

# Prolonged and Enhanced Secretion of Glucagon-like Peptide 1 (7-36 Amide) After Oral Sucrose Due to $\alpha$ -Glucosidase Inhibition (Acarbose) in Type 2 Diabetic Patients

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GLP-1, an incretin hormone of the enteroinsular axis with insulinotropic and glucagonostatic activity, is secreted after nutrient ingestion. GLP-1 is mainly produced by intestinal L-cells in the lower gastrointestinal tract (GIT); simple carbohydrates are absorbed in the upper GIT and  $\alpha$ -glucosidase inhibition leads to augmented and prolonged GLP-1 release in normal subjects. In a cross-over study, 100 mg acarbose or placebo was administered simultaneously with 100 g sucrose to 11 hyperglycaemic Type 2 diabetic patients poorly controlled with diet and sulphonylureas. Plasma levels of GLP-1, insulin, C-peptide, glucagon, GIP, glucose and H<sub>2</sub>-exhalation were measured over 6 h. Differences in the integrated responses over the observation period were evaluated by repeated measurement analysis of variance with fasting values used as covariates. With acarbose, sucrose reached the colon 60–90 min after ingestion as indicated by a significant increment in breath hydrogen exhalation ( $p=0.005$ ). After an early GLP-1 increment 15 min after sucrose under both conditions, GLP-1 release was prolonged in the acarbose group ( $p=0.001$ ; significant from 210 to 360 min.). Initially (0–150 min), glucose ( $p=0.001$ ), insulin ( $p=0.001$ ), and GIP ( $p<0.001$ ) were suppressed by acarbose, whereas later there were no significant differences. Glucagon levels were higher with acarbose in the last 3 h of the 6 h observation period ( $p=0.02$ ). We conclude that in hyperglycaemic Type 2 diabetic patients, ingestion of acarbose with a sucrose load leads to elevated and prolonged GLP-1 release. © 1998 John Wiley & Sons, Ltd.

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## Introduction

Glucagon-like peptide (7-36 amide, GLP-1) is a physiological incretin, produced by proteolytic processing of preproglucagon in intestinal L-cells of the lower gastro-intestinal tract (GIT).<sup>1–4</sup> After nutrient intake GLP-1, like gastric inhibitory polypeptide or GIP, another incretin hormone secreted from duodenal K-cells,<sup>5–7</sup> participates in the regulation of postprandial glucose homeostasis due to its insulinotropic and glucagonostatic activity.<sup>8–11</sup> As a result of the secretion and action of incretin hormones, glucose administered orally enhances insulin secretion in normal

subjects more than intravenous glucose—the incretin effect.<sup>12–14</sup>

Although the magnitude of the incretin effect has been reported to be less pronounced in Type 2 diabetic patients,<sup>15</sup> recent studies have shown that pharmacological doses of GLP-1 (7-36 amide) retain much of their insulinotropic action<sup>9,16</sup> and can normalize fasting hyperglycaemia in this population.<sup>17</sup>

Since L-cells are mainly located in the lower GIT (ileum, colon, rectum)<sup>1,2,18–20</sup> and can be stimulated by luminal glucose,<sup>21,22</sup> it is no surprise that a delay in carbohydrate absorption through inhibition of the luminal brush border enzyme  $\alpha$ -glucosidase potentiates GLP-1 (7-36 amide) release after sucrose loads in normal subjects.<sup>23,24</sup>

In metabolically healthy, normoglycaemic subjects, only a minor influence of elevated GLP-1 concentrations on insulin, glucagon or glucose concentrations can be expected, because both the insulinotropic<sup>8,17,25</sup> and the glucagonostatic<sup>17</sup> actions strongly depend on some degree

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of hyperglycaemia. This, however, might be different in patients with Type 2 diabetes mellitus (DM), where GLP-1 (7-36 amide) secretion induced by acarbose ingestion might explain the reduction in fasting glycaemia that has been reported to occur after acarbose treatment<sup>26,27</sup> which is not explained by its primary mode of action.

It was the aim of the present study to evaluate GLP-1 (7-36 amide) release and associated changes in plasma glucose, insulin, C-peptide, and GIP levels after oral sucrose combined with 100 mg acarbose in comparison to placebo in Type 2 diabetic patients. Preliminary results have been reported in abstract form.<sup>28</sup>

## Subjects, Materials and Methods

### Study Protocol

The study was approved by the ethics committee of the medical faculty of the Ruhr University Bochum on 20 March 1995 (registration number 608). Written informed consent was obtained from all participants.

### Subjects

Eleven Type 2 diabetic patients (6 male, 5 female with a mean age of  $63 \pm 9$  years) were studied. Fasting hyperglycaemia ( $\geq 7.8$  mmol L<sup>-1</sup>) on two occasions was an inclusion criterion. HbA<sub>1c</sub> was  $9.7 \pm 1.4$  % (non-diabetic range: 4.0–6.2 %) under therapy with diet alone ( $n = 3$ ) or glibenclamide ( $n = 8$ ). This medication was continued throughout the study. In 7 patients, the glibenclamide dose was 10.5 mg day<sup>-1</sup>; in the remaining patient, it was 7 mg day<sup>-1</sup>. Body mass index was  $30.1 \pm 2.5$  kg m<sup>-2</sup>. No patient had been on metformin or insulin treatment.

### Study Design

In randomized order, acarbose (100 mg) or placebo was administered orally together with a 100 g sucrose load on days 1 and 3, respectively. Previous data have indicated that almost all acarbose after a large single oral dose (300 mg) is excreted within a 24 h period, with negligible quantities remaining after 48 h.<sup>29</sup> A regular meal and drug schedule was allowed for 1 day between the experiments. All experiments were started in the morning after at least a 10 h fast.

### Experimental Procedures

The patients were in a semi-recumbent position throughout the tests. A teflon cannula (Vasofix, 1.2 mm diameter, B. Braun, Melsungen, Germany) was inserted into a distal forearm vein and kept patent with isotonic saline for blood sampling. Baseline samples were collected at -30, -15, and 0 min. One hundred grams sucrose, dissolved in 400 ml tap water, were ingested rapidly, simultaneously with 100 mg  $\alpha$ -glucosidase inhibitor

acarbose as tablets (Glucobay®, Bayer AG, Leverkusen, Germany) or with a placebo tablet. Venous blood samples were collected every 15 min during the first hour, every 30 min from the second to the fourth hour and every 60 min during the last 2 h of the 360 min sampling period.

Breath samples for measurement of forced end-expiratory H<sub>2</sub>-concentrations were collected simultaneously with blood sampling throughout the study as an indicator for bacteria-mediated decomposition of carbohydrate reaching the colon.<sup>30</sup> An electrochemical H<sub>2</sub> detector was used (GMI Medical Ltd, Stimotron, Windelstein, Germany).

Blood was drawn into tubes containing ethylenediaminetetraacetic acid (EDTA) and aprotinin (Trasylol®; 20.000 KIU ml<sup>-1</sup>, 200 µl 10 ml<sup>-1</sup> blood; Bayer AG, Leverkusen, Germany). A sample was stored in sodium fluoride (Microvette CB 300, Sarstedt, Numbrecht, Germany) for the subsequent measurement of glucose. After centrifugation, plasma for hormone analyses was kept frozen at -30°C.

### Laboratory Determinations

Glucose was measured using a glucose oxidase method (Beckman Instruments, Munich, Germany). Insulin was measured using an insulin microparticle enzyme immunoassay (MEIA, IMx Insulin, Abbott Laboratories, Wiesbaden, Germany). Results show a correlation coefficient of 0.982 in comparison to RIA 100 (Pharmacia, Freiburg, Germany), the assay used in our previous study in normal subjects.<sup>24</sup> Intra-assay coefficients of variation were less than 4.0 %. C-peptide was measured using an enzyme immunoassay (DRG Instruments GmbH Marburg, Germany). Human insulin and C-peptide were used as standard. GIP was determined using antiserum R65 and synthetic human GIP for the preparations of standards and <sup>125</sup>I-GIP tracer (purified by HPLC).<sup>31</sup> IR-GLP-1 was measured in ethanol-extracted plasma,<sup>32</sup> using antiserum 89390 (final dilution 1:150 000) and synthetic GLP-1 (7-36 amide) (Peninsula Laboratories, St Helens, UK) for tracer preparation and as standard. This antiserum reacts specifically with the amidated carboxy-terminus of GLP-1 (7-36 amide), and therefore the measurements largely represent the gut-derived, truncated GLP-1 (7-36 amide).<sup>33</sup> The intra-assay coefficient of variation was 6 %. Pancreatic glucagon was assayed in ethanol-extracted plasma using antibody 4305.<sup>34</sup> All plasma samples from one patient were assayed at the same time to avoid errors due to inter-assay variation.

### Statistical Analyses

Patient characteristics are given as mean  $\pm$  standard deviation (SD), results are reported as mean  $\pm$  standard error of the mean (SEM). Integration was carried out according to the trapezoidal rule. Glycaemic excursions and secretory responses were analysed as area under

curve. The significance of differences were tested using repeated measures analysis of variance (RM-ANOVA), with baseline values used as covariates. If appropriate, data were transformed logarithmically for this purpose. Significances of differences at individual time points were estimated from respective 95 % confidence intervals as calculated by the analysis of covariance (ANCOVA) model. A  $p$ -value  $<0.05$  was taken to indicate significant differences. Calculations were carried out using SAS Version 6.08 on a DEC VAX 3100 computer.

## Results

At 15–30 min after sucrose ingestion, plasma levels of GLP-1 (7-36 amide) reached their maximum (Figure 1). After the first hour, GLP-1 levels decreased but this was delayed with acarbose. With placebo, GLP-1 concentrations remained significantly ( $p<0.05$ ) elevated over mean baseline concentrations until 210 min after ingestion of sucrose, whereas with acarbose, they remained elevated for the whole study period (360 min). The timing of elevated GLP-1 levels after acarbose was correlated with an increased  $H_2$ -exhalation (Figure 1), indicating the presence of sucrose in the colon at that time. Acarbose maintained significantly higher GLP-1 (7-36 amide) plasma levels than placebo from 210 to 360 min (by repeated measures analysis of variance:  $p=0.03$ ).

The postprandial elevation in plasma glucose was lower after acarbose ( $p<0.001$ ) and this was accompanied by a smaller rise in insulin concentrations ( $p=0.001$ ). A similar trend was seen for C-peptide concentrations but the difference was not significant (Figure 2). There was no difference in plasma glucose, insulin or C-peptide levels at 150 min after sucrose ingestion and thereafter (Figure 2), while GLP-1 (7-36 amide) levels remained significantly elevated (Figure 1). The integrated incremental area for GLP-1 (7-36 amide) responses was significantly greater with acarbose, when calculated for the whole 6 h period ( $p=0.048$ ), whereas this difference was not significant for the first 4 h after sucrose ingestion.

The plasma levels of gastric inhibitory peptide (GIP) were clearly reduced ( $p=0.001$ ) through  $\alpha$ -glucosidase inhibition by acarbose (Figure 1). Plasma glucagon concentrations increased immediately after sucrose ingestion and remained higher ( $p<0.001$ ) from 180 min onwards with acarbose than with placebo (Figure 3).

The patterns of glucose, insulin, C-peptide, GLP, glucagon, and  $H_2$  responses (Figures 1,2 and 3) were confirmed by the comparison of integrated responses (Tables 1 and 2).

## Discussion

The present study demonstrates an augmented and prolonged release of GLP-1, when oral sucrose is administered together with acarbose in patients with Type 2 diabetes mellitus. These results are similar to

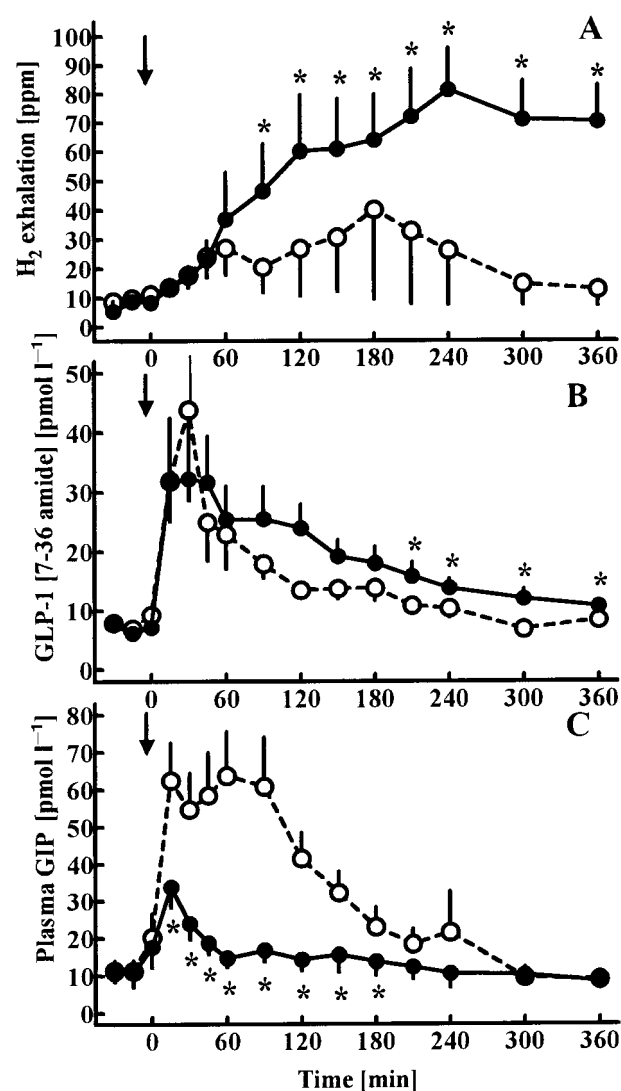


Figure 1. Breath hydrogen response (A), plasma GLP-1 (7-36 amide) (B), and gastric inhibitory peptide (GIP) concentrations (C) after oral sucrose (arrow) with (●—●) and without (○---○) the concomitant ingestion of the  $\alpha$ -glucosidase-inhibitor acarbose (100 mg). Mean  $\pm$  SEM,  $n=11$ . Repeated measures analysis of covariance indicated the following  $p$ -values regarding treatment (A), time (B) and the interaction of treatment and time (AB):  $H_2$ -exhalation: A:  $p=0.005$ , B:  $p=0.001$ , AB:  $p<0.001$ ; plasma GLP-1: A:  $p=0.001$ , B:  $p<0.001$ , AB:  $p=0.003$ ; plasma GIP: A:  $p<0.001$ , B:  $p<0.001$ , AB:  $p<0.001$ . Asterisks indicate significant differences ( $p<0.05$ ) at single time points

those obtained in a previous study performed in younger, non-diabetic subjects.<sup>24</sup> The most likely mechanism is that acarbose prevents rapid hydrolysis of sucrose, postponing the process of  $\alpha$ -glucosidase action to later time points and more distal areas of the gastrointestinal tract.<sup>35</sup> The consequence is the presence of chyme in the lower jejunum, ileum and eventually colon; locations with abundant L-cells.<sup>1,18,20</sup> Contrary to the normal situation, nutrients may, under the influence of acarbose, come into contact with L-cells and directly trigger release of GLP-1.<sup>21,24,36,37</sup> The presence of sucrose in the colon can be inferred from  $H_2$ -generation by colonic bacteria and occurred at approximately the same time as the

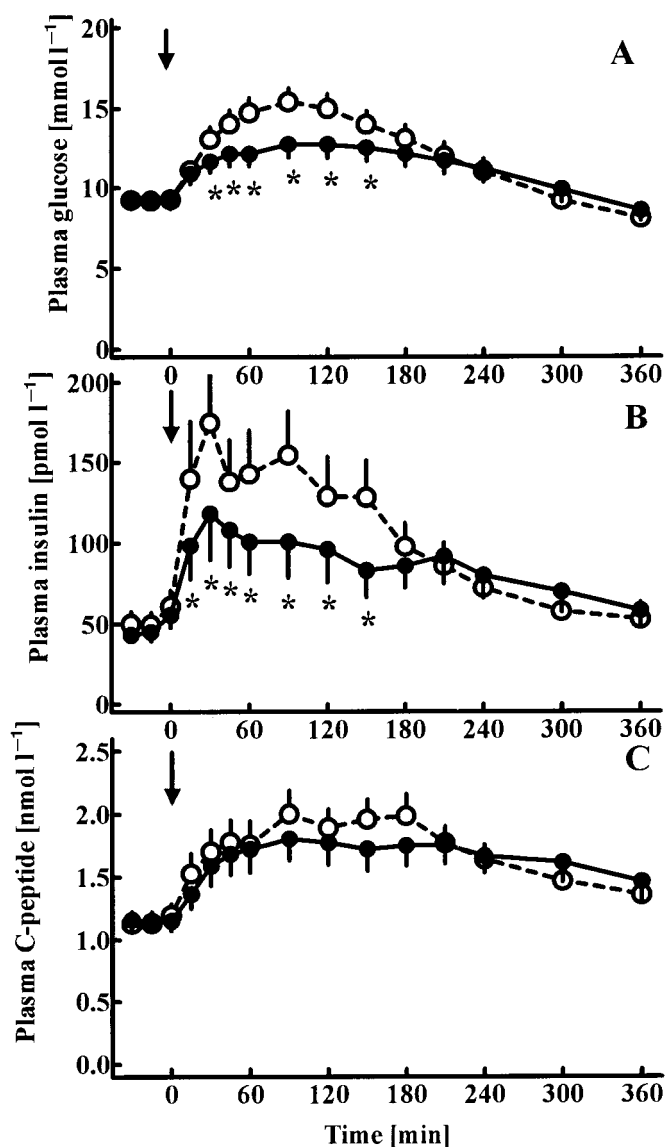


Figure 2. Responses of plasma glucose (A), insulin (B), and C-peptide (C) concentrations after oral sucrose (arrow) with (●—●) and without (○---○) the concomitant ingestion of the  $\alpha$ -glucosidase-inhibitor acarbose (100 mg). Mean  $\pm$  SEM;  $n = 11$ . Repeated measures analysis of covariance indicated the following  $p$ -values regarding treatment (A), time (B) and the interaction of treatment and time (AB): plasma glucose: A:  $p = 0.049$ , B:  $p < 0.001$ , AB:  $p < 0.001$ ; plasma insulin: A:  $p = 0.014$ , B:  $p < 0.001$ , AB:  $p < 0.001$ ; plasma C-peptide: A:  $p = 0.16$ , B:  $p < 0.001$ , AB:  $p = 0.54$ . Asterisks indicate significant differences ( $p < 0.05$ ) at single time points

onset of exaggerated GLP-1 responses. The situation is different in the case of GIP, which stems from the duodenum.<sup>5,6</sup> As shown in previous studies,<sup>24,38</sup> GIP release is reduced by acarbose because it is not the presence of appropriate nutrients in the GIT lumen but their absorption<sup>39</sup> that leads to GIP secretion.  $\alpha$ -glucosidase inhibition prevents carbohydrate absorption in the upper gastrointestinal tract.<sup>35</sup>

In comparison to our previously studied group of healthy volunteers, the difference in GLP-1 concentrations was smaller in the diabetic patients (28 % based on 4 h

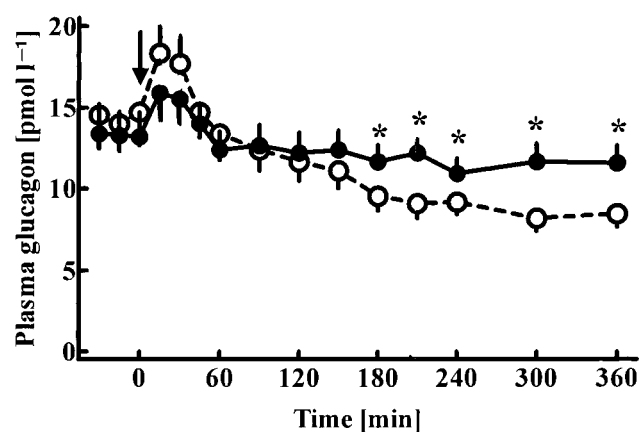


Figure 3. Plasma glucagon concentrations after oral sucrose (arrow) with (●—●) and without (○---○) the concomitant ingestion of the  $\alpha$ -glucosidase-inhibitor acarbose (100 mg). Mean  $\pm$  SEM;  $n = 11$ . Repeated measures analysis of covariance indicated the following  $p$ -values regarding treatment ( $p = 0.02$ ), time ( $p < 0.001$ ), and the interaction of treatment and time ( $p < 0.001$ ). Asterisks indicate significant differences at single time points

areas under the curve vs 200 % in normal subjects<sup>24</sup>). Rather, a prolonged effect of acarbose was observed, with a significant difference in 6 h AUCs, and a significant difference remained at the latest time point (6 h) studied (Figure 1). Since the previous study<sup>24</sup> was performed in younger ( $25 \pm 4$  years) subjects, this difference may relate in part to age, but there may be quantitative differences between acarbose effects on GLP-1 concentrations in normally glucose tolerant and Type 2 diabetic subjects. The most likely explanation is provided by the finding of enhanced  $\alpha$ -glucosidase activity in diabetic versus non-diabetic animals,<sup>40,41</sup> possibly due to non-enzymatic glycation and reduced turnover. A pre-existing excess of  $\alpha$ -glucosidase activity in the gut mucosa of Type 2 diabetic patients could compensate for acarbose effects or require a larger dose to get a similar degree of enzyme inhibition. This hypothesis, however, is only supported by animal experiments.

Exogenous administration of larger amounts of GLP-1 stimulates insulin and reduces glucagon.<sup>16,17,42</sup> There is no evidence for a similar effect of the smaller, endogenous elevation of GLP-1 from the present data, because during the period characterized by elevated GLP-1 plasma concentrations (210 to 360 min), insulin concentrations were not higher and glucagon levels were not lower in the experiments with acarbose. Furthermore, plasma glucose was not lower. This apparent discrepancy may be explained by insufficient increments in GLP-1 concentrations, by continuing inflow of glucose and fructose from the gut during the 6 h study period and by differences in hepatic glucose output.<sup>43,44</sup> It appears likely that acarbose treatment can lead to a decreased suppression of hepatic output after the ingestion of carbohydrate meals.

Acarbose will profoundly influence the time course of postprandial glucose metabolism, leading to prolonged absorption at a lower rate and a reduced stimulation of

Table 1. Integrated responses (AUC, trapezoid rule) over 6 h after a 100 g sucrose load with and without ingestion of 100 mg acarbose as calculated by the ANCOVA model (crossover design)

Parameter	Measured		Calculated by ANCOVA model		
	Acarbose	Placebo	Difference	(95 % confidence interval)	p-value
H <sub>2</sub> -exhalation (ppm·min)	20832 ± 4365	8478 ± 4636	14886	(5585 to 24186)	0.0062
GLP-1 (pmol·l <sup>-1</sup> min)	6786 ± 1021	5302 ± 738	1325	(13 to 2640)	0.048
GIP (pmol·l <sup>-1</sup> min)	4912 ± 849	11185 ± 2032	-5856	(-9084 to -2634)	0.003
Plasma glucose (mmol·l <sup>-1</sup> min)	4077 ± 255	4350 ± 261	-309	(-556 to -61)	0.02
Insulin (nmol·l <sup>-1</sup> min)	30.7 ± 5.4	36.5 ± 6.1	-4.8	(-10.0 to 0.5)	0.07
C-peptide (nmol·l <sup>-1</sup> min)	596.0 ± 59.5	593 ± 43.2	-21.8	(-72.7 to 30.3)	0.37
Glucagon (pmol·l <sup>-1</sup> min)	4425 ± 354	3941 ± 354	+576	(204 to 949)	0.007

Mean ± SEM.

Table 2. Integrated responses (AUC, trapezoid rule) over 4 h after a 100 g sucrose load with and without ingestion of 100 mg acarbose as calculated by the ANCOVA model (crossover design)

Parameter	Measured		Calculated by ANCOVA model		
	Acarbose	Placebo	Difference	(95 % confidence interval)	p-value
H <sub>2</sub> -exhalation (ppm·min)	12017 ± 3040	6433 ± 3521	7740	(332 to 15150)	0.042
GLP-1 (pmol·l <sup>-1</sup> min)	5333 ± 920	4331 ± 662	802	(-425 to 2026)	0.17
GIP (pmol·l <sup>-1</sup> min)	3810 ± 627	9723 ± 1573	-5574	(-8220 to -2880)	0.001
Plasma glucose (mmol·l <sup>-1</sup> min)	2889 ± 182	3224 ± 182	-367	(-554 to -179)	0.002
Insulin (nmol·l <sup>-1</sup> min)	22.4 ± 4.1	29.3 ± 5.2	-5.5	(-9.9 to -1.1)	0.02
C-peptide (nmol·l <sup>-1</sup> min)	405.0 ± 33.7	436.0 ± 33.3	-31.1	(-67.3 to 5.1)	0.08
Glucagon (pmol·l <sup>-1</sup> min)	3053 ± 253	2921 ± 274	270	(5.4 to 535.2)	0.047

Mean ± SEM.

insulin secretion, especially since GIP release is blunted. The latter incretin however may be less important in Type 2 diabetic patients, because physiological doses of GIP are nearly ineffective as an insulinotropic agent in Type 2 diabetic patients.<sup>9</sup> As a result, there is an effect of acarbose on postprandial plasma glucose concentrations which probably explains the differences in glucagon during the latter part of our study.

In conclusion, GLP-1 increments are augmented and prolonged by acarbose in hyperglycaemic Type 2 diabetic patients, too. The effect, however, appears to be somewhat smaller in hyperglycaemic Type 2 diabetic patients than in previously studied healthy volunteers.

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