

Exendin-4, but not dipeptidyl peptidase IV inhibition, increases small intestinal mass in GK rats

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Simonsen L, Pilgaard S, Orskov C, Rosenkilde MM, Hartmann B, Holst JJ, Deacon CF. Exendin-4, but not dipeptidyl peptidase IV inhibition, increases small intestinal mass in GK rats. *Am J Physiol Gastrointest Liver Physiol* 293: G288–G295, 2007. First published April 12, 2007; doi:10.1152/ajpgi.00453.2006.—Long-term treatment with dipeptidyl peptidase IV inhibitors (DPP-IV) or glucagon-like peptide (GLP)-1 analogs may potentially affect intestinal growth by down- or upregulating the intestinotrophic hormone GLP-2. This study compared the intestinotrophic effects of 12-wk administration of vehicle, exendin-4 (Ex-4; 5 nmol/kg bid sc), or DPP-IV (NN-7201, 10 mg/kg qd orally) in GK rats. Some animals were observed additionally for 9 wk after the end of treatment. Both treatments lowered glycated hemoglobin A1c at wk 12 vs. control (Ex-4, -0.8% ; DPP-IV, -0.4%). Body weight was reduced by Ex-4 compared with control (361 ± 4 vs. 399 ± 5 g; $P < 0.001$) because of reduced food intake, whereas neither parameter was affected by DPP-IV. Linear bone growth was unaffected by either treatment. After treatment end, food intake in Ex-4 animals increased, and, by wk 21, body weight was identical in all groups. The small intestine of Ex-4-treated animals was larger at wk 12 compared with control (length, 135.6 ± 1.6 vs. 124.5 ± 2.3 cm, $P < 0.001$; absolute weight, 8.4 ± 0.2 vs. 6.4 ± 0.4 g, $P < 0.001$), being most pronounced proximally, where the absolute cross-sectional area related to body weight increased by 24% because of increased mucosal thickness. These effects were reversible, and 9 wk after the end of treatment, no differences between Ex-4 and control were apparent. Plasma GLP-2 concentrations were unaltered by either treatment, and Ex-4 had no agonistic or antagonistic effects on the transfected GLP-2 receptor. DPP-IV had no intestinal effects. In conclusion, the continued presence of Ex-4 is necessary to maintain weight loss in GK rats. Effective antihyperglycemic treatment with Ex-4 increases intestinal mass reversibly, whereas DPP-IV lacks intestinal effects.

glucagon-like peptide-1; glucagon-like peptide-2; glucagon-like peptide-1 receptor; glucagon-like peptide-2 receptor; intestinotrophic

THE INTESTINAL PEPTIDE glucagon-like peptide (GLP)-2 has been identified as an endocrine regulator of small intestinal growth. Endogenous GLP-2, released in response to orally ingested nutrients, is involved in adaptive intestinal growth, whereas exogenous GLP-2 induces increased proliferation of both the small and large intestine (13). Thus the physiological effect of GLP-2 may be to adjust the absorptive capacity of the intestine to the supply of nutrients by stimulating mucosal growth while decreasing gastric emptying and increasing intestinal transit time (35). GLP-2 originates from the proglucagon gene and is cosecreted from the L-cells in equimolar amounts with its sister

peptide, the incretin hormone GLP-1 (27). GLP-1 has potent antidiabetic effects (8), but its rapid degradation by dipeptidyl peptidase IV (DPP-IV) (9) means the native peptide is not suitable as a therapeutic agent. Alternative approaches, such as longer-acting DPP-IV-resistant GLP-1 analogs and use of DPP-IV inhibitors to enhance levels of endogenously released GLP-1, are therefore being investigated for treatment of type 2 diabetes. The naturally occurring GLP-1 receptor agonist exendin-4 (Byetta) was launched in the United States in 2005, and the DPP-IV inhibitor sitagliptin (Januvia) was recently approved by the United States Food and Drug Administration, with others [e.g., vildagliptin (Galvus)] awaiting approval.

In addition to cleaving GLP-1, DPP-IV also cleaves GLP-2 (1–33) to generate a metabolite, GLP-2-(3–33), which is a partial agonist and may behave as a competitive antagonist (20). Accordingly, studies in rats and mice have shown that the intestinotrophic effect of exogenous GLP-2 is enhanced by concomitant administration of a DPP-IV inhibitor (22). However, the increase in intact GLP-1 promoted by the administration of a DPP-IV inhibitor may in itself affect L-cell secretion and thereby indirectly reduce GLP-2 secretion. Thus it has been suggested that a negative feedback loop exists, whereby GLP-1 inhibits further L-cell secretion, and in humans, infusion of GLP-1-(7–37) has been demonstrated to reduce endogenous GLP-1 secretion (36), whereas infusion of GLP-1-(7–36) amide reduces endogenous GLP-2 secretion (15). In dogs, DPP-IV inhibition reduces meal-induced incretin secretion (10), and similar findings have been reported in clinical trials with DPP-IV inhibitors (13a). In the isolated perfused porcine intestine in vitro, the same tendency for GLP-1 to inhibit L-cell secretion has also been confirmed, and it seems this negative feedback may be mediated by a GLP-1-stimulated increase in somatostatin secretion (19). Because GLP-2 originates from the same precursor as GLP-1, it follows that increased concentrations of intact endogenous GLP-1 induced by DPP-IV inhibition, as well as administration of long-acting GLP-1 receptor agonists, could initiate a negative feedback mechanism, whereby not only endogenous GLP-1 but also GLP-2 secretion is reduced. However, with inhibitor administration, reduced secretion may be counterbalanced by reduced degradation. With the GLP-1 receptor agonists, reduced endogenous GLP-1 secretion would be compensated for by the administered GLP-1 agonist, but the simultaneously reduced GLP-2 secretion may affect intestinal growth. In support of this, it has

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previously been shown (21) that adaptive intestinal growth in rats is compromised when the action of endogenous GLP-2 is blocked by immunoneutralization.

Thus, although both GLP-1 receptor agonists and DPPIV inhibitors show great promise as antihyperglycemic agents, it is hard to predict whether long-term administration will have intestinal effects and, if so, how intestinal growth will be affected. We therefore investigated the intestinotrophic effects of long-term administration of exendin-4 and a DPPIV inhibitor in the diabetic GK rat.

MATERIALS AND METHODS

Animals. The animal studies were in accordance with international guidelines (National Institutes of Health publication no. 85-23, revised 1985, and Danish legislation governing animal experimentation, 1987) and were carried out after permission had been granted by the Animal Experiments Inspectorate, Ministry of Justice, Denmark. The study was performed in the GK rat, which is a nonobese rat model that spontaneously develops Type 2 diabetes around 8 wk of age; the diabetes resembles the human Type 2 diabetes syndrome, with a multifactorial, partly genetic origin (17). Ten-week-old male animals (Taconic, Ejby, Denmark) weighing ~300 g were housed three animals per cage in plastic-bottomed wire-lidded cages on a 12:12-h light-dark cycle (lights on at 7 AM). The animals were kept in air-conditioned (21°C) and humidity-controlled (55%) rooms and had free access to standard rat food and water. All animals were acclimated at least 1 wk before use.

Compounds. Exendin-4, obtained from Bachem (Weil am Rhein, Germany), was administered subcutaneously, dissolved in saline containing 3.5 mg/ml Hemacel (Behringwerke, Marburg, Germany). A pilot study showed that exendin-4, at a dose of 5 nmol/kg sc, caused significantly raised plasma exendin-4 concentrations for 6–8 h following administration. The DPPIV inhibitor used was NN-7201, a generous gift from A. Kanstrup and R. D. Carr (Novo Nordisk, Maalov, Denmark), which was diluted in saline and administered orally by gavage. At an oral dose of 10 mg/kg, <25% DPPIV activity remains 9 h after administration and 50% DPPIV activity remains 12 h after administration (personal communication with B. Rolin, Novo Nordisk). Control animals received either saline containing 3.5 mg/ml Hemacel subcutaneously ($n = 6$) or saline dosed orally by gavage ($n = 9$). Because no significant differences were observed between the subcutaneously and the orally dosed control groups, these two groups were pooled and considered a single control group of $n = 15$ throughout the study. During wk 12–21, the control group was composed of three orally dosed and three subcutaneously dosed animals. For oral dosages, the volume was ~700 μ l/animal, and for subcutaneous injections it was ~200 μ l/animal.

Experimental protocol. Animals were allocated into three groups ($n = 15$) with similar areas under the glucose curve following an initial oral glucose tolerance test (2 g/kg). Animals received either vehicle, exendin-4 (5 nmol/kg sc twice daily at 8 AM and 6 PM), or DPPIV inhibitor (10 mg/kg, once daily orally at 6 PM for 12 wk). After 12 wk of treatment, 9 animals from each group (control group: 6 orally dosed and 3 subcutaneously dosed animals) were euthanized, and the remaining 6 animals in each group were observed for an additional 9 wk without further treatment. Glycemic control was assessed by means of the glycated hemoglobin A1c (HbA1c) measurements determined at wk 0 and wk 12. HbA1c is a measure of the degree of glycosylated hemoglobin and is related to the mean blood glucose over the past 2 mo, which is the approximate lifespan of erythrocytes in rats (normal value for nondiabetic rats varies between 2.2 and 2.9%). Body weight and food intake were monitored weekly. The food intake was monitored for each cage and was expressed as the food intake per 100 g body weight. At the time of death, the weight and length of the small intestine were measured after the luminal

contents and the mesenteric fat had been removed. The small intestine was suspended vertically with a 10-g weight to provide uniform tension during measurement of the length. Additionally, the tibia bone was isolated and its length was measured to give a measure of linear growth.

Histology. Tissue samples from the small intestine (proximal, middle, and distal) and colon (middle) were fixed by immersion in 4% paraformaldehyde. Subsequently, the pieces were dehydrated, embedded in paraffin, and cut with a microtome, perpendicularly to the axis of their length, into 5- μ m sections. The sections were stained with hematoxylin and eosin and were examined by using an Axioscope 2 Plus microscope. The absolute cross-sectional area, as well as the cross-sectional area of the mucosa and the muscularis layers, of each section were determined by using Image Pro Plus 5.0. The examination and the computer analysis of the histological sections were performed with the examiner blinded to the protocol.

Glucose control (HbA1c). The analysis was performed on blood collected from the tail vein. Whole blood suspended in hemolysis buffer was analyzed on a Cobas Mira Plus analyzer (Roche Diagnostic Systems), and the percentage of HbA1c was obtained as the proportion of glycosylated hemoglobin related to the total amount of hemoglobin.

GLP-1 and GLP-2 receptor signaling. Receptor signaling experiments were carried out by using COS-7 cells transfected with the human GLP-1 or GLP-2 receptor. The COS-7 cells were grown at 10% CO₂ and 37°C in Dulbecco's modified Eagle's medium with GlutaMAX (catalog no. 21885-025; GIBCO) adjusted with 10% fetal bovine serum, 180 U/ml penicillin, and 45 μ g/ml streptomycin. Transfection of the COS-7 cells was performed by the calcium phosphate precipitation method (33). For the cAMP accumulation assay, the transiently transfected cells (2.5×10^5 cells/well) were incubated for 24 h with 2 μ Ci/ml of [³H]adenine in 0.5 ml growth medium per well. Cells were washed twice in HBS buffer [25 mM HEPES, pH 7.2, supplemented with 0.75 mM NaH₂PO₄, 140 mM NaCl, and 0.05% (wt/vol) bovine serum albumin], and 0.5 ml HBS buffer supplemented with 1 mM of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (Sigma, St. Louis, MO) was added along with increasing concentrations of the different ligands. After 15 min of incubation at 37°C, the cells were placed on ice, the medium was removed, and the cells were lysed in 1 ml of 5% (wt/vol) trichloroacetic acid supplemented with 0.1 mM cAMP and 0.1 mM ATP for 30 min. The lysate mixtures were loaded onto Dowex columns (Bio-Rad, Hercules, CA), which were washed with 2 ml water and placed onto the top of alumina columns (Sigma) and washed again with 10 ml water. The alumina columns were eluted with 6 ml of 0.1 M imidazole into 15 ml scintillation fluid (Highsafe III). Columns were reused up to 15 times. Dowex columns were regenerated by adding 10 ml of 2 N HCl followed by 10 ml of water; the alumina columns were regenerated by adding 2 ml of 1 M imidazole, 10 ml of 0.1 M imidazole, and finally 5 ml of water. Determinations were made in duplicate.

L-cell secretion and intestinal proliferation in response to exendin-4. In a separate study, 10-wk-old GK rats were allocated into two groups ($n = 4$) and were subcutaneously dosed twice daily (at 8 AM and 6 PM) for 4 days with either exendin-4 (5 nmol/kg dissolved in saline containing 3.5 mg/ml Hemacel) or vehicle. On the fifth day, the L-cell response to acute exendin-4 administration and intestinal proliferation following 4 days of treatment with exendin-4 were assessed as follows.

Animals were anesthetized, and serial blood samples (1.5 ml) were obtained from the retroorbital plexus before and 15 min following exendin-4 or vehicle administration. At time (t) = 30 min, the rats were euthanized and blood was collected from the vena cava. Blood samples were collected into chilled tubes containing EDTA (7.4 mmol/l final concentration) and valine pyrrolidide (1 mmol/l final concentration) and were centrifuged at 4°C. Plasma samples were analyzed for total GLP-2 immunoreactivity by using the side-viewing

GLP-2 antiserum HYB 312-01, which cross-reacts with major glucagon fragment (38), and with antiserum 89390, which has an absolute requirement for the amidated COOH terminal of GLP-1 (29).

Two hours before euthanization, the rats were injected with 50 mg/kg ip bromodeoxyuridine (BrdU; Sigma-Aldrich, Broendby, Denmark). The intestines were removed, and pieces from the proximal, middle, and distal small intestine and the middle colon were treated and placed in 4% paraformaldehyde as described in *Histology*. Within 24 h, the tissue pieces were transferred to a 70% ethanol solution before being embedded in paraffin and cut as described in *Histology*. BrdU-positive nuclei were visualized by using monoclonal rat anti-BrdU (AH Diagnostics, Aarhus, Denmark) followed by biotinylated donkey anti-rat IgG, Vectastain ABC (Vector Laboratories, Burlingame, CA), and 3,3-diaminobenzidine containing nickel ammonium sulfate (Sigma-Aldrich). The number of BrdU-positive cells per crypt was estimated by using an Axioscope 2 Plus microscope by measuring the area of BrdU-positive cells in the epithelial lining (excluding all cells with a clear nonepithelial morphology) and dividing this with the mean size of the cells' nuclei and the number of crypts in the section analyzed.

Statistical analysis. Data are expressed as means \pm SE and were analyzed by ANOVA, followed by Bonferroni post hoc analysis where appropriate, using Statistica 6.0 for Windows (Stat Soft, Tulsa, OK). *P* values of <0.05 were considered significant.

RESULTS

Glucose control. Both treatments were effective antihyperglycemic agents and significantly reduced HbA1c levels compared with the control group after 12 wk of treatment (control HbA1c, $4.9 \pm 0.2\%$; change relative to control: exendin-4, $-0.8 \pm 0.1\%$, $P < 0.0001$; DPPIV inhibitor, $-0.4 \pm 0.2\%$, $P < 0.05$). The reduction in HbA1c was not significantly different between the two treatment groups.

Body weight and food intake. The body weight growth curve is illustrated in Fig. 1. There was no difference in body weight between the control and DPPIV inhibitor-treated groups at any time during the study. In contrast, exendin-4 treatment initially caused a significantly reduced weight gain, which was preserved throughout the treatment period and resulted in a 10% lower body weight for the exendin-4-treated animals compared with the control group after 12 wk of treatment (361 ± 4 vs. 399 ± 5 g; $P < 0.001$). During the follow-up period, animals

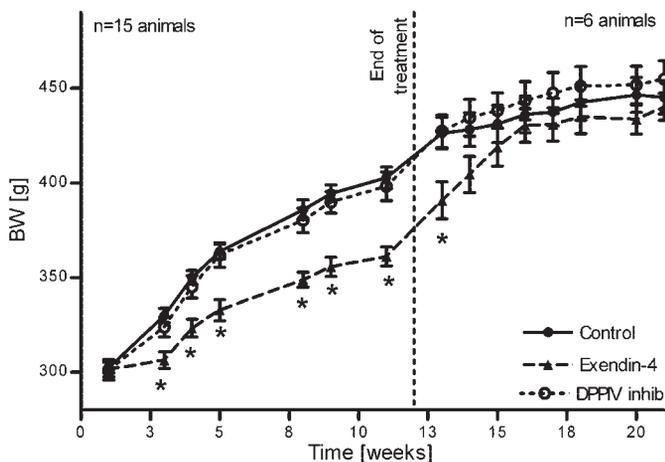


Fig. 1. Body weight (BW) development during 12 wk of treatment ($n = 15$ animals) and in the following 9 wk ($n = 6$ animals) in GK rats treated with exendin-4 (Ex-4) or a dipeptidyl peptidase IV (DPPIV) inhibitor. $*P < 0.05$ compared with control. Data are means \pm SE.

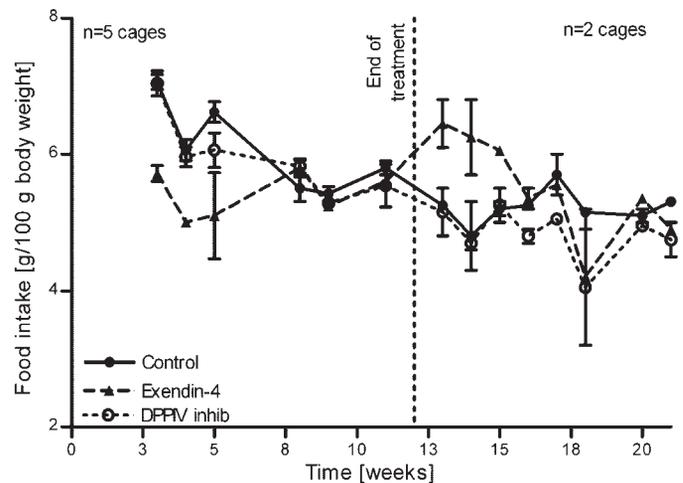


Fig. 2. Estimated food intake monitored per cage and expressed as g food intake/100 g BW during 12 wk of treatment ($n = 5$ cages) and in the following 9 wk ($n = 2$ cages). Data are means \pm SE.

previously receiving exendin-4 gained weight more rapidly in the first 4 wk after cessation of treatment, so that by the end of the study, there were no differences in body weight between the groups.

The estimated food intake is shown in Fig. 2, and although the small number of observations precluded statistical analysis, there was a clear tendency for food intake to be similar in the control group and the DPPIV inhibitor-treated group throughout the entire study. The food intake of the exendin-4-treated group, on the other hand, was clearly reduced in the first few weeks following initiation of treatment, whereas in the first weeks after cessation of the treatment there was a clear tendency for a transient increase in food intake.

The linear growth of the animals was assessed on the basis of the length of the tibia bone at euthanization. There was no difference between the groups in tibia length either immediately at the end of the 12-wk dosing period (3.93 ± 0.03 , 3.96 ± 0.04 , and 3.89 ± 0.03 cm for control, DPPIV inhibitor, and exendin-4, respectively) or at the end of the 9-wk follow-up period without treatment (4.15 ± 0.03 , 4.07 ± 0.03 , and 4.05 ± 0.02 cm for control, DPPIV inhibitor, and exendin-4, respectively).

Intestinal length and weight. There was no difference in length and weight of the small intestine between control and DPPIV inhibitor-treated animals after 12 wk of treatment (Fig. 3). In contrast, 12 wk of exendin-4 treatment significantly increased both the length and weight compared with control ($P < 0.001$; Fig. 3), and this was even more pronounced when related to body weight (small intestinal weight, $2.3 \pm 0.1\%$ compared with $1.6 \pm 0.1\%$ of total body weight in the control group; $P < 0.001$), corresponding to a 44% increase. This effect was reversible, in that after the additional 9 wk without treatment, no difference was observed in intestinal length or weight between the groups (Fig. 3).

Histology and intestinal cross-sectional area. DPPIV inhibitor treatment for 12 wk had no effect on intestinal morphology. However, exendin-4 treatment resulted in an increased cross-sectional area of the small intestine compared with the control animals, with a 16% increase being observed in the proximal part of the intestine, where the difference was most

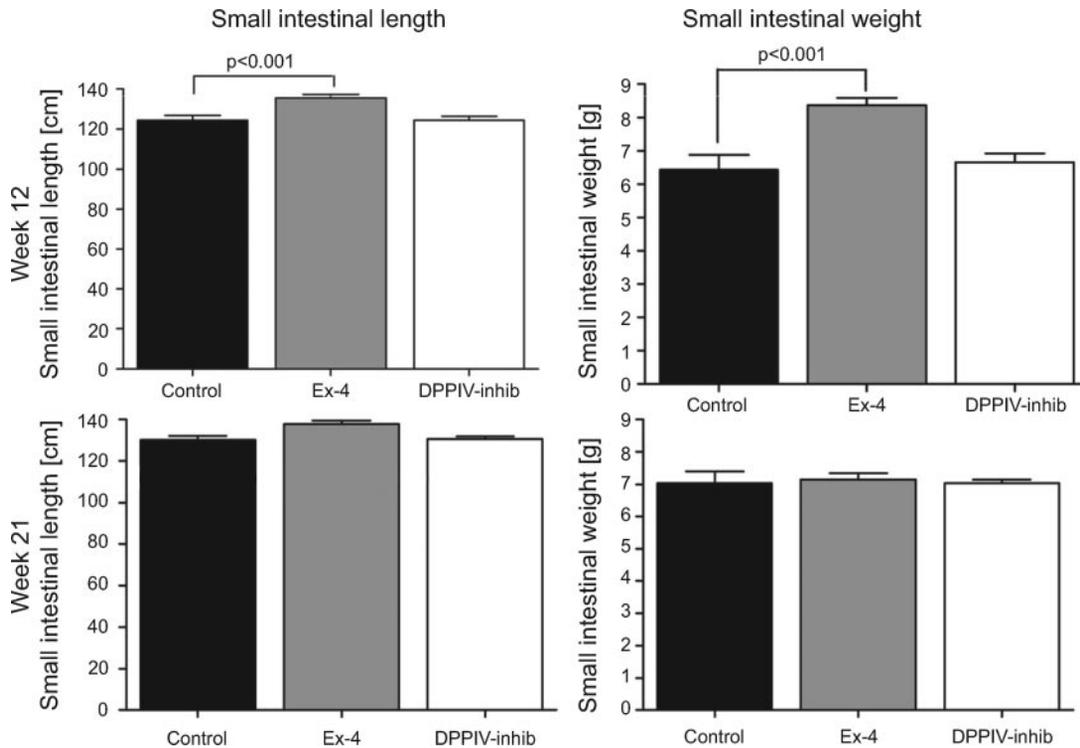


Fig. 3. Small intestinal length and weight immediately following 12 wk of treatment ($n = 9$) and after an additional 9 wk without treatment ($wk 21$; $n = 6$). Data are means \pm SE.

pronounced. The enlargement was even more pronounced when related to body weight, constituting 24% (Table 1), and was primarily due to changes in the mucosal area (Table 1 and Fig. 4), such that the area of the mucosal part of the proximal small intestine in the exendin-4-treated group was increased (26%, $P < 0.05$) compared with the control group. When related to body weight, the mucosal area in both the proximal part (33%, $P < 0.005$) and the middle part of the small intestine (33%, $P < 0.05$) was increased compared with control. In contrast, the muscularis layer was unaffected proximally, although an increase was evident in the distal part of the small intestine of exendin-4-treated animals compared with control. In the colon, no differences in total cross-sectional area or the individual mucosal or muscularis layers were observed between the groups (Table 1 and Fig. 5). Like its effects on length and weight, the morphological effects of exendin-4 were reversible, so that no differences between the groups were evident after the additional 9 wk without treatment.

Effects of exendin-4 on GLP-1 and GLP-2 receptor activation. As expected, both GLP-1 and exendin-4 were potent agonists of the GLP-1 receptor and stimulated cAMP accumulation with high potency (EC_{50} of 5.4 and 9.2×10^{-11} M, respectively; Fig. 6A). Likewise, GLP-2 was a potent agonist of the GLP-2 receptor (EC_{50} of 3.5×10^{-10} M; Fig. 6B). However, exendin-4 alone was unable to activate the GLP-2 receptor (Fig. 6B) and furthermore was unable to affect GLP-2-mediated stimulation of cAMP accumulation in an antagonistic manner, even when added in concentrations as high as $10 \mu\text{M}$ (Fig. 6C).

L-cell response to exendin-4 administration. Basal (before exendin-4 administration) plasma GLP-2 immunoreactivity (55.5 ± 2.9 pmol/l) was not changed at both 15 (60.0 ± 1.8

pmol/l) and 30 min (61.5 ± 2.6 pmol/l) following exendin-4 administration (nonsignificant by one-way ANOVA). Likewise, GLP-1 secretion was unaffected by acute exendin-4 administration in that the immunoreactivity of endogenous GLP-1 was 14.0 ± 5.2 , 16.5 ± 3.6 , and 7.0 ± 2.6 pmol/l at 0, 15, and 30 min following exendin-4 administration, respectively.

Intestinal proliferation. Exendin-4 treatment had no effect on intestinal proliferation as assessed by BrdU incorporation, with the numbers of BrdU-positive cells per crypt in each intestinal section in the exendin-4 group being similar to those in the control group (data not shown).

DISCUSSION

The present study investigated whether there were effects on intestinal growth connected with long-term treatment with the two GLP-1-based candidates for the treatment of Type 2 diabetes, exendin-4 and a DPPIV inhibitor. The rationale for the study is the involvement of DPPIV in the inactivation of the intestinotrophic hormone GLP-2, as well as the reported negative feedback mechanism of GLP-1 on its own secretion, which may also affect GLP-2 secretion because both peptides are derived from the same precursor and are cosecreted from the L-cells. Thus it could be speculated that intestinal growth could be affected in either an inhibitory or a stimulatory manner during a GLP-1-based treatment. The study was performed in the GK rat, which is a polygenic model of type 2 diabetes, to ensure that both treatment regimens were sufficient to improve glycemic control. The results presented indicate that long-term and equally effective antihyperglycemic treatment with either a DPPIV inhibitor or the GLP-1 receptor

Table 1. Morphometric estimates of the small intestine and the colon in control, exendin-4-treated, and DPPIV inhibitor-treated rats after 12 wk of treatment

	Control	Exendin-4	DPPIV-I
Cross-sectional area, mm ²			
Proximal small intestine	9.7±0.7	11.3±0.6	9.5±0.6
Middle small intestine	4.9±0.1	5.7±0.2*	5.0±0.2
Distal small intestine	5.1±0.5	5.0±0.2	4.2±0.4
Colon	7.0±0.3	5.6±0.4*	6.9±0.4
Mucosal area, mm ²			
Proximal small intestine	6.9±0.6	8.7±0.6*	7.0±0.5
Middle small intestine	3.6±0.1	4.3±0.3	3.5±0.3
Distal small intestine	3.1±0.5	2.9±0.2	2.9±0.5
Colon	3.9±0.3	3.1±0.2	4.2±0.3
Muscularis area, mm ²			
Proximal small intestine	2.0±0.1	2.3±0.2	1.8±0.2
Middle small intestine	1.1±0.1	1.2±0.1	1.1±0.1
Distal small intestine	1.2±0.1	1.8±0.1*	1.2±0.1
Colon	2.6±0.1	2.3±0.1	2.8±0.2
Cross-sectional area related to body wt, mm ² /100 g			
Proximal small intestine	2.5±0.2	3.1±0.2*	2.4±0.1
Middle small intestine	1.2±0.1	1.6±0.1*	1.3±0.1
Distal small intestine	1.1±0.1	1.4±0.1*	1.1±0.1
Colon	1.8±0.1	1.6±0.1	1.7±0.1
Mucosal area related to body wt, mm ² /100 g			
Proximal small intestine	1.8±0.2	2.4±0.2*	1.8±0.1
Middle small intestine	0.9±0.1	1.2±0.1*	0.9±0.1
Distal small intestine	0.8±0.1	0.8±0.1	0.7±0.1
Colon	1.0±0.1	0.9±0.1	1.1±0.1
Muscularis area related to body wt, mm ² /100 g			
Proximal small intestine	0.49±0.03	0.63±0.05	0.46±0.05
Middle small intestine	0.28±0.01	0.34±0.02	0.28±0.02
Distal small intestine	0.30±0.02	0.51±0.02*	0.30±0.02
Colon	0.66±0.02	0.65±0.03	0.71±0.06

Values are means ± SE; *n* = 9. DPPIV-I, dipeptidyl peptidase IV inhibitor. **P* < 0.05 compared with controls.

agonist exendin-4 has differential effects on the intestine, such that exendin-4 increases small intestinal mass but DPPIV inhibition has no intestinal effects.

The animals were weighed weekly, and their food intake was estimated, because weight is known to be affected by GLP-1 due to a combination of a centrally mediated depression of food intake and a decreased gastric emptying (39), leading to reduced body weight. In clinical trials, both short- and long-lasting exendin-4 formulations have shown a dose-dependent ability to lower body weight (11, 12), whereas DPPIV

inhibitor treatment is normally weight neutral (1, 30). This difference may simply be due to the inability of DPPIV inhibition to increase endogenous GLP-1 levels sufficiently to reduce food intake. Interestingly, recent clinical data (12) with the long-acting exendin-4 formulation, exenatide-LAR, also seem to indicate that higher agonist concentrations are required to have body-weight-lowering effects than to have beneficial effects on glycemic control. In the present study in GK rats, body weight changes agreed with these reports, with DPPIV inhibition having no effect on either food intake or body weight, whereas exendin-4 lowered food intake, leading to a reduction in body-weight gain throughout the treatment period. Similar findings have been reported in nondiabetic rats with diet-induced obesity, in which the GLP-1 derivative liraglutide, but not the DPPIV inhibitor vildagliptin, led to reduced food intake and loss of body weight (31). However, previous long-term studies have not addressed whether these food intake- and body weight-lowering effects are sustained in the absence of the drug. Here we report that after cessation of treatment, a clear compensatory increase in food intake was observed in the exendin-4-treated group until these animals attained the same weight as the control group, demonstrating that the continued presence of exendin-4 is necessary to maintain the weight loss. To ensure that changes in body weight were not simply due to effects on growth per se, linear growth was assessed by measuring tibia length, revealing that neither exendin-4 nor DPPIV inhibition had any direct effect on growth itself. Earlier reports have raised the question of a potential role of DPPIV in growth, since growth hormone-releasing hormone is a substrate in vitro (16), whereas CD26 (a.k.a. DPPIV) knockout animals actually are smaller than their wild-type counterparts (25). However, results from the present study suggest that the catalytic activity of DPPIV does not play a major physiological role in regulating growth, since neither skeletal growth nor body weight gain were affected by DPPIV inhibition. Very recent studies have also demonstrated that DPPIV inhibition does not alter circulating IGF-I concentrations in growing pigs, further supporting that DPPIV is not a physiological regulator of endogenous growth hormone-releasing hormone activity (14).

DPPIV inhibition could, in theory, affect intestinal growth in either a stimulatory or an inhibitory manner, due to its respective effects to reduce degradation of endogenous GLP-2 or to reduce overall GLP-2 secretion because of negative feedback from enhanced GLP-1 levels. However, no intestinal effects of

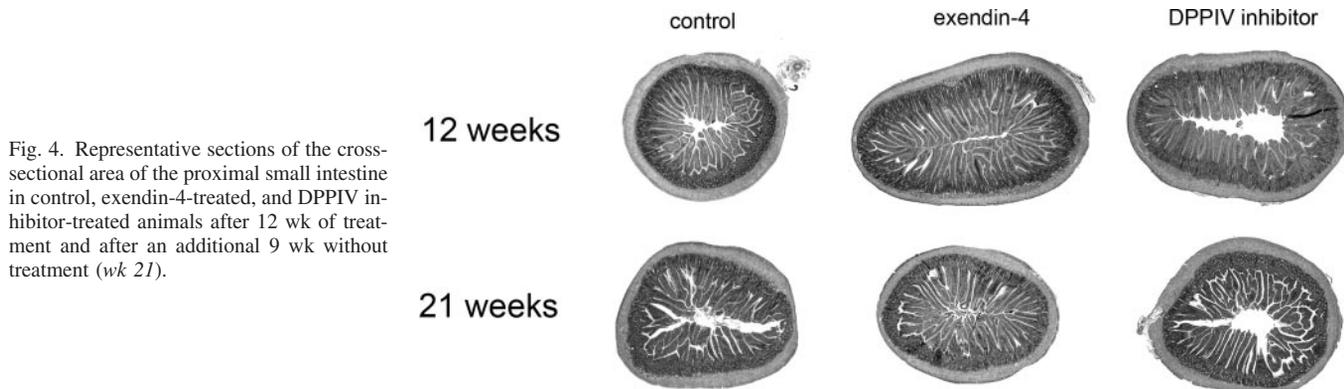


Fig. 4. Representative sections of the cross-sectional area of the proximal small intestine in control, exendin-4-treated, and DPPIV inhibitor-treated animals after 12 wk of treatment and after an additional 9 wk without treatment (wk 21).

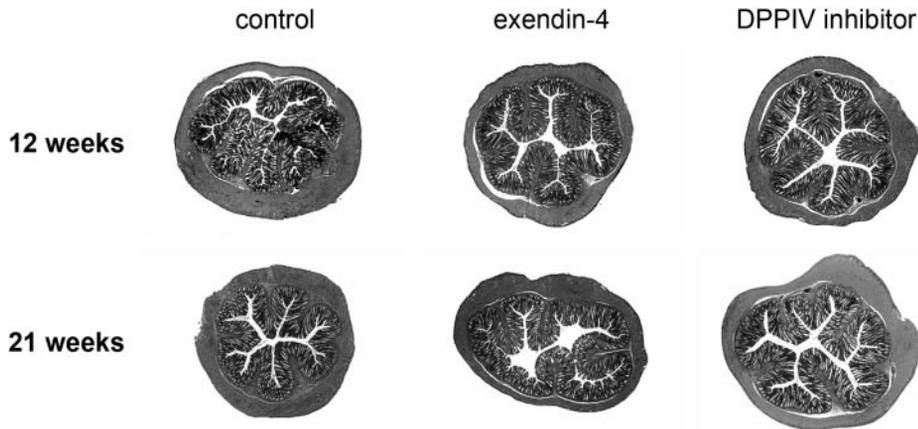


Fig. 5. Representative sections of the cross-sectional area of the colon in control, exendin-4-treated, and DPPIV inhibitor-treated animals after 12 wk of treatment and after an additional 9 wk without treatment (wk 21).

DPPIV inhibition were observed in the present study, in agreement with previous findings (22). It has previously been suggested that GLP-2's intestinotrophic effects are mediated locally via a paracrine route. In this case, DPPIV inhibition would not be expected to have any significant effect, because GLP-2 would have had its effect before it contacts DPPIV in the local capillary endothelium. However, the more distal localization of the intestinal L-cells, in relation to the primarily proximal promotion of growth induced by GLP-2 (22), could suggest the involvement of an endocrine mechanism, whereby reduced GLP-2 degradation following DPPIV inhibition might lead to increased intestinal growth. However, it could be argued that reduced degradation of endogenous GLP-2 is largely counterbalanced by reduced secretion as a result of the negative feedback from enhanced GLP-1 levels, leading to little or no overall change in active GLP-2 levels, thereby explaining the lack of intestinal effects of DPPIV inhibition observed in the present study. Indeed, this is supported by the recent report that although total GLP-2 secretion is reduced in rats after 12-wk treatment with a DPPIV inhibitor, levels of intact GLP-2 are not significantly changed (31).

In contrast to the DPPIV inhibitor, we found unexpectedly that exendin-4 significantly increased the size of the small intestine, although body weight was reduced, with the pattern of the increase being highly reminiscent of that induced by GLP-2 (most pronounced proximally and not affecting the colon). This increase in small bowel mass could be due to

hypertrophy, hyperplasia, or a combination of both. Protein and DNA measurements were not made in the current study, but there was no evidence that changes in cell size contributed to the increased size. Given the high rate of enterocyte proliferation, any major effect of exendin-4 to increase enterocyte cell number should become apparent within a matter of days. In the present study, administration of exendin-4 for 4 days had no detectable effect on the intestinal proliferation rate, as determined by the incorporation of BrdU, and a similar lack of increase in BrdU incorporation has been reported in some studies with GLP-2 (5, 34). However, it remains possible that a minor increase in proliferation may have occurred, which, over the course of the 12-wk study, could account for the increase in small intestinal mass. Furthermore, although it was not measured, an effect of exendin-4 to reduce apoptosis, as has been observed with GLP-2 (5, 37), cannot be excluded, and additional studies will be required to ascertain precisely which mechanisms are involved. An enlargement of the small intestine following exendin-4 treatment has not been reported before, and a long-term toxicology study involving administration of high doses of exendin-4 to rats and mice for their entire lifetimes did not report any tendency for proliferative lesions or hyperplasia of any investigated tissue, including the intestine (23). However, although possible, we do not believe that the effect observed in the current study is specific for the diabetic GK rat because a similar exendin-4-induced increase in small intestinal size has also been found in ongoing studies in the

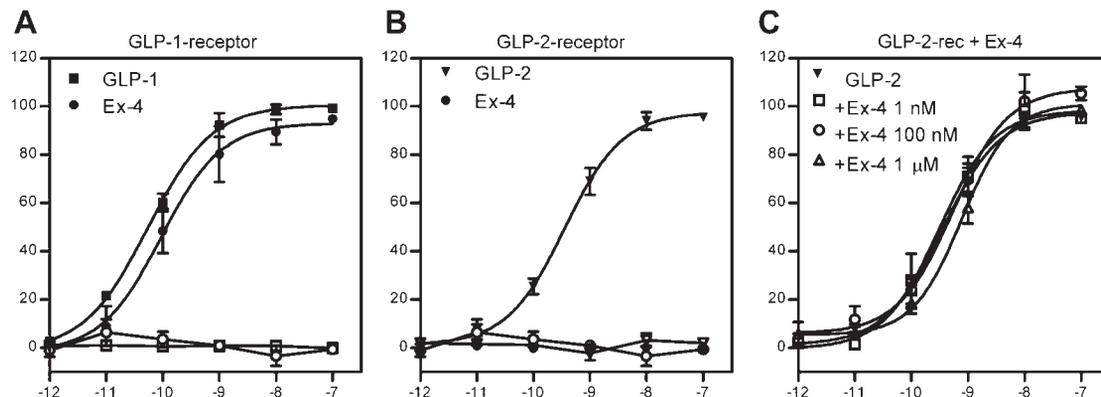


Fig. 6. Release of cAMP from isolated cells transfected with either the glucagon-like peptide (GLP)-1 or the GLP-2 receptor. In A and B, open symbols represent the stimulation of untransfected cells and closed symbols represent the activation of cells transfected with the respective receptor. C: activation of the GLP-2 receptor with GLP-2 in the absence or presence of exendin-4 in different concentrations. Results are means \pm SE; $n = 3-4$.

C57Bl/6 mouse (unpublished data). The intestinal effect of exendin-4 is interesting, because it could be speculated that the reduced food intake induced by exendin-4 treatment may indirectly reduce the adaptive intestinal growth, and likewise, a theoretical reduction in adaptive intestinal growth might be expected from long-term treatment due to activation of the negative feedback mechanism GLP-1 is reported to induce on L-cell secretion. On the other hand, the secretion of exendin-4 in its natural setting, the Gila monster, is increased in relation to chewing (7), which occurs with consumption of the very large and infrequent meals, characteristic for this animal, perhaps indicating that in the Gila monster, exendin-4 together with GLP-2 is responsible for an immediate adaptive intestinal growth. This raises the question of whether the intestinal effects are exendin-4 specific and independent of the close structural and functional relationship with GLP-1 or whether both GLP-1 and exendin-4 have the potential to exhibit GLP-2-like behavior.

One explanation could be that exendin-4, as well as being a GLP-1 receptor agonist, also has some agonistic effect on the GLP-2 receptor. Earlier *in vitro* reports on the ability of exendin-4 and GLP-1 to activate the GLP-2 receptor indicate that although GLP-1 is a weak agonist at the GLP-2 receptor, exendin-4 is only a very weak partial agonist (24). We performed a cAMP activation assay in isolated cells transfected with either the GLP-1 or the GLP-2 receptor, confirming that exendin-4 is a potent GLP-1 receptor agonist, whereas it was neither able to activate the GLP-2 receptor nor to affect the cAMP response induced by GLP-2, even in concentrations as high as 10 μ M. Thus it seems unlikely that exendin-4 could promote intestinal growth by direct activation of the GLP-2 receptor *in vivo*. It could, however, be speculated that instead of directly interacting with the GLP-2 receptor itself, exendin-4 may affect L-cell activity to promote GLP-2 secretion either directly or indirectly. L-cell secretion is regulated in a complex manner, involving both nutrients and a neuroendocrine loop (3). Potential mediators include somatostatin, which has an inhibitory effect (18); gastric inhibitory polypeptide, which both directly and through neural reflexes involving gastrin-releasing hormone has a stimulatory effect, at least in rodents (32); and leptin, which has also been suggested to stimulate L-cell secretion (2). However, in the present study, plasma GLP-1 and GLP-2 concentrations were unaltered following acute exendin-4 administration, and similar results have been reported after 12-wk treatment with the GLP-1 derivative liraglutide, suggesting that a changed pattern of GLP-2 secretion due to chronic exendin-4 treatment is unlikely to explain the increased intestinal size.

Alternatively, it may be speculated that the close relationship between GLP-1 and GLP-2 causes a greater overlap in effects than previously believed. In addition to the structural relationship between GLP-1 and GLP-2, the receptors share ~50% sequence homology and both belong to the family B glucagon-secretin G protein-coupled receptor superfamily, which signals primarily through G_s , activating adenylyl cyclase to increase intracellular cAMP (26). GLP-1 and GLP-2 together mediate the adaptive response to food intake, such that effects of GLP-2 are widely believed to be primarily aimed at intestinal adaptation, whereas GLP-1 results in more general metabolic adaptation. However, some overlap between the effects of the two peptides is already known to exist, and both,

for example, decrease gastric emptying and gastric acid secretion (35, 39). If this also extends to the intestinal effects, it could be speculated that GLP-1, via activation of the GLP-1 receptor, may be able to induce an intestinal response. It remains controversial whether a GLP-1 receptor is actually present in the intestine, because previously, no binding of 125 I-GLP-1 was detected in the small intestinal mucosa by using autoradiographic analysis (28), whereas GLP-1 receptor mRNA was only detected in the duodenum by *in situ* hybridization (4). Preliminary studies from this laboratory have, however, shown the presence of GLP-1 receptor mRNA in rat proximal intestine by using quantitative PCR (J. Petersen, C. Ørskov, and J. J. Holst, unpublished observations). Therefore, although existing data are not conclusive, there is some evidence to support the presence of a GLP-1 receptor in the gut, which would thereby enable an intestinotrophic effect to be mediated directly through the GLP-1 receptor. It should be reiterated that intestinotrophic effects of long-term treatment with GLP-1 or GLP-1 analogs have not previously been reported, but since no one had suspected such an effect, it may have been overlooked. Otherwise, the dose needed to induce an intestinal response could be beyond the pharmacological dose needed in an antidiabetic setting, which would minimize the clinical impact of intestinal effects related to GLP-1 analogs. Furthermore, it should also be kept in mind that the observed increase in small bowel mass observed in rats may not necessarily translate to a similar response in humans.

In conclusion, we have shown that, whereas effective antihyperglycemic treatment with a DPP-IV inhibitor has no intestinal effects in the GK rat, long-term treatment with the GLP-1 receptor agonist exendin-4 increases the size of the small intestine in a GLP-2-like manner. Further studies will be needed to examine whether treatment with GLP-1 elicits the same response and to elucidate the mechanisms of action underlying these effects.

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