2 meals induced similar responses. In healthy subjects, concentrations were still rising after the first 2 hours of the test, whereas in diabetes patients, the values had stabilized. However, absolute GLP-1 values did not differ between groups.

There was a remarkable difference in GIP response after consumption of the fat-rich meal as compared with CH-rich meals in both healthy subjects and those with diabetes (Fig. 2). Whereas GIP levels increased rapidly and reached significantly higher values after consumption of the CH-rich meals after the first 30 minutes and then declined, GIP continued to increase after the fat-rich meal in subjects with and without diabetes. ΔAUC_{GIP} was not significantly different between the meals (Table 3), implying similar total responses during the first 120 minutes of the test albeit with a different secretory pattern over time. There were no differences in absolute GIP response between the small and the large CH-rich meal. The GIP response did not seem impaired in these well-controlled patients with diabetes as compared with healthy individuals.

3.4. Estimates of β-cell function

For non–model-based assessments, in healthy subjects, the large CH-rich meal resulted in a significantly higher insulinogenic index than the small CH-rich meal (Table 4). In addition, the fat-rich meal also tended to lead to a higher

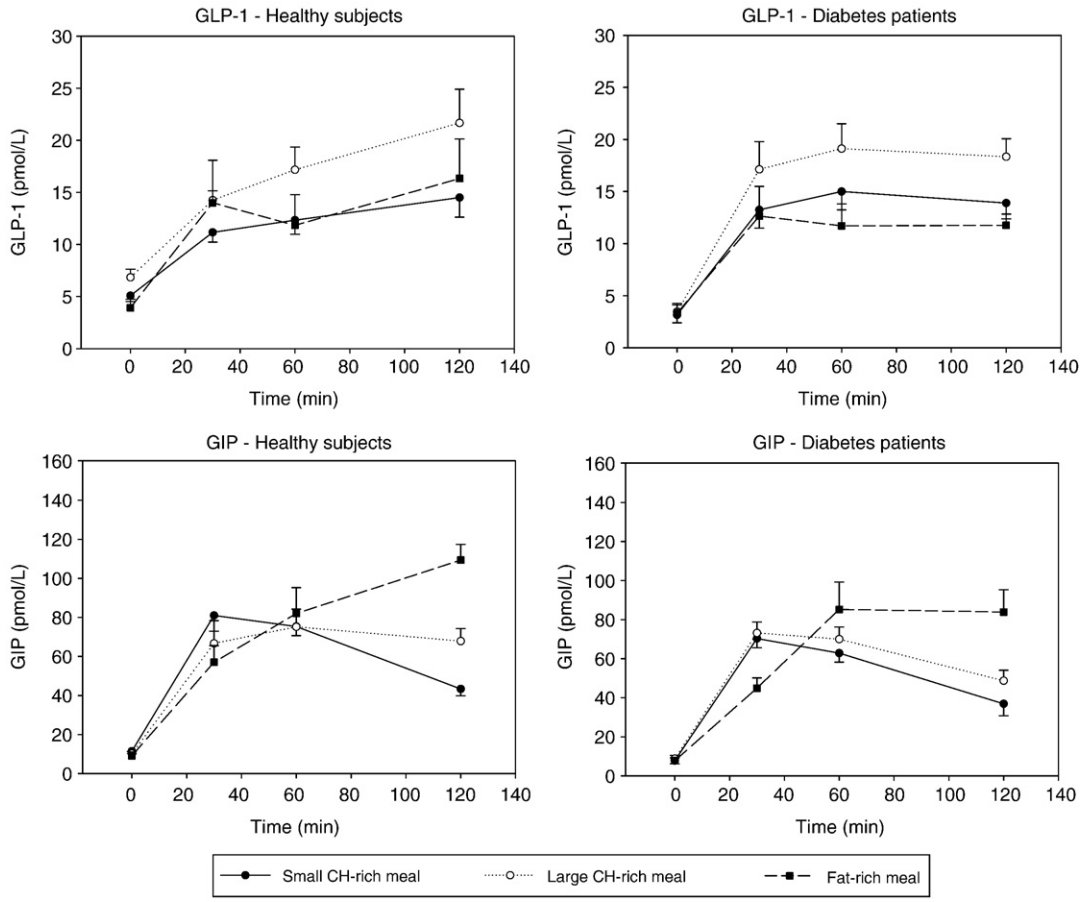

Fig. 2. The GIP and GLP-1 responses (mean ± SE) after ingestion of the small CH-rich meal (closed circles), large CH-rich meal (open circles), and the fat-rich meal (closed squares) in healthy subjects and diabetes patients (left and right hand panels, respectively).

insulinogenic index relative to the small CH-rich meal; but the difference did not reach statistical significance. Interestingly, the insulinogenic index was lower after the fat-rich meal type relative to the other meal types in diabetes patients (Table 4). As an index of overall insulin secretion relative to glucose excursion, the $AUC_{insulin}/AUC_{glucose}$ ratio also tended to be higher with the large CH-rich meal and the fat-rich meal relative to the small CH-rich meal both in healthy subjects and in those with diabetes (Table 4). In contrast, the measures of β-cell function derived from the model-based assessments did not substantively differ by meal type (Table 4). The β-cell glucose sensitivity was similar across all 3 meal types, and the rate sensitivity also was only modestly different across the 3 meals. The

Table 4
β-Cell function parameters during meals

	Small CH-rich meal	Large CH-rich meal	Fat-rich meal
Healthy subjects			
Insulinogenic index (pmol/mmol)	114.9 (83.2, 576.8)	215.2 (126.1, 312.8)*	192.2 (129.8, 1290.9)
$AUC_{insulin}/AUC_{glucose}$ ratio (pmol/mmol)	37.2 (25.2, 58.4)	43.8 (35.2, 62.3)	44.6 (28.0, 67.5)
β-Cell glucose sensitivity (pmol/(min m^2 mM))	113.9 (49.8)	126.8 (83.2)	116.5 (78.9)
Rate sensitivity (pmol/(m^2 mM))	1145.4 (440.1, 1786.6)	1365.8 (446.6, 2991.2)	891.4 (542.4, 2320.9)
Potentiation factor ratio (fold)	1.01 (0.49)	1.03 (0.20)	1.35 (0.99, 1.72)
Diabetes patients			
Insulinogenic index (pmol/mmol)	72.6 (33.5, 97.8)	64.4 (28.2, 93.8)	45.1 (20.7, 57.7)*
$AUC_{insulin}/AUC_{glucose}$ ratio (pmol/mmol)	27.3 (15.2, 33.1)	30.4 (18.5, 39.6)*	26.3 (19.6, 38.6)*
β-Cell glucose sensitivity (pmol/(min m^2 mM))	48.4 (28.6)	46.1 (26.7)	46.6 (27.4)
Rate sensitivity (pmol/(m^2 mM))	317.4 (45.6, 829.8)	415.4 (29.5, 558.2)	141.8 (47.3, 412.5)
Potentiation factor ratio (fold)	1.42 (0.31)	1.33 (1.15, 1.51)	1.47 (0.57)

Values are mean (SD) or median (interquartile range) when appropriate.
* $P < .05$ compared with small CH-rich meal.

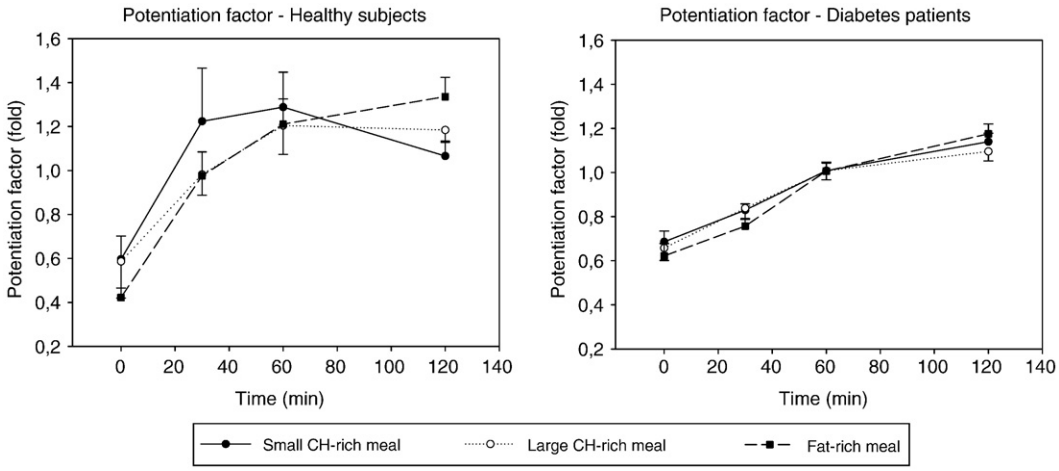

Fig. 3. Potentiation factor and GIP responses during the first 120 minutes after ingestion of the small CH-rich meal (closed circles), large CH-rich meal (open circles), and the fat-rich meal (closed squares) in healthy subjects and diabetes patients (left and right hand panels, respectively).

potentiation factor ratio tended to be higher after the fat-rich meal in healthy subjects, but the difference was modest.

3.5. Incretins in relation to β-cell function

Absolute incretin levels at $t = 30$ and ΔAUCs were not related to β-cell function estimates. However, when incretin *changes* (increment between 0 and 120 minutes) were taken into account, there was a tendency for a correlation between GIP and the potentiation factor ratio between 0 and 120 minutes ($r = 0.58$, $P < .05$ for healthy subjects after exclusion of 2 outliers; $r = 0.23$, $P > .05$ for diabetes patients). Indeed, the pattern of the potentiation factor (Fig. 3) resembles that of GIP (Fig. 2) during the first 120 minutes. For GLP-1, this tendency was not present ($r = 0.08$, $P > .05$ for healthy subjects; $r = 0.17$, $P > .05$ for diabetes patients).

4. Discussion

In patients with diabetes and age- and BMI-matched healthy subjects, more CH or more fat in a meal led to a larger late insulin response than a small CH-rich meal. In healthy subjects, this led to a slight (not statistically significant) reduction in the hyperglycemic period as compared with the small CH-rich meal. In the diabetes patients, glycemic excursions were similar after the 3 meals, possibly because the β-cells still have some capacity to adjust their insulin production to control the glucose levels. The fat-rich meal resulted in a significantly lower insulinogenic index in patients with diabetes. Model-based insulin secretion estimates did not show pronounced differences between meals within healthy participants or those with diabetes. Concerning the incretins, larger CH load resulted in a larger GLP-1 release regardless of diabetes status. The GIP release showed a different pattern for the high-fat meal (slow but continuous rise after the meal vs a hyperbola for the other 2 meals) and resembled the pattern of the potentiation factor. The initial glucagon response was stimulated by the large CH-rich meal, whereas the fat-rich meal induced a late glucagon response.

4.1. Strengths and limitations of the study

As one of the first studies, we compared β-cell and incretin responses after meals with different compositions, including not only differences in the amount of CH, but also differences in meal size and fat content. Several methodologies are available to assess insulin secretion and insulin sensitivity. The gold standard to assess β-cell function is the hyperglycemic clamp. This is a labor-intensive technique, however, and cannot be generally applied in population-based studies. The non–model-based β-cell function parameters that we have used in our study are easy to use in population-based studies, which give an impression of the overall β-cell function. In addition, the model-based parameters show different aspects of the β-cell function, including the glucose sensitivity of the β-cells [16].

Our study included a relatively small number of healthy subjects. However, because our primary aim was to study the effects of the different meals on the responses within patients with diabetes, the study as designed was sufficiently powered based on the confidence interval of glucose AUC for the diabetes patients. Although the number of healthy individuals was rather small, it provides an indication of the postprandial responses in healthy subjects as compared with diabetes patients. Most of the diabetes patients who participated in this study were well controlled. Although they may not be representative of all patients with diabetes, they are representative of a large part of the diabetic population in the Netherlands. Moreover, in patients with diabetes using insulin, it would not be possible to estimate β-cell function. Although patients on 2- or 3-times-a-day

treatment withheld the morning dose on the days of the tests, their medication may have influenced the results. Additional analyses (data not shown) pointed out that β-cell function parameters of patients using metformin only (n = 7) or sulfonylureas only (n = 8) were not significantly different. Patients using metformin had slightly (but not significantly) higher glucose levels during the meals and (borderline significant) higher insulin levels than patients using sulfonylureas. The study was performed in a Caucasian population in the Netherlands; it is unknown if our results would extend to other ethnic groups.

4.2. Incretin response and β-cell function during different meals

Besides glucose, several fatty acids [17] and proteins [18,19] have been reported to stimulate β-cell insulin secretion. Other than direct effects on the β-cell, an increased β-cell response could also be due to an increased incretin effect caused by the differences in macronutrients and/or caloric load between the meals [20]. Therefore, we did not expect a lower early insulin response after the fat-rich meal, which is also in contrast with our previous study [21]. This might be explained by the lower early GIP response as compared with the other meals. There have been contrasting results regarding the question of whether fatty acid saturation of a meal has an effect on incretin response [22-24]. Our meals consisted of a mixture of saturated and unsaturated fatty acids and did not allow us to distinguish effects of different fatty acids. Addition of whey proteins to a meal stimulates GIP and insulin release and reduces postprandial blood glucose excursion [25]. The large CH-rich meal and fat-rich meal contained similar amounts of proteins, and all our meals contained milk proteins; but small differences in protein type between the meals are still present.

Incretin concentrations at 30 minutes and ΔAUCs of the incretins did not seem to be related to β-cell function estimates. Fried et al [3] also showed no correlation between plasma insulin concentrations and plasma levels of GIP in healthy individuals after a mixed meal. With the mathematical model, we were able to determine if different components of β-cell function are affected by the composition of a mixed meal. Earlier, it has been shown that incretins increase glucose and rate sensitivity with minimal effects on potentiation [26], whereas potentiation is affected by direct GLP-1 administration [27]. We did not observe significant differential effects of the meals on the different β-cell function components. However, rate sensitivity was lowest after the fat-rich meal, possibly because GIP concentration was increasing only slowly. Furthermore, GIP excursion appears to play a specific role in increasing the potentiation factor in healthy subjects, as indicated by the finding that progressive GIP stimulation from the small CH-rich meal to the fat-rich meal produced an increasing rise of the potentiation factor. In patients with diabetes, in whom potentiation is somewhat slow compared with healthy subjects, this effect is less clear. Nevertheless, the results suggest that the incretin-mediated increase in potentiation represents a factor for the control of postprandial glucose.

GLP-1 secretion seems to be increased by the amount of CH and not by the quantity of the meal because the fat-rich meal was largest in quantity. The explanation of the increase of GIP secretion by the amount of fat in the fat-rich meal is not unequivocal in this study because the fat-rich meal also contained more calories, which might also increase GIP secretion. Furthermore, the different types of proteins in the meals may have affected GIP and GLP-1 secretion differently. To address this, additional studies are needed.

In insulin-resistant states and type 2 diabetes mellitus, it has been documented that GLP-1 response to a meal is impaired, leading to diminished potentiation of meal-stimulated insulin secretion [26,28]; but similar or elevated GLP-1 levels have also been reported [2,29]. In our study, we did not see an impaired GLP-1 or GIP response in individuals with diabetes. Our participants did not represent severe cases of diabetes; they were not using insulin therapy and were well controlled with oral hypoglycemic agents, which might partially explain our findings.

4.3. α-Cell response

Not many studies have reported postprandial glucagon concentrations. Recently, an association was shown between fasting glucagon and insulin resistance in the general population without diabetes, possibly indicating insulin resistance of the α-cells [30]. Impaired suppression of glucagon release has been observed in individuals with impaired glucose tolerance and type 2 diabetes mellitus after glucose ingestion [31]. Meier et al [32] showed that glucagon was suppressed after both intravenous and oral glucose administration in relatives of type 2 diabetes mellitus patients and healthy controls. Another study showed a late increase in glucagon after a meal or OGTT [33]. However, we found an increase already present in the first 30 minutes of the test for both healthy subjects and diabetes patients, which is in accordance with another study reporting glucagon concentrations after mixed meals [6]. This early response may be induced by the proteins in the meals. Claessens et al [34] showed in an experimental setup administering several beverages to 8 healthy subjects that glucagon responses are suppressed after a CH drink but increased when various types of amino acids were added to the drink. It appeared that protein stimulated glucagon secretion, although it was also suppressed by the corresponding insulin secretion. In the present study, the lower insulin after the small CH-rich meal was accompanied by a lower glucagon response, regardless of the presence or absence of diabetes. The large CH-rich meal seemed to be associated with a higher glucagon response in healthy individuals (not significant). Thus, in the present study, the amount of CH seemed to determine the

initial glucagon response; fat appeared to result in a late glucagon response.

5. Conclusions

In conclusion, fat seems to stimulate GIP secretion, whereas CH in particular increases GLP-1 secretion. Both the large CH-rich meal and the fat-rich meal resulted in a larger insulin response as compared with the small CH-rich meal. Whereas this led to a slightly shorter period of hyperglycemia in healthy subjects, this was not the case for diabetes patients. Thus, differences in caloric load and meal composition led to differences in insulin and incretin responses but not to differences in the postprandial glucose levels of well-controlled patients with diabetes.

Acknowledgment

The authors thank Jannet Entius, Lida Ooteman, Marianne Veeken, and Jolanda Bosman for their excellent assistance in data collection and organization of the study.

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