ORIGINAL ARTICLE

doi: 10.1111/j.1463-1326.2009.01066.x

Long-term exendin-4 treatment delays natural deterioration of glycaemic control in diabetic Goto–Kakizaki rats

L. Simonsen, S. Pilgaard, C. Orskov,* B. Hartmann,* J. J. Holst and C. F. Deacon

Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Aim: The glucagon-like peptide-1 (GLP-1) receptor agonist, exendin-4, has previously been shown to delay the onset of diabetes when administered to Goto–Kakizaki (GK) rats in the prediabetic period. The present study aimed to evaluate whether long-term administration of exendin-4 to GK rats in the diabetic period would improve their diabetes and how glycaemic control was affected following drug wash-out.
Methods: Glycaemic control was assessed in diabetic GK rats during 12 weeks of exendin-4 or vehicle treatment. Moreover, some animals were followed for an additional 9 weeks without treatment.
Results: Glycaemic control was seen to deteriorate in vehicle-treated animals, as assessed by increased glycated haemoglobin A1c (HbA1c), whereas HbA1c improved in exendin-4-treated animals. Following an additional 9 weeks without treatment, glycaemic control in exendin-4-treated animals remained below baseline value and thus remained significantly lower than that of vehicle-treated animals. Following exendin-4 administration, oral glucose tolerance tests revealed greatly reduced glucose and insulin excursions compared with vehicle-treated animals, whereas following overnight drug wash-out, only little difference was seen, suggesting that the improvement in glycaemic control may have been obtained primarily by increased postprandial control. No significant differences were observed in pancreatic islet morphology or islet hormone content.
Conclusions: Exendin-4 treatment improved glycaemic control in diabetic GK rats, independent of changes in β-cell mass. Additionally, progression of the disease seemed to be delayed because the improvement in HbA1c was still apparent 9 weeks after cessation of treatment.

Keywords: exendin-4, GLP-1, glycaemic control, HbA1c, type 2 diabetes
Received 29 January 2009; returned for revision 30 March 2009; revised version accepted 2 April 2009

Introduction

Exendin-4 is a 39 amino acid peptide found naturally in the saliva of the gila monster, *Heloderma suspectum* [1]. It shares 53% sequence homology with the mammalian incretin hormone glucagon-like peptide-1 (GLP-1) and is a potent GLP-1 receptor agonist [2]. Exendin-4 has a number of effects reported for GLP-1 including glucose-dependent stimulation of insulin secretion [3], glucose-dependent reduction of glucagon secretion [4], delay of gastric emptying and promotion of satiety [5]. Like GLP-1, exendin-4 may increase β-cell mass in rodents [6]. The major advantage of exendin-4 compared with native GLP-1 is its longer metabolic survival. GLP-1 is inactivated by the enzyme dipeptidyl peptidase-4 with a half-life of 1–2 min [7], which means that continuous administration

Correspondence:
Carolyn F. Deacon, Department of Biomedical Sciences, Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark.
E-mail:
deacon@mfi.ku.dk
*Present address: Novo Nordisk, Novo Nordisk Park 1, DK-2760 Maaloev, Denmark.

is required to obtain an antihyperglycaemic effect [8]. In contrast, exendin-4 is resistant to dipeptidyl peptidase-4-mediated cleavage and has a half-life in humans of approximately 30 min following intravenous administration [5] and 4–5 h after subcutaneous (s.c.) administration [9]. Twice-daily administration has, therefore, been associated with improvements in glycaemic control in type 2 diabetic subjects inadequately treated with existing antidiabetic agents [10,11], and exendin-4 (exenatide, Byetta®) has been implemented in the treatment of type 2 diabetes in order to exploit the beneficial effects of GLP-1.

In addition to improving glycaemic control in type 2 diabetic subjects, in rats, expansion of β-cell mass following exendin-4 administration early in life has been reported to improve the outcome in animals prone to develop diabetes. Thus, only 6 days of exendin-4 treatment in the newborn period of the intrauterine growth retarded Sprague–Dawley rat, completely prevented the development of diabetes by preventing the reduction in β-cell mass otherwise correlated to foetal growth retardation [12]. Likewise, Tourrel *et al.* reported that administration of exendin-4 for only 5 days in the prediabetic period leads to expansion of β-cell mass and thereby delays the onset of overt diabetes in the Goto–Kakizaki (GK) rat [13]. The GK rat is a lean model of type 2 diabetes, where the diabetes develops spontaneously at around 8 weeks of age, and although the GK rat differs from most type 2 diabetic animal models by being lean, it has a characteristic resemblance with the human syndrome because of the polygenic origin [14]. The primary defect in the GK rat is β-cell incapacity [15]. Based on the findings of Tourrel *et al.*, we wanted to investigate whether exendin-4 would have beneficial effects in overtly diabetic GK rats.

The objective of the present study, therefore, was to evaluate whether longer-term administration of exendin-4 to diabetic GK rats would improve the diabetes and possibly delay progression of the disease.

Methods

Animals

The animal studies were conducted in accordance with international guidelines (National Institutes of Health, publication number 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 male GK rats. Ten-week-old male animals (Taconic, Ejby, Denmark) weighing around 300 g were housed three animals per cage in plastic-bottomed wire-lidded cages on a 12 : 12-h light–dark cycle. The animals had free access to standard rat food and water. All animals were acclimatized at least 1 week before use. Animals were handled regularly prior to and during the experimental period in order to accustom them and minimize any stress because of handling in the subsequent oral glucose tolerance tests (OGTT).

Compounds

Exendin-4 (Bachem; Weil am Rhein, Germany) was administered s.c., dissolved in saline containing 10% (v/v) Hemaccel (Behringwerke, Marburg, Germany). A pilot study showed that exendin-4, at a dose of 5 nmol/kg s.c., caused significantly raised plasma exendin-4 concentrations for 6–8 h following administration in the GK rat. Vehicle-treated animals received saline containing 10% (v/v) Hemaccel.

Experimental Protocol

Animals were allocated into two groups (n = 15) with similar mean area under the glucose curve ($AUC_{glucose}$) based on an initial OGTT. Animals received either vehicle or exendin-4 (5 nmol/kg s.c. twice daily) for 12 weeks (200 μl/animal). After 12 weeks of treatment, nine animals from each group were euthanized and the remaining six animals in each group were observed for an additional 9 weeks without treatment. At the time of death, the pancreas was excised and divided with one piece for morphometric analysis and one for determination of insulin and glucagon content.

Glycaemic Control

Overall glycaemic control was assessed by means of glycated haemoglobin A1c (HbA1c) measurements determined at weeks 0, 8, 12 and 21. HbA1c is a measure of the degree of glycated haemoglobin and is related to the mean blood glucose over the previous 2 months, which is the approximate lifespan of erythrocytes in rats (normal value for non-diabetic rats varies between 2.2 and 2.9%). In addition, OGTTs were performed at weeks 1 and 10 following an overnight fast. Animals were dosed with their usual treatment 30 min before the glucose load. At time 0 min, an oral load of glucose (2 g/kg, 50% glucose solution) was given by gavage. Additionally, at weeks 12 and 21, OGTTs were performed without prior treatment (at week 12, the animals were dosed in the evening and the OGTT performed following an overnight fast and drug wash-out). Blood samples from all OGTTs were taken from the tail vein into heparinized capillary tubes at −30, 0, 30, 60, 120 and 180 min and kept on ice until

the plasma was separated by centrifugation. Plasma for glucose determination was analyzed immediately, whereas plasma for insulin determination was stored at −20 °C until analysis. The stimulatory effect on β cells was assessed by means of the insulin : glucose ratio.

Analytical Techniques

Glycated Haemoglobin A1c

The analysis was performed on blood collected from the tail vein. Whole blood suspended in haemolysis buffer was analyzed on a Cobas Mira Plus analyzer (Roche Diagnostic Systems, Basel, Switzerland), and HbA1c was presented as the percentage of glycated haemoglobin related to the total amount of haemoglobin.

Plasma Glucose

Plasma glucose levels were measured using a Vitros DT60II (Ortho-Clinical Diagnostics, Birkerod, Denmark) according to the manufacturer's instructions.

Plasma Insulin

Insulin concentrations were analyzed using the Lincoplex multiplex kit (Linco Research, St Charles, MO, USA) and measured with Luminex 100 (Bio-Rad Laboratories, Osterbro, Denmark).

Pancreas Extraction

Pieces of pancreas were extracted as described for neutral and basic peptides [16]. Briefly, frozen pieces of pancreas (50 mg) were homogenized using a Polytron homogenizer in 1 ml of acid ethanol. The homogenate was centrifuged, and the supernatant was analyzed for insulin and glucagon immunoreactivity using specific radioimmunoassays. Insulin concentrations were determined using guinea pig antiserum raised against porcine insulin (code 2006-3), which cross-reacts with both rat insulin I and II and human ^{125}I-labelled insulin labelled at position A14. A mixture (2 : 1) of rat insulin I and II was used as standard (Novo Nordisk, Bagsvaerd, Denmark) [17]. Glucagon immunoreactivity was measured using highly purified porcine glucagon as standard and the COOH-terminally directed antiserum 4305, which mainly detects glucagon of pancreatic origin [18].

Pancreas Histology

Pieces of pancreas were fixed by immersion in 4% paraformaldehyde. Subsequently, the pieces were dehydrated, embedded in paraffin and cut with a microtome into 5-μm sections. For immunohistochemistry, the sections were incubated for 30 min in 10% normal rabbit serum (code number X0902; DakoCytomation, Glostrup, Denmark) and then for 18 h at room temperature with the primary antisera; insulin antiserum (number 2004) and glucagon antiserum (number 4304) diluted 1 : 400 and 1 : 1600 respectively. For visualization of the immunoreactions, the sections were incubated for 1 h with biotinylated porcine anti-rabbit immunoglobulins (code number E353; DakoCytomation) diluted 1 : 40 as the second layer, followed by StrepABComplex/horseradish peroxidase (code number E 353; DakoCytomation) diluted 1 : 100 and as the third layer and finally stained by means of 3,3-diaminobenzidine for 30 min. The sections were examined using a Zeiss Axioscope 2 Plus microscope (Brock and Mickelsen A/S, Birkeroed, Denmark), and the average size of four islets and the percentage of α cells and β cells were determined 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.

Calculations and Statistical Analysis

Composite insulin sensitivity index (CISI) was used as a measure of insulin sensitivity and was calculated as $10\,000/[(\mathrm{FPG} \times \mathrm{FPI}) \times (\text{mean OGTTglu} \times \text{mean OGTT ins})]^{-½}$, where FPG is the fasting plasma glucose concentration and FPI is the fasting plasma insulin concentration. Mean OGTT is taken as the mean concentration from time 0 to 180 min [19]. Areas under the curve are calculated according to the trapezoidal method. The insulin : glucose ratios were determined from the plasma insulin concentration (pM) divided by the plasma glucose concentration (mM) at the 60-min time point of an OGTT.

Results are given as means ± s.e.m. Statistical analysis were performed as one-way ANOVA, and $p < 0.05$ is considered significant. Statistics were performed using STATISTICA 8.0 for Windows (StatSoft, Tulsa, OK, USA).

Results

Blood Glucose

All animals were diabetic at the beginning of the treatment period with mean HbA1c around 4.4%. In vehicle-treated animals, glycaemic control deteriorated over the course of the study; thus, HbA1c levels were higher at week 12 than at week 0 (+0.5 ± 0.1% points, $p < 0.005$). At week 21 (9 weeks after cessation of treatment), HbA1c levels had increased further with vehicle treatment compared with week 0 (+0.7 ± 0.4% points, $p < 0.01$)

(figure 1). In contrast, exendin-4 treatment led to an improvement in glycaemic control, with lower HbA1c after 12 weeks of treatment ($-0.3 \pm 0.1\%$ points, $p < 0.01$) and a significantly lower HbA1c than seen in vehicle-treated animals ($-0.8 \pm 0.2\%$ points, $p < 0.001$). After an additional 9 weeks without treatment, the HbA1c of the exendin-4-treated animals was still below the baseline value and remained significantly lower than that of vehicle-treated animals ($-0.9 \pm 0.1\%$ points lower, $p = 0.001$) (figure 1).

The groups were well matched with respect to glucose tolerance at the start of the study, with similar mean areas under the glucose curve in response to the initial OGTT (data not shown). Following the OGTTs with prior treatment, glucose excursions were significantly reduced by exendin-4 compared with vehicle (figure 2). There was no further reduction in the blood glucose excursion at week 10 compared with week 1. After 12 weeks of treatment when OGTTs were performed following an overnight drug wash-out, a small but significant difference was observed between the glucose excursions of exendin-4 and vehicle-treated animals (week 12 incremental area under the glucose curve ($iAUC_{glucose}$): vehicle 1120 ± 56 mM $\times$ min, exendin-4 884 ± 87 mM $\times$ min; $p < 0.01$). Following additional 9 weeks without treatment, the glucose excursions were not significantly different between vehicle- and exendin-4-treated animals (week 21 $iAUC_{glucose}$: vehicle 1148 ± 92 mM $\times$ min, exendin-4 993 ± 66 mM $\times$ min; $p = 0.2$).

Insulin and Insulin : Glucose Ratios

Following an OGTT with prior treatment, the insulin excursions were significantly decreased for the exendin-4-treated animals (figure 2). Calculation of the insulin : glucose ratios revealed that (although not significant, $p = 0.2$) there was a tendency for this to decrease with exendin-4 (9.6 ± 1.0) compared with vehicle treatment (13.7 ± 2.5). After 12 weeks of treatment when OGTTs were performed following an overnight drug wash-out, the insulin excursions were reduced with exendin-4 treatment (week 12 $iAUC_{insulin}$: vehicle 24.3 ± 3.9 nM $\times$ min, exendin-4 13.0 ± 2.4 nM $\times$ min; $p < 0.05$). After additional 9 weeks without treatment, the insulin excursions were similar between the groups (week 21 $iAUC_{insulin}$: vehicle 17.6 ± 6.1 nM $\times$ min, exendin-4 20.2 ± 4.1 nM $\times$ min). The insulin : glucose ratios were unchanged from week 12 to week 21 and were at both time points similar for exendin-4- and vehicle-treated animals (week 12: vehicle 12.9 ± 1.2, exendin-4 11.2 ± 1.2; week 21: vehicle 11.1 ± 2.2, exendin-4 13.0 ± 2.0). The insulin sensitivity at week 12 was calculated according to the CISI, and no difference was seen between the groups (vehicle 5.7 ± 0.4, exendin-4 5.9 ± 0.6).

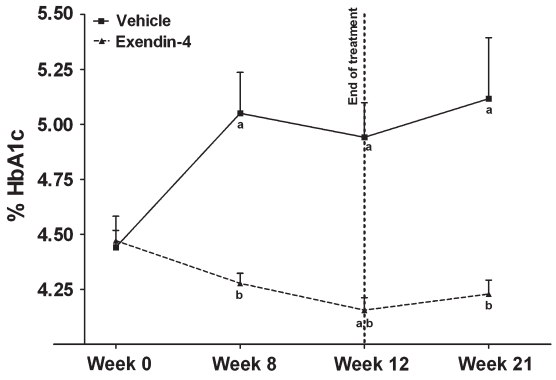

Fig. 1 Glycated haemoglobin A1c (HbA1c) assessed during 12 weeks of treatment with exendin-4 or vehicle ($n = 15$) and again at week 21 following 9 weeks of drug wash-out ($n = 6$). a, $p < 0.05$ vs. baseline value; b, $p < 0.05$ vs. vehicle-treated animals. Data are means $\pm$ s.e.m.

Pancreas Morphology and Islet Hormone Content

There were no morphological differences between animals treated with vehicle or exendin-4 and no significant differences in the proportion of α cells (vehicle $0.14 \pm 0.01\%$, exendin-4 $0.13 \pm 0.02\%$) and β cells (vehicle 0.55 ± 0.03, exendin-4 $0.63 \pm 0.04\%$; $p = 0.09$). The α/β-cell ratio was likewise similar between the groups (vehicle $26.0 \pm 2.0\%$ vs. exendin-4 $22.1 \pm 5.5\%$). There were no differences between the pancreatic contents of insulin (vehicle 84.7 ± 16.7 μmol/g, exendin-4 79.3 ± 11.8 μmol/g) and glucagon (vehicle 2.98 ± 0.42 μmol/g, exendin-4 1.96 ± 0.29 μmol/g; $p = 0.1$).

Discussion

The present study evaluated the effect of long-term exendin-4 treatment after the development of diabetes in the GK rat. It has previously been demonstrated that administration of exendin-4 for only a few days to GK rats in the prediabetic period delays the onset of diabetes [13], whereas it is unknown whether exendin-4 can ameliorate the loss of glycaemic control if treatment is first started in the postdiabetic period. We found that while glycaemic control deteriorated over time in the vehicle-treated animals, 12 weeks of exendin-4 treatment led to a significant reduction in HbA1c. Furthermore, the improvement obtained after 12 weeks of treatment with exendin-4 was still evident after an additional 9 weeks without treatment. Thus, at week 21, HbA1c in exendin-4-treated animals was still not significantly different

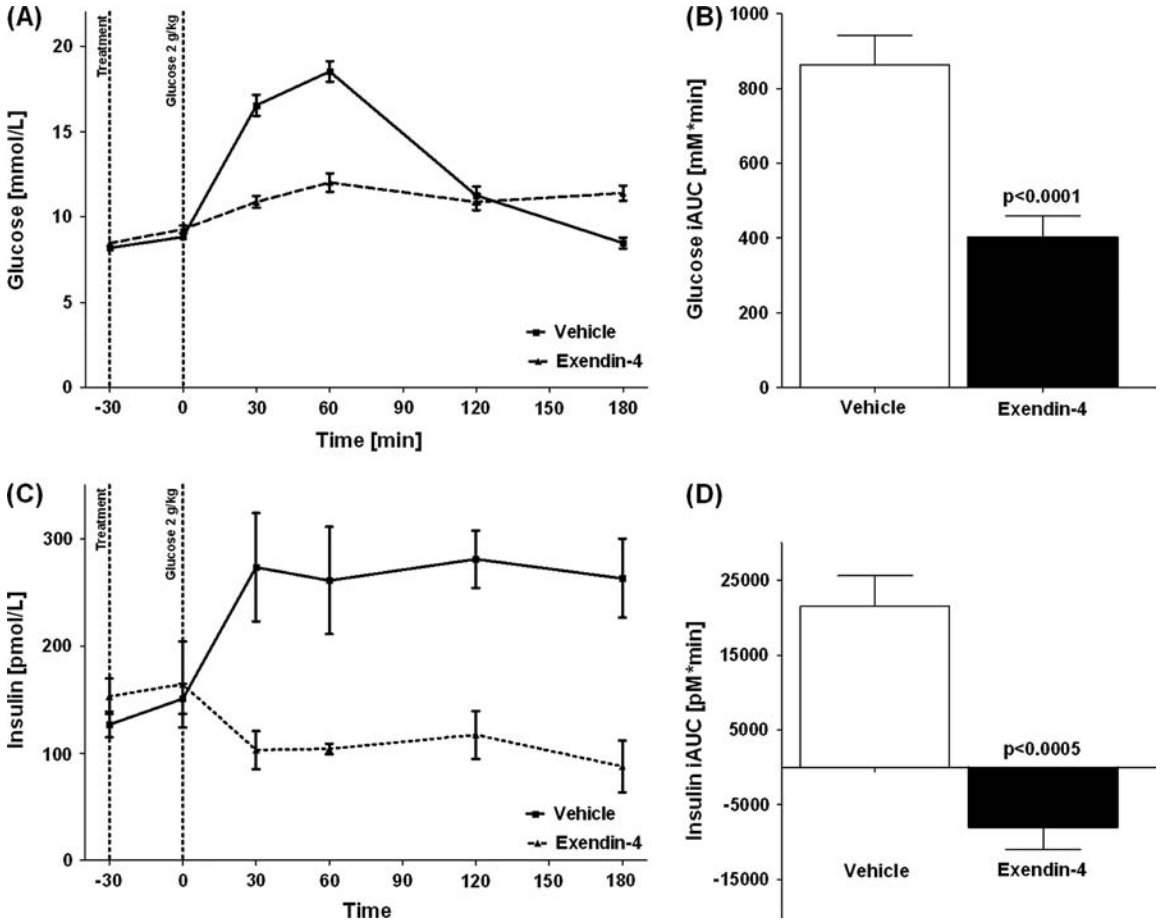

Fig. 2 Plasma glucose (A) and insulin (C) concentrations prior to and during the 180 min following an oral glucose load. Thirty minutes prior to the oral glucose load, animals received exendin-4 or vehicle treatment. Incremental areas under the glucose ($iAUC_{glucose}$) (B) and the insulin (D) curves, which are significantly lower for both glucose and insulin with exendin-4 administration. Data are means ± s.e.m.

from the baseline value and was significantly improved compared with vehicle-treated animals, which had shown further deterioration. Thus, it seems that in addition to improving the diabetic state during the treatment period, exendin-4 may delay the further development of diabetes in the GK rat. Corresponding to the results obtained in the present study, a number of preclinical studies performed in diabetic animal models show reduced HbA1c following long-term treatment with exendin-4 [20–22]. No preclinical studies have addressed the issue of whether the improvement obtained with exendin-4 treatment is maintained following drug wash-out. A recent clinical study, however, found that the improvements in HbA1c and β-cell function, observed after 1 year of therapy with exenatide in patients with type 2 diabetes, were rapidly lost following cessation of treatment [23]. It may therefore be speculated that the same phenomenon would occur if the GK rats had been followed for a longer period of time, but it is also possible that 1 year of treatment in humans is insufficient to cause lasting improvements because of the much slower turnover of β cells.

The improved glycaemic control obtained in the present study with exendin-4 treatment seems to be mediated primarily by increased postprandial control of the blood glucose because there was only little effect on blood and insulin excursions when an OGTT was performed following an overnight drug wash-out, whereas the glucose and insulin excursions following an oral glucose load with prior exendin-4 treatment were significantly reduced compared with vehicle. The minimal rise in blood glucose and insulin resulted in a tendency for the insulin : glucose ratio to decrease. The insulin : glucose ratio is normally used as a measure of the stimulatory effect on β cells, with

an increased ratio indicating improved β-cell stimulation at a given blood glucose level. An unchanged insulin : glucose ratio has previously been reported with exendin-4 administration [24] and is most likely a reflection of a profound inhibition of gastric emptying, which is one of the mechanisms of action of exendin-4 [5,25,26]. The minimal excursions of both glucose and insulin following an oral glucose load seen in the present study have also previously been shown by Ionut *et al.* following a meal test in dogs [27]. In that study, the mechanisms underlying the antihyperglycaemic effect of exendin-4 were investigated in more detail, and they found that although exendin-4 induced a significant delay in gastric emptying, not only it was not the sole parameter involved but also mechanisms, independent of gastric emptying and pancreatic hormones, contributed. It was suggested that these mechanisms may involve the hepatoportal glucose sensor, which is an activation of GLP-1 receptors hypothesized to be expressed on neural fibres in the portal vein [28]. Additionally, other parameters such as hepatic glucose uptake and increased insulin sensitivity may contribute [26]. Reduced glucagon secretion may also be a contributing factor, but because the plasma glucagon concentrations in the GK rats were below the detection limit of the assay, this contribution could not be assessed.

After administration of exendin-4 in the prediabetic period, the delay in progression to overt diabetes was associated with an expansion of the β-cell mass [13]. We were, however, unable to detect any significant differences in the pancreas following exendin-4 treatment, with both α- and β-cell mass and the content of insulin and glucagon being unchanged compared with vehicle-treated animals. The lack of effect in the present study may be because of the complex mechanisms involved in β-cell mass regulation because normoglycaemia, itself, may induce normalization of the increased proliferation and thereby increased β-cell mass correlated with hyperglycaemia [29]. Thus, in the present study, an increased proliferation resulting from exendin-4 treatment may be counterbalanced by the improved glycaemic control in exendin-4-treated animals compared with vehicle-treated animals. Several studies have shown effects of exendin-4 in the pancreas. Thus, exendin-4 induces increased β-cell mass in db/db mice, both with and without prior pancreatectomy [30]. Likewise, in db/db mice, Wang and Brubaker demonstrated increased β-cell mass concomitant with increased pancreatic insulin content and increased β-cell sensitivity [31]. Lamont and Drucker were unable to see an effect on β-cell mass after 8 weeks of treatment in high fat–fed db/db mice [24], and one study showed reduced β-cell mass concomitant with increased insulin sensitivity in Zucker rats [32].

Insulin resistance, which is caused by obesity and lack of physical activity, is also a contributing factor in human type 2 diabetes. Many rodent models of type 2 diabetes are obese models in which obesity develops spontaneously or is induced by high-fat feeding and leads to insulin resistance and the development of type 2 diabetes. The GK rat, however, differs in that the primary defect is β-cell incapacity [15], and although they exhibit some degree of insulin resistance, it is not as pronounced as in obese models of type 2 diabetes. Thus, improved insulin sensitivity is not expected to be a contributing factor to the increased glycaemic control in the GK rat. In accordance, the CISI, which is a measure of insulin sensitivity, revealed no change in the exendin-4-treated animals compared with vehicle-treated animals.

In conclusion, we have shown that 12 weeks of exendin-4 treatment improves glycaemic control in the diabetic GK rat and may delay progression of the disease with the improvement in glycaemic control still being apparent following 9 weeks of drug wash-out.

Acknowledgements

We acknowledge Sofia Svensson for technical assistance and Jorgen Bogh Stormm and Kirsten Hansen for great animal care. Thóra Bödvarsdóttir and Bente Stallknecht are acknowledged for allowing us access to Luminex 100 analyzers. This study was supported by grants from the Danish Medical Research Council, the Novo Nordisk Foundation, the Velux Foundation, Aase and Ejnar Danielsens Foundation and the Toyota Foundation.

References

1 Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. J Biol Chem 1992; **267:** 7402–7405.

2 Goke R, Fehmann HC, Linn T *et al.* Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. J Biol Chem 1993; **268:** 19650–19655.

3 Parkes D, Pittner R, Jodka C, Smith P, Young A. Insulinotropic actions of exendin-4 and glucagon-like peptide-1 in vivo and in vitro. Metabolism 2001; **50:** 583–589.

4 Silvestre RA, Rodriguez-Gallardo J, Egido EM, Marco J. Interrelationship among insulin, glucagon and somatostatin secretory responses to exendin-4 in the perfused rat pancreas. Eur J Pharmacol 2003; **469:** 195–200.

5 Edwards CM, Stanley SA, Davis R *et al.* Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. Am J Physiol Endocrinol Metab 2001; **281:** E155–E161.

6 Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. J Biol Chem 2003; **278:** 471–478.
7 Deacon CF, Nauck MA, Toft-Nielsen M, Pridal L, Willms B, Holst JJ. Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. Diabetes 1995; **44:** 1126–1131.
8 Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 1993; **36:** 741–744.
9 Kolterman OG, Kim DD, Shen L *et al.* Pharmacokinetics, pharmacodynamics, and safety of exenatide in patients with type 2 diabetes mellitus. Am J Health Syst Pharm 2005; **62:** 173–181.
10 DeFronzo RA, Ratner RE, Han J, Kim DD, Fineman MS, Baron AD. Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. Diabetes Care 2005; **28:** 1092–1100.
11 Kendall DM, Riddle MC, Rosenstock J *et al.* Effects of exenatide (exendin-4) on glycemic control over 30 weeks in patients with type 2 diabetes treated with metformin and a sulfonylurea. Diabetes Care 2005; **28:** 1083–1091.
12 Stoffers DA, Desai BM, DeLeon DD, Simmons RA. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. Diabetes 2003; **52:** 734–740.
13 Tourrel C, Bailbe D, Lacorne M, Meile MJ, Kergoat M, Portha B. Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the beta-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. Diabetes 2002; **51:** 1443–1452.
14 Goto Y, Suzuki K, Ono T, Sasaki M, Toyota T. Development of diabetes in the non-obese NIDDM rat (GK rat). Adv Exp Med Biol 1988; **246:** 29–31.
15 Movassat J, Bailbe D, Lubrano-Berthelier C *et al.* Follow-up of GK rats during prediabetes highlights increased insulin action and fat deposition despite low insulin secretion. Am J Physiol Endocrinol Metab 2008; **294:** E168–E175.
16 Holst JJ, Bersani M. Assays for peptide products of somatostatin gene expression. In: Conn PM ed. Methods in Neurosciences. New York: Academic Press, 1991; 3–22.
17 Brand CL, Jorgensen PN, Knigge U *et al.* Role of glucagon in maintenance of euglycemia in fed and fasted rats. Am J Physiol 1995; **269:** E469–E477.
18 Orskov C, Jeppesen J, Madsbad S, Holst JJ. Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. J Clin Invest 1991; **87:** 415–423.
19 Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999; **22:** 1462–1470.
20 Szayna M, Doyle ME, Betkey JA *et al.* Exendin-4 decelerates food intake, weight gain, and fat deposition in Zucker rats. Endocrinology 2000; **141:** 1936–1941.
21 Young AA, Gedulin BR, Bhavsar S *et al.* Glucose-lowering and insulin-sensitizing actions of exendin-4: studies in obese diabetic (ob/ob, db/db) mice, diabetic fatty Zucker rats, and diabetic rhesus monkeys (Macaca mulatta). Diabetes 1999; **48:** 1026–1034.
22 Greig NH, Holloway HW, De Ore KA *et al.* Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. Diabetologia 1999; **42:** 45–50.
23 Bunck MC, Diamant M, Corner A *et al.* β-cell function and glycemic control following one year exenatide therapy, and after 12 week wash out, in patients with type 2 diabetes. Diabetes 2008; **57** (Suppl. 1): A32.
24 Lamont BJ, Drucker DJ. Differential antidiabetic efficacy of incretin agonists versus DPP-4 inhibition in high fat fed mice. Diabetes 2008; **57:** 190–198.
25 Linnebjerg H, Park S, Kothare PA *et al.* Effect of exenatide on gastric emptying and relationship to postprandial glycemia in type 2 diabetes. Regul Pept 2008; **151:** 123–129.
26 Cervera A, Wajcberg E, Sriwijitkamol A *et al.* Mechanism of action of exenatide to reduce postprandial hyperglycemia in type 2 diabetes. Am J Physiol Endocrinol Metab 2008; **294:** E846–E852.
27 Ionut V, Zheng D, Stefanovski D, Bergman RN. Exenatide can reduce glucose independent of islet hormones or gastric emptying. Am J Physiol Endocrinol Metab 2008; **295:** E269–E277.
28 Vahl TP, Tauchi M, Durler TS *et al.* Glucagon-like peptide-1 (GLP-1) receptors expressed on nerve terminals in the portal vein mediate the effects of endogenous GLP-1 on glucose tolerance in rats. Endocrinology 2007; **148:** 4965–4973.
29 Sturis J, Gotfredsen CF, Romer J *et al.* GLP-1 derivative liraglutide in rats with beta-cell deficiencies: influence of metabolic state on beta-cell mass dynamics. Br J Pharmacol 2003; **140:** 123–132.
30 Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. Diabetes 1999; **48:** 2270–2276.
31 Wang Q, Brubaker PL. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. Diabetologia 2002; **45:** 1263–1273.
32 Gedulin BR, Nikoulina SE, Smith PA *et al.* Exenatide (exendin-4) improves insulin sensitivity and {beta}-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. Endocrinology 2005; **146:** 2069–2076.

