Comparative Effects of the Long-Acting GLP-1 Receptor Ligands, Liraglutide and Exendin-4, on Food Intake and Body Weight Suppression in Rats

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The glucagon-like-peptide-1 receptor (GLP-1R) agonists, liraglutide (Victoza) and the synthetic product of exendin-4 (Byetta), are approved for type II diabetes mellitus (T2DM) treatment and may be efficacious in obesity treatment as well, in part, due to the drugs' resistance to enzymatic degradation and prolonged half-life relative to endogenous GLP-1. To address the need to directly compare the food intake- and body weight-suppressive effects of these two GLP-1R ligands, acute and chronic dosing experiments were performed. Once-daily (q.d.) exendin-4 (0, 0.33, 1.5, and 3.0 µg/kg) and liraglutide (0, 50, 100, and 300 µg/kg, q.d.) both reduced the chow intake in nonobese rats in a dose-dependent fashion following either intraperitoneal (IP) or subcutaneous (SC) administration, whereas only liraglutide reduced 24 and 48 h body weight in nonobese, chow-maintained rats. Chow intake and body weight suppression by liraglutide were of greater magnitude and shorter latency following IP compared to SC delivery, whereas for exendin-4, the magnitude of intake-suppression was similar for IP and SC administration. The effects of chronic delivery (7 consecutive days; IP) of liraglutide (25 and 50 µg/kg; q.d.) and exendin-4 (3 µg/kg; q.d. and twice-daily (b.i.d.)) on food intake and body weight were also examined in diet-induced obese (DIO) rats. Liraglutide (50 µg/kg q.d.) and exendin-4 (3 µg/kg b.i.d.) were comparable in suppressing overall high fat/sucrose diet (HFS; 60% kcal from fat) intake. Both drugs regimens yielded marked weight loss over the 7-day period. The weight loss effect of liraglutide was achieved in the first 2 days and remained stable for the duration of the experiment; weight loss with exendin-4 appeared more linear over the 7-day period. In conclusion, administration of the GLP-1R ligands, exendin-4 (b.i.d.) and liraglutide (q.d.), lead to comparable and pronounced suppression of food intake and body weight in DIO rats, suggesting a potential role for these drugs as a clinical tool for obesity treatment.

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INTRODUCTION

The pharmaceutical development of drugs aimed at enhancing the glucoregulatory actions of the incretin hormone, glucagon-like-peptide-1 (GLP-1), has proven efficacious in the treatment of type II diabetes mellitus (T2DM) (see refs. 1,2 for review). Within minutes following food ingestion, GLP-1 is secreted from the "L" cells of the gastrointestinal tract resulting in the activation of GLP-1 receptors (GLP-1R) expressed on both dendritic terminals of vagal afferent fibers innervating the organs of the peritoneal cavity, as well as the pancreatic β-cells (3,4). Activation of these GLP-1R populations promotes glucose-dependent insulin secretion, slowing of gastric emptying, and glucose-dependent inhibition of glucagon secretion, together facilitating the rapid clearance, storage, and normalization of blood glucose (see refs. 3,4 for review). Under normal physiological conditions, however, degradation of endogenous GLP-1 by the enzyme dipeptidyl-peptidase-4 (DPP-IV) is very rapid and limits the half-life of GLP-1 to ~1–2 min (4). The development of GLP-1R agonists that are resistant to rapid enzymatic degradation (e.g., exendin-4 (Byetta), liraglutide (Victoza)), with prolonged half-lives in the order of hours, has further enhanced the merits of pharmacotherapies targeting the GLP-1 system for treatment of T2DM (5,6). Long-acting GLP-1R agonists such as exendin-4 and liraglutide may also prove efficacious for the treatment of obesity, a notion that is directly supported by accumulating clinical and preclinical animal studies

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showing that acute peripheral administration of these GLP-1R agonists reduces food intake and body weight (7–12). However, to date there have been no detailed studies comparing the food intake- and body weight-suppressive effects of the two leading long-acting GLP-1R ligands, exendin-4 and liraglutide.

Liraglutide is a potent synthetic GLP-1 analog with an additional amino acid-based spacer and 16-carbon fatty acid tail that enhances its binding affinity to albumin (13,14). Consequently, liraglutide has reduced renal excretion compared to endogenous GLP-1, has some resistance to degradation by ubiquitous endogenous enzymes (e.g., DDP-IV and neutral endopeptidases) (15), and has an approximate half-life of 13 h (13,14,16). Together with a potent incretin-response profile (7,17), the aforementioned properties have led to the recent US Food and Drug Administration-approved use of once-daily subcutaneous (SC) administration of liraglutide for the treatment of T2DM (5). Exendin-4, a naturally occurring 39 amino acid peptide extracted from the saliva of the Gila monster (*Heloderma suspectum*), is resistant to enzymatic degradation, accounting for its duration of action (approximate half-life of 2.5 h) (18,19). The US Food and Drug Administration-approved synthetic product of exendin-4, Byetta, is administered SC twice-daily in humans for T2DM treatment. While previous reports have compared the efficacy of liraglutide and exendin-4 to regulate blood glucose levels following various doses and routes of drug administration (6,16,18,20,21), there have been no studies reported that provide a dose–response comparison with varying routes of administration between these two GLP-1 agonists on food intake and body weight suppression.

Using a standard chow-maintained, nonobese rat model, we first compared the efficacy of acute liraglutide and exendin-4 administration to suppress food intake and body weight at varying doses and routes of administration (intraperitoneal (IP) vs. SC). Given that activation of GLP-1Rs expressed on vagal afferent fibers innervating the gastrointestinal tract and hepatoportal-bed appear to play a major role in mediating the intake-suppressive effects of systemic endogenous GLP-1, as well as exogenous GLP-1R agonists (see ref. 3 for review), we hypothesized that the intake-suppressive effects of the GLP-1R agonists would be greater and have a shorter latency following IP compared to SC administration. Having established dose–response comparisons for liraglutide and exendin-4 for both routes of administration, we next evaluated, in a separate experiment, the food intake and body weight suppression for each drug in high-fat diet-maintained, diet-induced obese (DIO) rats via repeated daily IP administrations of liraglutide (administered once-daily (q.d.) at two doses) and exendin-4 (administered either once- or twice-daily (b.i.d.) at one dose) over 7 days.

METHODS AND PROCEDURES

Subjects

Adult male Sprague–Dawley rats (Charles River Laboratories), housed individually in hanging metal cages under a 12-h light/dark cycle (lights on 1,200 h), had *ad libitum* access to rodent chow (Purina 5001; Purina St Louis, MO) and water except where noted. All procedures conformed to the institutional standards of The University of Pennsylvania Animal Care and Use Committee.

Procedures (experiment 1: acute dosing)

Rats weighing ~400 g were matched according to body weight and assigned to one of two groups differing by drug treatment: liraglutide ($n = 13$; gift of Novo Nordisk, Bagsvaerd, Denmark) or exendin-4 ($n = 12$; American Peptide, Sunnyvale, CA). Rats fed *ad libitum* were given one SC injection (volume = 1 ml/kg, 0.9% NaCl vehicle) immediately before dark cycle onset in a counterbalanced design using the following liraglutide doses: 0, 50, 100, and 300 μg/kg (1 μg = 266 pmol; liraglutide group), and the following exendin-4 doses: 0, 0.33, 1.5, and 3.0 μg/kg (1 μg = 246 pmol; exendin-4 group). Dose selections were designed to include a dose of each drug that produced a comparable intake-suppressive effect at the 6-h postadministration measurement. Subsequent chow intake was recorded at 1, 3, 6, 24, and 48 h (recorded to the nearest 0.1 g, spillage collected and accounted for), and body weights were recorded at 24 and 48 h postinjections. Injection treatments were separated by 3–4 days. Following the last SC injection treatment, the rats were given a 1-week rest period before beginning the acute IP injection phase of the experiment. IP liraglutide (liraglutide group) and exendin-4 (exendin-4 group) injections were again given in a counterbalanced fashion using the same doses and procedures that were used for SC administration, with the only difference being the route of drug administration.

Procedures (experiment 2: chronic dosing)

To examine the effects of chronic IP liraglutide and exendin-4 treatment in the obese DIO rat model, doses of each drug (50 μg/kg liraglutide, 3 μg/kg exendin-4) were chosen based on a comparable magnitude of chow intake-suppression at the 6 h time point following acute IP administration (experiment 1). Given that 6 h intake suppression was of a slightly larger magnitude following 50 μg/kg liraglutide compared to 3 μg/kg exendin-4, we also included a lower dose of liraglutide (25 μg/kg) for chronic treatment in experiment 2. Furthermore, based on the finding that the IP 50 μg/kg liraglutide dose also significantly suppressed intake and body weight at longer-term periods (i.e., 24 and 48 h), whereas IP 3 μg/kg exendin-4 did not, we included a treatment group in experiment 2 that received twice-daily (b.i.d.) IP administration of 3 μg/kg exendin-4 (injections separated by 6 h). Thus, this design includes treatments resembling clinical strategies for human T2DM treatment for Victoza (once-daily (q.d.)) and Byetta (b.i.d.); however, higher doses of liraglutide than that used in this experiment are being pursued in clinical trials for human obesity (7).

A separate group of rats weighing ~450 g were switched from standard lab chow (Purina 5001) to a high fat/sucrose (HFS) pelleted diet (60% kcal fat; Research Diets D12492, New Brunswick, NJ). Following 19 days of *ad libitum* HFS diet maintenance the rats were matched according to body weight (average body weight = 571 g) and divided into five groups ($n = 7$/group): (i) saline, (ii) liraglutide 25 μg/kg q.d., (iii) liraglutide 50 μg/kg q.d., (iv) exendin-4 3 μg/kg q.d., and (v) exendin-4 3 μg/kg b.i.d. For 7 consecutive days, rats were given a single IP injection (volume = 1 ml/kg) of their respective drug treatments immediately before dark cycle onset. A second daily IP injection was delivered 6 h later; this second injection was saline for all groups except for the exendin-4 3 μg/kg b.i.d. group, who received a second daily IP injection of exendin-4 (3 μg/kg). HFS diet intake was recorded on each of the 7 days at 1, 3, 6, and 24 h following the first daily injection. Body weight was recorded each day before the first daily injection.

Statistical analyses

Repeated measures ANOVAs were conducted across treatments (doses) and routes of administration (IP and SC) to assess the effects of acute IP and SC liraglutide and exendin-4 injections on subsequent food intake and body weight in experiment 1. Linear regression analyses using drug dose as a predictor variable were conducted to assess the dose-related nature of food intake and body weight suppression following liraglutide and exendin-4 administration. Experiment 2 analyses employed ANOVA with group as a between-subjects factor and day as a within-subjects factor to assess the effects of continuous daily administration of liraglutide and exendin-4 on HFS diet intake and body weight

change across days. To compare the intake-suppresive effects of liraglutide and exendin-4 in DIO, HFS-maintained rats with that observed in nonobese, chow-maintained rats, one-way ANOVA was employed comparing the percentage of chow intake suppression following drug treatments relative to saline treatments (experiment 1) with the percentage of HFS diet intake suppression on day 1 relative to the vehicle-treated group (experiment 2).

RESULTS

Experiment 1

Chow intake and body weight suppression following acute SC liraglutide and exendin-4 administration. Chow intake following acute SC liraglutide administration was suppressed relative to vehicle in a dose-related manner at 6, 24, and 48 h, with maximal intake suppression achieved at the 24 h time point (~18–63% depending upon dose) (**Figure 1a,b**). Intake suppression at each of these three time points was significantly different from vehicle for all three doses of liraglutide ($F(1,12) > 5.13$, $P < 0.05$). Linear regression analyses across doses supported the conclusion that intake suppression by liraglutide was dose-related at 6, 24, and 48 h ($R > 0.48$, $P < 0.001$). Following SC exendin-4 administration, intake suppression was significant only at the 3 and 6 h time points (1.5 and 3 µg/kg doses, $F(1,11) > 8.58$, $P < 0.01$) in a dose-related manner ($R > 0.54$, $P < 0.001$) with maximal intake suppression at 3 h after injections (~25–46%) (**Figure 1d,e**).

Body weight relative to saline was significantly reduced at 24 h for all three doses of SC liraglutide and the magnitude of weight loss was dose-related ($F(1,12) > 36.87$, $P < 0.001$) ($R = 0.92$, $P < 0.001$), and also at 48 h for the 100 and 300 µg/kg doses ($F(1,12) > 5.07$, $P < 0.05$) ($R = 0.78$, $P < 0.001$) (**Figure 1c**). By contrast, SC exendin-4 delivery did not significantly affect body weight at either 24 or 48 h ($F(1,11) < 1$) (**Figure 1f**).

Chow intake and body weight suppression following acute IP liraglutide and exendin-4 administration. Following acute IP administration, all doses of liraglutide significantly suppressed food intake relative to saline at 3, 6, 24, and 48 h ($F(1,12) > 11.33$, $P < 0.001$), with maximal intake suppression achieved at 24 h (~38–66%) (**Figure 2a,b**). Regression analyses supported a dose-related suppression of intake by IP liraglutide at each of these time points ($R > 0.43$, $P < 0.01$). Intake suppression following acute IP exendin-4 was significant relative to vehicle at 3 h (1.5

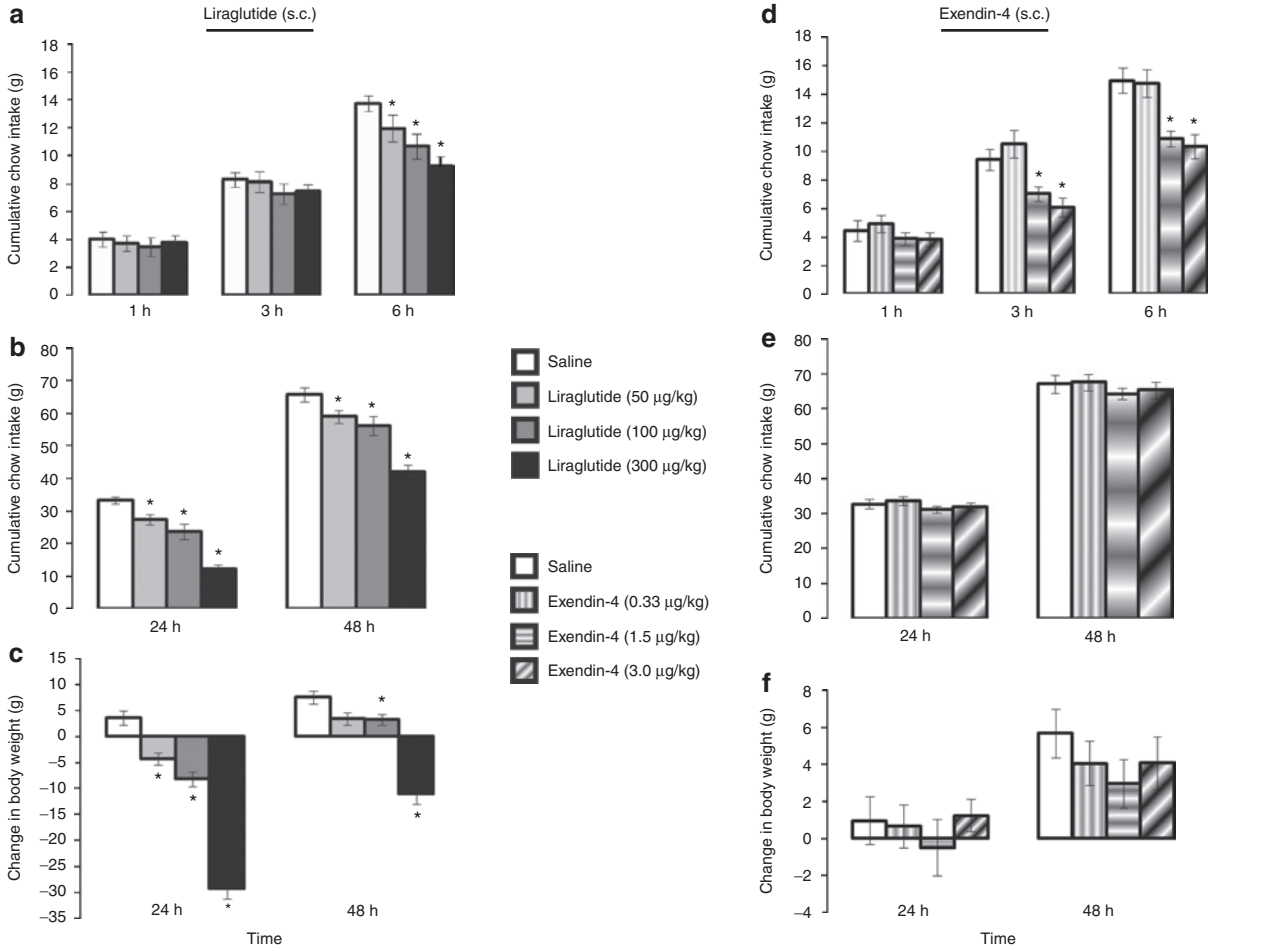

Figure 1 Cumulative chow intake at (**a**) 1 h, 3 h, 6 h, (**b**) 24 h, and 48 h, as well as (**c**) 24 and 48 h body weight change following subcutaneous (SC) liraglutide (0, 50, 100, and 300 µg/kg) administration at dark cycle onset. Cumulative chow intake at (**d**) 1 h, 3 h, 6 h, (**e**) 24 h, and 48 h, as well as (**f**) 24 and 48 h body weight change following SC exendin-4 (0, 0.33, 1.5, and 3.0 µg/kg) administration at dark cycle onset. *$P < 0.05$ from within-subject saline (vehicle) condition.

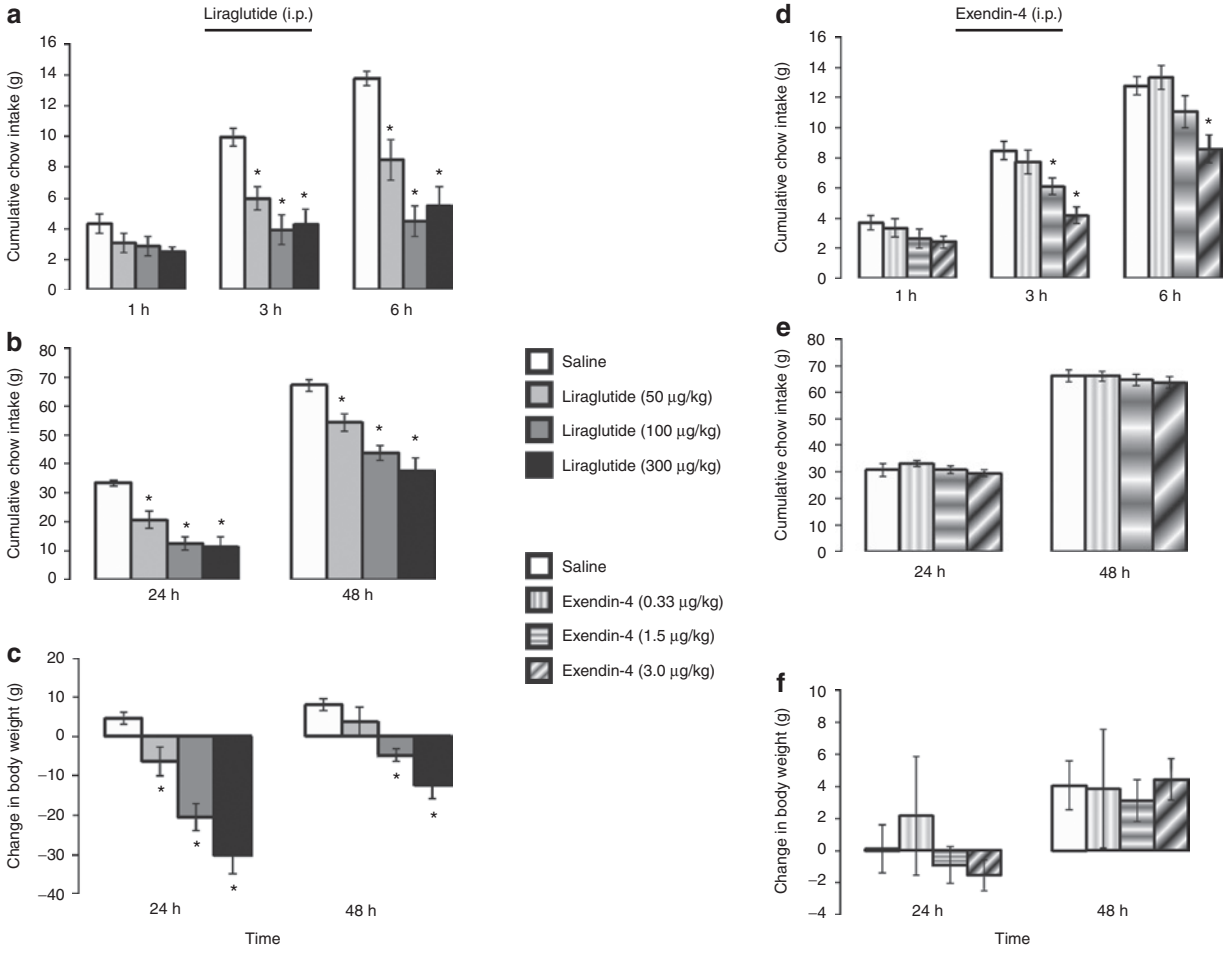

Figure 2 Cumulative chow intake at (**a**) 1 h, 3 h, 6 h, (**b**) 24 h, and 48 h, as well as (**c**) 24 and 48 h body weight change following intraperitoneal (IP) liraglutide (0, 50, 100, and 300 µg/kg) administration at dark cycle onset. Cumulative chow intake at (**d**) 1 h, 3 h, 6 h, (**e**) 24 h and 48 h, as well as (**f**) 24 and 48 h body weight change following IP exendin-4 (0, 0.33, 1.5, and 3.0 µg/kg) administration at dark cycle onset. $^*P < 0.05$ from within-subject saline (vehicle) condition.

and 3 µg/kg doses, $F(1,11) > 10.39$, $P < 0.01$) and at 6 h (3 µg/kg dose, $F(1,11) = 21.14$, $P < 0.001$) following a dose-related function ($R > 0.52$, $P < 0.001$), with the largest suppression by IP exendin-4 achieved at the 3 h time point (~28–50%) (**Figure 2d,e**). IP exendin-4 had no effect on 24 or 48 h food intake.

IP liraglutide significantly reduced body weight at 24 h (all doses, $F(1,12) > 7.71$, $P < 0.05$) ($R = 0.68$, $P < 0.0001$) and at 48 h (100 and 300 µg/kg, $F(1,12) > 34.44$, $P < 0.001$) ($R = 0.67$, $P < 0.0001$) (**Figure 2c**), whereas 24 and 48 h body weight were not influenced by IP exendin-4 ($F < 1.9$) (**Figure 2f**).

Comparison of the intake-suppressive effects for liraglutide and exendin-4 depending on peripheral route of administration. Food intake and body weight suppression by liraglutide were of greater magnitude and had a shorter latency following IP compared to SC delivery, whereas food intake suppression following IP exendin-4 was comparable to that following SC exendin-4. This conclusion was supported by multifactorial repeated measures ANOVA. For the liraglutide group, ANOVA combining the SC and IP food intake data yielded a significant interaction between route of administration (SC vs. IP) and drug treatment (0, 50, 100, and 300 µg/kg) at the 3 and 6 h time points ($F(3,27) > 4.66$, $P < 0.01$). Furthermore, this interaction showed a trend for significance at the 24 h ($F(3,27) = 2.73$, $P = 0.06$) and 48 h ($F(3,27) = 2.67$, $P = 0.067$) time points. Similarly, ANOVA combining SC and IP body weight data for the liraglutide group yielded a significant interaction between route of administration and drug treatment at 48 h ($F(3,30) = 4.99$, $P < 0.01$), and a trend for a significant interaction at 24 h ($F(3,33) = 2.74$, $P = 0.059$). When similar analyses were conducted for the exendin-4 group, however, this interaction was not significant at any time point for either food intake or body weight data (all $F(3,30) < 1$). Collectively, these analyses support the conclusion that the peripheral route of drug administration had a differential effect of food intake and body weight suppression for liraglutide (IP > SC), but not for exendin-4.

Experiment 2

HFS diet intake suppression in DIO rats following IP administration of liraglutide and exendin-4. In rats made obese through HFS diet maintenance, daily IP liraglutide (25 and 50 µg/kg q.d.) and exendin-4 (3 µg/kg q.d. and b.i.d.) significantly suppressed HFS

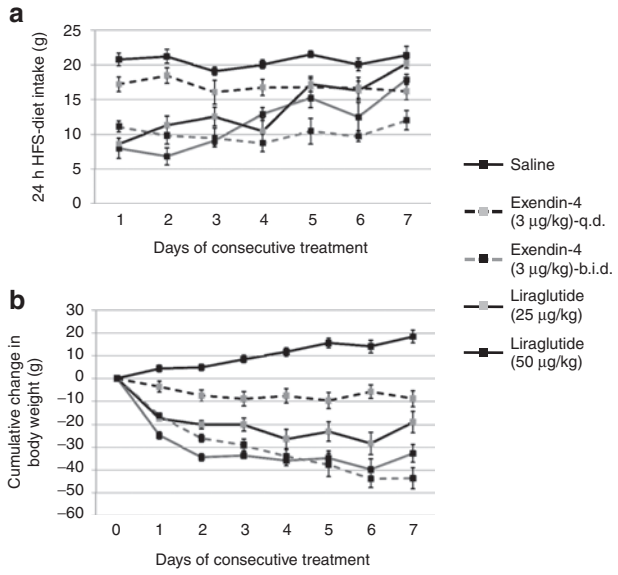

Figure 3 Daily (24 h) intake of (**a**) high fat/sucrose (HFS) diet and (**b**) cumulative daily change in body weight in diet-induced obese (DIO) rats following once-daily (q.d.) consecutive intraperitoneal (IP) administration of liraglutide (25 and 50 μg/kg) and exendin-4 (3 μg/kg-q.d.) or twice-daily (b.i.d.) IP administration of exendin-4 (3 μg/kg-b.i.d.) compared to saline (vehicle)-treated rats. Both doses of IP liraglutide (25 and 50 μg/kg q.d.) and exendin-4 (3 μg/kg q.d. and b.i.d.) significantly suppressed 7-day-overall HFS diet intake relative to saline-treated rats.

diet intake relative to saline-treated rats across the 7-day treatment period ($F(1,12) > 9.85$, $P < 0.01$) (Figure 3a). Intake suppression, however, was of a lesser magnitude for the exendin-4 3 μg/kg q.d. group relative to the other three groups, whereas the magnitude of the overall 7-day average intake suppression was comparable between the exendin-4 3 μg/kg b.i.d. group, liraglutide 25 μg/kg q.d. group, and liraglutide 50 μg/kg q.d. group. Planned comparisons between groups using repeated measures ANOVA across days showed that food intake for the exendin-4 3 μg/kg q.d. group significantly differed from the exendin-4 3 μg/kg b.i.d. group ($F(1,12) = 20.17$, $P < 0.001$) and liraglutide 50 μg/kg q.d. group ($F(1,12) = 15.64$, $P < 0.01$), and showed a nearly significant difference from liraglutide 25 μg/kg q.d. group ($F(1,12) = 4.37$, $P = 0.058$). These latter three groups did not significantly differ in overall intake. The pattern of intake suppression across the 7-day treatment period, however, was different for liraglutide-treated groups compared to exendin-4-treated groups. For the liraglutide 25 μg/kg q.d. and liraglutide 50 μg/kg q.d. groups, the magnitude of 24 h intake suppression was larger initially and then gradually reduced across the 7-day treatment period. On the other hand, the magnitude of food intake suppression remained relatively stable across the 7 days for the exendin-4 3 μg/kg q.d. and exendin-4 3 μg/kg b.i.d. groups. These observations were supported by repeated measures ANOVA that demonstrated significant group × day interactions between the liraglutide 25 μg/kg q.d. and exendin-4 3 μg/kg q.d. groups, liraglutide 25 μg/kg q.d. and exendin-4 3 μg/kg b.i.d. groups, liraglutide 50 μg/kg q.d. and exendin-4 3 μg/kg q.d. groups, and liraglutide 50 μg/kg q.d. and exendin-4 3 μg/kg b.i.d. groups ($F(6,72) > 6.6$, $P < 0.001$).

Body weight suppression in DIO rats following IP administration of liraglutide and exendin-4. Daily administration of both liraglutide (25 and 50 μg/kg q.d.) and exendin-4 (3 μg/kg q.d. and b.i.d.) suppressed body weight relative to saline-treated rats across the 7-day treatment period ($F(1,12) > 32.11$, $P < 0.001$) (Figure 3b). Similar to the results obtained for food intake, the magnitude of body weight suppression was smallest for the exendin-4 3 μg/kg q.d. group (significantly different from the three other groups, $F(1,12) > 12.16$, $P < 0.01$). Furthermore, body weight suppression for the liraglutide 25 μg/kg q.d. group was significantly less than that observed for the liraglutide 50 μg/kg q.d. and exendin-4 3 μg/kg b.i.d. groups ($F(1,12) > 6.7$, $P < 0.05$), whereas body weight suppression was comparable between the liraglutide 50 μg/kg q.d. and exendin-4 3 μg/kg b.i.d. groups ($F(1,12) < 0.1$), each producing an approximate of overall 50–60 g of weight loss relative to saline-treated rats by the end of the 7-day treatment period. As with food intake, the 7-day overall pattern of body weight suppression differed for liraglutide-treated groups compared to exendin-4-treated groups. Body weight was substantially suppressed for the liraglutide 25 μg/kg q.d. and liraglutide 50 μg/kg q.d. groups at the beginning of the 7-day treatment; that degree of weight loss remained stable over the remainder of the treatment period. On the other hand, body weight suppression was more gradual and linear for the exendin-4 3 μg/kg q.d. and exendin-4 3 μg/kg b.i.d. groups. These conclusions were supported by significant group × day interactions between the liraglutide 25 μg/kg q.d. and exendin-4 3 μg/kg q.d. groups, liraglutide 25 μg/kg q.d. and exendin-4 3 μg/kg b.i.d. groups, liraglutide 50 μg/kg q.d. and exendin-4 3 μg/kg q.d. groups, and liraglutide 50 μg/kg q.d. and exendin-4 b.i.d. groups ($F(6,72) > 2.39$, $P < 0.05$).

Comparison of intake suppression between nonobese, chow-maintained rats and DIO, HFS-maintained rats. To compare the intake-suppressive effects of liraglutide and exendin-4 in DIO rats consuming a HFS diet with that observed in nonobese rats consuming standard rodent chow, the % intake suppression (relative to saline treatments) of chow (experiment 1) and HFS diet (day 1 of experiment 2) following IP administration of 50 μg/kg liraglutide was compared across four time points (1, 3, 6, and 24 h) via repeated measures ANOVA. Similar analyses were conducted for percentage of intake suppression following IP exendin-4 (3 μg/kg q.d.) treatment in experiment 1 and experiment 2 (group exendin-4 3 μg/kg q.d). These analyses revealed that IP liraglutide (50 μg/kg) had a shorter latency for food intake suppression in nonobese, chow-maintained rats compared to DIO, HFS-maintained rats (Figure 4). A significant within-subjects intake suppression was observed following liraglutide 50 μg/kg at 3 h in chow-maintained rats ($F(1,11) = 9.55$, $P < 0.05$), whereas significant between-subjects intake suppression was not observed until the 6 h time point in HFS-maintained rats ($F(1,12) = 6.72$, $P < 0.05$). Following IP exendin-4 (3 μg/kg q.d.) administration, the pattern and latency of intake suppression across the time points did not differ between nonobese, chow-maintained and DIO, HFS-maintained rats. It should be noted, however, that the overall

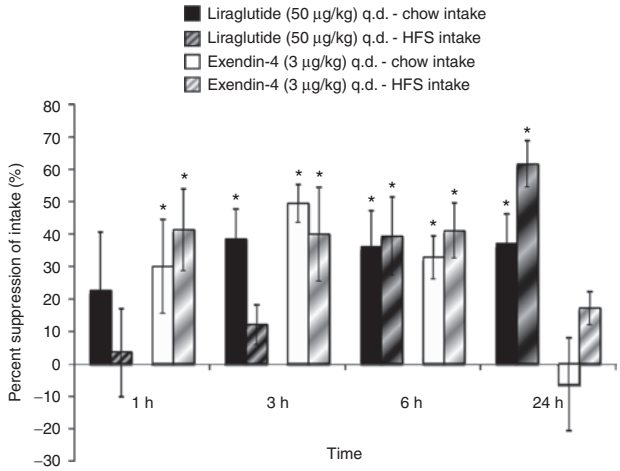

Figure 4 Comparison of the percent intake suppression (relative to saline treatments) of standard rodent chow and high fat/sucrose (HFS) diet following acute once-daily (q.d.) intraperitoneal (IP) administration of liraglutide (50 μg/kg) and exendin-4 (3 μg/kg). *$P < 0.05$ from intakes following saline treatment.

magnitude of intake suppression was comparable following liraglutide and exendin-4 administration for rats maintained on HFS diet compared to those maintained on chow.

DISCUSSION

The development of long-acting GLP-1R ligands, such as liraglutide and exendin-4, holds promise for pharmacological obesity treatment in humans. A number of preclinical studies have evaluated the intake and body weight suppressive effects of liraglutide (7,21,22) and exendin-4 (8,12,23–25) using various dosing and obesity-inducing strategies. However, a direct dose–response comparison of food intake and body weight suppression by these two GLP-1R ligands in normal weight, as well as obese animal models is lacking; the present studies addressed this need. Both ligands reduced food intake in a dose-related fashion following different peripheral routes of acute administration (IP and SC) in normal weight, chow-fed rats. Additionally, liraglutide and exendin-4 reduced food intake and body weight following chronic IP administration in rats made obese by maintenance on food high in fat and sucrose. The marked weight loss achieved with chronic treatment of liraglutide (q.d.) and exendin-4 (b.i.d.) in DIO rats highlights the potential for these long-acting GLP-1R ligands to be used as a pharmacological tool in the treatment of human obesity.

Liraglutide and exendin-4 suppressed food intake and produced a pronounced body weight loss in DIO rats across a 7-day treatment period involving continuous daily IP injections, a route of administration that readily accesses peripheral physiological GLP-1 sites of action (e.g., GLP-1R expressed on vagal afferents of gastrointestinal origin) (10). Results show that the overall intake and body weight suppression observed for the two drugs was comparable using treatment strategies that resemble clinical use of liraglutide (q.d.) and exendin-4 (b.i.d.) for human T2DM treatment. The pattern of intake suppression and body weight loss over time differed for the two drugs. For liraglutide-treated rats, the magnitude of intake suppression was larger during the first 2 days of treatment compared to the last 2 days; an observation that is consistent with previous reports (21). Yet, despite this relative reduction in the magnitude of intake suppression by liraglutide, the magnitude of body weight suppression was stable over the 7-day treatment period. This differs from the pattern of intake and weight suppression for exendin-4, where the magnitude of intake suppression was stable across the 7 days, contributing to a progressive reduction in body weight that was maximal at the end of the 7-day period.

It is unclear why the pattern of intake suppression but not body weight suppression changed across the 7-day liraglutide treatment in DIO rats. One possible explanation for the stability of the weight loss despite reduced food intake suppression is that daily liraglutide treatment led to a progressive reduction in water intake. Although water intake was not measured here, this explanation seems unlikely given that Larsen *et al.* (21) reported that the magnitude of both food and water intake suppression by chronic peripheral liraglutide treatment reduced across a 10-day treatment period. Another possible explanation for the reduction in the magnitude of food intake, but not body weight suppression by daily liraglutide administration is that this compound may also increase in energy expenditure. Liraglutide administered peripherally has been shown to produce a small, but nonsignificant increase resting metabolic rate in rats assessed by indirect calorimetry (21,26). Additional study of the energy expenditure effect of liraglutide is therefore needed. For exendin-4, acute peripheral and central administration in rats produces a mixed energetic picture that includes hypothermia (27) and tachycardia (27,28). Whether or not continuous daily treatment alters the direction of these effects is not known. Further examination of the effects of acute vs. chronic liraglutide and exendin-4 treatment on a common set of energy expenditure parameters in the DIO and the chow-fed rodent models is warranted.

Intake suppression following acute IP administration of liraglutide and exendin-4 were compared with that following acute SC administration, the route of administration used in humans for T2DM treatment by Victoza and Byetta (6). We hypothesized that the intake suppressive effects of the GLP-1R agonists would have a shorter latency following IP compared to SC administration based on the direct accessibility and activation of GLP-1R expressed on gastrointestinal vagal afferents that contribute to mediating intake suppression by GLP-1 and GLP-1R agonists (see refs. 3,4 for review). Results show that for liraglutide, but not for exendin-4, the latency of significant intake suppression was shorter for IP (3 h) relative to SC (6 h) administration. One explanation for this difference between ligands is that relative to exendin-4, the high albumin-binding-affinity of liraglutide may delay access to relevant vagal afferent GLP-1R populations within the peritoneal cavity when administered SC compared to IP. This notion is supported by research showing that the absorption kinetics of Byetta are much more rapid than that of liraglutide (29).

However, despite the differing latency of intake suppression following acute q.d. IP vs. SC administration of liraglutide, the overall magnitude of long-term (e.g., 24 and 48 h) food intake and body weight suppression for q.d. liraglutide did not differ by route of administration. Whether or not increased SC adiposity accompanying obesity alters the latency and efficacy of intake suppression following SC administration of these ligands requires further research. Also requiring further research is the need to determine the relevant GLP-1R site-of-action for IP and SC liraglutide and exendin-4-mediated suppression of intake. It is interesting to note that suppression of short-term intake (first meal) following GLP-1(7-36) administration into either the portal vein or vena cava, sites that may be of relevance to pharmacological GLP-1R ligand delivery, does not require vagal afferent mediation (10). These findings suggest the possibility that exendin-4 and liraglutide may reduce food intake by simultaneous activation of both peripheral (vagal) and central GLP-1 systems. However, to date, there is no report that directly examines such a hypothesis.

One limitation to the present study is that we did not find a range of doses for the two ligands that were equivalent in terms of food intake suppression. However, achieving such an equivalent dose range across multiple time points in an experiment employing an acute, once-daily administration procedure is nearly impossible given the large difference between the half-lives of the drugs (~2.5 vs. 13 h). For this reason, direct statistical comparisons for route of drug delivery were only done based on within-drug comparisons. Nevertheless, the present results are informative with regard to drug comparisons on the duration of action of these two GLP-1R ligands given that the dose ranges employed did include doses that produced comparable levels of intake suppression 6 h after administration (3.0 μg/kg exendin-4 and 50 μg/kg liraglutide).

For both liraglutide and exendin-4, the magnitude of intake suppressive effectiveness following acute IP administration was comparable between nonobese, chow-fed rats and rats made obese by HFS diet maintenance. One limitation of the comparative analysis used to support this conclusion is that intake suppression was contrasted across experiments, as opposed to a direct, within-study comparison. Nevertheless, the data suggest that for both liraglutide and exendin-4, the obese state does not result in any "resistance" to the intake suppressive effects of GLP-1R activation. Thus, whereas other reports have shown that maintenance on a high-fat diet and/or development of obesity leads to reduced sensitivity to the intake suppressive effects of other classic anorectic hormones, such as leptin (30–32) and cholecystokinin (33,34), no such alteration in sensitivity appears to occur for liraglutide and exendin-4. Despite the equivalent degree of intake suppression in chow-fed, nonobese rats compared to HFS-fed, DIO rats in response to either liraglutide and exendin-4 administration, the latency of intake suppression following acute IP liraglutide administration was shorter for chow-fed, nonobese rats (3 h) compared to that observed for HFS-fed, DIO rats (6 h), whereas this was not observed following exendin-4 administration. Whether or not this difference is based on metabolic status (nonobese vs. obese), overall maintenance diet (chow vs. HFS), or macronutrient composition of the diet requires further investigation.

In conclusion, the present research provides a direct comparison of the food intake and body weight suppressive effects of the long-acting GLP-1R agonists, liraglutide and exendin-4. Results show that both ligands suppressed food intake following acute IP and SC administration in a dose-related manner in nonobese rats. Intake and body weight were suppressed at 24 and 48 h after acute peripheral q.d. administration of liraglutide, whereas acute exendin-4 q.d. suppressed intake from 3 to 6 h, without influencing intake and body weight at 24 or 48 h. For liraglutide, but not for exendin-4, the latency of intake suppression was shorter and the magnitude of intake suppression was larger for IP compared to SC administration. In DIO rats consuming a HFS diet, continuous q.d. administration of liraglutide and b.i.d. administration of exendin-4 produced comparable, pronounced weight loss and comparable overall average magnitudes of food intake suppression. The present findings complement data from several other recent reports (7,22–24) and collectively highlight the therapeutic potential of liraglutide and exendin-4 in the treatment of human obesity.

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DISCLOSURE

The authors declared no conflict of interest.

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