Effects of the DPP-4 Inhibitor, Linagliptin, in Diet-Induced Obese Rats: A Comparison in Naïve and Exenatide-Treated Animals

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SUMMARY

Background: To assess the chronic effect of the DPP-4 inhibitor, linagliptin, alone, in combination with exenatide, and during exenatide withdrawal, in diet-induced obese (DIO) rats.

Methods: Female Wistar rats were exposed to a cafeteria diet to induce obesity. Animals were then dosed with vehicle or linagliptin (3 mg/kg PO) orally once-daily for a 28 day period. In a subsequent study, rats received exenatide (either 3 or 30 μg/kg/day) or vehicle by osmotic mini-pump for 28 days. In addition, groups of animals were dosed orally with linagliptin either alone or in combination with a 3 μg/kg/day exenatide dose for the study duration. In a final study, rats were administered exenatide (30 μg/kg/day) or vehicle by osmotic mini-pump for eleven days. Subsequently, exenatide-treated animals were transferred to vehicle or continued exenatide infusion for a further ten days. Animals transferred from exenatide to vehicle were also dosed orally with either vehicle or linagliptin. In all studies, body weight, food and water intake were recorded daily and relevant plasma parameters and carcass composition were determined.

Results: In contrast to exenatide, linagliptin did not significantly reduce body weight or carcass fat in DIO rats versus controls. Linagliptin augmented the effect of exenatide to reduce body fat when given in combination but did not affect the body weight response. In rats withdrawn from exenatide, weight regain was observed such that body weight was not significantly different to controls. Linagliptin reduced weight regain after withdrawal of exenatide such that a significant difference from controls was evident.

Conclusions: These data demonstrate that linagliptin does not significantly alter body weight in either untreated or exenatide-treated DIO rats, although it delays weight gain after exenatide withdrawal. This finding may suggest the utility of DPP-4 inhibitors in reducing body weight during periods of weight gain.

INTRODUCTION

A recent report from the World Health Organization estimates that approximately 400 million adults are obese and 1.6 billion are overweight worldwide [1]. The metabolic consequences of obesity are drivers of other life-threatening disorders including dyslipidemia, hypertension, atherogenesis, and type 2 diabetes mellitus (T2DM) [2]. Indeed, there is an exponential relationship between increasing body mass index (BMI; kg/m²) and the relative risk of developing diabetes such that women with a BMI >35 have approximately 60-fold greater probability of being diagnosed with T2DM than women with a BMI <22 [3]. T2DM is a progressive disease characterized by hyperglycemia, which arises from insufficient pancreatic in-
sulin secretion, insulin resistance in peripheral tissues, and inadequate suppression of glucagon production [4]. Dipeptidyl peptidase-4 (DPP-4) is an enzyme that mediates the degradation of incretin hormones such as glucagon-like peptide-1 (GLP-1) [5]. Incretins are secreted from the gut in response to a meal [6] and, in the case of GLP-1, the dual effects of the stimulation of insulin secretion from β cells of the pancreas and the suppression of glucagon secretion from α cells [7-9], contribute equally to its glucose-lowering action [9]. Furthermore, GLP-1 reduces energy intake and acts as a meal-related satiety factor in laboratory animals [10] and humans [11,12]. In addition, reports have suggested that GLP-1 may have a role in islet neogenesis, differentiation, and the concomitant regulation of β cell mass and preservation [13,14]. For example, des-fluoro-sitagliptin, the analog of the DPP-4 inhibitor sitagliptin, is reported to preserve β cell mass and to correct changes in glycated hemoglobin (HbA1c) and plasma glucose in an animal model of T2DM [15]. Inhibitors of DPP-4 activity were predicted to augment the activity of GLP-1 and, therefore, exhibit a major role in glucose metabolism and, accordingly, DPP-4 became a target for the discovery of novel drugs with potential for the treatment of T2DM [16].

DPP-4 inhibitors approved for the treatment of T2DM either in the United States or Europe include sitagliptin, vildagliptin, and saxagliptin. These agents significantly reduce HbA1c and fasting plasma glucose when they are used as monotherapy or in combination with traditional antidiabetic agents. DPP-4 inhibitors are generally well tolerated and have a weight-neutral effect in the clinic [16]. Linagliptin is regarded as having a benign toxicological profile [17] and is an approved drug for the treatment of T2DM [18,19]. In contrast to other DPP-4 inhibitors, linagliptin has a long duration of action in vivo [20], a long terminal half life [21] and, moreover, linagliptin has a unique non-renal elimination pathway [22]. In addition, it is highly potent for the DPP-4 enzyme (IC₅₀ = 1nM), structurally distinct from other DPP-4 inhibitors, and chronic, once-daily, administration improves glycemic control in diabetic rodent models [23].

Not only is obesity a major driver for the development of T2DM, but effective treatment is complicated further since many antidiabetic agents are associated with weight gain in patients [24]. Accordingly, clinically effective drugs that are either weight-neutral or decrease body weight (e.g., the GLP-1 receptor agonist, exenatide [synthetic exendin-4]) may be of increased utility in the successful treatment of diabetes. The present studies were undertaken to assess the effect of linagliptin on body weight, carcass composition, and the plasma levels of relevant markers in a rodent model of obesity with insulin resistance [25]. Specifically, the effect of linagliptin in female cafeteria diet-induced obese (DIO) rats was determined. Since DPP-4 inhibitors and exenatide are both incretin-based therapies, subsequent studies in the DIO rat investigated whether chronic adminis-

**MATERIALS AND METHODS**

The work reported in this manuscript was performed in accordance with United Kingdom law as detailed in the Animals (Scientific Procedures) Act 1986.

**Animals**

Female Wistar rats (weight range 250 - 300 g upon arrival) were obtained from Charles River (Margate, UK) and housed in pairs at a temperature of 21 ±4°C and 55 ±20% humidity. Animals were maintained on a reverse phase light-dark cycle (lights off for 8 hours from 9:30 AM to 5:30 PM) during which time the room was illuminated by red light. Animals had free access to powdered high-fat diet (VRF1 plus 20% lard; Special Diet Services [SDS], Witham, UK), ground chocolate (Cadbury’s Dairy Milk®), ground peanuts (Big D®), and tap water at all times unless specified otherwise. The three different diets were contained in separate glass feeding jars with aluminum lids. Each lid had a 3 - 4 cm hole to allow access to the food. Animals were housed in pairs for at least 19 weeks for the induction of obesity. Approximately 2 weeks before the start of the studies, animals were housed singly.

**Experimental Procedures**

In all studies, animals underwent a baseline phase where each animal was dosed once daily orally with vehicle. Dosing was timed shortly prior to the onset of the 8 hour dark period. The body weights and daily food and water intakes were also recorded. Towards the end of the baseline period, animals were allocated by a statistician into treatment groups; balanced in regard to body weight, daily food, and water intake. In the case of the exenatide withdrawal, experimental animals were also allocated to drug treatment on the basis of baseline levels of glucose, insulin, and triacylglycerol (TAG). As in the baseline phase, oral administration of linagliptin (or vehicle) was timed to begin at the start of the 8 hour dark period in the study phase.

**Mini-Pump Implantation**

Exenatide (or sterile saline vehicle) was dosed via an osmotic mini-pump (model 2ML4; Alzet, Durect Corp, Cupertino, CA, USA) implanted subcutaneously under anesthesia. Animals received carprofen (5 mg/kg SC) as an analgesic. Approximately 30 minutes after carprofen administration, anesthesia was induced using isoflurane (5%), O₂ (2 L/min), and N₂O (2 L/min). During surgery,
anesthesia was maintained with isoﬂurane at 2% with O₂ (1 L/min) and N₂O (1 L/min). The implant site was shaved and an incision sited at the flank. A pocket of suitable size was created, the pump inserted, and the wound secured by wound clips. Betadine and Opsite (Smith and Nephew, Hull, UK) dressing sprays were applied. Because of the large weight range of the DIO rats at the time of surgery, the concentration of exenatide in the minipumps was adjusted for each rat so that at the time of implantation the dose was appropriate (i.e., 3 or 30 μg/kg/day). At the time of mini-pump replacement in the exenatide withdrawal experiment, animals that received further exenatide (30 μg/kg/day) infusion did so on the basis of their body weight 2 days before replacement surgery. Control animals and animals that were withdrawn from exenatide received a pump filled with sterile saline. On veterinary advice, the second pump was inserted into a separate pocket from the first mini-pump.

**Blood Collection**

In the initial study investigating the effect of 28 days of linagliptin on body weight in the DIO rat (Study 1), blood samples were taken in the freely fed state 2 hours after dosing in the baseline phase and on day 29. In the subsequent study combining the efficacy of a low dose of exenatide with linagliptin (Study 2), blood samples (4 hours fasted) were taken 4 hours after dosing during the baseline phase and on day 29. In the exenatide withdrawal study (Study 3), blood samples (4 hours fasted) were taken 4 hours after dosing during the baseline phase and at termination.

For GLP-1 determination, an initial blood sample was collected into ethylenediaminetetraacetic acid (EDTA)-coated collection tubes (Sarstedt, Leicester, UK) containing a protease inhibitor cocktail (Sigma, Poole, UK) and DPP-4 inhibitor (Millipore, St. Charles, MO, USA). A further blood sample was collected into EDTA-coated tubes and inverted to mix. An aliquot of whole blood was used to determine HbA1c. The remainder was immediately spun in a cooled centrifuge and the plasma fraction frozen prior to determination of glucose, insulin, glycerol, TAG, leptin, non-esterified fatty acids (NEFAs), C-reactive protein (CRP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) content using commercially available kits and reagents.

**Plasma Analysis**

Enzyme-linked immunosorbenet assays (ELISAs) and colorimetric kits were used to assay glucose (Thermo Electron Corp., San Jose, CA, USA), insulin (Merckodia, Uppsala, Sweden), glycerol and triglycerides (Sigma, St. Louis, MO, USA), leptin (Assay Designs, Ann Arbor, MI, USA), GLP-1 (Millipore, St. Charles, MO, USA), AST and ALT (Thermo Electron Corp., San Jose, CA, USA), CRP (Alpco Diagnostics, Salem, NH, USA), and NEFAs (Wako Chemicals, Neuss, Germany).

**Drugs**

Exenatide (exendin-4, American Peptide Company Inc., Sunnyvale, CA, USA) was dissolved in sterile saline. Linagliptin (provided by the Department of Medicinal Chemistry, Boehringer Ingelheim Pharma GmbH and Co. KG, Biberach an der Riss, Germany) was dosed orally in a vehicle of aqueous 0.5% natrosol w/v in a volume of 3 mL/kg. In all studies, oral dosing was once daily at the onset of the dark phase of the light-dark cycle. The doses of exenatide used were based on the literature [25] and previous in-house experience. The dose of 3 mg/kg PO linagliptin was selected for use in the present studies since this dose is reported to improve glucose control when given once daily in animal models of diabetes [23].

**Body Composition Assessment**

Carccass composition (body fat, protein, and water) was determined using the FoodScan™ NIR (near infra-red) meat analyzer (Foss, Warrington, UK). Carcasses were milled at the temperature of liquid nitrogen and stored at -20°C in sealed containers until required. Subsequently, a portion of the milled carcass was allowed to thaw to room temperature and was then placed in the FoodScan analyzer. This method has been demonstrated to produce highly comparable results (correlation coefficient: \( r^2 = 0.95 \)) to those obtained with the gold standard chemical analysis method of carcass composition [27].

**Statistical Analysis**

Data analysis was performed by a statistician. Variations in the energy levels of the different types of food were accounted for by expressing the food intake results in kJ. Effects on body weight, food and water intake were assessed by analysis of covariance (ANCOVA) with treatment as a factor and baseline data as the covariate. In the case of body weight analysis, day 1 body weight (i.e., the weight immediately before the first drug treatment) was the covariate. In the case of the food and water intake analysis, the covariate was the average daily intake during the baseline phase of the study. Plasma data were analyzed by a general linear model with treatment as a factor. Where appropriate, data underwent a log transformation prior to analysis. Baseline plasma data and day 1 body weight were included as covariates. HbA1c data were analyzed by a robust regression model using M estimation (Huber weighting, using the default parameter \( c = 1.345 \)). Baseline HbA1c levels and day 1 body weight were included as covariates. Carcass composition data were analyzed by robust regression with treatment as factors. Day 1 body weight was included as a covariate. Means detailed in figures and tables are adjusted for differences at baseline (see above). Standard errors of the mean (SEM) are calculated from the residuals of the statistical model. Animals were withdrawn from the mini-pump study at an earlier stage if adverse events occurred, e.g., the
opening of the wound because of excessive biting/scratching/licking at the site. Such animals were humanely killed. All available body weight, food and water intake data, and body composition data are included in the statistical analyses and figures, with the exception that body composition was determined only in animals that finished the study and those that were removed from the study up to 48 hours prior to the planned termination.

All comparisons were by the multiple t test, with the exception of Study 2 where the Williams’ test was used to compare the effect of exenatide dose to vehicle controls. A value of \( p < 0.05 \) was regarded as being statistically significant. Statistical analysis was performed using SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

**Study 1: The Effect of Linagliptin in DIO Rats**

Linagliptin (3 mg/kg PO) did not significantly affect body weight over the study duration (Table 1). Consistent with the lack of effect on body weight, linagliptin had no marked effect on average daily food or water intake compared with vehicle-treated controls (data not shown). Hence, linagliptin did not significantly affect either overall average daily food intake \( (p = 0.582, \text{not significant [n.s.]} \) or daily water intake \( (p = 0.137, \text{n.s.}) \) when compared with control animals. Linagliptin had no significant effect on plasma levels of glucose, insulin, leptin, TAG, glycerol, AST, ALT, or blood HbA1c, after 4 weeks of dosing (day 29; Table 1). However, plasma GLP-1 was significantly increased \( (p<0.05) \) in animals treated with linagliptin, an effect consistent with the action of linagliptin to inhibit DPP-4 in vivo. Carcass composition was not altered by linagliptin administration (Table 1).

**Study 2: The Effect of Linagliptin in Combination with a Low Dose of Exenatide**

As in Study 1, 28-day dosing with linagliptin (3 mg/kg PO) was not associated with a statistically significant change in body weight (0% from vehicle-treated controls; \( p = 0.983; \text{Figure 1} \) in the DIO rats. Similarly, no significant changes in daily food (nor food preference for the individual diets) or water intake were observed (Figures 2 and 3) with linagliptin. Continuous SC infusion of exenatide had a dose-dependent effect on body weight (Figure 1). Hence, a low dose of exenatide (3 \( \mu \text{g/kg/day} \) ) reduced body weight by 1.8% \( (p = 0.31; \text{Figure 1}) \) and significantly reduced daily food intake over the 48-hr period after pump implantation, but not thereafter (Figure 2). No significant effect on daily water intake (Figure 3) was observed at any stage of the study at the 3 \( \mu \text{g/kg/day} \) dose (Figure 3). In contrast, infusion of a higher exenatide dose (30 \( \mu \text{g/kg/day} \) ) significantly reduced body weight by 8.4% over the study duration compared with vehicle-treated controls \( (p<0.001; \text{Figure 1}) \). These effects were associated with statistically significant reductions in food intake (Figure 2) and plasma leptin (Table 2). The magnitude of the exenatide-induced reduction in food intake was most marked during the initial 4 days of drug delivery (Figure 2) and was associated with a selective and statistically significant reduction in the consumption of the chocolate diet \( (p<0.001) \). Exenatide did not significantly reduce daily food intake compared with vehicle-treated controls from days 14 to 28, (Figure 2).

In contrast to the stable daily water intake of control animals and animals treated with the 3 \( \mu \text{g/kg/day} \) exenatide dose, animals treated with 30 \( \mu \text{g/kg/day} \) exenatide exhibited a bi-phasic response with significant reductions in daily water intake evident over the first days of treatment succeeded by significantly increased water intake over days 4 to 9 (Figure 3). Body composition revealed that the significant reduction in body weight was associated with a statistically significant and selective reduction in body fat \( (p<0.001; \text{Table 2}) \) with carcass protein and water unaffected by treatment (Table 2).

Combined treatment of linagliptin and the low (3 \( \mu \text{g/kg/day} \) ) dose of exenatide reduced body weight in the DIO rats by 2.7% \( (p = 0.13; \text{Figure 1}) \) though the associated reduction in body fat almost achieved statistical significance \( (p = 0.050; \text{Table 2}) \) compared with vehicle-treated controls. However, neither the additional weight loss \( (p = 0.639) \) nor the reduction in average daily food intake \( (p = 0.939) \) observed with the drug combination were significantly different from the effects of exenatide (3 \( \mu \text{g/kg/day} \) ) given alone. In contrast, animals treated with the combination exhibited a significant increase in average daily water intake \( (p<0.05) \) and plasma GLP-1 \( (p<0.05; \text{Table 2}) \) compared with animals treated with exenatide alone.

**Study 3: The Effect of Linagliptin in DIO Rats Previously Treated with Exenatide**

Continuous infusion of 30 \( \mu \text{g/kg/day} \) exenatide by osmotic mini-pump significantly reduced body weight compared with controls \( (p<0.01 \text{ at day 11}; \text{Figure 4}) \). These effects were associated with statistically significant reductions in food intake (Figure 5) and a bi-phasic change in daily water intake (for clarity, data not shown) similar in nature to the temporal profiles observed in Study 2. As observed in Study 2, exenatide infusion was associated with a selective and statistically significant reduction in chocolate intake \( (p<0.001) \).

Subsequent to replacing mini-pumps on day 11, animals that continued to receive exenatide maintained a statistically significant reduction in body weight compared with controls \( (p<0.05; \text{Table 3}) \). Accordingly, at the end of the study exenatide-treated animals weighed 6.3% less (approximately 27 g) than vehicle-treated controls (Table 3). As observed after implantation of the initial pump containing exenatide, the hypophagic effect of exenatide delivered from the second pump was most marked over the first days of infusion (Figure 5). Specifically, average daily food intake between days 11 and 17 was significantly reduced \( (p<0.001) \), whereas aver-
EFFECTS OF THE DPP-4 INHIBITOR, LINAGLIPTIN, IN DIET-INDUCED OBESE RATS: A COMPARISON IN NAÏVE AND EXENATIDE-TREATED ANIMALS

Table 1 Effect of linagliptin on body weight and terminal (day 29) plasma parameters of diet-induced obese rats.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Linagliptin (3 mg/kg PO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>470.6 ±8.3</td>
<td>471.4 ±5.4</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>8.42 ±0.34</td>
<td>8.34 ±0.32</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>5.27 ±0.74</td>
<td>5.08 ±0.82</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>26.5 ±2.0</td>
<td>26.5 ±1.9</td>
</tr>
<tr>
<td>TAG (mM)</td>
<td>0.37 ±0.05</td>
<td>0.36 ±0.07</td>
</tr>
<tr>
<td>Glycerol (mM)</td>
<td>0.42 ±0.03</td>
<td>0.41 ±0.02</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>47.9 ±3.8</td>
<td>44.6 ±2.8</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>24.9 ±1.6</td>
<td>27.6 ±2.3</td>
</tr>
<tr>
<td>GLP-1 (pM)</td>
<td>3.98 ±0.53</td>
<td>6.42 ±0.85*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.43 ±0.09</td>
<td>6.47 ±0.09</td>
</tr>
<tr>
<td>Carcass protein (g)</td>
<td>70.6 ±2.2</td>
<td>70.3 ±2.1</td>
</tr>
<tr>
<td>Carcass water (g)</td>
<td>215.4 ±6.0</td>
<td>219.2 ±5.1</td>
</tr>
<tr>
<td>Carcass fat (g)</td>
<td>147.4 ±7.8</td>
<td>145.0 ±7.2</td>
</tr>
</tbody>
</table>

Data are mean ±SEM (n = 10). *p<0.05 by multiple t test.

Table 2 Effect of linagliptin and exenatide combination on plasma parameters and body composition in diet-induced obese rats at day 29.

<table>
<thead>
<tr>
<th>Pump treatment (SC)</th>
<th>Vehicle</th>
<th>Exenatide (3 µg/kg/day)</th>
<th>Vehicle</th>
<th>Exenatide (30 µg/kg/day)</th>
<th>Vehicle</th>
<th>Exenatide (3 µg/kg PO)</th>
<th>Vehicle</th>
<th>Exenatide (3 µg/kg PO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral treatment</td>
<td>Vehicle</td>
<td>Vehicle</td>
<td>Vehicle</td>
<td>Linagliptin (3 mg/kg PO)</td>
<td>Linagliptin (3 mg/kg PO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>8.14 ±0.33</td>
<td>8.06 ±0.31</td>
<td>7.70 ±0.21</td>
<td>8.72 ±0.22</td>
<td>8.51 ±0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>2.12 ±0.34</td>
<td>1.89 ±0.32</td>
<td>1.61 ±0.64</td>
<td>1.82 ±0.36</td>
<td>1.89 ±0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>26.7 ±2.6</td>
<td>20.5 ±2.3</td>
<td>14.4 ±1.2d</td>
<td>23.9 ±2.5</td>
<td>21.1 ±1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-1 (pM)</td>
<td>3.67 ±1.1</td>
<td>3.52 ±0.3</td>
<td>4.58 ±0.5</td>
<td>5.72 ±0.9b</td>
<td>5.44 ±1.3b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcass protein (g)</td>
<td>60.8 ±4.6</td>
<td>55.6 ±5.2</td>
<td>54.3 ±3.5</td>
<td>63.8 ±4.0</td>
<td>63.7 ±5.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcass water (g)</td>
<td>206.8 ±5.9</td>
<td>209.9 ±7.7</td>
<td>207.5 ±3.5</td>
<td>212.6 ±3.4</td>
<td>213.6 ±3.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcass fat (g)</td>
<td>161.1 ±5.9</td>
<td>144.7 ±10.0</td>
<td>127.3 ±9.3c</td>
<td>151.1 ±7.3</td>
<td>139.5 ±7.1a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ±SEM (n = 7 - 10). Multiple comparisons versus vehicle are by Williams’ test for groups treated solely with exenatide, and the multiple t test for all other groups: *p = 0.050, **p<0.05, ***p<0.01, ****p<0.001. *p<0.05 from the exenatide (3 µg/kg/day) group.
GLP-1: glucagon-like peptide-1.

Age daily food intake between days 18 and 20 was not (p = 0.186, n.s.). Animals that were switched from exenatide to vehicle (both as an infusion from the mini-pump and as an oral dose) increased in weight from day 11 onwards such that their body weight was not significantly different to vehicle-treated controls at the conclusion (13.8 g; Table 3). During this withdrawal period, chocolate intake remained significantly below that of controls (p<0.05), though the intake of high fat chow was significantly increased (p<0.05). In contrast to the exenatide withdrawal group, the weight gain of animals switched from exenatide treatment to vehicle but dosed once daily with linagliptin was reduced (Figure 4) such that at the con-
Table 3. Effect of linagliptin on terminal parameters of diet-induced obese rats withdrawn from continuous exenatide infusion.

<table>
<thead>
<tr>
<th>Pump Treatments</th>
<th>Day 1 - 10</th>
<th>Exenatide</th>
<th>Exenatide</th>
<th>Exenatide</th>
</tr>
</thead>
<tbody>
<tr>
<td>From Day 11 onward</td>
<td>Vehicle</td>
<td>Exenatide</td>
<td>Vehicle*</td>
<td>Exenatide*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oral dose</th>
<th>From Day 11 onward</th>
<th>Vehicle</th>
<th>Exenatide</th>
<th>Vehicle*</th>
<th>Linaglaptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mM)</td>
<td>9.10 ±0.56</td>
<td>10.00 ±0.98</td>
<td>8.72 ±0.37</td>
<td>9.52 ±0.63</td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>3.31 ±0.05</td>
<td>2.90 ±1.52</td>
<td>3.00 ±0.49</td>
<td>3.10 ±0.46</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>16.5 ±3.0</td>
<td>7.4 ±1.2b</td>
<td>11.5 ±1.7</td>
<td>11.1 ±1.0a</td>
<td></td>
</tr>
<tr>
<td>TAG (mM)</td>
<td>1.01 ±0.27</td>
<td>1.25 ±0.46</td>
<td>0.64 ±0.23</td>
<td>0.75 ±0.12</td>
<td></td>
</tr>
<tr>
<td>Glycerol (mM)</td>
<td>0.53 ±0.06</td>
<td>0.57 ±0.03</td>
<td>0.43 ±0.04</td>
<td>0.44 ±0.03</td>
<td></td>
</tr>
<tr>
<td>NEFA (µM)</td>
<td>366 ±28</td>
<td>388 ±31</td>
<td>388 ±42</td>
<td>386 ±32</td>
<td></td>
</tr>
<tr>
<td>CRP (µg/mL)</td>
<td>326 ±23</td>
<td>319 ±19</td>
<td>276 ±26</td>
<td>293 ±31</td>
<td></td>
</tr>
<tr>
<td>Carcass protein (g)</td>
<td>62.7 ±1.4</td>
<td>61.4 ±1.5</td>
<td>59.7 ±1.8</td>
<td>61.6 ±2.1</td>
<td></td>
</tr>
<tr>
<td>Carcass water (g)</td>
<td>204.2 ±5.3</td>
<td>202.1 ±5.2</td>
<td>194.6 ±4.3</td>
<td>198.0 ±4.4</td>
<td></td>
</tr>
<tr>
<td>Carcass fat (g)</td>
<td>123.0 ±6.5</td>
<td>103.9 ±7.5a</td>
<td>132.0 ±6.3</td>
<td>117.6 ±5.0</td>
<td></td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>438.2 ±4.6</td>
<td>410.5 ±8.3a</td>
<td>424.4 ±4.5</td>
<td>419.1 ±5.7a</td>
<td></td>
</tr>
<tr>
<td>Weight change from vehicle control (g)</td>
<td>N/A</td>
<td>-27.7</td>
<td>-13.8</td>
<td>-19.1</td>
<td></td>
</tr>
</tbody>
</table>

*aFrom Day 11 onward, animals withdrawn from exenatide treatment received a pump filled with sterile saline. Data are mean ±SEM (n = 5 - 10). Exenatide infused at 30 µg/kg/day. Linagliptin dose at 3 mg/kg PO. Comparisons against control were by the multiple t test: *p<0.05, **p<0.01. Comparisons against the vehicle-treated exenatide withdrawal group by multiple t test: *p<0.01. CRP: C-reactive protein; NEFA: non-esterified fatty acids; TAG: triacylglycerol.

Conclusion of the experiment the body weight of this group of animals was significantly different to vehicle-treated controls (19.1 g; p<0.05; Table 3). However, although the body weight of linagliptin-treated animals was significantly reduced at the conclusion of the study compared with vehicle-treated controls, the difference in body weight of this group from the body weight of vehicle-treated animals withdrawn from exenatide was not significant (5.3 g; p = 0.52; Table 3). The daily food intake (Figure 5) of animals switched from exenatide to vehicle and dosed with either vehicle or linagliptin was not significantly different to vehicle-treated controls (animals that received pumps delivering saline throughout and that were dosed with vehicle orally from day 11, the day of pump replacement, onwards). Although animals dosed with linagliptin during exenatide withdrawal showed a marked decrease in chocolate intake (p<0.001), this decrease was offset by an increased intake of high fat chow (p<0.01). Hence, there was no significant difference in the average daily food intake of either linagliptin-treated (p = 0.62, n.s.) or vehicle-treated (p = 0.95, n.s.) animals withdrawn from exenatide compared with vehicle-treated controls between days 11 and 20. Similarly, there were no statistically significant differences between the daily water intakes of these groups and vehicle-treated controls (data not shown).

At the study conclusion, exenatide significantly reduced plasma leptin (p<0.001) compared with controls but all other parameters were not significantly changed (Table 3). Animals that were withdrawn from exenatide on day 11 and dosed once daily with linagliptin also exhibited a statistically significant reduction in plasma leptin (p<0.05) compared with vehicle-treated controls (Table 3). In the case of exenatide treatment, the significant reduction in body weight was accounted for almost entirely by a reduction in body fat (p<0.05), with no significant differences in carcass water (p = 0.80) or protein (p = 0.71) evident in comparison with vehicle-treated controls (Table 3). The body fat of animals switched from exenatide to vehicle and receiving vehicle orally was in excess of vehicle-treated controls at termination, though this difference was not statistically significant. In contrast, the body fat of these animals was significantly greater than that of animals treated with exenatide for the study duration (p<0.01; Table 3). The reduction in body fat observed in animals withdrawn from exenatide and dosed with linagliptin was not significantly different from animals withdrawn from exenatide and dosed with vehicle (p = 0.07; Table 3).
EFFECTS OF THE DPP-4 INHIBITOR, LINAGLIPTIN, IN DIET-INDUCED OBESE RATS: A COMPARISON IN NAÏVE AND EXENATIDE-TREATED ANIMALS

**Figure 1.** Body weight means + SEM; \( n = 7 - 10 \). Data analyzed by analysis of covariance with body weight on day 1 as a covariate. Multiple comparisons are by Williams' test for groups receiving exenatide (3, 30 \( \mu \)g/kg) alone and the multiple \( t \) test for all other comparisons. Significant differences from vehicle control: *\( p<0.05 \), **\( p<0.01 \), ***\( p<0.001 \).

**Figure 2.** Mean daily food intake + SEM; \( n = 7 - 10 \). Data analyzed by analysis of covariance with average baseline food intake as a covariate. Multiple comparisons are by Williams' test for groups receiving exenatide (3, 30 \( \mu \)g/kg) alone and the multiple \( t \) test for all other comparisons. Significant differences from vehicle control: *\( p<0.05 \), **\( p<0.01 \), ***\( p<0.001 \).
Figure 3. Mean daily water intake + SEM; \( n = 7 - 10 \). Data analyzed by analysis of covariance with average baseline water intake as a covariate. Multiple comparisons are by Williams’ test for groups receiving exenatide (3, 30 μg/kg) alone and the multiple t test for all other comparisons. Significant differences from vehicle control: *\( p<0.05 \), **\( p<0.01 \), ***\( p<0.001 \).

Figure 4. Mean change in body weight of diet-induced obese rats treated with exenatide for either a 10 or 21 day period (\( n = 5 - 11 \); day 1 - 11 data with exenatide are pooled and include all data for animals treated with exenatide over this period). Pumps were replaced on day 11 and oral dosing commenced. Multiple comparisons versus the vehicle control group were by the multiple t test. Significant differences from vehicle control: **\( p<0.01 \). Differences from the exenatide withdrawal group: #\( p=0.07 \) (multiple t test).
EFFECTS OF THE DPP-4 INHIBITOR, LINAGLIPTIN, IN DIET-INDUCED OBESE RATS: A COMPARISON IN NAÏVE AND EXENATIDE-TREATED ANIMALS

**DISCUSSION**

The present study demonstrates that the DPP-4 inhibitor, linagliptin, does not significantly alter body weight in an established animal model of obesity, insulin resistance, and impaired glucose tolerance [25,28]. In addition, linagliptin did not significantly affect food or water intake during the studies and no significant changes in carcass composition were evident at termination. Such findings are consistent with both preclinical [20] and clinical [16,29,30] reports which suggest that DPP-4 inhibitors are weight-neutral. Accordingly, the present data reinforce suggestions that DPP-4 inhibition may prove to be a useful strategy for the treatment of diabetes since, in contrast to other drug classes (e.g., thiazolidinediones, sulphonylureas, insulin, etc. [31]), DPP-4 inhibitors are unlikely to promote weight gain, a major causative factor in the development of diabetes.

Although exhibiting dietary-induced obesity, the rats used in the present studies did not exhibit a diabetic phenotype. Hence, in contrast to the reported effect of DPP-4 inhibition in diabetic animals [15,20,32], it was not unexpected that linagliptin had no significant effect on plasma glucose levels and HbA1c in the present studies. Similarly, exenatide also failed to significantly reduce plasma glucose in the present studies. As expected after long-term exposure to a cafeteria diet, the animals exhibited insulin resistance characterized by a moderate hyperinsulinaemia. Linagliptin treatment did not significantly reduce plasma insulin levels after chronic dosing, though performance of glucose tolerance tests may have detected drug-induced improvements in insulin sensitivity. That said, the principal goal of the present studies was to assess the effect of linagliptin on body weight and not on glucose control, which has been previously reported [20,23].

The GLP-1 receptor agonist, exenatide, is approved in the United States and Europe for the treatment of T2DM in patients whose glycaemia is inadequately controlled by metformin and/or a sulfonylurea [33]. In addition to improving glucose control, exenatide also reduces body weight in both animals [26,34] and humans [33]. In agreement with the results from a previous study [26], the present data demonstrate that not only does exenatide reduce body weight in DIO rats, but that this weight loss is attributable to a selective reduction in body fat. These results are in apparent contrast to...
the weight-neutral effect of linagliptin although both drugs are incretin-based therapies. However, the differential effect of linagliptin and exenatide on body weight may reflect the ability of the different drugs to affect central GLP-1 receptor activation in the hypothalamus which is linked to reductions in food intake and body weight [10]. Certainly, there is evidence that exenatide is brain penetrant [35], and the continuous infusion protocol used in the present study may lead to sufficient CNS exposure of exenatide to activate GLP-1 receptors and so reduce food intake and body weight. Although linagliptin does not enter the brain when given orally [36], circulating GLP-1 in the periphery may be expected to activate central GLP-1 receptors [37,38]. Whilst linagliptin significantly increased plasma levels of GLP-1 in the present studies, it may be the case that the exposure achieved in the periphery was not sufficient to affect central GLP-1 levels, such that reductions in food intake or body weight were evident. An alternative hypothesis is linked to the fact that there are other substrates for DPP-4, including PYY, a hormone reported to reduce food intake in laboratory animals and humans [39]. Specifically, PYY3-36 is produced by the action of the enzyme DPP-4 and, accordingly, administration of a DPP-4 inhibitor such as linagliptin may be expected to reduce circulating levels of PYY3-36. As a result, the endogenous action of PYY3-36 to inhibit food intake, and therefore reduce body weight, may be abolished in the presence of a DPP-4 inhibitor.

To extend the investigation of the effect of DPP-4 inhibition in the DIO model, the effect of a DPP-4 inhibitor on the body weight of animals either concurrently treated with, or withdrawn from, exenatide was assessed. Linagliptin did not augment the effect of a low (i.e., sub-maximal) dose of exenatide on body weight, food intake, or various plasma parameters. As previously discussed, the reason for this may be that the ability of exenatide to reduce food intake and body weight in rats is because of activation of centrally located GLP-1 receptors, and oral linagliptin treatment is unlikely to affect central levels of GLP-1.

To our knowledge, the present study is also the first investigation into the potential rebound weight gain in animals that have been withdrawn from a dose of exenatide that reduces body weight. Once transferred from exenatide to saline, animals tended to consume a greater amount of food each day than exenatide-treated counterparts and, accordingly, exhibited increased weight gain. This weight gain was characterized almost exclusively by the deposition of fat. Interestingly, during this withdrawal phase, body weight levels never exceeded those of vehicle-treated controls. This is consistent with a number of compounds that reduce body weight prior to withdrawal in the DIO rat model (e.g., [25]). Animals treated with linagliptin during the withdrawal phase also exhibited an increase in body weight gain. However, this body weight gain and the concomitant increase in body fat tended to be reduced compared with controls. This profile may be attributable to effects of drug treatments on the individual components of the cafeteria diet presented. Hence, the exenatide-induced reduction in body weight was associated with a selective reduction in the intake of the palatable chocolate diet. During exenatide treatment both vehicle- and linagliptin-treated animals exhibited a maintained reduction in chocolate intake although the reduction was more marked in the case of linagliptin (148 kJ less per day on average). Whilst high fat chow and peanut intake were increased in the linagliptin group, this was calculated only as an average of 124 kJ per day. These differences in the diet consumption may account for the delayed weight gain and reduced carcass fat of animals treated with linagliptin during exenatide withdrawal. If this hypothesis is true then it may illustrate the importance in using cafeteria diets to model human obesity since such changes may not be evident using a single diet high in fat. Why a DPP-4 inhibitor such as linagliptin would have an effect on diet preference during exenatide withdrawal but not in naïve animals is unclear.

At the conclusion of the study, while animals treated with linagliptin continued to exhibit significant reductions in both body weight and plasma leptin compared with vehicle-treated controls, the group of animals withdrawn from exenatide and dosed with vehicle did not. Furthermore, at termination, carcass fat was reduced by more than 10% in linagliptin-treated animals compared with vehicle-treated animals withdrawn from exenatide. Interestingly, reductions in carcass fat with linagliptin treatment were evident in both studies where animals were dosed with exenatide (either previously or concurrently), which may suggest that DPP-4 inhibitors potentiate fat loss when dosed with another incretin-based therapy. Such a finding is of particular interest in light of a recent study that has identified DPP-4 as a novel adipokine released by human adipocytes [40]. Indeed, not only is the expression of DPP-4 increased in the visceral adipose tissue of obese patients but also the release of DPP-4 from adipose tissue explants of obese patients is significantly greater than that seen in lean controls [39]. Furthermore, morbidly obese men exhibit elevated serum levels of DPP-4 compared with lean controls [40]. Accordingly, it is postulated that DPP-4 may exhibit a causative “link” between adipose tissue (i.e., obesity) and the development of metabolic diseases such as T2DM.

Since exenatide is not degraded by DPP-4 in mammals [41], reasons for the reduced weight gain following exenatide withdrawal in animals treated with linagliptin are unlikely to include linagliptin maintaining the activity of circulating exenatide by reducing its degradation. Linagliptin was found to increase endogenous plasma levels of GLP-1 in the present study; however, the reasons why it does not affect body weight when given alone, or in combination with a low dose of exenatide, but inhibits the body weight gain of animals putting weight on after treatment with a GLP-1 receptor agonist, are unclear. One explanation is that the effect of DPP-4 inhibitors on body weight is dependent upon weight status.
(i.e. whether an animal is weight-stable or actively putting on weight). Specifically, DPP-4 inhibitors may act to reduce body weight in animals actively increasing body weight through the deposition of fat. Such a condition may arise not only where animals have been withdrawn from a weight-reducing agent such as exenatide or sibutramine, but may also be evident where body weight is increased through the action of other clinically effective diabetes treatments (e.g., thiazolidinediones, sulphonylureas, etc).

Consistent with published data in both lean [26] and obese Zucker rats [34], the maximal effect of exenatide to reduce daily food intake in the present study was shortly after the onset of infusion (e.g., day 2) and, with time, the magnitude of the hypophagic effect of the drug reduced. This apparent tolerance is similar to that observed in preclinical studies with clinically effective anti-obesity agents such as sibutramine, fenfluramine, and rimonabant [42-44]. In the case of the present study, this profile may at least in part be due to the lack of stability of exenatide in the mini-pumps. Specifically, animals that continued to receive exenatide after pump replacement on day 11 would be predicted to exhibit a similar daily food intake to that on the day before implantation of the second pump. In contrast, subsequent to implantation of a second pump containing exenatide, animals showed a marked drop in food intake which was, as before, reduced with time. Accordingly, with increased duration in the mini-pump, it appears that the ability of exenatide to inhibit food intake, and potentially therefore body weight, is reduced. As a result, this dosing approach may underestimate the true efficacy of exenatide to reduce body weight in preclinical studies. Reasons for this are unlikely to include “dose dumping”, where all the exenatide is released over the first few days. It is only upon exenatide withdrawal that body weight increases towards control levels (i.e., importantly, exenatide is still maintaining effects on body weight up to and including 4 weeks after administration). Despite these caveats, the use of osmotic mini-pumps in the delivery of exenatide in laboratory rodents is widely used in scientific praxis [26,34] and may be superior to daily injection regimens where effects on body weight are often not evident [45].

Daily water intake was reduced during the first days of exenatide treatment but, subsequently, rebounded above vehicle levels before stabilizing at a daily intake similar to controls. To our knowledge this effect of exenatide has not been reported previously, since most published studies do not report water intake [25,35,46]. However, these changes in water intake confirm previous studies in our laboratory using the same dose of exenatide in the same cafeteria-fed DIO rat model (unpublished data). Interestingly, this bi-phasic response was evident after implantation of a second pump containing exenatide, again suggesting that exenatide may not be fully stable in the mini-pumps. Reasons for the bi-phasic effect of exenatide on water intake are unclear. Drinking behavior is often prandial in nature such that where an animal eats less food it also drinks less water. Hence, significant reductions in daily water intake are often observed with clinically effective obesity agents in preclinical studies [42,43]. However, a rebound water intake above control levels is not typically observed with such compounds [42,43] and this hyperdipsic effect with exenatide is unexpected.

In conclusion, the present work suggests that chronic administration of the novel DPP-4 inhibitor linagliptin does not have a major effect on body weight, food intake, or carcass composition in the DIO rat. As reported with other DPP-4 inhibitors, the data suggest that linagliptin is likely to be weight-neutral in the clinic. The present data are the first to demonstrate that DIO rats treated with exenatide lose weight compared with vehicle controls but regain this weight once withdrawn from the drug. Importantly, this body weight gain does not increase beyond the level of vehicle-treated controls and there is evidence that this weight gain, especially the increase in fat, may be reduced by treatment with a DPP-4 inhibitor. In the case that these findings also transfer to the clinical situation, the present data suggest that a treatment regimen inducing initial weight loss via GLP-1 receptor agonism is subsequently replaced by a DPP-4 inhibitor may have benefits for patients who may be experiencing unwanted side effects with exenatide. Although DPP-4 inhibition does not appear to augment the weight loss induced by exenatide, in light of the practice of polypharmacy for the treatment of diabetes, future studies investigating the combined effect of a DPP-4 inhibitor and exenatide on glycemic control may be warranted. Such studies may elucidate improved strategies for the treatment of T2DM in clinical practice.

CONCLUSION

Linagliptin does not significantly affect the body weight of DIO rats and does not augment the weight loss effect of a low dose of exenatide. Such a finding is consistent with the weight-neutral effect of other DPP-4 inhibitors in the clinic [16,29,30]. However, the present data suggest that a DPP-4 inhibitor such as linagliptin inhibits the weight regain (principally fat) of animals previously treated with a dose of exenatide that significantly reduced body weight. The mechanism responsible is currently unknown but may be attributable to effects of linagliptin on food preference during exenatide withdrawal. This is of special interest since DPP-4 has recently been identified as a novel adipokine released by human adipocytes [40]. Given also the beneficial cardiac and renal profile of Linagliptin (22,23,47), the present data suggest that a treatment regimen inducing initial weight loss via GLP-1 receptor agonism subsequently replaced by a DPP-4 inhibitor may have benefits for those patients experiencing unwanted side effects with exenatide.
Acknowledgment:
Editorial assistance was provided by Jennifer Edwards of Envision Scientific Solutions during the preparation of this paper and was supported by Boehringer Ingelheim. The authors were fully responsible for all content and editorial decisions, and were involved at all stages of manuscript development.

Declaration of Interest:
Rolf Grempler, Michael Mark, and Thomas Klein are employees of Boehringer Ingelheim Pharma GmbH & Co. Steven P. Vickers, Keith Dickinson, Gareth D. Birmingham, Helen L. Rowley, Katie R. Headland, and Sharon C. Cheetham are employees of RenaSci Consultancy Ltd., which is a fee-for-service contract research organization (CRO).

References:
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