

Liraglutide, a Long-Acting Glucagon-Like Peptide-1 Analog, Reduces Body Weight and Food Intake in Obese Candy-Fed Rats, Whereas a Dipeptidyl Peptidase-IV Inhibitor, Vildagliptin, Does Not

Kirsten Raun,¹ Pia von Voss,¹ Carsten F. Gotfredsen,² Valeria Golozoubova,¹ Bidda Rolin,¹ and Lotte Bjerre Knudsen¹

Metabolic effects of the glucagon-like peptide-1 analog liraglutide and the dipeptidyl peptidase-IV inhibitor vildagliptin were compared in rats made obese by supplementary candy feeding. Female Sprague-Dawley rats were randomized to 12-week diets of chow or chow plus candy. The latter were randomized for 12 further weeks to continue their diet while receiving 0.2 mg/kg liraglutide twice daily subcutaneously, 10 mg/kg vildagliptin twice daily orally, or vehicle or to revert to chow-only diet. Energy expenditure was measured, and oral glucose tolerance tests (OGTTs) were performed. Body composition was determined by dual-energy X-ray absorptiometry scanning, and pancreatic β -cell mass was determined by histology. Candy feeding increased weight, fat mass, and feeding-associated energy expenditure. Liraglutide or reversal to chow diet fully reversed weight and fat gains. Liraglutide was associated with decreased calorie intake and shifted food preference (increased chow/decreased candy consumption). Despite weight loss, liraglutide-treated rats did not decrease energy expenditure compared with candy-fed controls. Vildagliptin affected neither weight, food intake, nor energy expenditure. OGTTs, histology, and blood analyses indirectly suggested that both drugs increased insulin sensitivity. Liraglutide and vildagliptin inhibited obesity-associated increases in β -cell mass. This was associated with weight and fat mass normalization with liraglutide, but not vildagliptin, where the ratio of β -cell to body mass was low. *Diabetes* 56:8–15, 2007

Glucagon-like peptide-1 (GLP-1) is an incretin hormone released from intestinal L-cells into the circulation in response to dietary nutrient intake (1,2). In recent years, considerable interest has focused on the potential of GLP-1 as a novel

treatment strategy for type 2 diabetes (3,4). In this context, GLP-1 exerts a number of potentially useful endocrine actions. First, it is a potent antihyperglycemic hormone, inducing glucose-dependent stimulation of insulin secretion (1,2) while suppressing glucagon secretion (5,6). Such glucose-dependent action is particularly attractive because when the plasma glucose concentration is in the normal fasting range, GLP-1 no longer stimulates insulin to cause hypoglycemia (7). GLP-1 appears to restore the glucose sensitivity of pancreatic β -cells (8), with the mechanism possibly involving the increased expression of GLUT2 and glucokinase (9). GLP-1 is also known to inhibit pancreatic β -cell apoptosis and stimulate the proliferation and differentiation of insulin-secreting β -cells (10–12). In addition, GLP-1 inhibits gastric secretion and motility (13–15). This delays and protracts carbohydrate absorption and contributes to a satiating effect (14,16–19). Furthermore, GLP-1 reduces feelings of hunger and caloric consumption in humans, including overweight individuals with type 2 diabetes (18). GLP-1 reduces body weight when administered by intracerebroventricular route in animals (20) and by subcutaneous route in humans (21). In conclusion, GLP-1 offers the promise of providing a treatment for type 2 diabetes that could stabilize or reverse disease progression.

However, despite very encouraging results with intravenous infusion (22), early attempts to use GLP-1 as a therapeutic tool in subcutaneous injection met with limited success (6) because of the rapid, extensive metabolism of GLP-1 that occurs via the ubiquitous enzyme dipeptidyl peptidase IV (DPP-IV) (23). Consequently, recent attempts to modulate the level of GLP-1 to therapeutic advantage have concentrated on two broad strategies, namely the development of analogs of GLP-1 with protracted action or the development of DPP-IV inhibitors (24). The latter have proven glucose-lowering potential (25,26), but this action involves mechanisms other than rescue of GLP-1 (27). This is because DPP-IV is a nonspecific enzyme that acts on a number of additional peptide substrates, including GLP-2, glucose-dependent insulinotropic polypeptide, neuropeptide Y, peptide YY, growth hormone-releasing hormone, and various paracrine chemokines (28). Therefore, the antidiabetic pharmacological profiles of GLP-1 analogs and DPP-IV inhibitors may differ substantially (24). For example, the relative effects of these agents on appetite and body weight may differ. In this respect DPP-IV inhibition seems to have a limited

From the ¹Department of Pharmacology Research 3, Novo Nordisk A/S, Maaloev, Denmark; and the ²Department of Pharmacology Research 4, Novo Nordisk A/S, Maaloev, Denmark.

Address correspondence and reprint requests to K. Raun, Diabetes Pharmacology, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Maaloev, Denmark. E-mail: kira@novonordisk.com.

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AUC, area under the curve; DEXA, dual-energy X-ray absorptiometry; DIO, diet-induced obesity; DPP-IV, dipeptidyl peptidase-IV; GLP-1, glucagon-like peptide-1; OGTT, oral glucose tolerance test.

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effect (29), whereas a GLP-1 analog has been shown to lower body weight in animals (30), and GLP-1 has been shown to reduce body weight in humans (21). This contrast may be attributable to a strong correlation between the pharmacokinetic profile and pharmacodynamic effects of GLP-1 (31), whereas DPP-IV inhibition mainly impacts GLP-1 levels when it is secreted in the postprandial period (27).

In the current study, we compared a GLP-1 analog, liraglutide, and a DPP-IV inhibitor, vildagliptin, with respect to their effect on body weight, food ingestion, body composition, energy expenditure, and other parameters in rats. Liraglutide is a GLP-1 analog to which a 16-carbon fatty acid is attached, enabling reversible albumin binding. This results in a metabolically stable compound, partially resistant to DPP-IV, with a sufficient duration of action to enable once-daily administration by subcutaneous injection in the treatment of type 2 diabetes (32–34). Vildagliptin is an orally bioavailable *N*-substituted glycyl-2-cyanopyrrolidine DPP-IV inhibitor (25,35).

RESEARCH DESIGN AND METHODS

A total of 59 Sprague-Dawley female rats (Møllegaard Breeding and Research Centre, Skensved, Denmark) aged ~4 months at baseline were randomized into a series of groups. A control group (Chow) of 15 animals were fed on rat chow ad libitum throughout the experiment and were given vehicle orally for the final 12 weeks. The remaining animals, termed diet-induced obesity (DIO) rats, were fed ad libitum on rat chow and up to 20 g of candy per day for a period of 12 weeks. This “cafeteria diet” (36) represented a crude model of excess calorie consumption in human obesity. A series of different candies (sugar cubes, Droste chocolate [Droste Nederland, Vaassen, the Netherlands], Toffifee chocolate [Storck Danmark, Brøndby, Denmark], Marabou chocolate [Kraft Foods Danmark, Glostrup, Denmark], grape sugar, and chocolate biscuit) were provided on a daily rotational basis to tempt the rats to maximize their energy intake. After the initial 12 weeks, the candy-fed (DIO) rats were further randomized into treatment groups for another 12 weeks: 1) liraglutide 0.2 mg/kg s.c. twice daily ($n = 10$), 2) vildagliptin 10 mg/kg twice daily orally ($n = 10$), 3) control vehicle twice daily subcutaneously ($n = 7$), 4) control vehicle twice daily orally ($n = 7$), and 5) DIO-Chow (candy excluded from diet, reverting to chow only) plus vehicle twice daily orally ($n = 10$).

Previous experiments with vildagliptin showed DPP-IV activity <25% for >9–12 h (data not shown); therefore, we dosed vildagliptin twice daily to obtain 24-h inhibition. Liraglutide is dosed once daily in humans, but here it was administered twice daily because of its slightly shorter half-life in rodents. During the study, rats were caged two-by-two but kept separate by a dividing wall and fed individually ad libitum via a hopper system. All injections were given at 0730 and 1930 h. Laboratory lights were on from 0730 to 2030 h. The study was carried out in accordance with permission given by the experimental animal committee in Denmark.

Data collection and end points

Body weight and food consumption. Body weight was measured twice weekly, and food intake (chow and candy) was monitored every day. The calorie density of food (chow and candy) was obtained from the manufacturers.

Energy expenditure. Energy expenditure was measured at the end of treatment by indirect calorimetry (Oxymax equal flow system; Columbus Instruments, Columbus, OH). The system was calibrated daily, with 12 rats tested per day. Oxygen and carbon dioxide concentrations in reference air and in the chambers were measured every 18 min. Energy expenditure (oxygen consumption) was calculated per metabolic weight ($\text{kg}^{0.75}$). At 1330, animals were weighed and placed in metabolic chambers with constant ventilation and access to water but not food. At 1930, animals received their assigned treatment and preweighed food. Lights were off at 2030. Next morning, at 0730 (lights on), remaining food was removed and weighed. Animals received scheduled treatment, and measurement continued until 1130, when they were returned to their home cages. Energy expenditure was measured and calculated for 10 rats of the liraglutide group, 9 of the vildagliptin group, 7 of the DIO subcutaneous control group, 7 of the DIO oral vehicle group, 10 of the DIO-Chow group, and 5 of the Chow controls. The means for each group were expressed relative to the means for the DIO control animals. Energy expenditure for the whole 20-h-long period (excluding the initial 1.5 h of adaptation) was calculated as the area under the curve (AUC) for each individual animal. The resting metabolic rate was calculated as the lowest oxygen consumption at any time during the period between 1500 and 1930 h (resting period).

Oral glucose tolerance test. At the end of the study, after an overnight fast (17 h), and immediately after their final drug doses, rats received 2 g/kg orally of 50% glucose solution (0.5 g/ml). Blood samples were taken (from the tail tip) just before dosing and at 15, 30, 45, 60, 90, 120, and 180 min. For glucose measurement, blood samples (10 μl) were diluted in 500 μl of buffer solution (Eppendorf, Hamburg, Germany) and kept on ice until analysis by immobilized glucose oxidase methodology (EBIO Plus autoanalyzer; Eppendorf). Plasma insulin measurement was also performed: 70 μl of tail tip blood was collected in glass capillary tubes containing heparin and kept on ice until centrifugation; 15 μl plasma samples were transferred to cooled micronic racks containing 30 μl bovine calf serum and stored at -20°C until analysis by in-house enzyme-linked immunosorbent assay.

Body composition and pancreatic histology. At the end of the study, rats were anesthetized and body composition measured using whole-body dual-energy X-ray absorptiometry (DEXA) scanning (pDEXA Sabre; Stratec Medintechnik, Norland Medical Systems, Pozheim, Germany). Rats were then killed, laparotomy was performed, and abdominal organs were macroscopically inspected.

The pancreas/intestinal block was removed and fixed in 4% paraformaldehyde overnight. Pancreata were then dissected, weighed, and fractionated. Tissue samples were dehydrated and embedded in paraffin before blinded observation for histological differences. Sections 3 μm thick were cut on a microtome. Sections were deparaffinized in xylene and rehydrated, and antigen retrieval treatment was carried out in 0.01 mol/l citrate buffer, pH 6.0 (preheated to 98°C). Sections were then cooled, rinsed, and endogenous peroxidase blocked by 20 min of incubation with 0.5% H_2O_2 . Finally, the sections were washed in water followed by Tris-buffered saline plus 0.01% Triton X-100 and then ringed. The remaining immunohistochemical staining reactions were carried out in an Autostainer (Dako Danmark, Glostrup, Denmark). β -Cell mass was estimated using stereological point counting on sections immunohistochemically stained for insulin, counting two sections 250 μm apart. The same sections immunohistochemically stained for glucagon, somatostatin, and pancreatic polypeptide were used to estimate the mass of the non- β islet cells (sum of α -cells, δ -cells, and pancreatic polypeptide cells). The estimated cell volumes were expressed as a percentage of the total pancreatic volume, and they were also converted to milligrams of total cells by multiplication by pancreas weight or as milligrams of cells per kilogram body weight (37,38).

Plasma parameters. Blood samples were obtained 2 h after last injection and before death (rats were not fasted). Blood from the retro-orbital plexus was analyzed for A1C, free fatty acids, glucose, insulin, erythrocyte count, hematocrit, platelets, white blood cells, and hemoglobin. Aortic blood was analyzed for DPP-IV activity, adiponectin, GLP-1, intact and total GLP-2, fructosamine, glucagon, leptin, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides.

Statistical methods. Data are the means \pm SE. Weight change, food intake, and energy expenditure parameters were compared across groups using one-way ANOVA, with Bonferroni multiple comparison subtests. Quantified histological differences in the pancreata were tested by Student's *t* test. All energy expenditure-related parameters of rats receiving vehicle, either subcutaneous or orally, were calculated separately and compared, and, because no between-group differences were observed, these groups were pooled (DIO control) for subsequent analyses. For similar reasons, data for the DIO control groups were also pooled for analyses of body composition.

RESULTS

Body weight and food intake. Mean body weight values were similar across the candy-fed groups at the initiation of the 12-week treatment period (liraglutide: 314.92 ± 11.16 g; vildagliptin: 316.56 ± 8.86 ; DIO control [pooled control]: 317.71 ± 6.53 ; and DIO-Chow: 316.16 ± 8.45). During the treatment period, this between-group parity changed, as shown in Fig. 1, with liraglutide and DIO-Chow rats showing a relative decline in weight. Liraglutide-treated rats weighed less than all other DIO groups, and there was a significant difference in absolute mean weight at end point comparing liraglutide with DIO control and with vildagliptin (Table 1).

The effects of treatment on accumulated total food intake are shown in Fig. 2. DIO rats preferred candy over chow and obtained 75% of their calories from candy, gaining 15–20% more weight than lean controls on chow. Compared with DIO control, vildagliptin had no significant

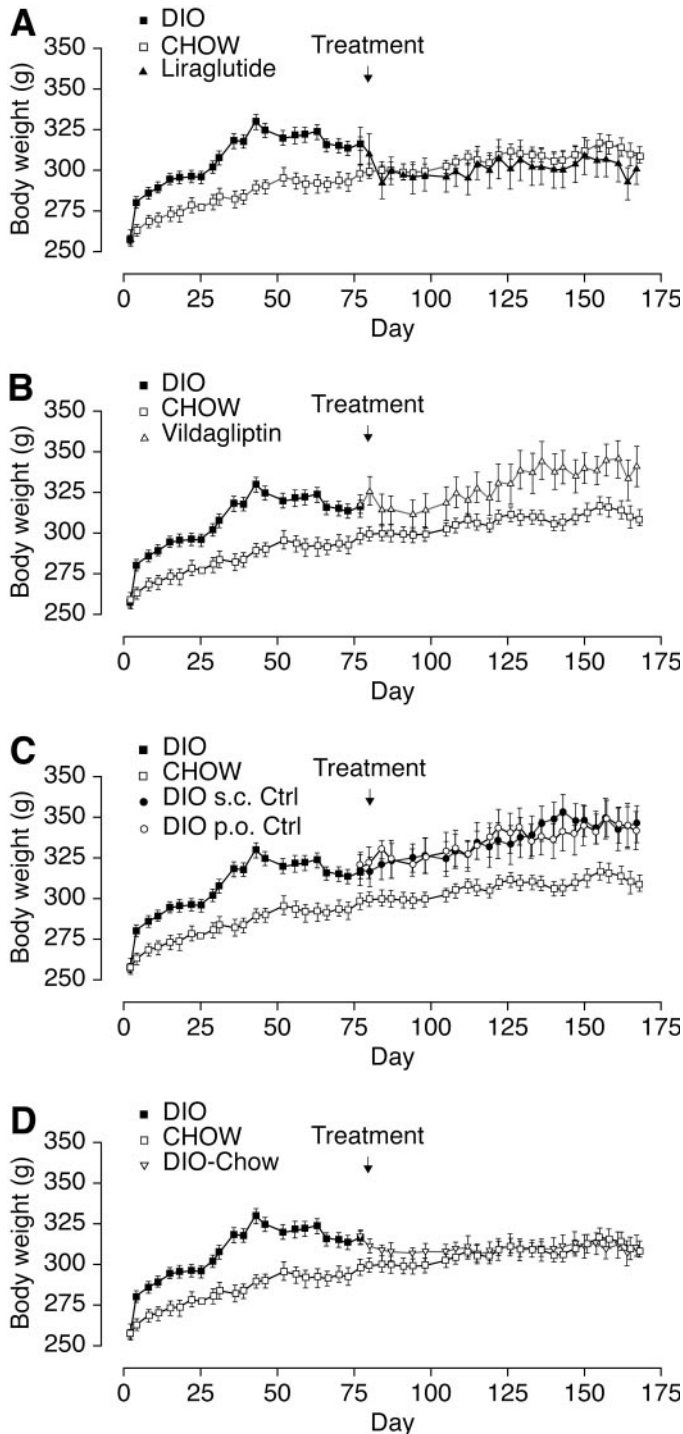


FIG. 1. Weight change over time in candy-fed obese rats treated with liraglutide and vildagliptin and in candy-fed obese (DIO), candy-fed chow-treated (DIO-Chow), and lean control rats (Chow). **A:** Liraglutide (from day 80) compared with candy-fed and chow control rats. **B:** Vildagliptin (from day 80) compared with candy-fed and chow control rats. **C:** Candy-fed and chow control rats and vehicle treatment (from day 80). **D:** Candy-fed obese rats, fed only chow (from day 80), compared with candy-fed and chow control rats. Data are means \pm SE.

effect on calorie intake. Liraglutide administration was associated with a significant reduction in total calorie intake as well as significant changes in the choice of food, with a significant reduction in candy consumption (compared with DIO control and vildagliptin), accompanied by a relative increase in chow consumption (compared with DIO control).

Energy expenditure. Calculated per metabolic weight, the energy expenditure of DIO control rats was somewhat higher than Chow rats, the difference being most pronounced during the active (dark) period (Fig. 3). In the DIO-Chow rats, the return to normal body weight was associated with a reduction in the level of energy expenditure comparable with animals fed chow throughout, so that total energy expenditure was significantly lower ($11 \pm 2\%$) in the DIO-Chow compared with DIO control animals. The energy expenditure of liraglutide or vildagliptin rats did not differ from DIO controls (Fig. 3), despite a significant weight reduction in the liraglutide group. There were no significant between-group differences in resting metabolic rates, although a nonsignificant relative increase in resting energy expenditure of $\sim 10\%$ was observed in the liraglutide-treated rats compared with other groups.

OGTT. No significant between-group differences were seen in the OGTT, although there was a trend ($P = 0.09$) toward a reduction in AUC_{glucose} for both liraglutide and vildagliptin compared with DIO controls (Fig. 4). Insulin levels during the OGTT were elevated in all DIO rats compared with the Chow group, with no significant difference between either liraglutide or vildagliptin and DIO control rats.

Body composition. DEXA scanning showed that DIO was associated with an increase in the percentage of fat mass (Table 2), which correlated with body weight ($R = 0.6981$, $P < 0.001$). Compared with DIO controls, Chow and DIO-Chow rats had a lower percentage of fat mass. The liraglutide group also showed a relative reduction in fat mass (when compared with DIO controls and vildagliptin), but there were no body compositional differences between vildagliptin and DIO control rats. In terms of bone mineral content (percent), there was a small but significant relative increase comparing liraglutide with vildagliptin. There were no between-group differences in the length of tibia, suggesting longitudinal growth was unaffected by treatment.

Pancreatic histology. Histological examination of pancreata showed that candy feeding without pharmacological intervention increased total β -cell mass (Fig. 5). In the liraglutide and vildagliptin groups, these values were normalized, and in the liraglutide group they were associated with a significant weight loss, whereas in the vildagliptin group, the weight remained the same as in the DIO control rats. Thus, at equivalent body weights, vildagliptin-treated rats had a 32% lower β -cell mass than DIO control rats. Neither candy feeding nor liraglutide or vildagliptin had any effect on pancreatic non- β -cell mass (data not shown). Figure 5 also shows representative fields of pancreas sections double stained for β -cells and non- β -cells, with inserts of low power overviews. There were no significant differences between the groups with respect to the overall islet morphology or staining intensities for β -cells and non- β -cells.

Hematology and plasma parameters. No major differences were noted in the hematologic parameters, although a few minor differences reached statistical significance. The relative reduction in hematocrit in the liraglutide group (42%) was statistically significant compared with the vildagliptin and DIO control groups (44%, $P < 0.01$ for both comparisons) and in the Chow groups ($P < 0.05$). Hemoglobin was also significantly lower in the liraglutide group (8.6 mmol/l) compared with DIO control (9.1 mmol/l, $P < 0.01$) and Chow (9.2 mmol/l, $P < 0.001$) groups. There were no major differences in glucose or A1C, although a small relative increase with liraglutide (3.9%) compared with

TABLE 1

Body weight at end point and weight change by study group in candy-fed obese (DIO) and lean control rats (CHOW), and in candy-fed obese liraglutide, vildagliptin, or chow-only treated rats (DIO-Chow), all for 12 weeks

	CHOW	Liraglutide	Vildagliptin	DIO-Ctrl	DIO-CHOW
Mean (g)	309 ± 5	301 ± 10*	341 ± 12	344 ± 8	312 ± 7
Significant differences		**			
	*	***			
Δ (g)	—	-14.2 ± 4.2	24.3 ± 6.0	26.0 ± 2.5	-4.2 ± 3.8
Significant differences		***			

		*			

Data are means ± SE, analyzed by ANOVA and Bonferroni multiple comparison substest. The rounded lines indicate between which groups the significance levels were obtained. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

Chow (3.8%) was statistically significant. Fructosamine levels were significantly increased in the chow-fed control rats (757 $\mu\text{mol/ml}$, $P < 0.05$ –0.01) compared with the other groups (582–586 $\mu\text{mol/ml}$). However, all of these values were within normal ranges.

DPP-IV activity was significantly decreased in vildagliptin-treated rats (2%, $P < 0.001$) compared with the other groups (~41–45%), as expected. There were no major differences in GLP-1 levels, and there were no major changes in intact GLP-2, although total GLP-2, surprisingly, was significantly decreased in vildagliptin-treated rats (130 pmol, $P < 0.01$) compared with the other groups (141–214 pmol).

Insulin levels were significantly higher in the vildagliptin group (112 pmol) compared with DIO control (84 pmol, $P < 0.01$), Chow (79 pmol, $P < 0.05$), and liraglutide (70 pmol, $P < 0.01$) groups. Leptin levels showed a significant relative increase in the vildagliptin (6.2 ng/ml, $P < 0.05$) and DIO control (6.2 ng/ml, $P < 0.05$) groups compared

with the other groups (1.8–3.6 ng/ml). Adiponectin was also increased in the vildagliptin (7.1 ng/ml, $P < 0.05$) and DIO control (7.7, $P < 0.05$) groups relative to the others (4.3–4.9 ng/ml). Increased leptin is consistent with the relative excess in fat mass in the DIO control and vildagliptin groups. Adiponectin was also increased in the DIO animals, in contrast to the decreased levels normally associated with obesity (39–41). However, several previous studies in DIO rats have found an increase in plasma adiponectin (42,43), indicating that this could be an initial response to increased adipose tissue and that the later decrease in adiponectin levels might be a consequence rather than a cause of obesity. There were no major differences in glucagon, although levels were higher in the DIO-Chow group (98 pg/ml) compared with vildagliptin (59 pg/ml, $P < 0.05$) and DIO controls (63 pg/ml, $P < 0.05$).

Compared with Chow (0.11 mmol/ml), free fatty acid levels were significantly higher in association with vildagliptin (0.18 mmol/ml, $P < 0.01$) and DIO controls (0.17 mmol/ml, $P < 0.05$). There were no major differences in HDL, LDL, and total cholesterol levels, although there were minor significant relative increases in total and HDL cholesterol in the Chow group. Triglycerides were significantly increased in the liraglutide (1.0 mmol/ml) and vildagliptin (1.0 mmol/ml) groups compared with vehicle-treated lean rats (0.6 mmol/ml, $P < 0.05$ both comparisons).

DISCUSSION

This study has highlighted differences that might be of clinical significance in terms of the influences of liraglutide and vildagliptin on metabolic responses to a high-calorie diet. The study showed that the high-candy diet caused rats to gain weight, and this was mostly attributable to an increase in fat mass, with corresponding relative increases in levels of the adipose-derived hormones adiponectin and leptin. There was an accompanying slight increase in energy expenditure, particularly during the dark phase of the cycle (Fig. 3). Vildagliptin did not influence the effect of the diet on these processes. In contrast, body weight in the liraglutide-treated group was normalized within 1 week to the level of Chow-fed lean control rats, and it remained at this level throughout the remainder of the

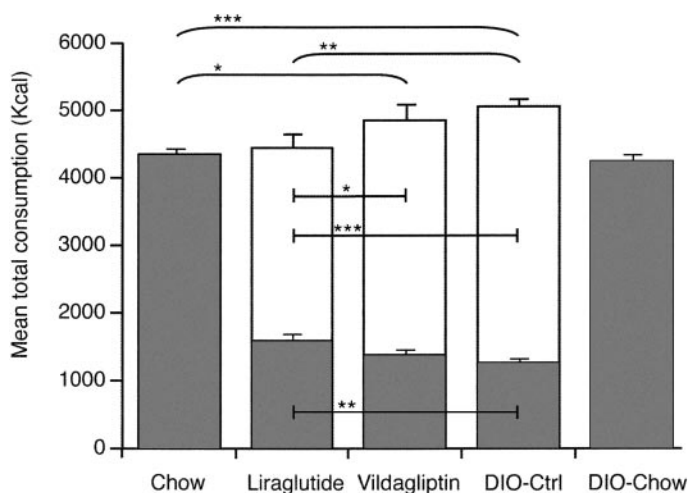


FIG. 2. Accumulated total, chow, and candy food intake by treatment group during the treatment period. Shown in grey is chow intake and in white total candy intake. Data are the means ± SE, analyzed by ANOVA and Bonferroni multiple comparison substest. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Ctrl, control.

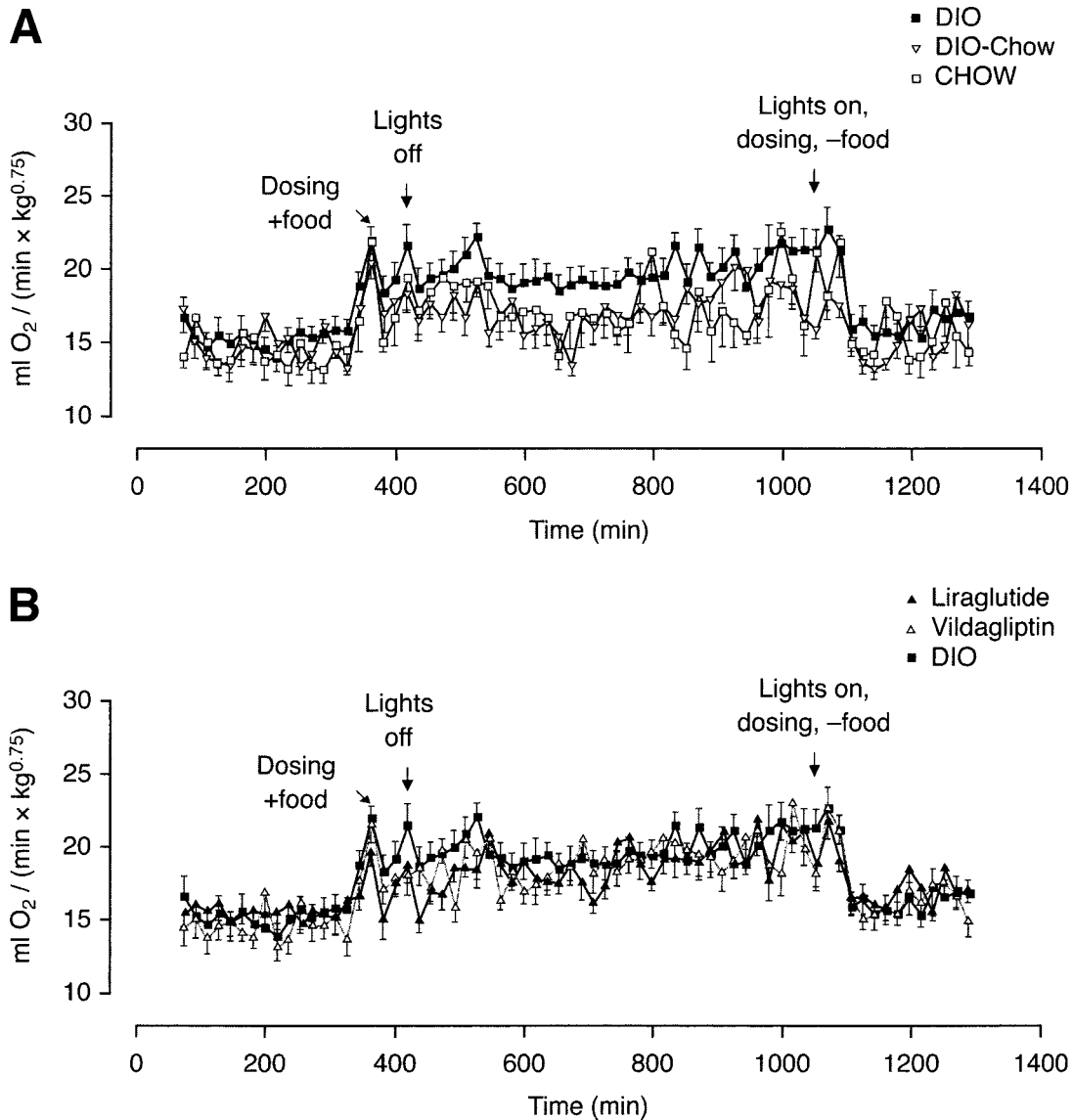


FIG. 3. Energy expenditure in liraglutide- and vildagliptin-treated rats, compared with candy-fed obese rats and control rats, measured during 22 h on the last day of 12 weeks treatment. *A:* Candy-fed obese rats (DIO); candy-fed obese rats, fed chow only in the treatment period (DIO-Chow); and chow-fed control rats (Chow). *B:* Liraglutide- and vildagliptin-treated rats and candy-fed obese (DIO) rats. Data are means \pm SE.

study. DEXA scanning showed that most of the weight loss in liraglutide-treated rats was attributable to a relative decrease in fat mass (Table 2). Thus, the effect of the candy diet on body weight and composition was essentially reversed by liraglutide.

Interestingly, this weight reduction appeared to result, at least in part, from an liraglutide-induced influence on choice of food; total calorie intake decreased during liraglutide treatment, and there was a marked shift during this treatment period in favor of chow rather than candy consumption. Other studies have shown liraglutide to be associated with anorectic and weight-losing properties in animal models of obesity (30,44) and in human type 2 diabetes (33), but this appears to be the first evidence of a qualitative effect of the drug on appetite, suggestive of a reduced craving for simple carbohydrates and/or fat.

It seems possible from the current data that some of the liraglutide-associated weight loss was attributable to a relative increase in energy expenditure. The resting metabolic rate was $\sim 10\%$ greater in liraglutide-treated rats in

comparison to all other groups, although this difference did not reach statistical significance. In addition, nocturnal (feeding-associated) energy expenditure was maintained at the same levels as in DIO control rats and vildagliptin-treated rats, even though liraglutide-treated animals were eating less. In contrast, the chow-fed rats and those switching from candy to the chow diet had relatively reduced feeding-associated energy expenditure. Thus, there was evidence that energy expenditure adjusted for weight increased with liraglutide. A previous, shorter study (30) showed liraglutide to reduce both weight and total energy expenditure (not adjusted for weight) in rats, so that energy expenditure adjusted for body weight was unaffected.

A treatment effect on the OGTT result is not to be expected in this study because the rats were not glucose intolerant and the GLP-1 effect on insulin secretion is glucose dependent. The lack of glucose intolerance is most likely attributable to our feeding regime, where the rats do not eat high-fat diet only, as other groups have

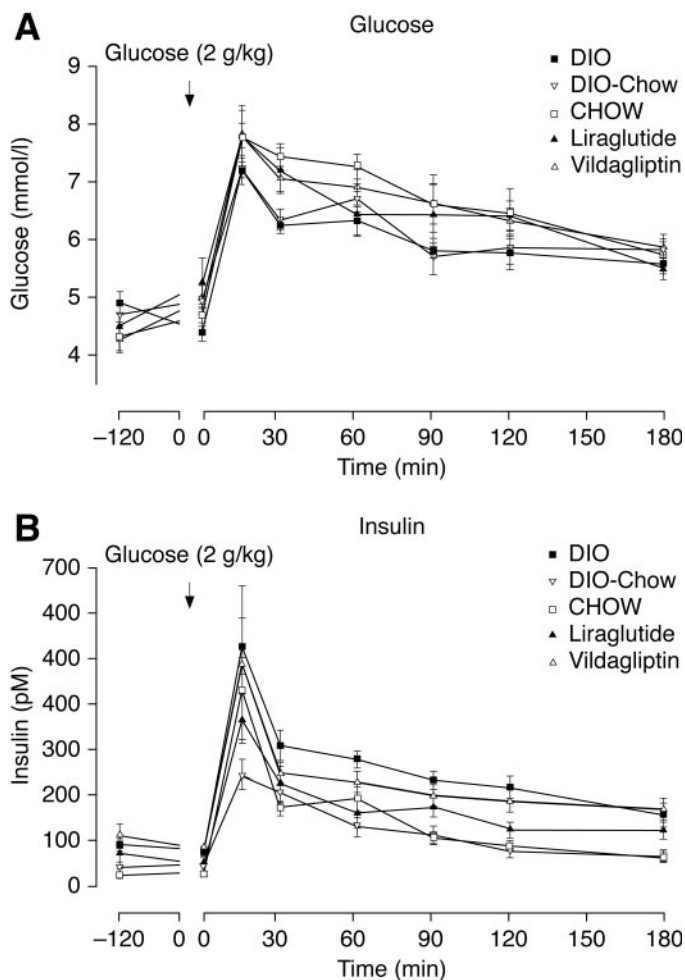


FIG. 4. Glucose (A) and insulin (B) responses to an OGTT immediately after the final drug dose, after a 17-h fasting period. Data are the means \pm SE.

shown, but can choose freely between candy and chow diet. Also of importance may be the observation that female rats become less obese than males. Whereas the OGTT showed no statistically significant between-group differences, the trend for a reduced AUC_{glucose} in both of the drug-treated groups, together with a similar insulin response in the DIO control group, point to an increased insulin sensitivity in drug-treated rats (Fig. 4). This investigation also suggested that the two drugs led to similar insulin-to-glucose ratios.

A close and dynamic correlation is known to exist between β -cell mass and total body weight throughout the lifetime of rodents, with changes in β -cell mass believed to reflect changes in metabolic demands (45). Both liraglutide and vildagliptin were associated with reductions in

the extent of this response, with the β -cell fraction of pancreas volume in drug-treated rats being smaller than that of DIO controls. The current observation of an attenuation in the obesity-associated increase in β -cell mass is therefore suggestive of a relative increase in insulin sensitivity or in β -cell function. It should be noted, however, that in experimental models of overt diabetes like *db/db* mice and ZDF rats, liraglutide has increased β -cell mass significantly (37,38), whereas no such data exist for vildagliptin. In overtly diabetic insulin-resistant rats, β -cells are degranulated, and liraglutide treatment and concomitant weight loss partially restore the insulin staining intensity (38), whereas in the current study, neither DIO nor the treatment with liraglutide or vildagliptin changed the general impression of a normal insulin staining intensity (content) of islets in all groups.

It should be noted that liraglutide-treated, but not vildagliptin-treated, rats lost weight, so the mechanism of action might differ fundamentally between the two drugs. In the case of liraglutide, it is possible that an increase in insulin sensitivity or β -cell function could occur at least partly as a result of normalization of weight and body composition. In contrast, vildagliptin might improve insulin sensitivity or β -cell function, despite an increase in total weight and fat mass, leading to a low ratio of β -cell mass to body mass. In fact, pharmacological studies have suggested that there are important differences between these drug classes with regard to their effect on glucose disposal. DPP-IV inhibition has been shown to have a direct effect on insulin sensitivity in rats (46). GLP-1 supplementation, in contrast, has been shown to enhance non-insulin-mediated glucose disposal while simultaneously increasing glucose-dependent insulin secretion in mice and humans, thereby mimicking enhanced insulin effectiveness (47–49).

DPP-IV activity was significantly decreased in vildagliptin-treated rats ($P < 0.001$) compared with the other groups, and there was a trend (though not significant) suggesting that this was associated with a preservation of GLP-1 and intact GLP-2 (data not shown). Another interesting observation was that vildagliptin was uniquely associated with a relative increase in the plasma insulin concentration. It can therefore be speculated that vildagliptin was acting to increase insulin secretion and sensitivity (presumably via preservation of several glucoregulatory peptides) and hence glucose disposal in the face of a diet-induced, obesity-associated state of insulin resistance. Liraglutide normalized body composition through effects on appetite and food selection in this model, possibly coupled with a relative increase in energy expenditure. Because of the glucose dependency of GLP-1 action on insulin release, an effect of a GLP-1 analog on insulin release would not be expected in a nondiabetic model. This is in contrast to the appetite-regulating effects

TABLE 2

Body composition (%) at end point in candy-fed obese (DIO) and lean control rats (Chow) and in candy-fed obese liraglutide-, vildagliptin-, or chow-only-treated rats (DIO-Chow)

	Chow	Liraglutide	Vildagliptin	DIO	DIO-Chow
Whole-body lean mass	83.3 \pm 1.2*	80.2 \pm 1.6†	72.9 \pm 3.2‡	70.9 \pm 1.6§	84.7 \pm 1.7
Whole-body fat mass	11.3 \pm 1.1*	15.5 \pm 1.7†	22.5 \pm 2.9‡	24.6 \pm 1.7	9.2 \pm 1.4
Whole-body bone mineral content	3.3 \pm 0.1	3.6 \pm 0.1†	3.3 \pm 0.1	3.5 \pm 0.1	3.3 \pm 0.1

Data are means \pm SE, analyzed by ANOVA and Bonferroni multiple comparison substest. * $P < 0.001$ vs. DIO control; † $P < 0.05$ vs. vildagliptin; ‡ $P < 0.001$ vs. Chow; § $P < 0.001$ vs. liraglutide; || $P < 0.01$ vs. liraglutide.

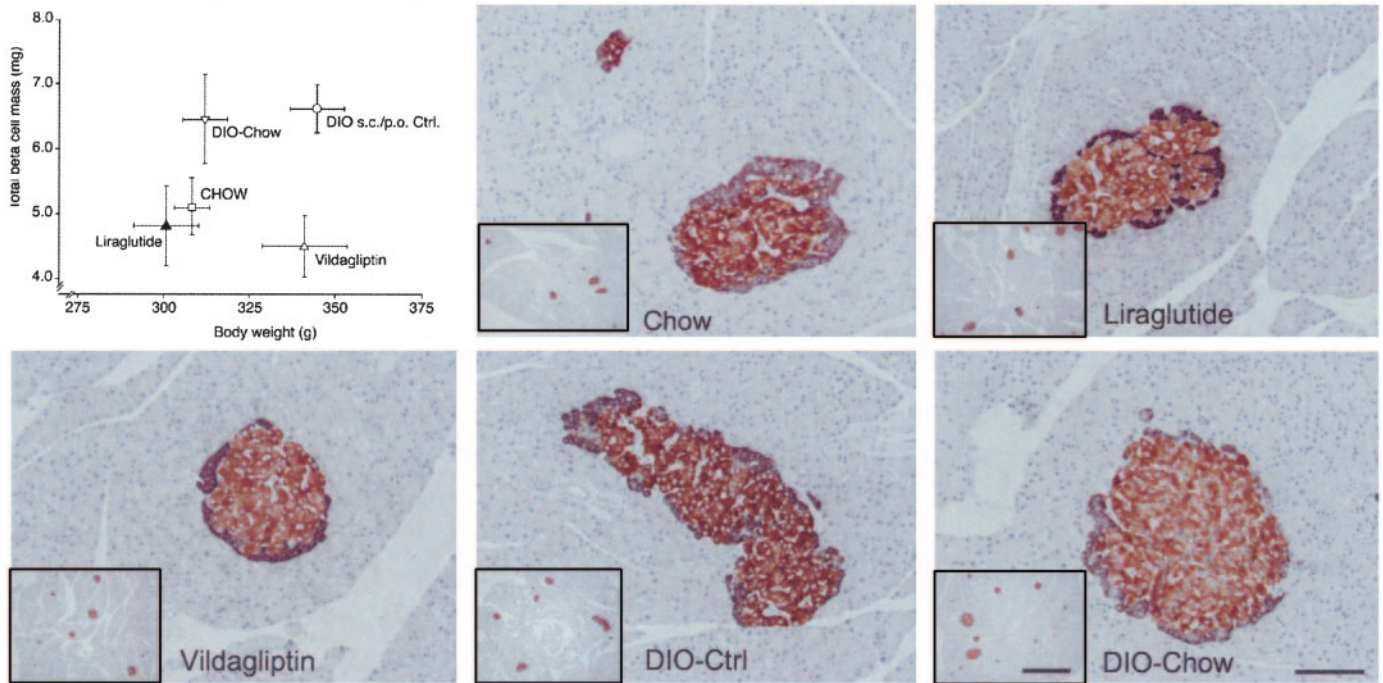


FIG. 5. Relative β -cell mass and weight. β -Cell mass and histology in candy-fed obese rats (DIO) treated with liraglutide, vildagliptin, or chow feeding (DIO-Chow) and compared with chow-fed nonobese rats (Chow). The top left line graph shows the total β -cell mass measured by stereology in relation to body weight, both shown as the means \pm SE values. The five plates with photomicrographs show representative fields of pancreas sections from the five treatment groups with β -cells stained reddish-brown and the sum of non- β -cells stained black. The large pictures were taken with a 20 \times objective, and the black bar shown in the DIO-Chow plate represents 100 μ m, the overviews shown in the inserts were taken with a 4 \times objective, and here the black bar represents 1,000 μ m. Ctrl, control.

of GLP-1, which do not seem to be glucose dependent, and therefore are operative in nondiabetic obese animals.

In summary, liraglutide normalized weight and body composition and reversed or limited most of the influences of the candy diet. In contrast, vildagliptin had no effect on weight, body composition, or energy expenditure. These observations exemplify differences in the profile of metabolic effects between GLP-1 analogs and DPP-IV inhibitors that may be of clinical relevance. Indeed, although the published clinical studies have not reported effects on body weight with vildagliptin (29,35), liraglutide has demonstrated a marked decrease in body weight in rats and pigs in preclinical studies (30,44), and a phase 2 clinical study showed liraglutide to be associated with a significant body weight decrease in comparison to glimepiride (33). Further research is required to elucidate the pharmacological mechanisms involved in these differences and to determine their clinical significance. The obvious inference of the current observations is that administration of an exogenous GLP-1 analog might represent a more promising treatment strategy than DPP-IV inhibition for type 2 diabetes when concurrent obesity is present.

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