The role of GLP-1 in the postprandial effects of acarbose in type 2 diabetes

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Abstract

Aims: The alpha-glucosidase inhibitor acarbose is believed to reduce plasma glucose by delaying hydrolysis of carbohydrates. Acarbose-induced transfer of carbohydrates to the distal parts of the intestine increases circulating glucagon-like peptide 1 (GLP-1). Using the GLP-1 receptor antagonist exendin(9–39)NH₂, we investigated the effect of acarbose-induced GLP-1 secretion on postprandial glucose metabolism in patients with type 2 diabetes.

Methods: In a double-blinded, placebo-controlled, randomized, crossover study, 15 participants with metformin-treated type 2 diabetes (age: 57–85 years, HbA1c: 40–74 mmol/mol) were subjected to two 14-day treatment periods with acarbose or placebo, respectively, separated by a 6-week wash-out period. At the end of each period, two randomized 4-h liquid mixed meal tests with concomitant infusion of exendin(9–39)NH₂ and saline, respectively, were performed.

Results: Compared to placebo, acarbose increased postprandial GLP-1 concentrations and decreased postprandial glucose. We observed no absolute difference in the exendin(9–39)NH₂-induced increase in postprandial glucose excursions between placebo and acarbose periods, but relatively, postprandial glucose was increased by $119 \pm 116\%$ (mean $\pm$ s.d.) during exendin(9–39)NH₂ infusion in the acarbose period vs a $39 \pm 27\%$ increase during the placebo period ($P = 0.0163$).

Conclusions: We confirm that acarbose treatment stimulates postprandial GLP-1 secretion in patients with type 2 diabetes. Using exendin(9–39)NH₂, we did not see an impact of acarbose-induced GLP-1 secretion on absolute measures of postprandial glucose tolerance, but relatively, the effect of exendin(9–39)NH₂ was most pronounced during acarbose treatment.

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Introduction

The gastrointestinal tract contributes to the regulation of glucose metabolism by several mechanisms including nutrient-induced secretion of the incretin hormones, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (1). These hormones are released postprandially from enteroendocrine cells; GIP from K cells with high density in the proximal small intestine, and GLP-1 from L cells with higher density more

distally in the gut (2). Both hormones are insulinotropic and contribute importantly to normal postprandial glucose tolerance (3). In addition to its insulinotropic effect, GLP-1 potentiates glucose-induced suppression of glucagon (4), decelerates gastric emptying and reduces appetite and food intake (5). These effects are currently being utilized in the management of type 2 diabetes by treatment with GLP-1 receptor agonists and oral

dipeptidyl peptidase 4 (DPP-4) inhibitors, respectively. It has been speculated that stimulation of GLP-1 secretion might represent a superior alternative to existing GLP-1-based treatments, and strategies promoting GLP-1 secretion without increasing caloric intake are warranted (6). Pharmacological inhibition of the oligosaccharide, trisaccharide and disaccharide-hydrolyzing intestinal enzyme alpha-glucosidase, delays carbohydrate digestion in the proximal gut causing a transfer of carbohydrates to the distal and L cell-rich part of the small intestine (7). Alpha-glucosidase inhibitors represent well-known oral antidiabetic agents used for the treatment of type 2 diabetes. The most widely used compound, acarbose, also inhibits salivary and pancreatic amylase, and it is believed that acarbose primarily lowers postprandial glucose excursions by delaying the rate of carbohydrate digestion and absorption (7); however, mechanistic studies establishing the precise glucose-lowering mode of action of acarbose are lacking. Acarbose treatment is recognized for increasing circulating concentrations of several gut hormones in the postprandial state including GLP-1 (8, 9, 10, 11, 12), cholecystokinin (CCK) and the satiety-promoting peptide tyrosine tyrosine (PYY) while decreasing postprandial GIP, insulin and ghrelin levels (11, 12, 13). Acarbose, thus, seems to represent an indirect non-nutritional GLP-1 secretagogue that work by promoting monosaccharide absorption in the distal gut (11). We used the specific GLP-1 receptor antagonist exendin(9–39)NH₂ to investigate and quantify the potential postprandial effects of GLP-1 released as a result of acarbose treatment in patients with type 2 diabetes.

Research design and methods

The study was performed at Center for Clinical Metabolic Research, Gentofte Hospital, University of Copenhagen, Hellerup, Denmark, after approval of the Ethics Committee of the Capital Region of Denmark (record no. H-17007893). The study was registered at ClinicalTrials.gov (ID: NCT03241303) and the Danish Data Protection Agency (local no. HGH-2018-041, I-Suite no. 6.802).

Participants

Inclusion criteria were diagnosis of type 2 diabetes at least 3 months prior to inclusion, metformin treatment, age >18 years and BMI between 23 and 35 kg/m². Exclusion criteria included acute or chronic illnesses including known hepatobiliary disorders, alanine aminotransferase

>2 times normal values, gastrointestinal disease, reduced kidney function (estimated glomerular filtration rate <60 mL/min/1.73 m²), active/recent malignant disease, pregnancy, thyroid disease, use of medication that could not be paused for 12 h alongside use of glucose-lowering drugs other than metformin and intake of antibiotics within 2 months prior to inclusion.

Peptides and study drugs

Oral acarbose (Glucobay®, Bayer Pharma AG, Berlin, Germany) or matching placebo tablets (50 mg) were administered as study drugs. Exendin(9–39)NH₂ (catalog number H-8740, Bachem, Bubendorf, Switzerland) was demonstrated to be identical to the natural peptides by HPLC, mass and sequence analysis with a purity of 97%. Exendin(9–39)NH₂ was formulated for infusion by the Central Pharmacy of the Capital Region of Denmark, Herlev, Denmark, using 1% human albumin (CSL Behring 5%, infusion fluid, 50 g/L, CSL Behring GmbH, Marburg, Germany) and sterilized water, with subsequent sterile filtration and testing for endotoxins and microbiological contamination before experimental use. After formulation, vials with exendin(9–39)NH₂ were kept at -20°C under sterile condition until use. On the study days, vials with the required amount of exendin(9–39)NH₂ were thawed and diluted in sterile isotonic saline (9 mg/mL, B. Braun, Melsungen, Germany) with 0.5% human albumin in a total volume of 250 mL before infusion. For placebo infusions 250 mL of isotonic saline with 0.5% human albumin were used.

Study design

The study was designed as a randomized, double-blinded, placebo-controlled, crossover study. For each study participant, the study consisted of two 14-day treatment periods with double-blinded administration of either placebo or acarbose in addition to their usual metformin doses, separated by a wash-out period of 6 weeks. Before each treatment period, study participants received the tablets (placebo or acarbose) and an uptitration schedule (with uptitration 2 days prior to the first experimental day to a maximum dose of three tablets of placebo or 100 mg acarbose at the start of main meals). Otherwise, the study participants were instructed to continue their usual lifestyle and diet. In the end of each treatment period, two liquid mixed meal tests separated by one full day and with administration of either acarbose or placebo depending on the treatment period were performed concomitantly

with either continuous i.v. infusion of isotonic saline (placebo) or exendin(9–39)NH₂ in a double-blinded manner. The primary endpoint was difference between the acarbose-induced change in postprandial plasma glucose excursions observed with and without blockade of GLP-1 receptor signaling.

Experimental procedures

The study participants arrived at the laboratory after an overnight 10-h fast (including water, coffee, medicine and use of tobacco). They had been instructed not to perform strenuous physical activity or to consume alcohol for 24 h before each study day. Two cannulas were inserted into the cubital veins of both arms for collection of blood samples and infusions, respectively (the forearm used for blood sampling was wrapped in a heating pad (-45°C) for arterialization of venous blood). Afterwards, the participants rested 30 min before start of the infusions. At time -20 min, the infusion of exendin(9–39)NH₂ (1000 pmol/kg of body weight/min) or isotonic saline was started (depending on randomization schedule). At time 0 min, the infusion rate was lowered to 450 pmol/kg/min. From time 0 min to 5 min, the patient ingested a liquid mixed meal (200 mL, 1260 kJ (36.8 g carbohydrate, 11.6 g protein and 12.0 g of lipid); Nutricia, Danone, Denmark) to which was added 100 mg of acarbose or placebo powder as well as 100 mL of water with dissolved paracetamol (1500 mg) (Pinex®, Actavis, Dublin, Ireland) for indirect assessment of gastric emptying.

Data collection and laboratory methods

Blood samples were collected at time -30, -15 and 0 min before and at time 15, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 min after the start of infusion. For bedside measurements of plasma glucose, blood was sampled into sodium-fluoride tubes, centrifuged immediately (at room temperature and 7400 g for 30 s) and analyzed on a glucose analyzer (YSI 2900D STAT glucose analyzer, Xylem Inc., Yellow Springs, OH, USA). For the analysis of plasma concentrations of GLP-1, GIP, glucagon, PYY, neurotensin, CCK and gastrin, blood was collected in chilled tubes (on ice) containing EDTA together with a specific DPP-4 inhibitor (final concentration 0.01 mmol/L valine-pyrrolidide) and a serine protease inhibitor (500 KIE/mL aprotinin). For the analysis of serum insulin and C-peptide concentrations, blood was sampled in plain tubes and left for 10 min at room temperature for coagulation. For analysis of plasma paracetamol

concentrations, blood was sampled in tubes containing lithium–heparin. All tubes were then centrifuged for 15 min at 2000 g and 4°C, after which they were stored at -20°C until analysis. At time -60, 0, 30, 55, 75, 105, 135, 180, 210 and 240 min, the gallbladder was measured ultrasonographically (LOGIQ, GE Healthcare) in three dimensions (height × width × length) for determination of gallbladder volume by the ellipsoid method (14). At time -60, -15, 15, 30, 55, 90, 150, 210 and 240 min, the study participants rated hunger, satiety, fullness, prospective food consumption, comfort, nausea and thirst on 100 mm visual analog scales (VAS). At time 240 min, the study participants received a standardized meal consisting of pasta Bolognese (50 energy (E)% carbohydrate, 37 E% fat, 13 E% protein) served together with 500 mL of water *ad libitum*, and were instructed to eat until they felt comfortably full. The food was weighed before and after the meal, and the difference in weight was used to calculate the total energy intake. Serum insulin and C-peptide were measured with two-sided electrochemiluminescence assays (Roche/Hitachi Modular Analytics; Roche Diagnostic). Total GLP-1 (15), total GIP (16), PYY (17), neurotensin (18), exendin(9–39)-NH₂ (19), CCK (20) and gastrin (21) were measured in plasma by radioimmunoassays as previously described. Plasma glucagon was measured by ELISA (cat no. 10-1271-01; Mercodia, Uppsala, Sweden).

Statistical analyses and calculations

Based on an s.d. of 661 mmol/L × min of plasma glucose excursions during the liquid mixed meal test (assessed by area under curve (AUC) for plasma glucose) obtained from a previous study investigating the glucose-lowering effect of metformin-induced GLP-1 secretion using similar methodology (22), an estimated minimal relevant difference (MIREDIFF) of 25%, a power of 80% and a level of statistical significance of 5%, we calculated the required number of participants (*n*) using the formula, $n = (Z_{\alpha} + Z_{\beta})^2 \times \text{s.d.}^2 / \text{MIREDIFF}^2$, where Z_{α} and Z_{β} are standardized deviations corresponding to the selected significance levels, $Z_{\alpha} = 1.96$ and $Z_{\beta} = 1.28$. According to this calculation, 14 study participants had to be included to detect a minimal difference of 25% in AUC for plasma glucose with and without blockade of GLP-1 signaling by exendin(9–39)NH₂. To ensure power of the primary endpoint and reduce the likelihood of statistical type II errors regarding secondary endpoints, 15 participants were included. We applied repeated measures ANOVA (rmANOVA) with Greenhouse-Geisser correction and Tukey multiple comparison test for

significance of changes and differences between endpoints. Statistical analyses were performed with GraphPad Prism 8.1.1 (GraphPad Software). A two-sided P value of < 0.05 was used as significance level. Results are reported as mean $\pm$ s.d. unless otherwise stated. Calculation of AUC was based on the trapezoidal rule. As our objective was to examine postprandial effects of acarbose, baseline-subtracted AUCs (bsAUCs), which describe meal-induced changes independently of potential baseline differences caused by acarbose and exendin(9–39)NH₂ infusion, are reported if nothing else is stated. GLP-1-mediated effects on bsAUC for plasma glucose were calculated in absolute terms with and without exendin(9–39)NH₂ (differences in bsAUC_{glucose} for placebo treatment period compared to acarbose treatment period), and relative terms (exendin(9–39)NH₂-induced differences relative to placebo infusions) and, for each data set, compared using paired t -tests. C-peptide:glucose ratios were calculated for evaluation of beta cell sensitivity to glucose. Gastric emptying was evaluated by the use of the paracetamol absorption method (23).

Results

Study participants

Two female and thirteen male study participants with metformin-treated type 2 diabetes (Table 1) were included after a screening visit. Written informed consent was obtained from all study participants before inclusion. In general, study participants reported increased flatulence in the acarbose treatment period; otherwise no side effects were reported. All participants completed the intervention periods, but due to poor ultrasonographic projection of the gallbladder, three participants were not included in the gallbladder volume analysis.

Exendin(9–39)NH₂ levels

During exendin(9–39)NH₂ infusions, similar mean plasma exendin(9–39)NH₂ levels were achieved following placebo treatment and acarbose treatment (162 ± 23 vs 169 ± 23 nmol/L, $P=0.4082$) (Fig. 1).

Glucose

No difference was observed in fasting plasma glucose levels measured before each infusion ($P=0.1335$) (Fig. 2A and Table 2). In the placebo period, during the liquid meal test with saline infusion, plasma glucose

Table 1 Baseline characteristics of study participants. Data are shown in median with range in parenthesis unless otherwise indicated.

Variable (unit)	Values
Age (years)	71 (57–85)
Male/female (n/n)	13/2
Height (m)	1.78 (1.53–1.85)
Weight (kg)	87.6 (66.3–111)
BMI (kg/m ²)	29.7 (23.6–34.6)
Body fat (%)	35.4 (24.6–45.1)
Fasting plasma glucose (mmol/L)	7.8 (6.6–11.6)
HbA _{1c} (%)	6.5 (5.8–11.6)
HbA _{1c} (mmol/mol)	48 (40–74)
Systolic blood pressure (mmHg)	136 (111–160)
Diastolic blood pressure (mmHg)	77 (36–89)
Heart rate (bpm)	61 (55–87)
HOMA-IR	2.9 (0.64–9.7)

bpm, beats per minute.

concentrations increased to a mean maximum of 12 ± 2.1 mmol/L at time 87 ± 20 min resulting in a bsAUC of 470 ± 128 mmol/L $\times$ min. During exendin(9–39)NH₂ infusion, postprandial plasma glucose levels increased to a $16 \pm 5.6\%$ higher mean peak value (14 ± 2.4 mmol/L, $P < 0.0001$, compared to saline) resulting in a $39 \pm 27\%$ greater bsAUC ($P < 0.0001$ compared to saline) (Fig. 2A, B

Figure 1

Exendin(9–39)NH₂ levels. Plasma exendin(9–39)NH₂ (A) levels in 15 patients with type 2 diabetes during four liquid mixed meal tests (initiated at time 0, dotted line) with continuous i.v. infusion of exendin(9–39)NH₂ (closed symbols) and saline (open symbols), respectively, performed after 14-day treatment periods with placebo (circles) and acarbose (squares). Data are presented as mean $\pm$ s.e.m.

Figure 2

Glucose. Plasma glucose levels (A) and corresponding bsAUC (time 0–240 min) (B) during four liquid mixed meal tests (initiated at time 0, dotted line) with continuous i.v. infusion of exendin(9–39)NH₂ (closed symbols) and saline (open symbols), respectively, performed after 14-day treatment periods with placebo (circles) and acarbose (squares). Data were compared by rmANOVA with Greenhouse-Geisser correction and Tukey multiple comparison. Significant differences for the *post hoc* analyses are marked with asterisks: *** $P < 0.001$, **** $P < 0.0001$. Data are presented as mean $\pm$ S.E.M. ex(9–39)NH₂, exendin(9–39)NH₂.

and Table 2). During the acarbose treatment period, the postprandial plasma glucose peak was $17 \pm 10\%$ higher during exendin(9–39)NH₂ infusion compared to saline.

Furthermore, exendin(9–39)NH₂ infusion resulted in higher mean bsAUC compared to saline ($119 \pm 116\%$, $P < 0.0001$) (Fig. 2B and Table 2). No absolute difference was seen in exendin(9–39)NH₂-induced bsAUC increase between placebo and acarbose treatment, but relatively, the increase in bsAUC of plasma glucose (i.e. the impairment of glucose tolerance) during exendin(9–39)NH₂ infusion was significantly greater in the acarbose treatment period than in the placebo treatment period ($119 \pm 116\%$ vs $39 \pm 27\%$, $P = 0.0163$).

GLP-1 and GIP

During the acarbose and placebo treatment periods, no difference was observed in fasting plasma GLP-1 levels ($P = 0.1212$) (Fig. 3A and Table 2). In the placebo period, both postprandial peak GLP-1 concentrations ($P = 0.003$) and bsAUCs for GLP-1 ($P < 0.0001$) were higher during exendin(9–39)NH₂ infusions compared to saline. In the acarbose period, postprandial GLP-1 levels were significantly higher than in the placebo period (Fig. 3A, B and Table 2). Fasting plasma GIP levels were similar on all four study days (Fig. 3C and Table 2), and exendin(9–39)NH₂ infusion did not affect the postprandial plasma GIP levels in any of the treatment periods. As expected, in the acarbose treatment period, postprandial GIP responses were significantly lower than in the placebo period ($P < 0.001$) (Fig. 3C, D and Table 2).

Gastric emptying

Compared to placebo treatment, treatment with acarbose decreased peak concentration of plasma paracetamol (0.100 vs 0.116 mmol/L, $P = 0.0124$) and delayed the time to peak concentration of plasma paracetamol (95 ± 44 vs 120 ± 47 min, $P = 0.0395$) during saline infusions (Fig. 4A and B). Furthermore, compared to saline infusion, exendin(9–39)NH₂ shortened time to peak of paracetamol in plasma in both treatment periods, however only significantly during placebo treatment ($P = 0.0275$).

Insulin, C-peptide, C-peptide: glucose ratios and glucagon

No significant differences in baseline values of insulin, C-peptide, C-peptide:glucose ratios or glucagon were observed (Fig. 5A, C, E, G and Table 2). Compared to placebo, acarbose treatment diminished bsAUC for insulin significantly during saline (26 ± 16 vs 15 ± 8.4 nmol/L min) ($P = 0.0038$) as well as exendin(9–39)NH₂

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Table 2 Overview of plasma and serum measurements. Overview of plasma and serum measurements from the four liquid mixed meal tests (with concomitant i.v. exendin(9–39)NH₂ infusion and saline infusion, respectively) performed during the two 14-day treatment periods (placebo treatment and acarbose treatment, respectively). Data are presented as mean ± s.d. Differences between groups were compared with rmANOVA with Greenhouse-Geisser correction and Tukey multiple comparison.

	Placebo treatment		Acarbose treatment		P value rmANOVA
	+ saline*	+ ex(9–39)NH ₂ [†]	+ saline [‡]	+ ex(9–39)NH ₂ [§]	
Glucose					
Baseline (mmol/L)	7.8 ± 1.9	8.1 ± 1.8	7.5 ± 1.7	7.7 ± 1.7	0.13
Peak (mmol/L)	12 ± 2.1 ^{†‡§}	14 ± 2.4 ^{*†§}	9.3 ± 1.9 ^{*†§}	11 ± 1.9 ^{*†‡}	<0.0001
Time to peak (min)	87 ± 20	74 ± 14 [§]	89 ± 30	97 ± 21 [†]	0.019
bsAUC (mmol/L × min)	470 ± 128 ^{†‡}	650 ± 200 ^{*†§}	214 ± 98 ^{*†§}	395 ± 115 ^{†‡}	<0.0001
GLP-1					
Baseline (pmol/L)	18 ± 3.2	18 ± 3.9	21 ± 4.4	20 ± 4.4	0.12
Peak (pmol/L)	30 ± 5.4 ^{†‡§}	46 ± 13 ^{*§}	40 ± 8.8 ^{*§}	65 ± 16 ^{*†‡}	<0.0001
Time to peak (min)	61 ± 41	61 ± 39	61 ± 59	83 ± 53	0.38
bsAUC (pmol/L × min)	1421 ± 709 ^{†‡§}	2537 ± 1444 ^{*§}	2657 ± 655 ^{*§}	5150 ± 1835 ^{*†‡}	<0.0001
GIP					
Baseline (pmol/L)	9.7 ± 3.4	11 ± 3.2	12 ± 4.8	12 ± 6.8	0.36
Peak (pmol/L)	72 ± 19 ^{†‡§}	92 ± 28 ^{*§}	45 ± 13 ^{*†}	46 ± 16 ^{*†}	<0.0001
Time to peak (min)	56 ± 25	47 ± 12	60 ± 55	61 ± 52	0.61
bsAUC (pmol/L × min)	6571 ± 1727 ^{†‡§}	7071 ± 2152 [§]	3351 ± 1943 ^{*†}	3899 ± 1123 ^{*†}	<0.0001
Insulin					
Baseline (pmol/L)	76 ± 52	80 ± 46	82 ± 53	75.13 ± 41	0.77
Peak (pmol/L)	283 ± 176	304 ± 186	184 ± 81	181 ± 96	0.015
Time to peak (min)	78 ± 30	64 ± 25	62 ± 34	83 ± 53	0.3
bsAUC (nmol/L × min)	26 ± 16 [§]	28 ± 18 [§]	15 ± 8.4 ^{*†}	18 ± 11 ^{*†}	<0.0001
C-peptide					
Baseline (pmol/L)	884 ± 413	961 ± 431	914 ± 405	862 ± 331	0.26
Peak (pmol/L)	2,031 ± 803 ^{†§}	1,948 ± 763 ^{†§}	1,552 ± 613 [†]	1,534 ± 700 [†]	<0.0001
Time to peak (min)	111 ± 37 [†]	110 ± 26 ^{†§}	146 ± 55 ^{*†}	141 ± 46 [*]	0.0026
bsAUC (nmol/L × min)	178 ± 81 [§]	170 ± 97 ^{†§}	109 ± 57 ^{*†}	116 ± 63 ^{*†}	<0.0001
C-peptide:glucose ratio					
Baseline (pmol/mmol)	117 ± 51	123 ± 55	126 ± 60	117 ± 51	0.29
Peak (pmol/mmol)	222 ± 105 ^{†§}	184 ± 95 [*]	202 ± 94	172 ± 91 [*]	0.0002
Time to peak (min)	171 ± 59	166 ± 61	192 ± 45	166 ± 55	0.34
bsAUC (nmol/mmol × L ⁻¹ min)	15 ± 9.7 ^{†‡§}	12 ± 9.8 [*]	12 ± 7.8 [*]	9.5 ± 7.6 [*]	<0.0001
Glucagon					
Baseline (pmol/L)	15 ± 4.7	17 ± 6.4	16 ± 7.8	15 ± 6.1	0.23
Peak (pmol/L)	27 ± 8.5 ^{†§}	40 ± 13 [*]	28 ± 11 ^{*§}	44 ± 11 [†]	<0.0001
Time to peak (min)	41 ± 14 [§]	57 ± 36	50 ± 53	85 ± 52 [*]	0.074
bsAUC (pmol/L × min)	639 ± 419 ^{†§}	1361 ± 806 ^{*§}	958 ± 874 [§]	2751 ± 1317 ^{*†‡}	<0.0001
PYY					
Baseline (pmol/L)	21 ± 8	23 ± 9	27 ± 10	23 ± 10	0.22
Peak (pmol/L)	35 ± 11 ^{†§}	48 ± 12 [*]	38 ± 8 [§]	60 ± 16 ^{*†}	0.0001
Time to peak (min)	84 ± 64	97 ± 51	96 ± 47	118 ± 57	0.34
bsAUC (pmol/L × min)	1771 ± 1545 [§]	3431 ± 2530	1956 ± 1264 [§]	5531 ± 2276 ^{*†}	0.0001
Neurotensin					
Baseline (pmol/L)	19 ± 10	18 ± 12	23 ± 11	22 ± 11	0.37
Peak (pmol/L)	47 ± 26 [§]	62 ± 27	55 ± 23 [§]	85 ± 39 ^{*†}	0.0026
Time to peak (min)	51 ± 43	65 ± 44	54 ± 54	58 ± 55	0.8
bsAUC (pmol/L × min)	2940 ± 3207	4601 ± 2875	3373 ± 2601 [§]	6366 ± 4739 [†]	0.013
Gastrin					
Baseline (pmol/L)	14 ± 22	14 ± 23	19 ± 34	16 ± 22	0.68
Peak (pmol/L)	22 ± 23	23 ± 22	22 ± 30	19 ± 20	0.65
Time to peak (min)	58 ± 56	61 ± 58	66 ± 57	48 ± 56	0.78
bsAUC (pmol/L × min)	839 ± 826	886 ± 676	555 ± 477	817 ± 1134	0.52
CCK					
Baseline (pmol/L)	1.3 ± 1.2	1.3 ± 1.1	1.8 ± 2.4	1.5 ± 1.2	0.21
Peak (pmol/L)	8.8 ± 4.6	9.0 ± 4.2	12 ± 8.3	10 ± 6.6	0.33
Time to peak (min)	24 ± 20	34 ± 45	22 ± 14	31 ± 15	0.45
bsAUC (pmol/L × min)	449 ± 394	397 ± 431	643 ± 482	539 ± 409	0.23

Symbols for $P < 0.05$ by Tukey multiple comparison compared with: *placebo treatment + saline; [†]placebo treatment + exendin(9–39)NH₂; [‡]acarbose treatment + saline; or [§]acarbose treatment + exendin(9–39)NH₂.

bsAUC, baseline-subtracted AUC; CCK, cholecystokinin; ex(9–39)NH₂, exendin(9–39)NH₂; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; PYY, peptide tyrosine tyrosine; rmANOVA, repeated measures ANOVA.

Figure 3

GLP-1 and GIP. Plasma levels of GLP-1 (A) and GIP (C) and corresponding bsAUC (time 0–240 min) (B and D) during four liquid mixed meal tests (initiated at time 0, dotted line) with continuous i.v. infusion of exendin(9–39)NH₂ (closed symbols) and saline (open symbols), respectively, performed after 14-day treatment periods with placebo (circles) and acarbose (squares). Data were compared by rmANOVA with Greenhouse-Geisser correction and Tukey multiple comparison. Significant differences for the *post hoc* analyses are marked with asterisks: * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$. Data are presented as mean $\pm$ S.E.M. ex(9–39)NH₂, exendin(9–39)NH₂.

infusion (28 ± 18 vs 18 ± 11 nmol/L min) ($P = 0.0012$) (Fig. 5A, B and Table 2). We observed no difference in insulin responses between the two infusions (with and without exendin(9–39)NH₂) during acarbose treatment ($P = 0.1860$) or during placebo treatment ($P = 0.4537$). Similar results were observed for C-peptide (Fig. 5C, D and Table 2). Postprandial C-peptide:glucose ratios were generally higher in the placebo period compared to the acarbose period (Fig. 5E, F and Table 2); however, only significantly different during saline infusions ($P = 0.0458$). Compared to saline, exendin(9–39)NH₂ infusion reduced C-peptide:glucose ratios ($P = 0.0034$) in the placebo period and tended to do so also in the acarbose period ($P = 0.22$). In each of the two treatment periods, postprandial peak plasma glucagon levels were highest during exendin(9–39)NH₂ infusion compared to saline infusion (Fig. 5G and Table 2). Postprandial glucagon responses as assessed

by bsAUC were also greater during exendin(9–39)NH₂ infusion than during saline infusion in both treatment periods with the greatest responses being observed during exendin(9–39)NH₂ infusion in the acarbose treatment period (Fig. 5H and Table 2).

PYY, neurotensin, *ad libitum* meal intake, and appetite and satiety measures

Fasting plasma levels of PYY were similar on all four study days (Fig. 6A and Table 2). Postprandial plasma PYY peak levels were significantly higher during exendin(9–39)NH₂ infusion, compared to saline infusion in both treatment periods (Fig. 6A, B and Table 2). Also, postprandial PYY responses (as assessed by bsAUC) were augmented during exendin(9–39)NH₂ infusion compared to placebo in both treatment periods, but significantly so only in the acarbose treatment period ($P = 0.1319$ and $P < 0.0001$) (Fig. 6A, B and Table 2). No differences were observed between placebo and acarbose treatment with regard to neurotensin; however, levels were generally higher during exendin(9–39)NH₂ infusion in the acarbose period (Fig. 6C, D and Table 2). No differences were observed in *ad libitum* food intake (for saline days in placebo treatment 451 ± 269 g vs 343 ± 176 g in acarbose period, $P = 0.3642$) (Supplementary Fig. 1A, see section on supplementary materials given at the end of this article) or appetite measures assessed by VASs (hunger, satiety, fullness, prospective food consumption, comfort, nausea and thirst) (Supplementary Fig. 2 and Supplementary Table 2).

Gastrin, CCK and gallbladder volume

We observed no significant differences in gastrin and CCK levels or gallbladder volume when comparing the two treatment periods or when comparing the effects of exendin(9–39)NH₂ and saline infusion (Supplementary Figs 3, 4 and Supplementary Table 2).

Discussion

We confirm that acarbose improves postprandial glucose tolerance compared to placebo; shows acarbose-induced doubling of postprandial GLP-1 responses; using the GLP-1 receptor antagonist exendin(9–39)NH₂, we did not see an impact of acarbose-induced GLP-1 secretion on absolute measures of postprandial glucose tolerance,

Figure 4

Gastric emptying assessed by paracetamol. Plasma paracetamol levels (A) and corresponding gastric emptying (B) in minutes after intake of paracetamol with liquid mixed meal expressed as mean percentage of the meal retained in stomach (time 0–240 min) during four liquid mixed meal tests (time 0 to 240 min) with continuous i.v. infusion of exendin(9–39)NH₂ (closed symbols) and saline (open symbols), respectively, performed after 14-day treatment periods with placebo (circles) and acarbose (squares). Data are presented as mean $\pm$ S.E.M. ex(9–39)NH₂, exendin(9–39)NH₂.

but relatively, the effect of exendin(9–39)NH₂ was most pronounced during acarbose.

Alpha-glucosidase inhibition induces GLP-1 secretion

Our data confirm that acarbose-induced transfer of polysaccharides to the more distal parts of the small intestine affects several of the gut hormones including GLP-1 (6, 8, 10). In our design, acarbose treatment for 14 days almost doubled the postprandial GLP-1 response after liquid mixed meal intake both during saline and exendin(9–39)NH₂ infusion, compared to placebo. The effect of acarbose on GLP-1 secretion has been investigated quite extensively with differing results. Some single-dose studies or studies investigating shorter acarbose treatment courses have not been able to measure an effect of acarbose on GLP-1 secretion (9, 24, 25, 26). The effect of acarbose could depend on longer treatment as illustrated by the present study, and a study by Zheng *et al.* who observed higher levels of both fasting and postprandial GLP-1 during a solid mixed meal test after 24 weeks of acarbose treatment in drug-naïve individuals with prediabetes (11). In addition, the dose of acarbose seems to constitute an important determinant of GLP-1 secretion; when comparing 25, 50 and 100 mg single-doses, only the 100 mg dose significantly increased GLP-1 levels during solid

Figure 5

Insulin, C-peptide, C-peptide:glucose ratio and glucagon. Plasma levels of insulin (A), C-peptide (C), C-peptide:glucose ratio (E) and glucagon (G) and corresponding bsAUC (time 0–240 min) (B, D, F and H) during four liquid mixed meal tests (initiated at time 0, dotted line) with continuous i.v. infusion of exendin(9–39)NH₂ (closed symbols) and saline (open symbols), respectively, performed after 14-day treatment periods with placebo (circles) and acarbose (squares). Data were compared by rmANOVA with Greenhouse-Geisser correction and Tukey multiple comparison. Significant differences for the *post hoc* analyses are marked with asterisks: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Data are presented as mean $\pm$ S.E.M. ex(9–39)NH₂, exendin(9–39)NH₂.

Figure 6

PYY and neurotensin. Plasma levels of PYY (A) and neurotensin (C) and corresponding bsAUC (time 0–240 min) (B and D) during four liquid mixed meal tests (initiated at time 0, dotted line) with continuous i.v. infusion of exendin(9–39)NH₂ (closed symbols) and saline (open symbols), respectively, performed after 14-day treatment periods with placebo (circles) and acarbose (squares). Data were compared by rmANOVA with Greenhouse-Geisser correction and Tukey multiple comparison. Significant differences for the *post hoc* analyses are marked with asterisks: **P* < 0.05; ****P* < 0.001; *****P* < 0.0001. Data are presented as mean ± S.E.M. ex(9–39)NH₂, exendin(9–39)NH₂.

mixed meal ingestion (25, 27). Another factor affecting acarbose-induced GLP-1 secretion seems to be the diet composition. A single dose of 100 mg acarbose during an oral sucrose (disaccharide) load resulted in elevated GLP-1 levels compared to placebo (8, 10, 28, 29) whereas no effect was seen during oral glucose tolerance test (24), emphasizing that acarbose needs relevant carbohydrates to ‘act on’ in order to increase GLP-1 secretion (24). In line with this notion, the glucose-lowering efficacy of acarbose has been shown to depend on the amount of polysaccharides/starch in the diet (30). In this study, we used a liquid mixed meal test with 49% polysaccharides (primarily maltodextrin); thus one could speculate that our estimate is conservative compared to what could be expected from a solid meal meal with a greater content of polysaccharides and fibers (12). Taken together, longer treatment periods with maximum dosing of acarbose

and a high amount of starch and fibers in the diet seem important for the GLP-1-inducing action of acarbose.

Alpha-glucosidase inhibition improves glucose metabolism

Acarbose treatment is generally thought to reduce postprandial hyperglycemia by inhibiting carbohydrate hydrolysis, and, thus, retarding and inhibiting glucose absorption. In order to delineate whether acarbose-induced GLP-1 secretion contributes to the glucose-lowering effect of acarbose during a meal, we investigated the effect of acarbose on postprandial glucose excursions with and without blockade of the GLP-1 receptor using exendin(9–39)NH₂. To our knowledge, no previous studies have utilized exendin(9–39)NH₂ to isolate GLP-1-mediated effects induced by acarbose. The study was powered to detect a minimal difference of 25% in plasma glucose excursions during a standardized mixed meal test. This minimal difference was based on the judgment that a ≥25% change in plasma glucose excursions during a standardized mixed meal test is clinically and physiologically relevant. We chose to express the minimal relevant difference in percent (relative difference) as no reference material exist, and because we expected a high degree of inter-individual variation in absolute AUC values for plasma glucose during a standardized mixed meal test in patients with type 2 diabetes. We observed that exendin(9–39)NH₂ more than doubled bsAUC for glucose during the mixed meal test in the acarbose treatment period (a 119% increase) – significantly more than the exendin(9–39)NH₂-induced increase in bsAUC of 39% during the placebo treatment period. In absolute terms, nevertheless, exendin(9–39)NH₂ affected postprandial glucose tolerance (as assessed by bsAUC) similarly during the acarbose and placebo treatment periods. Using exendin(9–39)NH₂, we were not able to show a greater GLP-1-mediated insulin secretion or beta cell sensitivity to glucose during the acarbose treatment period. Whether this relates to the much smaller postprandial glucose excursions and insulin secretory responses during acarbose treatment or the well-known compensatory rise in postprandial glucagon levels during exendin(9–39)NH₂ infusions (which in the present study was highest during the acarbose treatment period), or other factors, is uncertain. Similar observations have been made previously in studies using exendin(9–39)NH₂, and together these observations indicate that results obtained with exendin(9–39)NH₂ regarding beta cell function *in vivo* should be interpreted with care (31). Specifically,

the markedly increased glucagon secretion may be responsible for a considerable paracrine stimulation of insulin secretion via the unblocked glucagon receptors (presumably due to inhibition of GLP-1-mediated effects in the pancreatic islets) (32).

Alpha-glucosidase inhibition does not affect CCK and gallbladder volume

Acarbose has previously been shown to increase CCK secretion (12, 33), and we have previously demonstrated that CCK-induced gallbladder emptying stimulates GLP-1 secretion (34, 35). In the present study we found no difference in CCK release or gallbladder emptying between treatment periods and, thus, no indications that acarbose-induced GLP-1 secretion involves augmented CCK secretion and gallbladder emptying.

Alpha-glucosidase inhibition delays gastric emptying and results in lower GIP levels

Our study confirms previous findings that acarbose delays gastric emptying (12, 28). When we inhibited GLP-1 receptor signaling with exendin(9–39)NH₂, gastric emptying of the liquid meal was equally accelerated in both treatment periods, and, thus, acarbose-induced deceleration of gastric emptying does not seem to rely – at least not entirely – on acarbose-induced GLP-1 secretion. As previously reported (24), postprandial GIP levels were diminished by acarbose treatment, consistent with the notion that most enteroendocrine GIP-secreting K cells are located in the duodenum and proximal jejunum, which during acarbose treatment absorb less glucose than under normal circumstances (2). Acarbose-induced deceleration of gastric emptying may also contribute to the lower postprandial GIP response observed in the acarbose treatment period.

Alpha-glucosidase inhibition does not affect appetite sensations or ad libitum food intake

It has been shown that release of GLP-1 in combination with the other L cell product PYY exerts a satiety-promoting effect (36). In contrast to previous studies (12, 37), the potentiation of the postprandial PYY response during acarbose treatment was not statistically significant. In line with this, we were not able to detect any significant changes in appetite sensations or food intake *ad libitum* with acarbose treatment. However, the power of our study with regard to these exploratory

endpoints is inadequate to rule out potentially relevant differences.

Limitations

Despite rejection of the null hypothesis, we cannot rule out that with a larger sample size, differences in secondary endpoints, for example, postprandial plasma responses of PYY and neurotensin, could have been detected. The liquid mixed meals were identical and standardized, and throughout both treatment periods, the study participants were instructed to continue their usual diets. However, food intake was not logged (neither qualitatively or quantitatively), and it is possible that irregular diets or varying content of carbohydrates could have influenced the postprandial glucose excursions in this study. We used a crossover design and randomisation of treatment sequences to minimize these potential biases.

Conclusion

We confirm that acarbose treatment stimulates postprandial GLP-1 secretion in patients with type 2 diabetes. Using exendin(9–39)NH₂, we did not see an impact of acarbose-induced GLP-1 secretion on absolute measures of postprandial glucose tolerance, but relatively, the effect of exendin(9–39)NH₂ was most pronounced during acarbose treatment.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-20-1121>.

Declaration of interest

J J H serves on advisory boards for Novo Nordisk. T V has served on scientific advisory panels, been part of speaker's bureaus for, served as a consultant to and/or received research support from Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Gilead, Mundipharma, MSD/Merck, Novo Nordisk, Sanofi and Sun Pharma. F K K has served on scientific advisory panels and/or been part of speaker's bureaus for, served as a consultant to and/or received research support from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Carmot Therapeutics, Eli Lilly, Gubra, MedImmune, MSD/Merck, Mundipharma, Norgine, Novo Nordisk, Sanofi and Zealand Pharma. Filip K Knop is on the editorial board of EJE. Filip K Knop was not involved in the review or editorial process for this paper, on which he/she is listed as an author. The other authors have nothing to disclose.

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Guarantor statement

All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Author contribution statement

N B D, L S H and N L H performed the study. B H, S S and L S G performed the biochemical analysis. N B D, L S G and F K K designed the study and wrote the study protocol. F K K conceptualized and academically supervised the study. J J H, J F R and B H generated data. N B D, L S G and F K K drafted the manuscript. All authors contributed substantially to the acquisition, analysis or interpretation of data for the work, critically revised the manuscript for important intellectual content, and approved the final version.

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